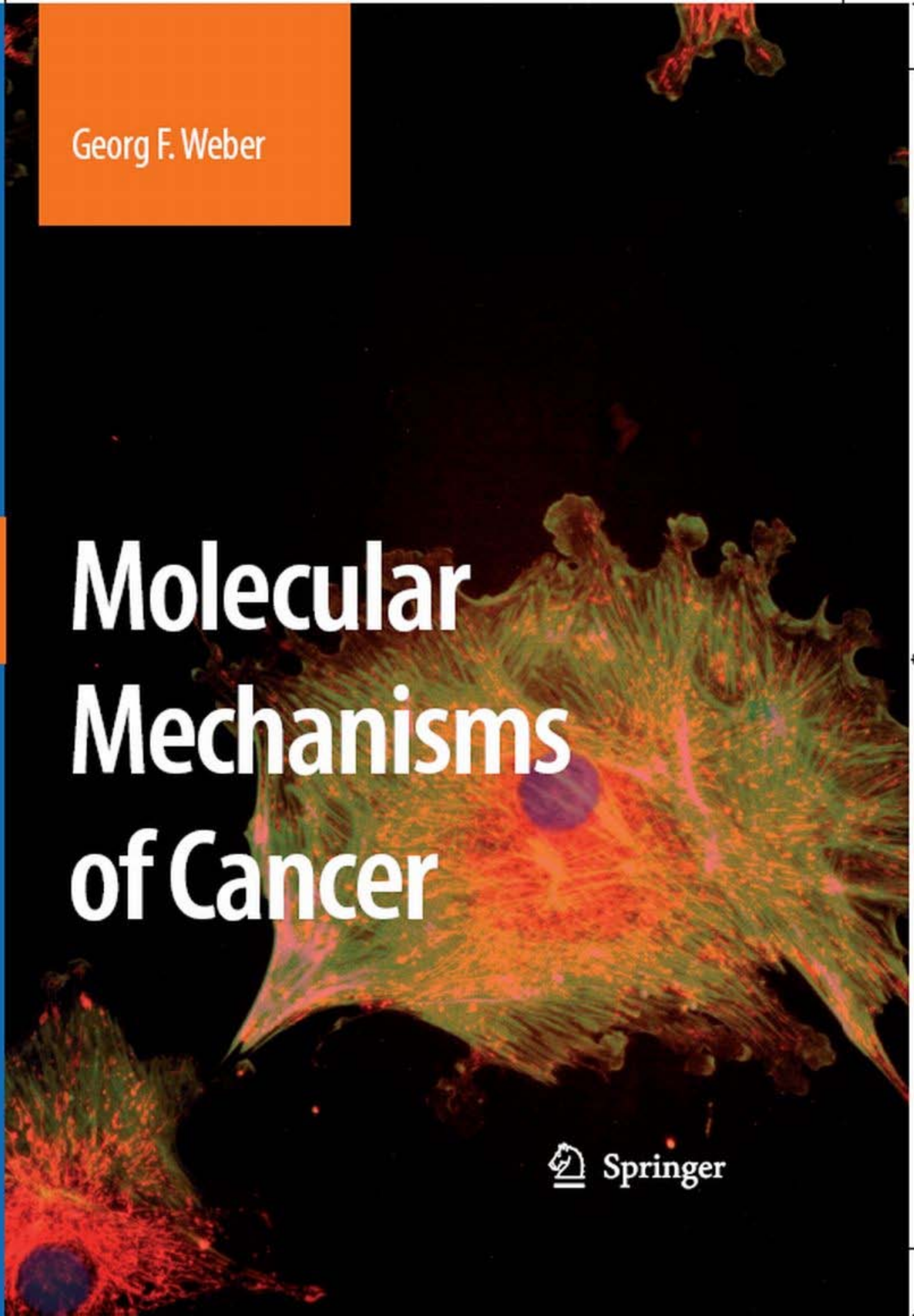
Georg F. Weber

Molecular Mechanisms of Cancer



Springer

MOLECULAR MECHANISMS OF CANCER

Molecular Mechanisms of Cancer

Georg F. Weber

University of Cincinnati Academic Health Center
Cincinnati, Ohio
USA

Springer

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 978-1-4020-6015-1 (HB)
ISBN 978-1-4020-6016-8 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Printed on acid-free paper

To those whose efforts
throughout the history of medicine
have been directed to
understanding and treating cancer,
so as to prevent premature deaths

CONDENSED TABLE OF CONTENTS

Section V. Molecular Mechanisms of Individual Malignancies

CONTENTS

INTRODUCTION

Cancer may constitute the most extensively studied disease entity of our time. Nevertheless, our comprehension of the cellular and molecular pathology of malignant transformation is incomplete. In view of the diverse clinical presentations of various malignancies, doubts may be raised as to whether it is appropriate to refer to cancer as one group of disease states. The notion of malignant tumors as a pathologic and pathophysiologic class of conditions begs the question for defining criteria that characterize all malignant growths, regardless of their tissue of origin. Toward this goal, the recognition that tumor development is caused by the dysregulation of growth-controlling genes (oncogenes and tumor-suppressor genes) has advanced our mechanistic understanding of oncology (Chapter 3). However, unrestricted cell division is not the only defining characteristic of cancer. Replicative senescence, if not overcome, would limit the growth of any tumor. Malignant cells are not subject to this control mechanism due to loss of function of senescence pathways or to aberrant activation of senescence-suppressor genes, the most prominent of which is Telomerase (Chapter 4). The aberrant expression of certain homing receptors, their ligands, and secreted proteinases leads to metastasis formation. The expression of these genes for invasiveness is atypical for the tissue type in which the tumor originates, and determines the organ preference of the resulting metastases (Chapter 5).

Defects in DNA repair mechanisms or epigenetic regulators of homeostasis increase the likelihood of transforming mutations and hence increase the cancer risk (Chapters 6 and 7). These biological functions constitute a second line of defense that protects against transforming defects in oncogenes or tumor-suppressor genes and are here considered as metasuppressor genes. Advances in the molecular explanations of growth dysregulation, metastasis formation, extension of life span, and loss of maintenance of genomic and epigenetic integrity in cancer suggest models for their causal connection. The mechanisms of growth control, senescence, and anchorage dependence are linked on the molecular level (Chapter 8). In cells that are not fully differentiated, the overactivation of oncogene pathways also induces the expression of metastasis genes. Telomerase, the enzyme that prevents cell senescence, is expressed in these precursor cells and may be further activated by growth factor signaling.

Because malignant tumors occur in the context of the host environment, their development depends on tumor–host interactions. While transformation itself is caused mainly by intrinsic alterations on the level of genetic defects, the progression and manifestation of malignant disease are stringently dependent on interactions with the host tissue. Those interactions comprise structural growth control (Chapter 9), organotropism in dissemination (Chapter 10), blood vessel supply (Chapter 11), the immune system (Chapter 12), and endocrine factors (Chapter 13). Dormancy and minimal residual disease are also manifestations of tumor–host interactions (Chapter 14).

It may appear, particularly at later stages of the disease, that cancer disrupts every aspect of physiology. Likewise, so many differences between transformed and nontransformed cells have been

reported in the literature that they may seem to include every component of cell functions. Such observations do not distinguish between causative and consequential changes associated with the acquisition of malignancy. In fact, the disruption or aberrant activation of only a fraction of genetic programs, encompassing a limited number of genes and their gene products, is causative for malignant growth. The genetic programs affected in this manner comprise those that are associated with growth control and cell survival, senescence, and invasion. Defects in DNA repair or in the epigenetic control over the relevant genetic programs set the stage for transformation. Once their dysregulation has irreversibly rendered a cell susceptible, the homeostasis is altered and even basic metabolic processes are changed.

The concept that has emerged is one that does not focus on individual cancer-related genes, but on genetic programs that are characterized by the coordinated and interdependent expression of multiple genes, whose products execute defined cell physiologic tasks. The cell cycle is completed by the harmonized activation of multiple genes. It is under the control of proofreading mechanisms, whose activation causes cell cycle arrest or apoptosis, and whose inactivation may facilitate transformation. Because senescence is permanent cell cycle arrest, some tumor-suppressor genes are also active in the genetic programs of senescence. A key feature of cancer cells is the ability to colonize distal and differentially specialized organs. This reflects the expression of genetic programs that are physiologically executed by stimulated immune system cells or tissue-resident precursor cells, but are not available to other fully differentiated cells. This points to tissue-resident precursor cells as likely origins of malignant transformation, because cell division in these cells is induced by tissue damage and is inevitably associated with inflammation. In these precursor cells, growth signals induce a stress response that involves the expression of homing receptors. These cells are also exempt of cellular senescence. Therefore, cancer is caused in immortal precursor cells by aberrant activation of growth control pathways that converge with genetic programs for homing. This important framework is discussed in Chapter 2.

Cancers are originally described as clinical entities. They are characterized by their symptoms and course of disease progression. Accordingly, various names have been given to diverse manifestations, even if they are based on identical genetic defects. As a case in point, familial adenomatous polyposis and Gardner syndrome are variants of the same disease, as both are caused by loss-of-function mutations in *apc*. The tumor predisposition syndromes now summarized under the term PTEN hamartoma syndromes were previously considered to be separate disease entities, all with their own name. Conversely, some clinically apparent homogeneous conditions may derive from distinct disease mechanisms. Diffuse large B-cell lymphoma has been considered one disease although it consists of two distinct groups of gene expression profiles. At a different level of assessment, pathologists describe tumors on the basis of their macroscopic and microscopic appearance. Histology has revealed new categories of tumors that were previously not distinguishable. Furthermore, this examination has resulted in a system of staging and grading of tumors that has improved therapeutic decisions. However, the diagnosis of tumors on the basis of their morphology is not based on pathogenetic mechanisms and has resulted in a plethora of descriptive names for the broad spectrum of human neoplasias. The definition of these growths on the basis of their underlying genetic defects and the ensuing steps of transformation on the biochemical and cellular levels can provide a mechanistic connection from molecular defects to morphologic changes to clinical symptoms (Section V). It can also focus cancer therapy on the transforming gene products, thus enhancing its efficacy and minimizing toxicity. The prototypic new-generation anticancer agents Herceptin, Rituxan, Gleevec, and Iressa are testament to this possibility.

This investigation will concern itself with basic mechanisms of malignant transformation. It will not analyze the details of diverse modes of carcinogenesis, such as irradiation, chemicals, or viruses. Nor will it go into strategies for cancer treatment and prevention. This study aims at defining general molecular requirements for the occurrence of invasive tumors. Following a reductionist approach, Section II first discusses the molecular basis of the intrinsic characteristics of cancer cells, alterations in cell cycle control and apoptosis, overcoming of replicative senescence, and invasive potential. It then describes secondary lines of defense (Section III), which include DNA repair (prevents onco-

genic mutations from gaining permanence) and the broad range of epigenetic mechanisms that regulate the function of cancer-related gene products (including the activities of chaperones, ubiquitination, sumolation, nucleosome modifications, and the intermediary metabolism). Finally, cancer occurs in the context of a host environment. Therefore, the importance of host factors, including structural growth control, angiogenesis, the immune system, and hormones, is discussed in Section IV. Section V outlines how the mechanisms developed in the preceding chapters apply to individual malignancies. Here the molecular mechanisms of carcinogenesis are put into the context of clinical symptoms and histopathologic descriptions. Individual molecules may contribute to multiple genetic programs, either because of convergence of distinct signal transduction pathways or because of context-dependent variations in their functions. This is the case for P53, which may be activated by G_1 checkpoint pathways as well as G_2 checkpoint pathways and, depending on the circumstances that are created by transcriptional coactivators, may induce either cell cycle arrest or apoptosis. P53 also plays an important role in replicative senescence. Such molecules are discussed in multiple places in this book according to their roles in various pathways.

This book makes an effort to apply stringent rules for nomenclature. The full names of proteins start with capital letters, while the acronyms referring to proteins are entirely capitalized. Genes are referred to in lowercase italics. In some cases, this does not agree with common use, but scientific consistency has priority over convention. For historical reasons, many molecules have multiple names. In those cases, the systematic or most common name is used, with alternatives listed in parentheses at the first occurrence in the text. Chromosome locations are provided in curly brackets. References are given in square brackets, and journal titles are not abbreviated.

SECTION I

GENERAL MECHANISMS OF TRANSFORMATION

CHAPTER 1

THEORIES OF CARCINOGENESIS

The oldest description of human cancer, referring to eight cases of tumors of the breast, was found in the Egyptian Edwin Smith Papyrus, written around 3000–1500 BC. The oldest specimens of human cancers were detected in the remains of a female skull dating back to the Bronze Age (1900–1600 BC), and in fossilized bones of ancient Egypt. The mummified skeletal remains of Peruvian Incas, dating about 2,400 years ago, contained lesions suggestive of malignant melanoma. The term "cancer" goes back to Hippocrates (460–370 BC), who named a group of diseases καρκινοσ and καρκινομα, the ancient Greek word for crab. It is a metaphor for the hard center and spiny projections of the tumors he studied. Cancer is the Latin word for crab and its use has been traced back to Galen (AD 129–199). A snapshot of theories of carcinogenesis, devised in the course of the last two centuries, reflects the progress of insight from the cellular level via biochemistry to an understanding of damaging influences and oncogenes, and to a more wholistic approach in the regulatory theory. It shows the relative success of reductionism as well as the current need to put the insights of various research endeavors into broader paradigmatic contexts.

1.1 CELLULAR THEORIES

In 1665, Robert Hooke described walled cavities in his microscopic examination of cork and called them cells. In 1805, Lorenz Oken conceptualized a cell-based theory of life, arguing that plants and animals are assemblages of tiny living infusoria. This notion was later populated and refined by Matthias Schleiden and Theodor Schwann [Schleiden 1838; Schwann 1839; Nurse 2000]. In 1841, Robert Remak (1815–1865) described the phenomenon of cell division in chick embryos and in muscle development. Between 1850 and 1855, he extended these observations to embryonic development and proposed that tumor cells arose by cell formation from existing specific tissues [Remak 1852, 1855]. Like Giovanni Morgagni, who had performed the first autopsy in 1761 and had correlated illness to macroscopic pathology, Rudolf Virchow (1821–1902) correlated illness to microscopic pathology. After initial skepticism, Virchow acknowledged Remak's evidence for cell division. In 1858, he gave a series of 20 lectures to a group of physicians at the Institute of Pathology in Berlin, in which he summarized his experience in microscopic anatomy of tissues with special attention to those deviating from the healthy condition [Virchow 1858]. According to Virchow's dictum "omnis cellula e cellule" cells of diseased tissues are derived from normal tissues, implying that malfunction begets disease (significantly, Virchow had been a student of Müller's, who had demonstrated in 1838 that cancer is made up of cells, not lymph; but he was of the opinion that cancer cells arose from interstitial budding elements, blastema, not from normal cells). Hence, tumors are derived from cells that divide faster than they should. The average human body experiences around 10^{16} cell divisions in a lifetime. With an individual's risk to contract cancer being about 10%, malignant transformation occurs in 1 out of 10^{17} cell divisions [Weinberg 1998]. The mechanistic underpinning for this process was defined by the identification of key regulators of the cell division cycle by Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse.

The analysis of transformation has been guided substantially by the technical accomplishment to expand cells in culture. Tissue culture was developed in the early years of the 20th century [Harrison 1907; Burrows 1910]. Warren Lewis cultured rodent cancers [Lewis 1936]. In 1951 at the Johns Hopkins Hospital, George Gey established human cancer cell culture from the cervical adenocarcinoma of the 30-year-old, black Henrietta Lacks [Gey et al. 1952]. Although the resulting HeLa cells are among the cornerstones of cancer research, their high rate of proliferation caused a risk for cross-contamination of other cultures by them. This lead to the establishment of cell-typing techniques on the biochemical [Gartler 1967] and genetic [Nelson-Rees et al. 1973] levels. Generally, the human tumor cells that grow permanently in culture are a selected group of very aggressive cancers. Almost all of the continuous cell lines are derived from high-grade, high-stage cancers.

Programmed cell death (apoptosis, Greek: falling off of tree leaves) [Kerr et al. 1972] may be invoked by many organisms as a control mechanism to prevent unrestricted growth. Research during the 1960s through 1970s in the worm *Caenorhabditis elegans*, identified *ced-3* and *ced-4* as essential genes for programmed cell death, while *ced-9* was found to be a negative regulator of apoptosis. The 2002, Nobel Prize in Medicine and Physiology was awarded for these observations to Sydney Brenner, H. Robert Horvitz, and John E. Sulston. The first mammalian homolog for *ced-3* was described as *bcl-2*, a gene that is involved in B-cell lymphomata [Negrini et al. 1987; Vaux et al. 1988]. *bcl-2* transfected B-lymphocytes are resistant to apoptosis, which is typically induced by Interleukin-3 withdrawal. For the first time, it was demonstrated that the pathway to tumorigenesis depends not only on the ability to escape growth control but also on the ability to prevent cell death [Hockenbery et al. 1990].

1.2 BIOCHEMICAL THEORIES

According to the biochemical theory of cancer, a key process that governs cell proliferation goes awry and causes transformation. Various aspects of metabolism may be affected in a manner that could lead to cancer. Consequently, before the discovery of oncogenes, a large variety of theories was debated, which incriminated the malfunction of diverse biochemical processes as causative for malignant transformation.

During tumor progression, the enzymatic composition of the affected cells is simplified (described as the theory of convergence in cancer), so that various cancers resemble one another more than they resemble their tissue of origin [Greenstein 1954]. As one possible underlying reason, the biochemist Otto von Warburg [von Warburg 1930] had suggested that the oxidative metabolism in cancer cells is replaced by glycolysis and that the excessive proliferation of cancer cells reflects their ability to metabolize independently of oxygen. Later, it was found that the limiting substrates for tumor growth are oxygen and glucose. Hence, anaerobic glycolysis is not the cause, but the consequence of the accelerated growth, which cannot be satisfied by the reorganization of the micro-vasculature [Vaupel et al. 1976]. However, in a remarkable reversal toward supporting the Warburg model, a 2005 publication showed that in cells engineered to become cancerous glycolytic conversion started early and expanded as the cells became more malignant [Ramanathan et al. 2005]. This rekindled the discussion of bioenergetics in cancer cells.

Others attributed the simplified enzyme patterns of cancerous cells to a regression of the tumor tissues to early embryonal stages of development. Highly malignant cells tend to resemble fetal tissues more than their adult normal counterparts do. The idea of derepressive dedifferentiation in carcinogenesis found support in the occurrence of onco-fetal proteins during the disease. The expression of these genes should be repressed in differentiated tissues, but this repression is reversed in tumors. The description of tumor tissue in histopathologic analysis as dedifferentiated is derived from this concept. The alternative model of "oncogeny as partially blocked ontogeny" suggested that cancer is the result of a series of alterations in the genes and their gene expression, which prevent a stem cell from completing all the steps necessary for terminal differentiation, suggesting that the target cell for carcinogenesis is the pluripotent stem cell [Potter 1978].

The protein deletion theory, an extension of the dedifferentiation theory, is an epigenetic model of cancer. Based on the observation that a carcinogenic aminoazo dye covalently bound liver proteins in animals undergoing early carcinogenesis, whereas little or no dye binding occurred to the proteins of

tumors induced by this dye, Miller and Miller [1947] proposed the deletion hypothesis. They suggested that carcinogenesis resulted from permanent alterations or loss of proteins that are essential for the control of growth. Thus, carcinogens eliminate specific enzymes from the affected cells by binding covalently to water-soluble basic proteins (h_2 proteins according to electrophoresis nomenclature). This causes the elimination (deletion) of these proteins from the cells. Cancer originates because the water-soluble basic proteins contain several growth inhibitory components. Therefore, the initial step in carcinogenesis is the inactivation of endogenous inhibition.

Transformation can be associated with refraction to exogenous inhibitors of cell cycle progression. Potter [1964] suggested that the proteins lost during carcinogenesis may be involved in the feedback control of enzyme systems required for cell division, and he proposed the feedback deletion theory. In this model, repressors crucial to the regulation of genes involved in cell proliferation are lost or inactivated by the action of oncogenic agents on the cell, either by interacting with DNA to block repressor gene transcription or by reacting directly with repressor proteins and inactivating them. It was thought that experimental evidence, in which the fusion of cancerous cells with nontransformed cells resulted in the absence of transformation, supported the epigenetic theory. Later, this phenomenon was attributed to the functional dominance of tumor suppressor genes.

The demonstration of the presence of an ordered biochemical imbalance, linked to transformation and progression, in cancer cells led to the molecular correlation concept. Weber [1977] stated that the biochemical dysregulations underlying neoplasia could be identified by elucidating the pattern of gene expression as revealed in the activity, concentration, and isozyme aspects of key enzymes and their linking with neoplastic transformation and progression. Key enzymes are involved in the regulation of rate and direction of the flux of competing synthetic and catabolic pathways and are most likely affected in the malignant process. A number of enzyme activities found to be altered in malignant cells are those involved in nucleic acid synthesis and catabolism. In general, the key enzymes in the de novo pathways and salvage pathways of purine and pyrimidine biosynthesis are increased and the opposing catabolic enzymes are decreased during malignant transformation and tumor progression. These findings and concepts were further developed by the analysis of gene expression profiles and identification of gene expression signatures in cancer cells some 20 years later.

1.3 NOXIOUS THEORIES

The stigma that cancer equals death, originating in the experiences of Hippocrates, Galen, and Celsus, was attached to the disease for centuries. It led to the long-respected dictum that doctors should not inform their patients of the diagnosis to avoid agony. In view of progress in surgery, which allowed the removal of some tumors, the American Cancer Society was formed in 1913 to educate the public about the warning symptoms of cancer and to reduce their fatalistic fears. The increased public health awareness was helpful whenever carcinogenic mechanisms were identified and the need for lifestyle changes was publicized. The insight that malignancy may be caused by the influence of damaging agents forms the basis of the noxious theory of carcinogenesis. Among the influences that may cause cancer are chemicals, radiation, and viruses.

Chemical carcinogenesis. In 1775, chemical carcinogenesis was observed by the English surgeon Sir Percival Pott, who related the cause of scrotal skin cancer in a number of his patients to a common history of occupational exposure to large amounts of coal soot as chimney sweepers when they were boys. The connection between soot and cancer was confirmed in 1915 by the first controlled experimental induction of cancer in laboratory animals by Katsusaburo Yamagiwa. The experiment established chemical carcinogenesis, and specifically occupational exposure, as one possible cause for malignant growths. An unrelated form of occupational exposure was documented in the mid-19th century in silver miners from St. Joachimsthal, Bohemia (today Czech Republic). Silver had been extracted there since the mid-16th century and was manufactured into the Joachimsthaler silver coins that were predecessors of the German currency "Thaler" and later the American currency "dollar." These miners had a high incidence of lung cancer, which was otherwise extremely rare at that time. The cause was traced to their occupational exposure (Table 1.3.A).

Archeological evidence suggests that the Mayans smoked tobacco leaves as early as the 1st century BC.

Table 1.3.A. Occupational cancers. Certain occupations are associated with high levels of exposure to specific carcinogenic influences. These agents cause DNA damage through physical or chemical effects. Accordingly, the types of cancers induced by these carcinogens have a higher than normal incidence among exposed workers

Agent	Occupation	Site of cancer
X-rays	Radiologists, radiographers	Skin
Ultraviolet radiation	Farmers, sailors	Skin
Polycyclic hydrocarbons (soot, tar)	Chimney sweepers, manufacturers of coal gas	Skin, bronchus, scrotum
Asbestos	Insulation workers, shipyard workers	Bronchus, pleura, peritoneum
Radon	Underground miners for uranium or fluorspar	Bronchus
Bis(chloromethyl)ether	Ion-exchange resin manufacturers	Bronchus
Mustard gas	Poison gas manufacturers	Bronchus, larynx
Tobacco smoke	Flight attendants, bar tenders	Lung
Naphthylamine	Rubber workers, manufacturers of coal gas	Bladder
4-aminobiphenyl	Chemical workers	Bladder
Vinyl chloride	PVC manufacturers	Liver (angiosarcoma)
Benzene	Workers with glues or varnishes	Bone marrow (leukemia)
Radium	Luminous dial painters	bone
Arsenic	Sheep dip makers, gold miners, vineyard workers, ore smelters	Epidermoid and basal cells, bronchus, liver, bladder

Only in 1761, John Hill published a treatise that warned of unusual tumors of the nose consecutive to sniffing tobacco. By 1949, Ernst Wynder had conducted a survey of 684 lung cancers, which indicated a substantially elevated risk in smokers compared to nonsmokers. It was followed 6 months later by a similar analysis, authored by Richard Doll. About 188 years after the publication by John Hill, a connection between lifestyle choices and cancer risk was established. During the following years of the 20th century, chemical carcinogenesis by tobacco products became a major cause for an increasing incidence of lung cancers. (Table 1.3.B).

In Italy, Bernardino Ramazzini associated breast cancer with reproductive factors. He reported in 1713 the virtual absence of cervical cancer and relatively high incidence of breast cancer in nuns and suggested that this was in some way related to their celibate lifestyle. The key observations by Pott, Hill, and Ramazzini laid the foundation for the field of cancer epidemiology. This area of research was given another foundation between 1930 and 1932, when Fisher, Haldane, and Wright established the principles of population genetics. In the United States, the first hospital registry for cancer was established in 1926 at Yale-New Haven Hospital in Connecticut. In 1935 and 1946, the first central cancer registries were initiated in Connecticut and California. In 1941, the United States National Cancer Institute published a survey of 696 chemical compounds, 169 of which were found to be carcinogenic in animals. During the 1960s, environmental movements became prominent in most of the Western societies. Rachel Carson believed that the long-term ecological effects of synthetic chemical pesticides were not being researched adequately. Her book "Silent Spring" pointed to the pathogenic potential of environmental toxins, and the concept of carcinogens entered popular consciousness. In 1964, Rachel Carson succumbed to cancer at the age of 56. The National Cancer Act of 1971 (declared "war on cancer" by President Richard Nixon) mandated the collection, analysis, and dissemination of all data useful in the prevention, diagnosis, and treatment of cancer. It resulted in the establishment of the National Cancer Program, under which the Surveillance, Epidemiology, and End Results (SEER) Program was developed in 1973.

Over the years, the susceptibility to various cancers has been associated with nutritional habits. In 1981, Doll and Peto [1981] estimated that 35% of cancer deaths in the United States were attributable to dietary factors. The Western European diet is rich in meat and correlates with a high incidence of colon cancer. Nasopharyngeal cancer is among the most widespread tumors in Southeast Asia, possibly supported by the ingestion of salted fish. Esophageal cancer typically occurs in conjunction with alcoholism. The growing health conscience in the late years of the 20th century, combined with insights into the potential carcinogenic properties of reactive oxygen intermediates prompted multiple studies into cancer preventive capacities of antioxidants as nutrition supplements. It was soon found that while

Table 1.3.B. Chemical carcinogens. While all chemical carcinogens share the property of damaging DNA, various compounds cause the formation of tumors in diverse organs. Among many mechanisms, this may reflect the site of exposure (skin, lungs), the site of metabolism (liver), or the site of accumulation during excretion (bladder)

Chemical Compounds	Cancer
Pro-carcinogen	
Polycyclic aromatic hydrocarbons	
3,4-Benzopyrene	Lung and pancreas cancer
3-Methylcholanthrene	Lung carcinoma
Benzanthracene	Bladder and skin cancer
7,12-Dimethylbenzanthracene	Mammary carcinoma
Aromatic amines and azo dyes	
2-Naphthylamine	Bladder carcinoma
Benzidine	Bladder carcinoma
2-Acetylaminofluorene	Bladder, kidney, and liver cancer
4-Dimethylaminoazobenzene	Liver tumors
Mycotoxins	
Aflatoxins	Hepatocellular carcinoma
Mitomycin C	
Metals	
Arsenic	Skin cancer, lung cancer
Chromium (hexavalent compounds)	Lung cancer
Cadmium	Sarcomas, testicular cancer
Nickel	Lung cancer
N-nitroso compounds	
Nitrosamines	Liver cancer
N-nitroso-piperidin	Liver cancer, esophagus cancer
Nitrosourea	Intestinal cancer, squamous skin cancer
Other pro-carcinogens	
Chlordane	Liver cancer
Carbon tetrachloride	Liver cancer
Direct-acting carcinogens	
Alkylating agents	
Cyclophosphamide	Bladder cancer, skin cancer
Busulfan	Leukemia, kidney cancer, uterine cancer
Chlorambucil	
β-Propiolactone	Skin cancer, stomach cancer
Bis(chloromethyl)ether	Lung cancer
Acetylating agents	
1-Acetylimidazole	Lung cancer
Promoters	
12-Tetradecanoyl phorbol-13-acetate (TPA, PMA)	
Dichlorodiphenyl-trichloroethane (DDT)	Breast cancer
Phenobarbital	Liver cancer
2,3,7,8-Tetrachloro-*p*-dioxin	Lymphoma
Cyclosporin	Squamous cell carcinoma
Unknown function	
Vinyl carbamate	Lung cancer
4-(Methylnitroamino)-1-(3-pyridyl)-1-butanone	Lung cancer

the intake of some foods can increase the risk for specific malignancies, others – such as retinoids – can act in a chemopreventive [Sporn et al. 1976] fashion (Figure 1.3.A).

From their studies of oral cancer, Slaughter, Southwick, and Smejkal derived the concept of carcinogenesis as a process of field cancerization (field carcinogenesis, condemned mucosa syndrome). The repeated exposure of a region's entire tissue area to carcinogenic insult increases the risk for developing multiple independent premalignant and malignant foci in that tissue [Slaughter et al. 1953]. Increasingly, molecular mechanisms have been identified to link certain toxins to specific cancers. In 1975, Bruce Ames at the University of California in Berkeley developed a test for the mutagenicity of chemical compounds, which was used to confirm that carcinogens are mutagens. Further mechanistic insight was gained with the demonstration that aflatoxin causes the mutation G249T in *p53*, which is associated with hepatoma [Bressac et al. 1991]. Ultraviolet (UV) light induces pyrimidine dimers, which cause mutations in *p53* that lead to skin cancer [Brash et al. 1991; Pierceall et al. 1991].

The double-edged sword of mutagens became evident when their possible benefit in the treatment of neoplasias was discovered. Mustard gas had been used as a chemical warfare agent during World War I and was studied further in World War II. In 1917, Krumbhaar, a Captain in the US Medical Corps, noted the development of profound leukopenia in individuals who survived a gas attack for several days [Krumbhaar 1919]. Following up on this observation, a group of the US Office of Scientific Research and Development (OSRD) at Yale Medical School secretly studied the effects of nitrogen mustard on lymphomata. There, Lindskog successfully treated a radioresistant lymphosarcoma that compressed the patient's trachea with the injection of nitrogen mustard in December 1942. None of this was made public until 1946. During a military operation in World War II, allied ships in Bari harbor, Italy, were sunk in an air assault (2 December 1943). At the center of the destruction was the vessel John Harvey, laden with ammunition, supplies, and 2,000 mustard gas bombs. A large number of military personnel were accidentally exposed to mustard gas and were later found to have abnormally low white blood cell counts. It was reasoned that an agent, which damaged the rapidly growing white blood cells, might have a similar effect on cancer. Cornelius P. Rhoads served as chief of

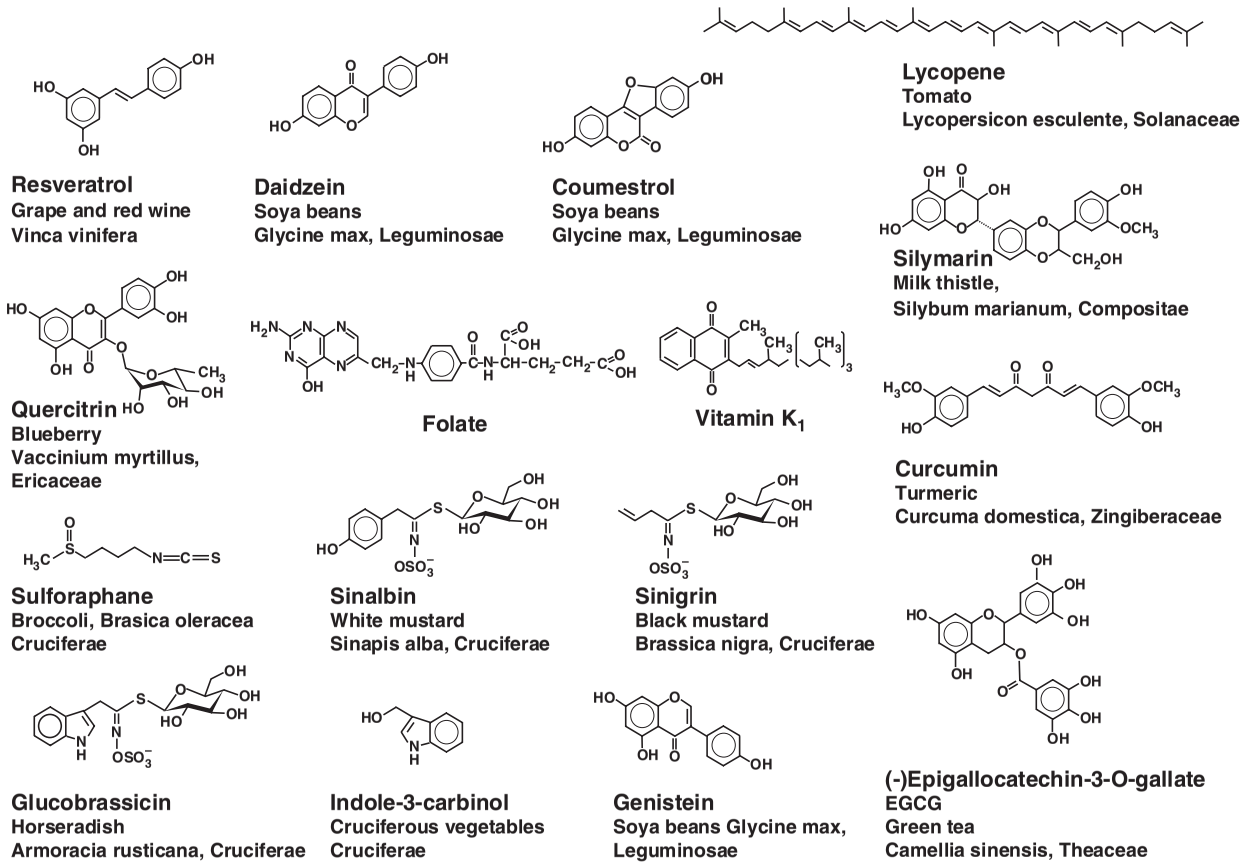


Figure 1.3.A. Dietary cancer chemopreventive compounds. Examples of compounds that have protective properties against certain cancers. Shown are the chemical structures, names, and food sources. [Reproduced from http://visiscience.com/free_powerpoint_slides.php]

the medical division of the US Army's chemical warfare unit during World War II. Based on his experience in the Bari incident, he investigated mustard gas as a tumor-killing agent. This presaged classical chemotherapy [Rhoads 1946]. Soon, the pharmacists Louis Goodman and Alfred Gilman, recruited by the US Department of Defense to investigate potential therapeutic applications of chemical warfare agents, observed that exposure to mustard gas caused profound lymphoid and myeloid suppression suggesting its utility for the treatment of lymphomata [Goodman et al. 1946]. Sidney Farber of Boston recognized that folic acid stimulated the proliferation of leukemia cells. In one of the first examples of rational drug design, he collaborated with Lederle Laboratories to devise folate analogs. He demonstrated that aminopterin produced remission in acute leukemia in children because it blocked a critical chemical reaction needed for DNA reduplication [Farber et al. 1948]. Aminopterin was the predecessor of methotrexate (developed by Lederle Laboratories in 1948), which in 1956 became the first compound cure of metastatic cancer, when it was used by Roy Hertz and Min Chiu Li to treat a case of choriocarcinoma. From 1942, research by George Hitchings and Gertrude Ellion at the Burroughs Wellcome Corporation had corroborated that it was possible to treat cancer with chemical compounds. Using one of them, 6-mercaptopurine, Joseph N. Burchenal (1912–2006) achieved a high percentage of complete remissions in childhood leukemias. Due to these early successes, the US Congress created a National Cancer Chemotherapy Service Center (NCCSC) at the National Cancer Institute in 1955. In 1965, cisplatin was discovered by Barnett Rosenberg, who explored the effects of electric fields on the growth of bacteria. He observed that the bacteria unexpectedly ceased to divide due to the exposure to an electrolysis product of the platinum electrodes. The discovery soon initiated studies into the effects of platinum compounds on cell division. This drug was later pivotal in the cure of testicular cancer. The often adverse effects of these agents were diminished when it was realized that they could be effectively used in combination [Frei et al. 1958; Frei et al. 1965]. This approach followed the strategy of antibiotic therapy for tuberculosis, which used combinations of drugs with different mechanisms of action. Frei, Freireich, and Holland hypothesized that cancer cells would be less likely to mutate and develop drug resistance to the drug combination (Table 1.3.C). The coalescence of efforts to eliminate compounds with intrinsic mutagenic potential from cancer therapy with increasing insights into the molecular pathways associated with growth signals led to the development of small molecule inhibitors, including STI571 (Gleevec) [Druker and Lydon 2000] and ZD1839 (Iressa).

Radiation carcinogenesis. In 1895, Wilhelm Conrad Röntgen (1845–1923), experimenting with electrical discharges in vacuum tubes (Crookes tubes), identified penetrating radiation that also produced

Table 1.3.C. Categories of conventional anticancer drugs. Chemotherapy is the use of chemical substances to treat cancer. The groups of classical anticancer agents comprises cytotoxic drugs that interfere with cell proliferation through various mechanisms

Alkylating agents: cross-link two DNA strands
Nitrogen mustards: Chlorambucil, Chlormethine, Cyclophosphamide, Ifosfamide, Melphalan
Nitrosoureas: Carmustine, Fotemustine, Lomustine, Streptozocin
Platinum: Carboplatin, Cisplatin, Oxaliplatin
Others: Busulfan, Dacarbazine, Mechlorethamine, Procarbazine, Temozolomide, ThioTEPA, Uramustine
Anti-metabolites: have affinity to enzymes of nucleic acid biosynthesis, "false building blocks"
Folic acid: Methotrexate, Pemetrexed, Raltitrexed
Purine: Cladribine, Clofarabine, Fludarabine, Mercaptopurine, Tioguanine. Pyrimidine: Capecitabine
Others: Cytarabine, Fluorouracil, Gemcitabine
Antibiotics: generate free radicals through redox cycles
Anthracyclines: Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mitoxantrone, Valrubicin
Others: Bleomycin, Hydroxyurea, Mitomycin
Alkaloids: inhibitors of mitosis
Taxanes: Docetaxel, Paclitaxel
Vinca alkaloids: Vinblastine, Vincristine, Vindesine, Vinorelbine
Topoisomerase inhibitors: interference with DNA transcription and replication Type 1: Topotecan, Irinotecan
Type 2: Etoposide, Teniposide

fluorescence, and named it X-rays ("X" symbolizing the unknown). He died from leukemia after years of working with these newly discovered rays. In 1896, Henri Becquerel observed that penetrating radiation was given off by uranium. Marie Curie (born Maria Sklodowska, 1867–1934) discovered the element radium, as well as methods for separating radium from radioactive residues in sufficient quantities to analyze its therapeutic properties. After a life time of research into radioactivity, Marie Curie succumbed to pre-leukemia. The hazards of exposure to ionizing radiation were soon recognized. Acute skin reactions were observed in many individuals working with the recently invented X-ray generators. In the early years of the 20th century, these researchers were frequently affected by skin cancers and leukemias. By 1902, a case of radiation-induced cancer was reported, arising in an ulcerated area of the skin. Within a few years, a large number of such skin cancers had been observed, and the first report of leukemia in five radiation workers appeared in 1911. The French physician Jean Bergonie developed the law of radiosensitivity. He died in 1925 from cancer caused by his research with X-rays. In 1927, Hermann J. Müller recognized that ionizing radiation, already known to be carcinogenic, is also mutagenic [Müller 1927]. X-rays break the sugar–phosphate backbone of DNA. Radiation damage may be exerted by directly and indirectly ionizing radiation. Photons and neutrons are not charged and are indirectly ionizing. Radiation of charged particles (α-rays, electron rays including β-rays, proton rays) bear a higher risk for cellular damage, including transforming events. The atomic bombs that exploded over Hiroshima and Nagasaki caused dramatic increases in the incidence of leukemias during the ensuing decades. By the 1950s, researchers at the Sloan-Kettering Institute in New York City became alarmed over thyroid cancers that were diagnosed in adolescents who had received radiation treatment of their thymus glands in childhood. Later reports began to document that thyroid cancers could develop about 20 years following childhood radiation therapy. Nevertheless, the use of radiation to fight cancer was under study early on. The work by Maude Menton (1879–1960), Simon Flexner, and J.V. Jobling at the Rockefeller Institute lead to the publication of the monograph "Influence of Radium Bromide on a Carcinomatous Tumor of the White Rat" in 1910.

Viral carcinogenesis. Tumor viruses were detected at the turn of the 20th century with the cell-free transmission of human warts [Ciuffo 1907] and of chicken leukemia [Ellermann and Bang 1908]. In 1911, Peyton Rous isolated a highly oncogenic retrovirus (Rous sarcoma virus) from a chicken sarcoma [Rous 1911]. In 1932, Shope and Hurst demonstrated that papillomavirus had oncogenic activity in rabbits. In the early 1940s, Clarence Cook Little argued that viruses had caused breast cancer in a strain of laboratory mice. These groundbreaking results had been met with skepticism, because transmissibility in chickens and tumorigenesis in rabbits were not seen as applicable to human disease. The doubts were dispelled in the 1950s, by the demonstration that a tumor induced by Rous sarcoma virus (RSV) could produce infected tumor cells [Rubin 1955]. In conjunction with the observation that murine leukemia viruses are transmissible to newborn animals [Gross 1950], it initiated two decades of intense research into animal viruses, including many retroviruses with tumorigenic properties in animals. In 1964, the Epstein-Barr virus (EBV) was observed by electron microscopy in cultured cells from Burkitt lymphoma [Epstein et al. 1964]. Studies of RSV lead to the identification of the first oncogene, *v-src*, in the 1970s [Martin 1970; Brugge and Erikson 1977] and its subsequent sequencing [Czernilofsky et al. 1980]. In general, the infection of cells with an oncogenic DNA virus may result either in productive lytic infection with cell death and the release of newly formed virus particles or in cell transformation to the neoplastic state with little or no virus production, but with the integration of viral genetic information into the cell DNA. The viral genes capable of causing transformation (viral oncogenes) typically belong to the latent group of genes, which allow the infected cells to stay alive. The viral oncogenes are then present in all of the resulting cancer cells. Transforming retroviruses carry oncogenes derived from cellular genes, that are involved in mitogenic signaling and growth control. Viral transforming genes are collectively called *v-onc*, and their normal cellular counterparts are collectively referred to as *c-onc*. DNA tumor viruses encode oncogenes of viral origin that are essential for viral replication and cell transformation. The long delay between infection and the occurrence of tumors suggested that viruses can act in tumor initiation, and that additional damaging influences are required for tumor promotion. It is estimated that

15% of all human tumors worldwide are caused by viruses. (Table 1.3.D)

Oncogenic DNA Viruses. Three major families of DNA viruses, including herpes viruses, hepadna viruses, and papilloma viruses, have oncogenic potential. Although the polyoma virus SV40 and adenoviruses induce tumors in some animal species they are not known to be causative for any human tumors.

The genomes of herpes viruses are double-stranded linear DNA molecules with sizes in the range of 140–170 kb. The initiation of transformation by oncogenic herpes viruses appears to depend on specific genes, although no single T antigens (tumor antigens) have been identified. EBV was discovered in 1964 by Epstein, Achong, and Barr in a biopsy from Burkitt lymphoma. It is a γ-1 herpes virus infecting all human populations, with a prevalence of over 90% in adults. Infection results in the establishment of a lifelong carrier state, characterized by the persistence of antibodies to several viral gene products and the secretion of infectious virus in the saliva, which is also the usual vehicle of transmission. The Epstein-Barr Virus, which is the agent of infectious mononucleosis, is causative for Burkitt lymphoma (described by English surgeon Denis Burkitt in Uganda in 1958) in Africa and sporadic cases elsewhere, for B-cell lymphomata in acquired immunodeficiency syndrome (AIDS), as well as for nasopharyngeal carcinoma with high prevalence in China. Viral DNA and various EBV antigens are detectable in the affected tumor cells. A herpes virus designated HHV type 8 (KSHV, Kaposi sarcoma-related herpes virus) has been implicated in AIDS associated Kaposi sarcoma [Chang et al. 1994], the most common malignant tumor in AIDS, and also in rare sporadic Kaposi sarcomata unrelated to AIDS. The herpes simplex virus type 2 (HSV-2) may be involved in the pathogenesis of cervical cancer.

Table 1.3.D. Tumor viruses. Viruses can cause transformation. Tumor viruses belong to various taxonomic families. Like chemical carcinogens, they typically display organ selectivity

Virus	Cancer	Size of genome (kb)
DNA tumor viruses		
Herpes viridae		100–200
Epstein-Barr virus	Burkitt lymphoma, B-cell lymphoma, nasopharyngeal carcinoma	172
Human herpesvirus 8	Kaposi sarcoma	165
Hepadna viridae		
Hepatitits B virus	Liver cancer	3
Papova viridae		
Papillomavirus	Cervical carcinoma	8
SV40	Mesothelioma	5
Polyomavirus		
Adeno viridae		35
RNA tumor viruses		3–9
Oncorna viridae		
HTLV-1	T-cell lymphoma	9
HTLV-2	Hairy T-cell leukemia	
Flavi viridae		
Hepatitis C virus	Liver cancer	10

Originally known as serum hepatitis, hepatitis B has only been recognized as such since 1947. It has caused epidemics in parts of Asia and Africa. Hepatitis B is recognized as endemic in China and various other parts of Asia. Hepatitis B viruses (HBV) specifically infect liver cells. Chronic infection with HBV may have a causal role in primary hepatocellular carcinoma, which is one of the most common forms of cancer in Asia. Viral DNA is integrated into the tumor cells in some of these cases. In 1963, Baruch Blumberg and Harvey Alter reported the discovery of the hepatitis B surface antigen (Aa, HBsAg, Australia antigen), and a specific antibody binding to it. In 1970, Dane visualized the hepatitis B virion. These discoveries paved the way for the development of a vaccine.

The genomes of the papova family members polyomavirus and SV40 are double-stranded circular DNA molecules with sizes of about 5 kb. They contain two main groups of genes that are associated with early and late events in the replication cycle. The early genes are transcribed soon after infection of a cell and their encoded proteins participate in viral DNA synthesis but are not structural components of the virions. The late genes encode proteins of the viral coat and capsid. In productive lytic infection, early proteins are formed transiently before the structural proteins are assembled into viral particles. When stable transformation takes place, viral DNA is integrated into the cellular chromosomal DNA and some of the early proteins are persistently synthesized, but viral particles are not produced. Approximately 60–120 distinct types of human papilloma viruses (HPV) have been identified, which infect epithelial cells. While several forms cause benign tumors, such as warts, some types of sexually transmitted HPV are associated

with precursor lesions to squamous carcinoma of the uterine cervix. In 1983, Harald zur Hausen and colleagues isolated HPV16 from a human cervical cancer specimen. HPV types 16 and 18 ("high risk HPV"), followed by HPV types 45 and 31, may cause invasive cervical carcinoma or anorectal cancers. HPV DNA is extrachromosomal in the precursor lesions and infectious virus is produced. Viral DNA is frequently integrated into the cancer cells, but additional agents or factors may be involved at various stages of the progression to invasive carcinoma. Cell transformation by HPV results from the expression of two early genes, *e6* and *e7*. *e6* binds to P53, while *e7* binds to RB, in both cases resulting in the degradation of their targets in the Ubiquitin-proteasome pathway. Acting together, *e6* and *e7* are sufficient to induce transformation in the absence of mutations in cell regulatory proteins. In 2006, a vaccine against high risk HPV strains came on the market.

While there is no evidence that SV40 can induce human tumors or that SV40 DNA is present in human tumor cells, it has been a valuable model in cancer research. The early proteins found in tumors induced by polyomavirus and SV40 are termed T (tumor) antigens. Polyomavirus produces large, middle, and small T antigens, of which the middle T antigen (55 kD) is necessary for transformation. This early protein is bound to the plasma membrane of transformed cells and activates signal transduction pathways that promote cell cycle progression. The two early proteins, T (large T, 94 kD) and t (small t, 17 kD), are formed from the same reading frame by alternative splicing. The large T antigen is located in the nucleus of infected cells and maintains the transformed state. Distinct domains of large T bind to P53 and RB, inhibiting their function. Because large T inhibits both proteins, expression of only the SV40 large T protein is sufficient to induce the transformation of certain cells.

Most adenoviruses only cause acute upper respiratory tract infections. Adenoviruses were discovered in adenomatous tissue in 1953 by Rowe. Their genomes are double-stranded linear DNA molecules with sizes of about 35–40 kb. In cells transformed by oncogenic adenoviruses, a region of the genome encoding early gene products, including the E1A and E1B oncoproteins, is transcribed. These transforming proteins inactivate the RB and P53 tumor suppressors, with E1A binding to RB and E1B binding to P53.

Oncogenic RNA Viruses. Hübner and Todaro postulated the existence of retroviral oncogenes [Hübner and Todaro 1969]. Among the many families of RNA viruses, only members of the retrovirus and flavivirus families are capable of transforming cells and inducing tumors. The genomes of retroviruses are single-stranded RNA molecules with a size range of 3–9 kb. All retroviruses contain a Reverse Transcriptase [Baltimore 1970; Temin and Mizutani 1970], and their reduplication requires the synthesis of a double-stranded DNA intermediate of the RNA genome. Some of the virally determined DNA becomes integrated into the host DNA as a provirus. Typically, there are three retroviral genes that encode proteins necessary for viral reduplication, but do not contribute to transformation:

– The *gag* gene encodes internal structural proteins of the virus.
– The *pol* gene encodes Reverse Transcriptase.
– The *env* gene encodes envelope proteins that enclose the virus particles and largely determine the host range.

Most oncogenic retroviruses also possess one, or rarely two, oncogenes, termed *v-onc*. Under the influence of the viral promoter sequence, the *v-onc* gene is transcribed along with other viral genes and is responsible for the neoplastic transformation of the infected cell. Some of them promote growth, while others inhibit programmed cell death. More than 20 such oncogenes have been isolated and characterized. They include:

– In the class of growth factors: *v-sis* (Simian sarcoma virus)
– In the class of receptor protein Tyrosine Kinases: *v-erbA* (avian erythroblastosis virus), *v-erbB* (avian erythroblastosis virus), *v-fms* (feline sarcoma virus), *v-kit*
– In the class of nonreceptor Tyrosine Kinases: *v-abl* (Abelson leukemia virus), *v-fes*, *v-fps*, *v-src* (Rous sarcoma virus)
– In the class of serine/threonine protein kinases: *v-mil*, *v-mos*, *v-akt*, *v-raf*
– In the class of G-Proteins: *v-H-ras* (rat sarcoma virus, Harvey strain), *v-K-ras* (rat sarcoma virus, Kirsten strain)
– In the class of transcription factors: *v-ets*, *v-fos*, *v-jun* (avian sarcoma virus), *v-myc* (avian myelocytomatosis virus), *v-myb* (avian myeloblastosis virus), *v-rel*
– In the class of inhibitors of apoptosis: *v-flip*, *v-bcl-2*

Two unique types of human retroviruses, human T-cell leukemia viruses (HTLV) types 1 and 2 take part in the etiology of leukemias [Ruscetti et al. 1977; Mier and Gallo 1980; Poiesz et al. 1980]. Human T-cell leukemia virus type 1 (HTLV-1), the first human retrovirus to be isolated and characterized, may be the causative agent of a relatively rare form of T-cell lymphoma that occurs mainly in Japan and the Caribbean Islands. HTLV-2 can cause hairy T-cell leukemia [Kalyanaraman et al. 1982]. All the known RNA-containing tumor viruses are classified as retroviruses, with the exception of the hepatitis C virus (HCV), which resembles a flavivirus. In 1989, Daniel Bradley provided Chiron with non-A/non-B hepatitis serum from chimpanzees. There, Michael Houghton and colleagues discovered a single virus and changed the name to HCV. The virus was then cloned from infectious sera of patients with posttransfusion hepatitis. Hepatitis C may lead to chronic liver disease and cirrhosis, which is a predisposing factor for liver cancer.

1.4 SOMATIC THEORIES

The encounter with a family, in which many members developed breast or liver cancer, led Pierre Paul Broca to hypothesize, in 1866, that an inherited abnormality within the affected tissue caused the tumor development [Broca 1866]. From 1895 through 1913, Warthin studied the pedigrees of cancer patients at the University of Michigan Hospital. He identified four multigenerational families with susceptibilities to specific cancer types that appeared to be transmitted as autosomal dominant Mendelian traits [Warthin 1913]. These observations were put on mechanistic footing by 1900, when Hugo de Vries, Carl Correns, and Erich von Tschermak rediscovered the laws of inheritance, previously formulated in 1865 by Gregor Mendel (1822–1884). The chromosomes had been discovered by Walther Flemming (1843–1905) in 1877. He had described cell division and in 1882 coined the term "mitosis". In 1890, David von Hansemann had advanced the hypothesis that irregularities of the mitotic process are responsible for disordered growth [von Hansemann 1890]. Theodor Boveri (1862–1915) then proposed that defects in chromosomes lead to malignancy [Boveri 1914]. He hypothesized that malignant tumors might be the result of a certain abnormal condition of the chromosomes, which may arise from multipolar mitosis. The main concepts of Boveri's theory are:

– The problem of tumors is a cellular problem
– Typically, every tumor arises form a single cell
– The primordial cells of tumors contain, as a result of an abnormal process, definite and wrongly combined chromatin contents
– Chromosome abnormalities are the cause to the tendency toward rapid cell proliferation, which is passed on to all decendents of the primordial cell.

In the 1950s, Sajiro Makino in Japan, Theodore Hauschka in the United States, and Albert Levan in Sweden observed that virtually all tumor cell lines have chromosomal aberrations. The discovery of the Philadelphia chromosome in chronic myeloid leukemia [Nowell and Hungerford 1960] later provided experimental evidence for Boveri's theories. It supported the hypothesis that damage to the chromosomes induced carcinogenesis. Aneuploidy, typically with elevated DNA content, is a frequent marker of cancerous cells. Providing more functional insight, the first description of a translocation was reported in 1973 by Janet D. Rowley [Rowley 1973]. Although the Philadelphia chromosome was among the first translocations to be discovered, the genes involved in the translocation that causes Burkitt lymphoma were the first to be molecularly characterized. In 1982, Carlo Croce and Bob Gallo showed that the *myc* proto-oncogene on chromosome 8 is affected by the translocation. Simultaneously, Phil Leder's group demonstrated that *myc* is translocated into the 5′ region of the *immunoglobulin heavy chain* (*igH*) gene [Dalla-Favera et al. 1982; Taub et al. 1982].

Cancers represent a large category of somatic cell genetic diseases [McKusick 1985]. The term "somatic mutation" was first applied to cancer by Ernest Tyzzer, who observed that tumors sequentially transplanted into mice developed a continuous broadening of host specificity among recipients from various inbred strains [Tyzzer 1916]. By the 1970s, Tyzzer's model had received a molecular underpinning and cancer was understood as a disease of genetic alterations. Tumor initiation and progression occurs through the accumulation of changes that begin when a single normal cell sustains a permanent genetic damage. The resulting dysregulation of gene function is responsible for the clonal expansion of a population of somatic cells that ultimately becomes dominant.

Progress in the understanding of DNA and genes has been a major determining factor for progress in cancer research. In 1869, Johann Friedrich Miescher had identified a weakly acidic substance of unknown function in the nuclei of human white blood cells, which later became known as deoxyribonucleic acid, or DNA. The term gene (derived from the Greek γενοσ = origin), attributed to Johanssen, first appeared in 1909 as an abstract concept to explain the hereditary basis of traits. Oswald Avery, Colin McLeod, and Maclyn McCarthy showed in 1944 that DNA constitutes the genetic material. In 1953, James Watson and Francis Crick deduced the double helical structure of DNA from X-ray diffraction data, generated by Rosalind Franklin. In 1961, Sidney Brenner and Francis Crick established that groups of three nucleotide bases, or codons, are used to specify individual amino acids. The genetic code of nucleotide triplets was worked out in final detail in 1966, mainly through work by Marshall Nierenberg and Heinrich Matthaei. This paved the way for the molecular analysis of gene damage.

One of the most important approaches for biotechnology is the cloning of genes inserted into plasmids. It was initiated through discussions between Stanley Cohen and Herb Boyer at a conference in Hawaii, and by March 1973 the feasibility of their new method was demonstrated. PCR was invented by Kary B. Mullis in spring of 1983. These techniques allowed for the large availability and easy manipulation of cancer related genes. In 1977, Frederick Sanger at the Medical Research Council in Cambridge, UK and Walter Gilbert at Harvard University in Boston, USA independently devised methods for sequencing DNA, which were further developed by Leroy Hood at the California Institute of Technology, who invented an automated DNA sequencer in 1985. In 1990, the Human Genome Project was launched to obtain the complete blueprint of human DNA, planned for 2005. In 1998, the competition by a private enterprise, led by Craig Venter, accelerated the process, so that both groups presented a draft sequence of the genome by June 2000. The genetic analysis of cancer experienced additional support from the technical accomplishment to manipulate individual genes in vivo. In 1982, a team led by Richard Palmiter and Ralph Brinster generated the first transgenic mouse. This was achieved through pronuclear microinjection of genetic material into the nuclei of fertilized eggs. From 1987 through 1989, teams led by Martin Evans, Oliver Smithies, and Mario Capecchi created knockout mice by selectively disabling a specific target gene in embryonic stem cells.

RNA tumor viruses can cause normal cells to adopt the characteristics of rapid uncontrolled growth that are typical of many tumors. The discovery of the human proto-oncogene *src* by Dominique Stéhelin, Harold Varmus, Michael Bishop, and Peter Vogt [Stéhelin et al. 1976] confirmed that viral oncogenes are derived from related genes of host cells. Their analysis implied that the cellular *src* sequence is involved in the normal regulation of growth. It also suggested that tumors could arise independently of viruses as a result of mutations in their related cellular genes. Consecutively in 1982, three publications in the journal Nature independently of one another identified a point mutation in the proto-oncogene *ras* as a defect associated with bladder cancer [Chang et al. 1982; Parada et al. 1982; McBride et al. 1982]. These discoveries revealed that a cellular transforming gene involved in human bladder and lung tumors was homologous to the transforming viral *ras* gene [Parada et al. 1982; Der et al. 1982], and that an activating point mutation affected the identical codon in all cases. Thus, it became apparent that the same cellular proto-oncogenes could be affected by viruses, by chemical carcinogens, or by nonviral somatic mutations, which brought together various previously independent lines of research.

The observation that the growth of murine tumor cells in vivo could be suppressed by fusion of the tumor cells with nontransformed cells provided evidence that the ability of cells to form a tumor is a recessive trait [Ephrussi et al. 1969]. Knudson [Knudson 1971] carried out an epidemiological study of retinoblastoma development in children. He postulated that "two hits" are required for the complete inactivation of a tumor suppressor gene. The gene *p53* was discovered independently by Linzer and Levine [1979] and by Lane and Crawford [1979] as a cellular protein that binds to the viral oncoprotein of SV40. Initially suspected as a cellular oncogene, due to mutations that act as dominant negative forms, the identification of loss of heterozygozity and loss of function mutations of *p53* confirmed its actual role as a tumor suppressor [Baker et al. 1990]. After this clarification, P53 became known as the guardian of the genome,

because it protects from the consequences of genetic damage by inhibiting cell division or inducing cell death. In 1983, loss of heterozygosity analysis was used to map the tumor suppressor gene *rb*, which was then cloned in 1986 [Friend et al. 1986].

Oxidative metabolism inevitably leads to DNA damage. This may occur by direct oxidation of bases, by induction of DNA strand breaks, or by mediation of frameshift mutations in microsatellite DNA. Each cell (of estimated 10^{14} in the human body) loses more than 10^4 bases (out of a total of 6×10^9 nucleotides) per day from the spontaneous breakdown of DNA at body temperature, mostly through the damage by reactive oxygen species. A similar number of lesions is generated by spontaneous depurination, resulting in miscoding by the residual apurinic site [Loeb 2001]. The deamination of 5-methylcytosine to thymine is among the most frequent causes for point mutations. It accounts for more than 20% of all base mutations that give rise to genetic disease [Krawczak et al. 1998]. It has been estimated that 5-methylcytosine deaminates at a rate of 5.8×10^{-17} s^{-1} at each CpG site (cytosine and guanine separated by a phosphate) [Shen et al. 1994], which corresponds to about four residues per cell per day. Mutation frequencies of the *hypoxanthine phosphoribosyl transferase* (*hprt*) gene, a commonly used marker for mutation frequency, in normal adult epithelial cells reach approximately 1.3×10^{-4} [Martin et al. 1996].

The reduplication of DNA during cell division introduces the possibility of errors at an estimated rate of 1.4×10^{-10} nucleotides/cell/division. Loeb and colleagues [Loeb et al. 1974] realized that it would be unlikely for tumor cells to acquire the number of mutations presumably needed for full transformation during the lifetime of the host and postulated the existence of mutator genes. Much later, the study of hereditary non-polyposis coli led to the discovery of defective DNA repair genes [Ionov et al. 1993; Thibodeau et al. 1993; Parsons et al. 1993]. Any mutation of cancer associated genes can be handed on to following generations and predispose the affected cells to malignant transformation in the case of additional DNA damage. The formation of cancer has been termed "clonal evolution" to describe how certain mutations enable cells to copy their damaged DNA and divide under conditions, which cause normal cells to stop replicating. The repetition of this process allows cells to accumulate cancerous mutations [Cavenee and White 1995].

In 1949, Berenblum and Shubik [1949] concluded that carcinogenesis is at least a two-stage process. Five years later, Armitage and Doll [1954] inferred from their analysis of age and cancer incidence a 6–7 step process. In 1983, Newbold and Overell observed that an activated *ras* gene failed to transform normal fibroblasts, unless they were first immortalized [Newbold and Overell 1983]. This led to the hypothesis that *ras* activation was only one step in a number of mutations necessary in the pathway to malignancy. The concept of multiple somatic mutations as underlying mechanism of carcinogenesis was further advanced by a multistep carcinogenesis model, conceived of by Foulds [1957] and refined by Fearon and Vogelstein [1990]. It also gave rise to the recognition of chromosome instability and microsatellite instability as two distinct pathogenetic mechanisms of carcinogenesis. The technical achievements of differential display [Liang and Pardee 1992; Liang et al. 1992], serial analysis of gene expression (SAGE) [Velculescu et al. 1995; Zhang et al. 1997], and DNA microarrays [Schena et al. 1995; DeRisi et al. 1996] further advanced these concepts to the definition of transformation on the basis of aberrant gene expression profiles [Kononen et al. 1998; Golub et al. 1999].

In addition to chromosome integrity and DNA sequence fidelity, the regulation of the chromatin structure is an important determinant in transformation. DNA methylation is a covalent modification of the C5 position in cytosine. This methylation pattern is stably maintained at CpG dinucleotides by a family of DNA Methyl Transferases that recognize hemi-methylated CpG dinucleotides after DNA replication. DNA hypo-methylation was identified as a characteristic of cancer cells in 1983 [Feinberg and Vogelstein 1983]. In 1964, Vincent Allfrey had realized that Histones were often chemically modified by acetylation, which caused them to relax their binding to DNA [Allfrey et al. 1964]. This implied the possibility of a role for histones in cancer [Roth 1965]. In 1974, Robert Kornberg proposed that chromatin was quite structured, consisting of repeated units of about 200 base pairs of DNA wrapped around 2–4 distinct Histones (later called nucleosomes) [Kornberg 1974; Kornberg and Thomas 1974]. The importance of acetylation for

the regulation of gene expression and gene silencing was, however, realized only many years later. In 1998, methylation and phosphorylation of Histones were observed by several investigators to contribute similarly [Bestor 1998]. Today, various enzymes that modify Histones are known to contribute to transformation [Horiuchi et al. 1981].

1.5 REGULATORY THEORIES

Beyond the development of cancer research from explanations on a cellular level to a molecular genetic level, there has been a development of dynamic models of carcinogenesis. Winge introduced the concept of selective cellular proliferation, realizing that selection must operate on a genotypically mixed population of proliferating cells as inevitably as it acts on a genotypically mixed population of reproducing organisms [Winge 1930]. Macfarlane Burnet conceptualized the clonal selection theory for immunity and applied it to cancer. It suggests that tumorigenesis represents the development of a clone of cells with the capacity to multiply excessively in the context of its relationships within the body [Burnet 1959]. In the 1960s, feedback control in biological systems was described by Francois Jacob and Jacque Monod. Cellular metabolism and proliferation are regulated by spatiotemporal circuits of mutual feedback control. They include extracellular and intracellular signals, rate limiting steps, and checkpoint controls. Cancer development has also been described with the algorithms of ecology [Michelson et al. 1987; Maley et al. 2006] and game theory [Tomlinson 1997]. The regulatory theory contends that cancer is not a morphologic entity, but an aberrant regulatory process among individual cells, their microenvironment, and the entire host. Genetically identical cells and organisms exhibit substantial diversity, even when they have identical histories of environmental exposure. Variation in gene expression, based in part on the stochastic nature of biochemical reactions, may contribute to this phenotypic variability [Raser and O'Shea 2005]. Genetic changes underlying growth control, senescence, invasion, and stromal–parenchymal interactions are part of a continuum of carcinogenesis that affects interrelated pathways. In malignant cells, the normal balance between the number of cells completing the cell cycle and the number of cells dying is changed. Likewise, the balance of adhesive versus migratory surface molecules on malignant cells is shifted in favor of the motility enhancing receptors.

Full transformation has two basic requirements:

– Genetic instability of the cell to drive tumor progression
– Selective advantage of the cell to allow for clonal expansion [Cairns 1975; Nowell 1976].

The genetic instability of tumor cells is reflected in the heterogeneity within individual tumors and among tumors of the same type. It is based either on chromosome instability, leading to aneuploidy, or on defective DNA repair, leading to microsatellite instability and gene mutations. Genomic destabilization is an early event in tumor development. The mean number of alterations in a cell that turns carcinomatous may amount to about 11,000 [Stoler et al. 1999]. Waves of clonal expansion give rise to daughter cells that have the growth advantage typical of cancer. Clonal selection drives this process. Tumors are clonal insofar as they are derived from the same stem cell precursor. Genetic instability generates a collection of coexisting subclones, each with the potential for future changes in the face of selective pressures [Cahill et al. 1999]. The relative importance of selective advantage versus genetic instability in tumor initiation and progression is still subject to debate.

Studies of cell senescence have led to a research focus on population dynamics, selection, and evolution. Hayflick recognized that there is a finite number of possible population doublings by nontransformed differentiated cells [Hayflick and Moorehead 1961]. After a limited number of divisions, a state of crisis is reached, in which most cells die. A few cells may be altered in a fashion that conveys a selective advantage, which allows them to grow out and dominate the population. These cells are selected and form an expanding population with potentially precancerous characteristics. The demonstration that HTLV-1 immortalizes normal T-lymphocytes [Popovic et al. 1983] led to additional investigations, which confirmed that tumor viruses can frequently immortalize human host cells. The shortening of the chromosome ends, telomeres [Szostak and Blackburn 1982; Moyzis et al. 1988], is an integral part of replicative senescence. The enzyme Telomerase [McKay and Cooke 1992; Chong et al. 1995] replenishes the chromosome ends and can prevent this shortening. Its activity is present in most cancer cells, but not

typically in nontransformed differentiated cells [Hastie et al. 1990].

The first cancer hospital was founded in the 18th century in Reims, France. French gynecologist Joseph Claude Anthelme Récamier (1774–1852) described the invasion of the bloodstream by cancer cells, coining the word "metastasis." In the 1850s, Pierre Paul Broca (1824–1880) and Karl von Rokitansky (1804–1878), independently of each other, observed the venous spread of cancer. Theories of metastasis formation have traditionally been based on concepts of population dynamics. In 1889, English surgeon Stephen Paget (1855–1926) described the propensity of various types of cancer to form metastases in specific organs. He stated that "the distribution of the secondary growth is not a matter of chance" and proposed that these patterns were due to the dependence of the "seeds" (the cancer cells) on the "congenial soil" (the target organ for metastasis) [Paget 1889]. This notion was challenged by American pathologist James Ewing (1866–1943), who suggested that circulatory patterns between a primary tumor and specific secondary organs were sufficient to account for most of the targeted metastasis [Ewing 1928]. This was relativized by Leonard Weiss, who demonstrated that the number of metastases in specific target organs, derived from certain tumors, could not be accounted for solely by blood flow patterns [Weiss 1992]. The first evidence that metastasis formation depends on intrinsic characteristics of the tumor cells came from experiments by Isaiah Fidler [Fidler 1975], who generated sublines with increasing invasive potential by serial passage of a melanoma cell line through mice. Soon, somatic cell fusion and microcell mediated chromosomal transfer suggested that the ability to disseminate was under positive and negative genetic control [Ramshaw et al. 1983; Sidebottom and Clark 1983; Layton and Franks 1986]. These observations placed ensuing research activities into metastasis on a deterministic footing. The secretion of proteases by tumor cells [Turpeenniemi-Hujanen et al. 1985; Matrisian et al. 1986] was recognized as one factor causing invasiveness. Homing receptors were identified on the cell surface, which are necessary and sufficient to mediate metastasis formation by specific tumors [Günthert et al. 1991]. In conjunction with the finding of metastasis suppressor genes [Steeg et al. 1988; Alvarez et al. 1990], the detection of metastasis genes has corroborated the existence of genetic programs intrinsic in the tumor cells, which regulate invasiveness. These observations have led to the development of a genetic theory of metastasis formation, according to which metastasis genes are developmentally nonessential genes that physiologically contribute to inflammation, wound healing, and stress-induced angiogenesis. Their dysregulation in cancer occurs on the level of aberrant expression and splicing [Weber and Ashkar 2000]. Tissue-specific molecular markers (Addressins) were identified in 1988 [Streeter et al. 1988], which implied the possibility that circulating cells could recognize target organs. This was corroborated by the identification of the contribution by Chemokines and their cognate receptors to tumor dissemination [Mueller et al. 2001].

In the evolution of research progress from a reductionist to a comprehensive understanding of cancer, interactions between the host and the cancer cells have recently received increasing attention. Mintz and Illmensee [1975] had demonstrated that the injection of undifferentiated embryonal carcinoma cells into mouse blastocysts suppressed their inherent tumorigenicity and led to the contribution by these cells to a variety of functional tissues. Around the same time, the Michigan radiologist John Wolfe recognized that women with dense breasts had an elevated risk of contracting breast cancer, implying a role for the stromal architecture. In 1990, it was realized that the tissue environment had a dramatic effect on the potential by tumors to metastasize [Nakajima et al. 1990]. Tumorigenic prostatic stroma and nontumorigenic prostatic epithelium can interact to induce the development of carcinosarcoma [Chung et al. 1988]. The concept that the stroma plays important roles in carcinogenesis has since been developed by Mina Bissell [Bissell and Radisky 2001], Judy Campisi [Krtolica et al. 2001], and Donald Ingber [Huang and Ingber 1999].

Early work in experimental carcinogenesis had shown vascularization and hyperemia around tumor transplants [Ide et al. 1939; Coman and Sheldon 1946] and similarities were seen between the vascular reactions to tumors and to tissue damage [Algire et al. 1945]. Cancer researchers became interested in angiogenesis factors in 1968, when the first hints emerged that tumors might release such substances to foster their own progression. Two groups, one led by Melvin Greenblatt in California with Phillipe Shubik in Chicago, and another by

Robert L. Ehrmann and Mogens Knoth in Boston, showed that burgeoning tumors can release a substance that induces existing blood vessels to grow into them [Rijhsinghani et al. 1968; Ehrmann and Knoth 1968]. Such vascularization promotes tumor growth because it ensures a sufficient supply of oxygen and nutrients. Folkman [1971; Folkman et al. 1971] recognized the important role of blood vessels in the growth of cancerous tumors. After more than a decade of research, mediators of angiogenesis that are secreted by some tumors were identified [Senger et al. 1983; Shing et al. 1984]. The inhibition of VEGF (Vascular Endothelial Growth Factor)-induced angiogenesis was shown to suppress tumor growth [Kim et al. 1993]. Today, a monoclonal antibody to VEGF is used in the treatments of some cancers. These investigations also led to the discovery of naturally secreted compounds that curtail the growth of new tumors [Taylor and Folkman 1982; O'Reilly et al. 1994; O'Reilly et al. 1997].

In the 17th and 18th centuries, some believed that cancer was contagious. In fact, the first cancer hospital in France was forced to move from the city in 1779 because of the fear that cancer could spread throughout the city. More than a century later, the potentially protective role of the immune system against transformed cells was recognized. In the 1890s, New York surgeon William B. Coley found a record of a young patient with round cell sarcoma on the neck, who had been listed as an utterly hopeless patient when he developed a severe infection of erysipelas. He survived the infection and his tumor went into remission. Based on this case, Coley devised a killed vaccine of *Streptococcus pyogenes* (the cause of erysipelas) with *Serratia marcescens*. After a few years of its use, he reported to have successfully treated some sarcoma patients with the application of his bacterial toxins (Coley's toxin) [Coley 1893, 1896]. After Coley's death, his daughter Helen Coley Nauts reviewed his records, published several reviews of his work, and founded the Cancer Research Institute, which promotes immune therapies for cancer. In 1909, Paul Ehrlich carried out immunizations in animals with tumor cells and suggested that tumors occur at high frequency in humans, but are kept under control by the immune system [Ehrlich 1909]. Further developments in tumor immunology have led to models of selection and evolution of cancer cells. Macfarlane Burnet coined the term immunosurveillance in 1967 [Burnet 1967]. In this conceptual framework, the host immune system constantly screens cells for signs of transformation and eliminates those that pose a threat to the body's integrity. The growth of a tumor reflects an escape from immunosurveillance. Cancer cells that can evade the immune system, be it by down-regulation of antigen presenting or co-stimulatory molecules, be it by expression of immunosuppressive cell surface molecules or cytokines, will grow out and form tumors. Three distinct theories were developed to interpret the nature of the tumor recognition by the immune system.

- Lewis Thomas described homograft rejection as a primary defense against neoplasia [Thomas 1959].
- According to concepts by Burnet, which are based on self/non-self discrimination, the immune system is active early in antitumor protection. The early surveillance mechanisms shape the tumor's immunological phenotype [Burnet 1967]. This was supported by the description of tumor specific antigens [Old and Boyse 1964]. Tumors mostly express self antigens, which may account for the incomplete protection from transformation by the immune system.
- The alternative proposal of the danger theory [Matzinger 1994] implies that the immune system is activated only at later stages of carcinogenesis. During the early stages, tumor cells appear immunologically as healthy growing cells that do not send out danger signals to activate the immune system because they express neither microbial immune recognition patterns nor release distress signals to alarm the innate immune system cells [Fuchs and Matzinger 1996]. In advanced growth, hypoxia and tissue damage induce stress responses, which activate the immune system. In the framework of the danger theory, the immune system is activated at later stages of tumor development, when tissue damage has occurred.

The possibility to direct the immune system to fight cancer cells in virtually any location within the body with minimal side effects has attracted increasing research efforts. The high specificity and high binding affinity of antibodies made them attractive as potential anticancer agents. For a long time, however, they were difficult to isolate in large quantities. The fusion of antibody producing cells with myeloma cells into hybridomas, accomplished by Cesar Milstein and Georges Koehler in the early 1970s, changed that. Yet, biotechnology had to advance to accomplish humanizing such antibodies before they became successful in therapy. In 1997,

the US Food and Drug Administration (FDA) approved Rituxan, a monoclonal antibody to CD20 (developed by IDEC Pharmaceuticals) to treat non-Hodgkin lymphoma [McLaughlin et al. 1998]. The process also led to the development of Herceptin, spearheaded by Dennis Slamon, an antibody that targets the receptor ERBB2 (HER-2/NEU) that is overexpressed on the surface of about 30% of breast cancers. Because antitumor immunity is predominantly cellular immunity, other research has been directed toward turning T-lymphocytes against tumors. Steven A. Rosenberg focused his efforts to generate antitumor vaccines on tumor associated antigens. In a similar approach, Martin Kast studied the development of peptide-based vaccines. Glenn Dranoff demonstrated the high effectiveness of irradiated tumor cells transfected with the cytokine GM-CSF in inducing antitumor immunity [Dranoff et al. 1993]. Over time it became clear, on the other hand, that the immune system could also impact negatively on cancer risk in the context of chronic inflammation. In 1876, Robert Koch and Louis Pasteur had shown independently of each other that microorganisms can cause disease. In the 1980s, Barry J. Marshall and J. Robin Warren demonstrated that gastric ulcers were caused by bacteria they called *Helicobacter pylori*. Infection results in widespread inflammation that predisposes to stomach cancer. Inflammation in the stomach mucosa is also a risk factor for MALT (mucosa-associated lymphoid tissue) lymphoma, a lymphatic neoplasm in the stomach.

Over the decades, the roles of hormones in carcinogenesis have received increasing attention. The observation by Bernardino Ramazzini in 1713 of a virtual absence of cervical cancer and relatively high incidence of breast cancer in nuns was an important step toward identifying and understanding the importance of hormonal factors, such as those associated with pregnancy, in modifying cancer risk. In 1878, Thomas Beatson discovered that the breasts of rabbits stopped producing milk after he removed the ovaries. He suggested to the Edinburgh Medico-Chirurgical Society in 1896: "This fact (. . .) pointed to one organ holding control over the secretion of another and separate organ." Beatson found that oophorectomy often resulted in the improvement of breast cancer patients and inferred the stimulating effect of a female ovarian hormone on breast cancer, before the hormone itself was discovered [Beatson 1896]. Allen and Doisy [1923] identified an ovarian hormone they referred to as "estrus stimulating principle," later called estrogen. From the late 1950s to the 1970s Elwood Jensen demonstrated that such hormones do not undergo redox modifications to become activated. Instead, they bind to a receptor protein within their target cells [Jensen and Jacobson 1962]. This hormone/receptor complex then travels to the cell nucleus, where it regulates gene expression. The first nonsteroidal antiestrogen to be reported in the literature, MER25, was described by Lerner and coworkers in 1958 [Lerner et al. 1958] as an agent that had no other hormonal or antihormonal properties. The drug failed in clinical trial because the large doses required caused serious central nervous system side effects. Tamoxifen, first discovered in 1962, is a nonsteroidal antiestrogen that serves a dual role as breast cancer treatment and preventive. It was approved for the treatment of advanced breast cancer by the US FDA in 1977. Awareness of the androgen dependence of prostate tissue can be traced back to the Scottish surgeon John Hunter, who observed in 1786 that castrated bulls had small prostates. In 1941, Charles Brenton Huggins (1901–1997), a urologist at the University of Chicago, with his students Clarence V. Hodges and William Wallace Scott, published three papers that demonstrated the relationship between the endocrine system and the normal functioning of the prostate gland. In the 1940s, Charles Huggins also reported a dramatic regression of metastatic prostate cancer following removal of the testes [Huggins and Hodges 1941]. Later, drugs that blocked male hormones were found to be effective treatments for prostate cancer. Androgen ablation with Gonadotropin Releasing Hormone agonists (GnRH-As) in prostate cancer patients was first reported in 1982 [Tolis et al. 1982]. In 1988, the Androgen receptor was cloned [Chang et al. 1988]. Iatrogenic causes for cancer predisposition were incriminated by a study published in 1971, which documented an association between clear-cell adenocarcinoma of the vagina and in utero exposure to diethylstilbestrol [Herbst et al. 1971] (Dodds and associates had characterized diethylstilbestrol as an extremely potent estrogen [Dodds et al. 1938]; it had been prescribed for close to 30 years to prevent certain complications of pregnancy and as a treatment for advanced breast cancer in postmenopausal women). In July 2002, the Women's Health Initiative study was stopped after more breast cancers and heart problems occurred

among women taking estrogen–progestin pills. In 2006, multiple clinical studies showed that breast cancer rates in the United States dropped in 2003, consecutive to a drastic reduction in the use of hormone replacement therapy. Some of the numbers came from the National Cancer Institute's surveillance database, which uses cancer registries around the country to project national incidence and death rates.

REFERENCES

Algire GH, Chalkely HW, Legallais FY, Park H. 1945. Vascular reactions of normal and malignant tumors in vivo: I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. Journal of the National Cancer Institute 6:73–85.

Allen E, Doisy EA. 1923. An ovarian hormone: preliminary report on its localization, extraction and partial purification and action in test animals. Journal of the American Medical Association 81:819–821.

Allfrey VG, Faulkner R, Mirsky AE. 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proceedings of the National Academy of Sciences USA 51:786–794.

Alvarez OA, Carmichael DF, DeClerck YA. 1990. Inhibition of collagenolytic activity and metastasis of tumor cells by a recombinant human tissue inhibitor of metalloproteinases. Journal of the National Cancer Institute 82:589–595.

Armitage P, Doll R. 1954. The age distribution of cancer and a multi-stage theory of carcinogenesis. The British Journal of Cancer 8:1–12.

Baker SJ, Markowitz S, Fearon ER, Wilson JK, Vogelstein B. 1990. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science 249:912–915.

Baltimore D. 1970. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature 226:1209–1211.

Beatson GT. 1896. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. The Lancet 2:104–107.

Berenblum I, Shubik PA. 1949. An experimental study of the initiating stage of carcinogenesis, and re-examination of the somatic cell mutation theory of cancer. The British Journal of Cancer 3:100–118.

Bestor TH. 1998. Gene silencing. Methylation meets acetylation. Nature 393:311–312.

Bissell MJ, Radisky D. 2001. Putting tumors in context. Nature Reviews Cancer 1:46–54.

Boveri T. 1914. Zur Frage der Entstehung maligner Tumoren. Jena: Verlag von Gustav Fischer.

Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Ponten J. 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proceedings of the National Academy of Sciences USA 88:10124–10128.

Bressac B, Kew M, Wands J, Ozturk M. 1991. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 350:429–431.

Broca PP. 1866. Traité des tumeurs, 2 vols., Paris.

Brugge JS, Erikson RL. 1977. Identification of a transformation-specific antigen induced by avian sarcoma virus. Nature 269:346–348.

Burnet FM. 1967. Immunological aspects of malignant disease. The Lancet 1:1171–1174.

Burnet M. 1959. The clonal selection theory of acquired immunity. Nashville, TN: Vanderbuilt University Press.

Burrows MT. 1910. The cultivation of tissues of the chick embryo outside the body. Journal of the American Medical Association 55:2057–2058.

Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. 1999. Genetic instability and darwinian selection in tumours. Trends in Cell Biology 9:M57–M60.

Cairns J. 1975. Mutation selection and the natural history of cancer. Nature 255:197–200.

Cavenee WK, White RL. 1995. The genetic basis of cancer. Scientific American 273:72–79.

Chang CS, Kokontis J, Liao ST. 1988. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324–326.

Chang EH, Furth ME, Scolnick EM, Lowy DR. 1982. Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus. Nature 297:479–483.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994. Identification of herpes-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869.

Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, de Lange T. 1995. A human telomeric protein. Science 270:1663–1667.

Chung LW, Chang SM, Bell C, Zhau H, Ro JY, von Eschenbach AC. 1988. Prostatic carcinogenesis evoked by cellular interaction. Environmental Health Perspectives 77:23–28.

Ciuffo G. 1907. Innesto positivo con filtrado di verrucae volgare. Giornale Italiano delle Malattie del Venerologia 48:12–15.

Coley W. 1896. Further observations upon the treatment of malignant tumours with the toxins of erysipelas and *Bacillus prodigiosus* with a report of 160 cases. Bulletin of the Johns Hopkins Hospital 7:57.

Coley WB. 1893. The treatment of malignant tumors by repeated inoculations of Erysipelas, with a report of ten original cases. American Journal of Medical Science 105:487–511.

Coman DR, Sheldon WF. 1946. The significance of hyperemia around tumor implants. American Journal of Pathology 22:821–831.

Czernilofsky AP, Levinson AD, Varmus HE, Bishop JM, Tischer E, Goodman HM. 1980. Nucleotide sequence of an avian sarcoma virus oncogene (src) and proposed amino acid sequence for gene product. Nature 287:198–203.

Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. 1982. Human c-MYC onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proceedings of the National Academy of Sciences USA 79:7824–7827.

Der CJ, Krontiris TG, Cooper GM. 1982. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proceedings of the National Academy of Sciences USA 79:3637–3640.

DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM. 1996. Use of cDNA microarray to analyze gene expression patterns in human cancer. Nature Genetics 14:457–460.

Dodds EC, Golberg L, Lawson W. 1938. Oestrogenic activity of esters of diethylstilboestrol. Nature 142:211–212.

Doll R, Peto R. 1981. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. The Journal of the National Cancer Institute 66:1191–1308.

Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC. 1993. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proceedings of the National Academy of Sciences USA 90:3539–3543.

Druker BJ, Lydon NB. 2000. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. Journal of Clinical Investigation 105:3–7.

Ehrlich P. 1909. Ueber den jetzigen Stand der Karzinomforschung. Nederlands Tijdschrift voor Geneeskunde 53:273–290.

Ehrmann RL, Knoth M. 1968. Choriocarcinoma. Transfilter stimulation of vasoproliferation in the hamster cheek pouch. Studied by light and electron microscopy. Journal of the National Cancer Institute 41:1329–1341.

Ellermann V, Bang O. 1908. Experimentelle Leukämie bei Hühnern. Centralblatt für Bakteriologie Abteilung I 46:595–609.

Ephrussi B, Davidson RL, Weiss MC, Harris H, Klein G. 1969. Malignancy of somatic cell hybrids. Nature 224:1314–1316.

Epstein MA, Achong BG, Barr YM. 1964. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. The Lancet I:702–703.

Ewing J. 1928. Neoplastic Diseases: a treatise on tumors. Philadelphia/London: W.B. Saunders.

Farber S, Diamond LK, Mercer RD, Sylvester RF, Wolff JA. 1948. Temporary remissions in acute leukemia in children produced by folic antagonist, 4-aminopteroylglutamic acid (aminopterin). The New England Journal of Medicine 238:787–793.

Fearon ER, Vogelstein B. 1990. A genetic model for colorectal tumorigenesis. Cell 61:759–767.

Feinberg AP, Vogelstein B. 1983. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301:89–92.

Fidler IJ. 1975. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Research 35:218–24.

Folkman J. 1971. Tumor angiogenesis: therapeutic implications. New England Journal of Medicine 285:1182–1186.

Folkman J, Mersler E, Abernathy C, Williams G. 1971. Isolation of a tumour factor responsible for angiogenesis. Journal of Experimental Medicine 133:275–288.

Foulds L. 1957. Tumor progression. Cancer Research 17:355–356.

Frei E 3rd, Holland JF, Schneiderman MA, Pinkel D, Selkirk G, Freireich EJ, Silver RT, Gold GL, Regelson W. 1958. A comparative study of two regimens of combination chemotherapy in acute leukemia. Blood 13:1126–1148.

Frei E 3rd, Karon M, Levin RH, Freireich EJ, Taylor RJ, Hananian J, Selawry O, Holland JF, Hoogstraten B, Wolman IJ, Abir E, Sawitsky A, Lee S, Mills SD, Burgert EO Jr, Spurr CL, Patterson RB, Ebaugh FG, James GW 3rd, Moon JH. 1965. The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. Blood 26:642–656.

Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. 1986. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323:643–646.

Fuchs EJ, Matzinger P. 1996. Is cancer dangerous to the immune system? Seminars in Immunology 8:271–280.

Gartler SM. 1967. Genetic markers as tracers in cell culture. National Cancer Institute Monographs 26:167–195.

Gey GO, Coffman WD, Kubicek MT. 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Research 12:264–265.

Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, Lander ES. 1999. Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. Science 286:531–537.

Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A and McLennan MT. 1946. Nitrogen mustard therapy. Use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia, and certain allied and miscellaneous disorders. The Journal of the American Medical Association 105:475–476.

Greenstein JP. 1954. Biochemistry of cancer. New York: Academic Press.

Gross L. 1950. Susceptibility of newborn mice of an otherwise apparently 'resistant' strain to inoculation with leukemia. Proceedings of the Society for Experimental Biology and Medicine 73:246–248.

Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. 1991. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 65:13–24.

Harrison RG. 1907. Observations on the living developing nerve fiber. Proceedings of the Society for Experimental Biology and Medicine 4:140–143.

Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. 1990. Telomere reduction in human colorectal carcinoma and with ageing. Nature 346:866–868.

Hayflick L, Moorehead PS. 1961. The limited in vitro lifetime of human diploid cell strains. Experimental Aging Research 25:585–621.

Herbst AL, Ulfelder H, Poskanzer DC. 1971. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. New England Journal of Medicine 284:878–881.

Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ. 1990. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334–336.

Horiuchi K, Fujimoto D, Fukushima M, Kanai K. 1981. Increased histone acetylation and deacetylation in rat ascites hepatoma cells. Cancer Research 41:1488–1491.

Huang S, Ingber DE. 1999. The structural and mechanical complexity of cell-growth control. Nature Cell Biology 1:E131–E138.

Hübner RJ, Todaro GJ. 1969. Oncogenes of RNA tumor viruses as determinants of cancer. Proceedings of the National Academy of Sciences USA 64:1087–1094.

Huggins C, Hodges CV. 1941. Studies on prostatic cancer, I: the effect of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Research 1:293–297.

Ide AG, Baker NH, Warren SL. 1939. Vascularization of the Brown-Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. American Journal of Roentgenology 42:891–899.

Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. 1993. Ubiquitous somatic mutations in simple repeat sequences reveal a new mechanism for colonic neoplasia. Nature 363:558–561.

Jensen EV, Jacobson HI. 1962. Basic guides to the mechanism of estrogen action. Recent Progress in Hormone Research 18:387–414.

Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. 1982. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571–573.

Kerr JFR, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biologic phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer 26:239–257.

Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N. 1993. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 362:841–844.

Knudson AG Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proceedings of the National Academy of Sciences USA 68:820–823.

Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. 1998. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nature Medicine 4:844–847.

Kornberg RD. 1974. Chromatin structure: a repeating unit of histones and DNA. Science 184:868–871.

Kornberg RD, Thomas JO. 1974. Chromatin structure; oligomers of the histones. Science 184:865–868.

Krawczak M, Ball EV, Cooper DN. 1998. Neighboring-nucleotide effects on the rates of germ-line single-base-pair substitution in human genes. American Journal of Human Genetics 63:474–488.

Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. 2001. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proceedings of the National Academy of Sciences USA 98:12072–12077.

Krumbhaar EB. 1919. Role of the blood and the bone marrow in certain forms of gas poisoning. The Journal of the American Medical Association 72:39–41.

Lane DP, Crawford LV. 1979. T antigen is bound to a host protein in SV40-transformed cells. Nature 278:261–263.

Layton MG, Franks LM. 1986. Selective suppression of metastasis but not tumorigenicity of a mouse lung carcinoma by cell hybridization. International Journal of Cancer 37:723–730.

Lerner LJ, Holthaus JF, Thompson CR. 1958. A nonsteroidal estrogen antagonist 1-(*p*-2 diethylaminoethoxyphenyl)-1-phenyl-2-*p*-methoxyphenyl-ethanol. Endocrinology 63:295–318.

Lewis WH. 1936. Malignant cells. The Harvey Lectures 31:214–234.

Liang P, Averboukh L, Keyomarsi K, Sager R, Pardee AB. 1992. Differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells. Cancer Research 52:6966–6968.

Liang P, Pardee AB. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967–971.

Linzer DI, Levine AJ. 1979. Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 17:43–52.

Loeb LA. 2001. A mutator phenotype in cancer. Cancer Research 61:3230–3239.

Loeb LA, Springgate CF, Battula N. 1974. Errors in DNA replication as a basis of malignant change. Cancer Research 34:2311–2321.

Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, Paulson TG, Blount PL, Risques RA, Rabinovitch PS, Reid BJ. 2006. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nature Genetics 38:468–473.

Martin GM, Ogburn CE, Colgin LM, Gown AM, Edland SD, Monnat RJ Jr. 1996. Somatic mutations are frequent and increase with age in human kidney epithelial cells. Human Molecular Genetics 5:215–221.

Martin GS. 1970. Rous sarcoma virus: a function required for the maintenance of the transformed state. Nature 227: 1021–1023.

Matrisian LM, Bowden GT, Krieg P, Furstenberger G, Briand JP, Leroy P, Breathnach R. 1986. The mRNA coding for the secreted protease transin is expressed more abundantly in malignant than in benign tumors. Proceedings of the National Academy of Sciences USA 83:9413–9417.

Matzinger P. 1994. Tolerance, danger, and the extended family. Annual Reviews in Immunology 12:991–1045.

McBride OW, Swan DC, Santos E, Barbacid M, Tronick SR, Aaronson SA. 1982. Localization of the normal allele of T24 human bladder carcinoma oncogene to chromosome 11. Nature 300:773–774.

McKay SJ, Cooke H. 1992. A protein which specifically binds to single stranded TTAGGGn repeats. Nucleic Acids Research 20:1387–1391.

McKusick VA. 1985. Marcella O'Grady Boveri (1865–1950) and the chromosome theory of cancer. Journal of Medical Genetics 22:431–440.

McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. 1998. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. The Journal of Clinical Oncology 16:2825–2833.

Michelson S, Miller BE, Glicksman AS, Leith JT. 1987. Tumor micro-ecology and competitive interactions. Journal of Theoretical Biology 128:233–246.

Mier JW, Gallo RS. 1980. Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media. Proceedings of the National Academy of Sciences USA 77:6134–6138.

Miller EC, Miller JA. 1947. The presence and significance of bound aminoazo dyes in the livers of rats fed p-dimethylaminoazobenzene. Cancer Research 7:468–480.

Mintz B, Illmensee K. 1975. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proceedings of the National Academy of Sciences USA 72:3585–3589.

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. 1988. A highly

conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proceedings of the National Academy of Sciences USA 85:6622–6626.

Mueller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56.

Müller HJ. 1927. Artificial transmutation of the gene. Science 46:84–87.

Nakajima M, Morikawa K, Fabra A, Bucana DC, Fidler I. 1990. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. Journal of the National Cancer Institute 82:1890–1898.

Negrini M, Silini E, Kozak C, Tsujimoto Y, Croce CM. 1987. Molecular analysis of mbcl-2: structure and expression of the murine gene homologous to the human gene involved in follicular lymphoma. Cell 49:455–463.

Nelson-Rees WA, Flandermeyer RR, Hawthorne PK. 1974. Banded marker chromosomes as indicators of intraspecies cellular contamination. Science 184:1093.

Newbold RE, Overell RW. 1983. Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. Nature 304:648–651.

Nowell PC. 1976. The clonal evolution of tumor cell populations. Science 194:23–28.

Nowell PC, Hungerford DA. 1960. Chromosome studies on normal and leukemic human leukocytes. Journal of the National Cancer Institute 25:85–109.

Nurse P. 2000. The incredible life and times of biological cells. Science 289:1711–1716.

Old LJ, Boyse EA. 1964. Immunology of experimental tumors. Annual Reviews in Medicine 15:167–186.

O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. 1997. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88:277–285.

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. 1994. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 79:315–328.

Paget S. 1889. The distribution of secondary growths in cancer of the breast. The Lancet 1:99–101.

Parada LF, Tabin CJ, Shih C, Weinberg RA. 1982. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature 297:474–478.

Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P. 1993. Hypermutability and mismatch repair deficiency in RER + tumor cells. Cell 75:1227–1236.

Pierceall WE, Mukhopadhyay T, Goldberg LH, Ananthaswamy HN. 1991. Mutations in the p53 tumor suppressor gene in human cutaneous squamous cell carcinomas. Molecular Carcinogenesis 4:445–449.

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proceedings of the National Academy of Sciences USA 77:7415–7419.

Popovic M, Sarin PS, Robert-Guroff M, Kalyanaraman VS, Mann D, Minowada J, Gallo RC. 1983. Isolation and transmission of human retrovirus (human t-cell leukemia virus). Science 219:856–859.

Potter VR. 1964. Biochemical perspectives in cancer research. Cancer Research 24:1085–1098.

Potter VR. 1978. Phenotypic diversity in experimental hepatomas: the concept of partially blocked ontogeny. The 10th Walter Hubert Lecture. The British Journal of Cancer 38:1–23.

Ramanathan A, Wang C, Schreiber SL. 2005. Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. Proceedings of the National Academy of Sciences USA 102:5992–5997.

Ramshaw IA, Carlsen S, Wang HC, Badenoch-Jones P. 1983. The use of cell fusion to analyse factors involved in tumour cell metastasis. International Journal of Cancer 32:471–478.

Raser JM, O'Shea EK. 2005. Noise in gene expression: origins, consequences, and control. Science 309:2010–2013.

Remak R. 1852. Über extracellulare Entstehung thierischer Zellen und über Vermehrung derselben durch Theilung. Müllers Archiv für Anatomie, Physiologie und wissenshcaftliche Medizin 19:47–72.

Remak R. 1855. Untersuchungen ueber die Entwickelung der Wirbelthiere. Berlin: Reimer.

Rhoads CP. 1946. The Edward Gemaliel Janeway Lecture: the sword and the ploughshare. The Journal of the Mount Sinai Hospital 13:299–309.

Rijhsinghani K, Greenblatt M, Shubik P. 1968. Vascular abnormalities induced by benzo[a]pyrene: an in vivo study in the hamster cheek pouch. Journal of the National Cancer Institute 41:205–216.

Roth JS. 1965. Histones in development, growth and cancer. Nature 207:599–601.

Rous P. 1911. A sarcoma of the fowl transmissible by an agent separable from the tumor cells. The Journal of Experimental Medicine 13:397–411.

Rowley JD. 1973. Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. Annals of Genetics 16:109–112.

Rubin H. 1955. Quantitative relations between causative virus and cell in the Rous No. 1 chicken sarcoma. Virology 6:669–688.

Ruscetti FW, Morgan DA, Gallo RC. 1977. Functional and morphologic characterization of human T cells continuously grown in vitro. Journal of Immunology 119:131–138.

Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. Science 270:467–470.

Schleiden MJ. 1838. Beiträge zur Pathogenesis. Müllers Archiv für Anatomie, Physiologie und Wissenschaftliche Medizin. 5:137–176.

Schwann T. 1839. Mikroskopische Untersuchungen über die übereinstimmung in der Struktur und dem Wachsthum der Thiere und der Pflanzen. Berlin: Verlag der Sanderschen Buchhandlung.

Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983–985.

Shen J-C, Rideout WM 3rd, Jones PA. 1994. The rate of hydrolytic deamination of 5-methylcytosine in double-stranded DNA. Nucleic Acids Research 22:972–976.

Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. 1984. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science 223:1296–1298.

Sidebottom E, Clark SR. 1983. Cell fusion segregates progressive growth from metastasis. British Journal of Cancer 47:399–405.

Slaughter DP, Southwick HW, Smejkal W. 1953. "Field cancerization" in oral stratified squamous epithelium: clinical implications of multicentric origin. Cancer 6:963–968.

Sporn MB, Dunlop NM, Newton DL, Smith JM. 1976. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Federation Proceedings 35:1332–1338.

Steeg PS, Bevilacqua G, Kopper L. 1988. Evidence for a novel gene associated with low tumor metastatic potential. Journal of the National Cancer Institute 80:200–204.

Stéhelin D, Varmus HE, Bishop JM, Vogt PK. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature 260:170–173.

Stoler DL, Chen N, Basik M, Kahlenberg MS, Rodriguez-Bigas MA, Petrelli NJ, Anderson GR. 1999. The onset and extent of genomic instability in sporadic colorectal tumor progression. Proceedings of the National Academy of Sciences USA 96:15121–15126.

Streeter PR, Berg EL, Rouse BT, Bargatze RF, Butcher EC. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. Nature 331:41–46.

Szostak JW, Blackburn EH. 1982. Cloning yeast telomeres on linear plasmid vectors. Cell 29:245–255.

Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P. 1982. Translocation of the c-MYC gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proceedings of the National Academy of Sciences USA 79:7837–7841.

Taylor S, Folkman J. 1982. Protamine is an inhibitor of angiogenesis. Nature 297:307–312.

Temin HM, Mizutani S. 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 226:1211–1213.

Thibodeau SN, Bren G, Schaid D. 1993. Microsatellite instability in cancer of the proximal colon. Science 260:816–819.

Thomas L. 1959. Discussion. In: Lawrence HS (ed.). Cellular and humoral aspects of the hypersensitive states. New York: Hoeber-Harper, pp. 529–532.

Tolis G, Ackman D, Stellos A, Mehta A, Labrie F, Fazekas ATA, Comaru-Schally AM, Schally AV. 1982. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. Proceedings of the National Academy of Sciences USA 79:1658–1662.

Tomlinson IP. 1997. Game-theory models of interactions between tumour cells. European Journal of Cancer 33:1495–1500.

Turpeenniemi-Hujanen T, Thorgeirsson UP, Hart IR, Grant SS, Liotta LA. 1985. Expression of collagenase IV (basement membrane collagenase) activity in murine tumor cell hybrids that differ in metastatic potential. The Journal of the National Cancer Institute 75:99–103.

Tyzzer EE. 1916. Tumor immunity. Journal of Cancer Research 1:125–156.

Vaupel V, Thews G, Wendling P. 1976. Kritische Sauerstoff-und Glucoseversorgung maligner Tumoren. Deutsche Medizinische Wochenschrift 101:1810–1816.

Vaux DL, Cory S, Adams JM. 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335:440–442.

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. 1995. Serial analysis of gene expression. Science 270:484–487.

Virchow R. 1858. Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre. Berlin: August Hirschwald.

von Hansemann D. 1890. Über asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. Virchows Archiv für Pathologische Anatomie 119:299–326.

von Warburg O. 1930. The metabolism of tumours. London: Constable.

Warthin AS. 1913. Hereditary with reference to carcinoma. Archives of Internal Medicine 12:546–555.

Weber G. 1977. Enzymology of cancer cells (first of two parts). New England Journal of Medicine 296:486–493.

Weber GF, Ashkar S. 2000. Stress response genes - the genes that make cancer metastasize. Journal of Molecular Medicine 78:404–408.

Weinberg RA. 1998. Racing to the beginning of the road. The search for the origin of cancer. New York: W.H. Freeman.

Weiss L. 1992. Comments on hematogenous metastatic patterns in humans as revealed by autopsy. Clinical and Experimental Metastasis 10:191–199.

Winge O. 1930. Zytologische Untersuchungen über die Natur maligner Tumoren. II. Teratokarzinome bei Mäusen. Zeitschrift für Zellforschung 10:683–735.

Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. 1997. Gene expression profiles in normal and cancer cells. Science 276:1268–1272.

CHAPTER 2

PHYSIOLOGIC CORRELATES OF MALIGNANCY

The essential features of cancer are uncontrolled growth, overcoming of replicative senescence, invasiveness, and tumor- or organ-specific neoplasm–host interactions. This raises the question whether all cells have the potential to transform into malignant tumors. The physiologic barriers preventing uncontrolled cell division are higher in differentiated cells than in undifferentiated ones. Stem cells have the potential to proliferate, whereas terminally differentiated cells typically have exited the cell division cycle. Stem cells also are not subject to replicative senescence, because they may still express Telomerase, thus preventing the shortening of the chromosome ends with every cell division. In contrast, Telomerase activity is typically absent from differentiated cells. Tumors derived from them would stop growing after a limited number of population doublings. Most importantly, tissue-specific cells would have to redifferentiate in order to adopt the genetic programs for metastasis formation, which are physiologically used for homing by lymphocytes and macrophages, but not by parenchymal cells in solid organs. The association between growth factor signaling and acquisition of invasiveness is preserved in precursor cells, because it is part of the genetic programs of stress responses. Stem cell expansion and activation are induced by tissue damage. After wounding, there is an accumulation of dividing precursor cells in the affected tissues [Flemming 1885]. Growth factor-dependent signal transduction in precursor cells, but not in differentiated cells, induces transcription factors that activate stress response genes [Witt et al. 2001; Laprise et al. 2002; Nollen and Morimoto 2002]. These characteristics raise the possibility that malignant transformation is not a condition of differentiated cells but rather of tissue-resident precursor cells or stem cells [Sell and Pierce 1994; Reya et al. 2001]. This possibility, referred to as "vitium primae formationis," was alluded to in 1875 in a study of a congenital myosarcoma of the kidneys [Cohnheim 1875]. Alterations in the cellular microenvironment, such as injury or carcinogens, may induce marked differentiative changes in tissue-resident precursor cells. In cancer, this mechanism accounts for the histologic appearance of dedifferentiation, in actuality reflecting the accumulation of stem cells that have become unresponsive to physiologic growth control mechanisms. Differentiation is an anticarcinogenic process.

Organs with high turnover of cells, including the epithelium and the lymphohematopoietic system, need a large pool of precursor cells throughout life. The cancer risk in those systems tends to increase with age due to the accumulation of DNA damage over time. In contrast, other organs, including the brain, are subject to low levels of turnover and regeneration in the mature organism. Their pool of precursor cells may decline with age. The cancer risk in those systems peaks in midlife, possibly reflecting a combination of accumulation of DNA damage and decline in the number of susceptible precursor cells. Osteosarcoma has an increasing incidence during adolescence, when the rate of growth of the long bones is highest. During this stage, precursor cell expansion is extensive and predisposes to the risk of malignant transformation. Ovulation may be important in the pathogenesis of ovarian cancer. Proliferation and migration of epithelial precursor

cells are required for the repair of defects during ovulation. Hence, ovulatory events could promote tumor progression by stimulating the proliferation of ovarian surface epithelial precursor cells. Consistently, early menarche, late menopause, nulliparity, and the use of fertility-stimulating drugs is associated with increased risk for ovarian carcinoma. In contrast, multiple pregnancies, prolonged breast-feeding, and the use of contraceptives decrease the risk.

The normal mammary gland is composed of three cell lineages, myoepithelial cells that form a basal cell layer, ductal epithelial cells, and milk-producing alveolar cells. They are derived from a common pluripotent tissue-resident precursor cell. Although most mammary cells have a limited capacity for self-renewal, these precursor cells can recapitulate the entire functional repertoire of the gland. Mammary cancer risk is positively correlated with the procreative life span of the tissue-resident epithelial stem cells. These cells age prematurely under the influence of TGF-β1, which suppresses Telomerase expression and activity in epithelial cells. Their senescence diminishes the pool of cells that are prone to transformation. Hence, the elevated expression of TGF-β1 in mammary epithelial cells is associated with a reduced susceptibility to cancer [Katakura et al. 1999; Boulanger and Smith 2001]. The EGR-1 (Early Growth Response 1, KROX24, NGFIA, ZIF268) gene product is a transcription factor with roles in differentiation. Its expression markedly reduces growth and tumorigenicity. Conversely, the suppression of *egr-1* expression enhances growth and promotes phenotypic transformation. The growth inhibitory effects of EGR-1 depend on the secretion and autocrine functions of TGF-β, the promoter of which contains two GC-rich EGR-1 binding sites [Liu et al. 1996a].

Epithelial cancers originate from the transformation of precursor cells. Infection with *Helicobacter pylori* causes chronic gastric inflammation and increases the risk for gastric cancer. The induced chronic inflammation and loss of parietal cells compromise the supply of tissue-resident stem cells. This leads to the repopulation of the stomach with bone marrow-derived precursor cells. In the continued presence of the noxious influence, these recruited precursor cells progress through metaplasia and dysplasia to intraepithelial cancer [Houghton et al. 2004].

In keratinocytes and epithelial cells, the levels of β-Catenin correlate with proliferative potential and decreased differentiation. The activation of the WNT singaling pathway, which β-Catenin is a part of, in epithelial stem cells leads to epithelial cancers [Nusse 1992; Reya et al. 2001]. The disruption of β-Catenin-mediated signals in intestinal cells induces a differentiation program. The control of *survivin* expression by the β-Catenin pathway may regulate colonic crypt cell renewal and crypt stem cell numbers by preventing stem cell apoptosis in the basal crypt colonocytes. TCF-4 is a transcription factor that normally associates with β-Catenin in response to WNT signaling. Its deficiency causes the rapid exhaustion of undifferentiated progenitors in the crypts of the gut epithelium during fetal development. The total absence of *tcf4* causes a lack of stem cells in the small intestine [Watt and Hogan 2000]. Conversely, the activity of β-Catenin and TCF-4 in colorectal cancer is essential to overcome rapid G_1 arrest and maintain a genetic program that is physiologically active in the proliferative compartment of colon crypts. The TCF-4 target gene *c-myc* plays a central role in the switch from proliferation to differentiation by direct repression of the $p21^{CIP1/WAF1}$ promoter. Following a disruption of β-Catenin/TCF-4 activity, the decreased expression of c-MYC releases $p21^{CIP1/WAF1}$ transcription, which in turn mediates G_1 arrest and differentiation. Thus, the β-Catenin/TCF-4 complex constitutes the master switch that controls proliferation versus differentiation in healthy and malignant intestinal epithelial cells [van de Wetering et al. 2002].

Certain molecular markers expressed by cancer cells are shared with precursor cells and may reflect the tumor origin. Brain tumors contain cells expressing the neural stem cell markers CD133 and Nestin while lacking the expression of neural differentiation markers. The proliferation rate of these cells correlates with the clinical aggressiveness of the tumors [Singh et al. 2003].

Specific forms of leukemia arise from mutations in hematopoietic stem cells. Chromosomal translocations that predispose to acute myeloid leukemia arise in hematopoietic stem cells in the bone marrow [Miyamoto et al. 2000]. Clonotypic, leukemia-associated chromosomal rearrangements may also occur in $CD34^+CD38^-$ stem cells in lymphoid and chronic myeloid leukemias [George et al. 2001; Mauro and Druker 2001], and most acute myeloid leukemia cells have a $CD34^+CD38^-$ phenotype [Bonnet and Dick 1997].

The precursor nature of the cells of origin for malignancy is evidenced by the frequent expression

of fetal proteins in cancer. They include the glycoprotein Carcinoembryonic Antigen (CEA), which may be elevated in gastrointestinal tumors, breast cancer, lung cancer, pancreas cancer, and ovarian cancer, and in cases of bronchogenic carcinoma and mammary carcinoma. Furthermore, CEA is excreted into the urine in papillary carcinoma of the bladder. There are six forms of CEAs. They are related glycoproteins in fetal intestinal epithelium that are expressed only in trace amounts in healthy adults. Another such tumor marker is the glycoprotein α-1-Fetoprotein (AFP), which is elevated in the blood in 60–90% of patients with hepatocellular carcinoma, 60% of patients with teratoid gonadal tumors, and 13% of patients with metastatic liver tumors. It is also expressed by some pancreas carcinomata and gastrointestinal tumors. Physiologically, α-1-Fetoprotein is synthesized at high levels in fetal hepatocytes and plasma, but in barely detectable amounts in the adult liver. An isoenzyme of Alkaline Phosphatase (Regan enzyme) has antigenic and biochemical properties that are identical to those of the normal human placenta. This enzyme is present in very low abundance in normal serum, but in 3- to 300-fold increased amounts in about 12% of patients with various forms of cancer. An oncofetal protein is TAG-72 (Tumor Associated Glycoprotein 72).

2.1 EXCLUSION BETWEEN PROLIFERATION AND DIFFERENTIATION

Repeated cycles of cell growth and division result in the development of the human body. The division of cells is extensively coordinated to ensure the formation of progeny cells that contain intact genomes and to accurately form anatomical structures. In adulthood, cell cycle progression is mostly active during normal tissue regeneration and during the repair of tissue damage.

Cell proliferation and differentiation are mutually exclusive, with the former typically preceding the latter. Proliferation is incompatible with the expression of a genetic program of terminal differentiation. Thus, irreversible arrest of cell division is a prerequisite for the expression of a terminally differentiated phenotype. In this way, differentiation protects from carcinogenesis. Consistently, most tumor cells display abnormalities in differentiation (anaplasia) and malignant tumors do not arise from fully differentiated cells (Table 2.1.A).

Table 2.1.A. Origins of tumors. Proto-oncogenes and tumor suppressor genes have physiologic roles in organ development and functions, which extend through all three germ layers. If they are defective, their lack often causes disease. The expression of these genes in development and in tumors and the consequences of their deficiencies give some insight into the mechanisms of transformation

Origin	Expression	Deficient phenotype	Cancer	Transforming genes
Ectoderm				
Apical ectodermal ridge	*fgf4*	Blocks development of inner cell mass		
Skin	*dcc*	Brain and spinal chord defects	Squamous cell carcinoma Basal cell carcinoma	*erbB,int-2*
Melanocytes	*kit*		Melanoma	*mts1*
Mammary gland			Breast carcinoma	*rasN, cdc25B, wnt-1, p53, rb, hst, brca1, c-myc, wt-1, erbB-2, int-2, notch*
Neural plate Notochord	*shh*	Deletion of distal limbs, holoprosencephaly, cyclopia		
Neuronal cells	*dcc* *int-1* *src*		Neuroblastoma Medullary blastoma	*ras, p53, p73, neu, c-myc, N-myc* *mts-1*
	wnt1	Underdevelopment of cerebellum		
	N-ras	Impaired antiviral immune response and T-cell function	Neurofibromatosis	*nf-1*
			Acoustic neuroma	*nf-2*
Glial cells			Glioblastoma	*neu, c-myc, erbB, mxi1*
Meninges			Meningioma	*nf-2*

continued

Table 2.1.A. (continued)

Origin	Expression	Deficient phenotype	Cancer	Transforming genes
Retinal cells			Retinoblastoma	*rb, N-myc*
			von Hippel–Lindau disease	*vhl*
Mesoderm				
Bone			Osteosarcoma	*mdm2, p53, rb, met*
Muscle	*myo-D*		Rhabdomyosarcoma	
			Leiomyosarcoma	
Kidney	*mxi1*	Polycystic kidney disease		
	myc		Renal carcinoma	*vhl*
			Wilms tumor	*wt-1*
Adrenal medulla			Pheochromocytoma	
Ovary	*mos*		Ovarian cancer	*rasK, dcc, erbB-2*
Prostate	*mxi1*	Hyperplasia, dysplasia	Prostate cancer	*kai1, mxi1*
Erythrocytes	*erbA*			
	kit		Erythrocytic leukemia	*c-myc, myb*
Granulocytes	*kit*		Myelotic leukemia	*rasK, c-myc, myb, abl*
Lymphocytes	*kit*		Lymphocytic leukemia	*rasK, c-myc, myb*
	myb			*abl*
	p21	Susceptibility to hematopoietic and endothelial tumors		
	mxi1	Enhanced proliferation	Lymphoma	*ras, dcc, ets-1, bcl-2*
	bcl-2		Lymphoid apoptosis, melanocyte, neuronal, intestinal lesions, kidney disease	
			Hodgkin's lymphoma	
			Burkitt's lymphoma	*c-myc*
Plasma cell	*kit*		Myeloma	
Endoderm				
Urinary bladder	*dcc*		Bladder carcinoma	*ras, rasH, rasK, int-2, hst, rb, wt-1*
Stomach			Stomach cancer	*c-myc, int-2, hst*
Colon	*dcc*		Colorectal carcinoma	*rasK, dcc, apc, trk, c-myc, myb, p53*
			Hereditary nonpolyposis	*msh2, mlh1, pms1*,
			Colorectal cancer	*pms2*
			Familial adenomatous polyposis	*apc*
Anus			Anal carcinoma	
Spleen	*mxi1*			
Liver	*mxi1*		Liver carcinoma	
Pancreas			Adenocarcinoma	*rasK, mts1*
Lung	*dcc*		Small cell carcinoma	*rasK, c-myc, N-myc, L-myc, rb, p53*
			Large cell carcinoma	
			Adenocarcinoma	
			Epithelial carcinoma	
Thyroid			Thyroid carcinoma	*B-raf*, *ret*, *ptc*

Various signal transduction pathways that lead to the proliferation of progenitor cells also govern cell divisions in tumors. Conversely, the mediators of cell differentiation also have tumor suppressor function. The terminal differentiation of a variety of cell types correlates with the expression of the Cyclin-Dependent Kinase Inhibitor $P21^{CIP1/WAF1}$, which is also a tumor suppressor [Weinstein et al. 1995; Harper 1997]. Vitamin D compounds are differentiating agents. Vitamin D-Binding Protein interacts with free vitamin D compounds, the biologic effects of which are then mediated by nuclear receptors. The expression of $p21^{CIP1/WAF1}$ can be induced by low concentrations of vitamin D and correlates with the induction of a differentiated phenotype. Vitamin D compounds induce the differentiation of

leukemic cells. The thyroid hormone receptor (eRBA) and the retinoic acid receptor (RARα) normally mediate cell differentiation. The vitamin A metabolite and RAR ligand retinoic acid supports cell differentiation in part by activating G_1 cell cycle regulatory proteins. Mutated forms of the receptors erbA and RARα may interfere with the actions of their normal homologs, thereby blocking differentiation and maintaining an actively proliferating state. Retinoic acid also has chemopreventive activity for certain neoplasms, such as head and neck cancer, cervical carcinoma, or neuroblastoma. All-trans-retinoic acid is particularly effective against acute promyelocytic leukemia, which is caused by a chromosome translocation that involves the RARα.

Cell differentiation and susceptibility to transformation are dependent on the microenvironment. There are tissue-specific profiles of Integrin expression. Integrins that do not efficiently activate FYN/SHC or FAK may facilitate differentiation by inducing exit from the cell cycle. Epidermal stem cells express high levels of Integrin β_1 and adhesion to the extracellular matrix mediated via this Integrin suppresses the onset of terminal differentiation. Integrin β_1 also regulates the differentiation of keratinocytes [Watt and Hogan 2000], and reduced expression of Integrin β_1 in organogenesis leads to altered neuroblast formation [Buck 1995].

Certain oncogenes, such as EWS-FLI1, inhibit tissue-specific differentiation. They may also activate genetic programs that are atypical for the cell type in which they are activated. In the case of EWS-FLI1, a degree of neuroectodermal features is induced [Hu-Lieskovan et al. 2005].

Epithelium. Keratin-1 and Keratin-10 are expressed during the commitment of keratinocytes to differentiate. The Cyclooxygenases COX-1 and COX-2 play important roles in limiting epidermal differentiation. In the absence of COX-1 or COX-2, Keratins −1 and −10 are expressed far more frequently in the epidermal basal cells. Due to the premature onset of keratinocyte terminal differentiation, deficiency of the *cox-1* or *cox-2* genes reduces the susceptibility to skin tumorigenesis [Tiano et al. 2002]. The terminal differentiation of skin also correlates with the expression of the CDKI and tumor suppressor *p21*.

The intestinal epithelium is patterned into crypts and villi. The position along the crypt-to-villus axis defines the stage of cell differentiation. It proceeds from the crypt bases to the villi, with apoptosis and cell shedding occurring upon reaching the villus tip. This process evolves over several days. The crypt-resident intestinal stem cells differentiate into four cell types, the absorptive epithelial cells (secrete Hydrolases and absorb nutrients), and the secretory Goblet cells (secrete protective Mucins), enteroendocrine cells (secrete hormones), and Paneth cells (proximal intestines, absent from the colon, reside in the bottom of the crypts). The high rate of self-renewal of the colon epithelium makes it susceptible to transformation. BMP-4 expression occurs exclusively in the intravillus mesenchyme. Its signaling is directed to the villus epithelial cells, resulting in the phosphorylation of SMADs −1, −5, and −8. This induces the crypt/villus structure of the colon wall. A lack of BMP signaling leads to the formation of numerous ectopic crypt units perpendicular to the crypt–villus axis. This may reflect an altered processing of mesenchymal BMP signals by the epithelial cells and lead to a cancer predisposition syndrome [Haramis et al. 2004]. Cases of juvenile polyposis frequently harbor mutations in BMP pathway genes.

Loss of imprinting of the *igf-2* (*insulin-like growth factor 2*) gene is an epigenetic alteration that results in a modest increase in IGF-2 expression. It generates a shift toward less differentiated cells in the colonic epithelium, reflected in an increase in crypt length and expression of progenitor cell markers. A loss of imprinting of *igf-2* is present in the colonic mucosa of about 30% of patients with colorectal cancer. The delayed maturation of the colon epithelial cells may constitute a predisposition for the development of cancer [Sakatani et al. 2005].

Central nervous system. Signal transduction induced by WNTs, BMPs, or FGFs is associated with neural crest formation and with the induction of neural tissue. WNT and LEF (TCF) family members are expressed in overlapping patterns in the developing brain. With overexpressed β-Catenin, Wnt, and LEF signaling is increased. This causes an increased proportion of precursor cells to reenter the cell cycle after mitosis, leading to an expansion of the precursor population. Therefore, β-Catenin can function in the decision of neural precursor cells to proliferate or differentiate during neuronal development [Chenn and Walsh 2002]. Gain-of-function mutations of genes whose products are active in the pathway WNT→APC→β-Catenin lead to an increased risk for medulloblastoma.

SHH-associated signaling contributes to germline and neural stem cell proliferation. In normal

tissues, the transcription factor and SHH target GLI (Glioblastoma) is mainly active in precursor cells. This implies that tumors are derived from such cells, which are unable to differentiate or to stop proliferating. SHH is dysregulated in medulloblastoma [Reya et al. 2001]. The tumor suppressor PTC inhibits SHH signaling. Its activity is essential for closure of the neural tubes and for reducing the risk for medulloblastomagenesis.

High-grade glioblastomata may have the characteristics of primitive neuroectodermal cells, which show consistent expression of the neural stem cell marker Nestin. The proliferation of neural stem cells can be stimulated by EGF, but not PDGF, while the inactivation of *pten*, a negative regulator of EGF signaling, causes their decreased proliferation. The EGF pathway is upregulated and *pten* is often lost in primary glioblastoma multiforme. PDGFR signaling is mitogenic for oligodendrocyte precursors, and likely for astrocyte precursors. The PDGFR signaling pathway is often active in tumors derived from these cells.

Secreted molecules contribute to the determination of stem cell fate. At least two members of the TGF-β family are important for the differentiation of neural crest stem cells [Watt and Hogan 2000]. TGF-β exerts a complex set of effects in cancers with tumor-suppressive functions through growth inhibition early in transformation. $P27^{KIP1}$ is a downstream effector of TGF-β and a universal CDKI. Its expression suppresses the malignant phenotype of brain tumors [Chen et al. 1996].

Liver. Chemically induced hepatoblast differentiation is accompanied by the cessation of cell growth [Rogler 1997]. Nucleolar proteins regulate cell proliferation and growth by controlling ribosome biosynthesis and P53 functions. The gene product of *nucleostemin* is a nuclear protein that contains an NH_2-terminal basic domain, which specifies its nuclear localization and interaction with P53, and contains two GTP-binding motifs. Nucleostemin is expressed in embryonic stem cells and primitive bone marrow cells. At the onset of differentiation, it is abruptly silenced [Tsai and McKay 2002]. Nucleostemin is involved in regulating the proliferation of liver cancer cells [Liu et al. 2004].

Blood. In the adult healthy organism, the hematopoietic stem cell pool is characterized by relative quiescence, while their progeny have strong proliferative ability. In the absence of the CDK inhibitor and tumor suppressor $P21^{CIP1/WAF1}$, hematopoietic stem cell proliferation is increased, leading to exhaustion of the precursor cells. Therefore, P21 is a molecular switch governing the entry of stem cells into the cell cycle [Cheng et al. 2000]. P21 is also essential for the terminal differentiation of hematopoietic precursor cells. The transcription of $p21^{CIP1/WAF1}$ is induced by the engagement of RARα, in conjunction with PML and Retinoid X Receptor (RXR), or by the engagement of the Vitamin D_3 Receptor [Liu et al. 1996b]. *pten* expression accompanies the terminal differentiation of myeloid cells. Vitamin D and retinoic acid increase the levels of $P27^{KIP1}$ and PTEN in myeloid cells. The cells from patients with acute promyelocytic leukemia (AML M3) undergo terminal differentiation when exposed to all-trans-retinoic acid. In this setting, *cathepsin G*, a promyelocyte stage-specific transcript that is perpetually synthesized in these cells, rapidly disappears. This indicates that the maturation arrest in AML M3 results in cells that may constitutively continue to produce proteins whose production is temporally confined during normal hemopoiesis [Seale et al. 1996].

Notch-dependent signal transduction is involved at various stages of hematopoietic cell development. Notch Receptors are expressed on bone marrow $CD34^+$ cells, a population enriched for hematopoietic precursors. Notch-1 (TAN-1) participates in mediating cell fate decisions during hematopoiesis. The exposure to the Notch ligand JAG-1 prevents the differentiation of $CD34^+$ cells. While Notch-1 is essential for T-lymphocyte lineage commitment, the continuous presence of its cleavage product NICD maintains the T-lymphocytes in the immature, $CD4^+CD8^+$ stage. Activating mutations of Notch-1 arise in more than 50% of T-cell acute lymphoblastic leukemias (ALLs) [Weng et al. 2004]. The overexpression of a fragment similar to NICD occurs following the chromosome translocation t(7;9)(q34;q34.2), which underlies a fraction of T-cell ALLs [Reynolds et al. 1987]. The prevention of complete T-cell differentiation in this setting is a tumor-initiating event that predisposes to full transformation.

WNT induces the rapid expansion of hematopoietic stem cells [Reya et al. 2003; Willert et al. 2003]. The overexpression of activated β-Catenin, a signaling mediator in the Wnt pathway, expands this pool. Conversely, expression of the WNT signaling

inhibitor Axin inhibits hematopoietic stem cell proliferation [Reya et al. 2001]. The translocation products AML1-ETO, PML-RARα, and PLZF-RARα, associated with acute myeloid leukemia, encode aberrant transcription factors. They regulate various genes associated with Wnt signaling. Among them, *plakoglobin* (*γ-catenin*) is induced by the fusion proteins and leads to the downstream transactivation of TCF- and LEF-dependent promoters, including *c-myc* and *cyclin* D_1. This enhances proliferation and accelerates the development of leukemia [Müller-Tidow et al. 2004]. Similarly, the *bmi-1* gene participates in normal hematopoietic development. BMI-1 synergizes with MYC to promote the self-renewal of bone marrow stem cells. The malfunction of BMI-1 is also linked to acute myeloid leukemia. The gene product is needed for the self-renewal of leukemia cells. In its absence the leukemia cells undergo apoptosis [Lessard and Sauvageau 2003].

Stem cell fate is determined by various transcription factors. The basic helix–loop–helix protein TAL-1 (T-Cell Acute Lymphocytic Lukemia-1, Stem cell Leukemia, SCL, T-Cell Leukemia-5, TCL-5) is essential for the formation of all blood cells [Watt and Hogan 2000]. *tal-1* is a common target for translocations in T-cell ALL. TAL-1 is a nucleation factor for a multifactorial complex that specifically enhances *c-kit* promoter activity without affecting the activity of myelomonocytic promoters. This complex contains hematopoietic-specific (TAL-1, LMO-2, GATA-1, GATA-2) and ubiquitous (E2A, LDB-1) factors. It is tethered to DNA via a SP1 (Specificity Protein-1) motif, through direct interactions between elements of the TAL-1 complex and the SP1 {12q13.1} zinc finger protein.

Muscle. IGFs can stimulate skeletal muscle differentiation. Phosphatidylinositol 3-Kinase, Protein Kinase B (AKT), and P70 S6 Kinase are crucial signaling molecules in this process. The Phosphatidylinositol 3-Kinase→Protein Kinase B pathway enhances the transcriptional activity of *myo-D*, *mef2*, and *myogenin*. In contrast, IGF-1 does not enhance *myogenin* expression in rhabdomyosarcoma cells due to a defect downstream of Protein Kinase B [Xu and Wu 2000]. Exposure to genotoxic agents causes a reversible inhibition of myogenic differentiation. Muscle-specific gene expression is suppressed by DNA-damaging agents if encountered prior to differentiation induction, but not after the differentiation program has been established. Specifically, the transcriptional activity of the myogenic determination factor MYO-D is inhibited by DNA damage. This inhibition requires a functional c-ABL tyrosine kinase, but not P53 or c-JUN [Puri et al. 2002]. The transcription factor MYO-D is sufficient to induce the muscle differentiation pathway and it may cause cell cycle arrest [Halevy et al. 1995].

– The induction of *rb* gene transcription by MYO-D is a key event in skeletal muscle differentiation [Adams and Kaelin 1998]. This process requires a cyclic AMP responsive element (CRE) in the *rb* promoter. CREB protein levels and CREB phosphorylation at serine 133 rapidly increase upon the onset of differentiation [Magenta et al. 2003].
– MYO-D induces $p21^{CIP1/WAF1}$ independently of P53. This inhibits cell cycle progression and facilitates differentiation.

The sustenance of a poor differentiation status under circumstances where MYO-D is inhibited predisposes affected cells to transformation. Myoblasts transformed by *myc* oncogenes are severely impaired in the accumulation of mRNAs encoding the myogenic transcription factors MYF-5, MYO-D, and Myogenin. v-MYC selectively interferes with the transcription of *myo-D* expression by targeting *cis*-regulatory elements involved in the auto-activation of *myo-D* [La Rocca et al. 2002]. TNF-α activates NF-κB, which suppresses the production of *myo-D* mRNA at the posttranscriptional level.

Notch signaling is a key determinant of muscle regenerative potential that declines with age. Quiescent skeletal muscle precursor cells (satellite cells) are positioned between the basal lamina and the plasma membrane of muscle fibers. In response to injury, these cells undergo proliferation. The efficacy of skeletal muscle regeneration is markedly impaired with age. This is due to insufficient upregulation of the Notch ligand Delta and thus diminished activation of Notch in aged regenerating muscle [Conboy et al. 2003].

Adipose tissue. The coordinated action of the Peroxisome Proliferator-Activated Receptor γ (PPARPγ) and the CCAAT/Enhancer Binding Protein (C/EBP) family of transcription factors regulates the adipocyte differentiation program. This differentiation may require the direct interaction between the tumor suppressor protein RB and C/EBP [Adams and Kaelin 1998]. The transcription factors GATA-2 and GATA-3 are specifically

expressed in white adipocyte precursors, where they directly suppress PPARPγ. Their downregulation sets the stage for terminal differentiation [Tong et al. 2000]. Subsequent to C/EBPβ and C/EBPδ expression during adipocyte differentiation, C/EBPα and PPARPγ production is stimulated. The mutual induction of expression between these two gene products drives the expression of a genetic program that is necessary for the generation of the adipocyte phenotype. PPARγ is expressed in each of the major histologic types of liposarcoma. Primary liposarcoma cells can be induced to undergo terminal differentiation by activation of PPARγ, suggesting that the differentiation block in these cells can be overcome by maximal induction of the PPAR pathway. RXR-specific ligands are potent adipogenic agents in cells expressing the PPARγ–RXRα heterodimer, and simultaneous activation of PPARγ and RXR in liposarcoma cells results in an additive stimulation of differentiation, which is characterized by the accumulation of intracellular lipid, induction of adipocyte-specific genes, and exit from the cell cycle [Tontonoz et al. 1997].

Bone. *rb* is essential for late ostoblast differentiation. RB binds to RUNX2 (CBFA1, AML3) and associates with osteoblast-specific promoters. This transactivating function is lost in certain RB mutants. Such loss of function mutations in the *rb* gene predisposes to osteosarcoma [Thomas et al. 2001].

2.2 EXTENDED REPLICATIVE POTENTIAL OF PRECURSOR CELLS

Stem cells are cells with unlimited, or prolonged, capacity for reduplication. Stem cells are normally quiescent until prompted to divide by external cues. They produce at least one type of highly differentiated descendant, usually via intermediate populations of committed progenitors. Nonsenescent cell division is physiologically restricted to germline cells and stem cells. The activation of the genetic programs that lead to cellular differentiation is accompanied by the activation of the process of cellular senescence. The number of possible population doublings by nontransformed differentiated cells is finite [Hayflick and Moorehead 1961], and senescence is reached when irreversible loss of the proliferative potential has occurred. The phenotype of replicative senescence is characterized by an irreversible arrest of cell proliferation, altered metabolism, and frequently increased resistance to apoptosis. Because the functional dominance of senescence genes is a part of terminal differentiation, the physiologic barriers preventing unlimited proliferation are higher in differentiated cells than in undifferentiated ones. The characteristics of quiescence and senescence have to be overcome in transformation.

Shortening of the chromosome ends, telomeres, occurs with every cell division. It is an integral part of replicative senescence. The enzyme Telomerase replenishes the chromosome ends and can prevent this shortening. Telomerase activity is present in germline cells [Kim et al. 1994] and Telomerase RNA is detected in all newborn tissues but decreases during postnatal development [Blasco et al. 1995]. In normal somatic tissues, Telomerase is active only in spermatogonial cells, bone marrow precursors, and cells within the thymus [Effros and Pawelec 1997]. These sources continuously generate mature cells with very limited life spans. Differentiated cells do not have Telomerase activity [Kim et al. 1994] and accordingly possess a limited potential to divide before reaching the state of crisis, characterized by genomic instability and massive cell death once the telomere length is reduced to a critical point. Telomerase is active in the vast majority of cancers, which reflects the importance of immortalization or overcoming of replicative senescence in carcinogenesis.

Germline cells may be the only totipotent cells and possibly not subject to malignant transformation. Embryonic stem cells are derived from the blastocyst inner cell mass and are presumed to be the most pluripotent cells. Their transformation gives rise to teratomata or teratocarcinomata [Stevens 1958; Stevens 1970]. Tissue-resident precursor cells are susceptible to the formation of particular types of cancer depending on their level and type of commitment, whereas fully differentiated cells are protected from malignant transformation.

2.3 THE GENETIC PROGRAMS OF INVASIVENESS ENCODE STRESS RESPONSES

It has been subject to intense debate whether the dissemination of cancer cells is an active process, driven by the malignancy, or the carriage of tumor cells to distant sites through the lymph or blood flow. (Table 2.3.A). The output of tumor cells from a primary site

Table 2.3.A. Evidence weighing genetic versus epigenetic mechanisms of metastasis. The mechanisms of metastasis formation have been subject to intense debate. Two opposing views contend that the ability of malignant, but not benign tumors to disseminate is either encoded in the genetic programs of the tumor cells or caused by complex interactions between the tumor cells and their microenvironment without genetic direction

Some observations seem to favor passive dislocation.
Video microscopy with methods to quantitate cell fate has suggested that tumor cells are effectively retained in proximal capillary beds [Chambers et al. 2002]. These studies are, however, limited to relatively short time frames of analysis and they depend on the injection of tumor cells rather than endogenous tumors.
The large size and low deformability of most cancer cells makes it more difficult for them than for blood cells to pass through small diameter capillaries.
Tumor cells that disseminate to the lungs stay inside the capillaries for extended periods of time before extravasating [Wong et al. 2002].
The process of metastatic dissemination is very inefficient due to negative regulation of cancer growth in secondary sites. This is accomplished mostly through apoptosis [Weiss 1990; Wong et al. 2001; Wong et al. 2002]. Millions of tumor cells are shed daily into the circulation. A very small percentage, estimated at 0.01%, of circulating tumor cells ultimately initiate successful metastatic colonies.
Individual tumor cells can be found disseminated, and frequently dormant, in peripheral tissues. However, normal cells also can survive in a dormant state for extended periods of time in peripheral tissues (e.g., microchimerism of fetal cells in mothers).
Active locomotion underlying metastasis is supported by other observations.
For various tumors, the number of metastases in specific target organs cannot be accounted for solely by blood flow patterns [Weiss 1992]. Metastases frequently do not form in proximal capillary beds. Breast cancer cells would be taken by the blood flow through the heart to the pulmonary capillaries. Any cells passing through would enter the systemic circulation to all capillary beds. Distal breast cancer metastases, however, form preferentially in the bones and brain. The lymphatic system also does not account for the pattern of distal metastases.
Incidence and distribution of experimental metastases in mice are affected by the genomic background in which they occur [Arguello et al. 1992].
Murine experimental melanoma metastasis targets preferentially the lungs. Ectopic lung tissue implanted into the thigh is also colonized by melanoma cells [Sandritter 1981].
Tumor cells with increased metastatic potential can be selected from parent cell lines by repeated passage of cells from the metastatic lesions [Fidler 1975; Bendre et al. 2002], supporting the hypothesis that metastatic potential is determined to a large degree by properties of the tumor cells.
Genes have been identified that are necessary and sufficient to mediate malignancy. Metastatic potential is conferred to cells that overexpress metastasis genes. The homing receptor CD44 does not affect early stages of transformation, but is essential for the dissemination of certain tumors [Günthert et al. 1991; Weber et al. 2002]. Osteopontin is essential for metastasis formation by some tumors. The overexpression of osteopontin increases the malignant phenotype of tumor cells [Denhardt and Guo 1993] while its repression yields populations with reduced malignant potential [Behrend et al. 1994; Gardner et al. 1994].
Homing receptors and their ligands have been found to be functionally important in metastasis. Their characteristics as cell surface molecules that mediate migration imply a process of active locomotion to underlie metastasis formation [Weber et al. 1996; Müller et al. 2001].
The secretion of extracellular matrix degrading enzymes by tumors supports the possibility that metastasis is an active process, which is directed by the invasive properties of the cancer cells.
The existence of metastasis suppressor genes [Steeg et al. 1988; Alvarez et al. 1990; Ray and Stetler-Stevenson 1994] points to the regulation of invasiveness by genetic programs those are intrinsic to the tumor cells.

appears to be stochastic and inefficient. In contrast, the cells that establish distant metastases are characterized by the completion of a process of active homing. Regardless of the apparent complexity of the event of metastasis formation, the activation of a limited number of genetic programs is sufficient for its implementation. The destruction of the basement membrane is accomplished by secreted enzymes, which also play roles during intravasation and extravasation. Dissemination is mediated by homing receptors on the tumor cell surface. Their ligation initiates cell migration and is often associated with the prevention of anoikis, the form of programmed cell death that occurs upon detachment. Metastasis associated gene products comprise a set of developmentally nonessential biomolecules that physiologically mediate the repertoire of stress responses, including inflammation, wound healing, and neovascularization, which are predominantly executed by macrophages and lymphocytes [Weber and Ashkar 2000]. Commonly, these gene products mediate leukocyte homing. (Table 2.3.B). In contrast to morphogenesis, invasiveness and tissue damage are in keeping with the normal functions of host defenses.

During wound healing, expression of the Integrin $\alpha_5\beta_1$ is upregulated even though it is not expressed in

Table 2.3.B. Genetic deficiency of metastasis-associated molecules. Gene-targeted mice, in which individual genes known to participate in tumor spread had been disrupted, turned out to be fertile and developmentally normal, arguing against a physiologic correlate to metastasis in morphogenesis during embryonic development. Consistently, the defects observed in the relevant gene-targeted mice are impairments of various features of stress responses. DTH = delayed type hypersensitivity

Gene	Types of cancer	Knockout mouse
Receptors		
upar	Prostate cancer, breast cancer, gastric carcinoma, brain tumors	Defect in leukocyte recruitment and adhesion [May et al. 1998]
cd44	Lymphomas, sarcomas colon cancer, breast cancer	Excessive granuloma formation, excessive lung inflammation after injury [Schmits et al. 1997; Teder et al. 2002]
L-selectin	Lymphoma	No DTH to cutaneous antigens [Xu et al. 1996]
lfa-1	Lymphoma	Impaired immune response to alloantigens [Shier et al. 1996]
icam-1	Melanoma, lymphoma, liver carcinoma	Granulocytosis, diminished DTH, impaired neutrophil homing [Sligh et al. 1993; Xu et al. 1994]
cd47 (iap)	Renal cell carcinoma, ovarian cancer	Impaired granulocyte activation [Lindberg et al. 1996]
Ligands		
osteopontin	Breast cancer, osteosarcoma	Defective wound healing, absence of DTH [Liaw et al. 1998; Weber et al. 2000]
thrombospondin-1	Breast cancer, pancreas cancer	Susceptibility to pneumonia [Lawler et al. 1998]
sE-selectin	Gastric cancer, breast cancer, head and neck cancer	Reduced stable adhesion of leukocytes in inflamed microvasculature [Kunkel and Ley 1996; Milstone et al. 1998]
P-selectin	Breast cancer, colon cancer	Impaired recruitment of immune cells [Subramaniam et al. 1995]
fgf-2	Glioma	Delayed excisional skin wound repair [Ortega et al. 1998]
Proteinases		
mmp-3 (stromelysin-3)	Breast cancer	Impaired wound healing [Shapiro 1997]
mmp-7 (matrilysin)	Colon cancer	Defective reepithelialization in wounded trachea [Dunsmore et al. 1998]
mmp-12 (macrophage elastase)	Glioma	Impaired macrophage recruitment [Shapiro 1997]

adult resting cells [Buck 1995]. It may contribute to the invasion of the wound by immune system cells and tissue precursor cells. Lymphoma cells that express the lymph node homing receptor L-Selectin metastasize extensively and exclusively to peripheral lymph nodes, a homing phenotype displayed by mature T-cells leaving the thymus [Bargatze et al. 1986].

Cancers of particular tissue origin show consistent preference for specific target organs to spread. Two principal mechanism may explain organ tropism.

– Tumor cells may disseminate stochastically, but selectively grow only in specific organs. Preferential growth and homing may be induced by the local microenvironment.
– Alternatively, circulating tumor cells may specifically home and adhere to the endothelial luminal surface only in the targeted organs.

The topology of metastasis formation by various malignant tumors is determined by the biochemical characteristics of the homing receptors on the tumor cell surface and their ligands. The first targets in metastatic spread are typically draining lymph nodes [Dukes 1932]. The homing to and expansion in the lymphoid system corroborate that cancer metastasis is based on mechanisms normally employed by immunocytes [Arch et al. 1992; Müller et al. 2001]. Differentiation of immune system cells proceeds in the context of their tissue of residence so that lymphocytes from the Peyer patches are distinct from cutaneous lymphocytes and Kupffer cells are distinct from alveolar macrophages. This implies that the recognition of topology is encoded in the surface molecules of immune cells and that organ preference by cancer is not derived from organogenesis but from a process, which the immune system uses to target its responses. This also applies to tumors with locally destructive growth, including chondrosarcoma, basalioma, and myelodysplastic forms of leukemia. The absence of distal metastases in those tumors is indicative of their homing receptors targeting them to their respective organs of origin [Weber and Ashkar 2000].

The biologic activity of metastasis-mediating gene products is extensively regulated by posttran-

scriptional mechanisms. Proteinases are typically secreted as precursors, whose activation requires proteolytic cleavage. Ligands for homing receptors often contain multiple domains. Posttranscriptional modification of function of these molecules may be beneficial in two ways:

– Activation by mechanisms like proteolytic cleavage or phosphorylation can be accomplished quickly in stress situations; some of the precursor molecules are widely expressed and can acutely be converted to their active forms at a site of damage.
– Diversity in structure may encode organ specificity in homing and metastasis formation.

While these genes have not been observed to be mutated in malignancies like the classical oncogenes (frequently through point mutations, deletions, frameshifts, or translocations) they are subject to dysregulation:

– Cancer cells aberrantly express metastasis genes that are typically silent in healthy cells of the same differentiation.
– Cancer cells display splice variants of metastasis genes that are not detected on their nontransformed counterparts.

Therefore, aberration of genes for cancer spread occurs frequently on the levels of transcription or splicing. Without this dysregulation tumors could not become malignant.

The frequency of coincidence between tumorigenesis and invasiveness in cancers (as opposed to tumorigenesis without invasive potential in benign tumors) suggests a linkage between signaling mechanisms that regulate the activity of oncogene products and the expression of molecules responsible for cell homing. In most differentiated cells, growth signals do not activate the genetic programs for invasiveness. In contrast, growth factor signaling also induces increased motility in cells of the immune system and in tissue-resident precursor cells, due to their physiologic activation in response to tissue damage. The genetic programs of proliferation and invasion are physiologically activated in the adult organism only after macrophage or lymphocyte activation [Arch et al. 1992] and during the repair of injury. Cancer does not originate from mature cells of organ-specific differentiation. Those cells have silenced the genetic program that is normally selectively used in stress responses by immune system cells and tissue-resident precursor cells.

2.4 THE MICROENVIRONMENT DETERMINES THE FATE OF TRANSFORMED CELLS

The multicellular composition of the organism necessitates coordination among cells of varied specializations through intercellular communication. Growth factor, hormone, and Integrin signaling pathways all contribute to maintaining tissue architecture. The extracellular matrix is a network of macromolecules that provides contextual information and an architectural scaffold for cell adhesion and migration. The condition of the stroma is an important factor to determine the susceptibility of cells to transformation. The loss of spatial control leads to the disorganization of normal tissue architecture that is the hallmark of neoplastic transformation.

Mechanical forces on cell shape, generated by insoluble extracellular matrix molecules, contribute, and may be rate limiting to the progression through G_1 [Huang and Ingber 1999]. Several important cell cycle events are dependent on cell–substratum adhesion in nontransformed cells, including the activation of G_1 CDKs and the expression of Cyclin A. Integrin occupation and clustering leads to stimulation of multiple early mitogenic events associated with the transition from G_0 to G_1 in the cell cycle, including EGR genes. Unanchored fibroblasts remain arrested in mid-G_1 and display a marked reduction of Cyclin E/CDK2 kinase activity and increased levels of the CDK Inhibitors, $P27^{KIP1}$ and $P21^{CIP1/WAF1}$ [Fang et al. 1996; Huang and Ingber 1999]. The adhesion requirement of these events is abrogated in cells transformed by *ras*, which may be due to the downregulation of $P27^{KIP1}$.

Breast cancer cells can colonize bone because they activate genetic programs that mediate cell–cell communication with osteoblasts and osteoclasts. Plasticity of the bone structure is maintained by a balance between osteoblastic and osteoclastic activities. This balance is regulated by various signals between the two cell types. Important interactions in the regulation of bone resorption include the induction and activation of osteoclastogenesis, induced by binding of RANK Ligand on osteoblasts to RANK on osteoclast progenitors, and the suppression of osteoclastogenesis, mediated by binding of RANKL to soluble Osteoprotegerin, which interferes with the engagement of RANK. Osteoprotegerin is a member of the TNFR family and a negative regulator of osteoclast

function. Bone metastases of breast cancer typically express RANKL and thus activate bone resorption.

REFERENCES

Adams PD, Kaelin WG jr. 1998. Negative control elements of the cell cycle in human tumors. Current Opinion in Cell Biology 10:791–797.

Alvarez OA, Carmichael DF, DeClerck YA. 1990. Inhibition of collagenolytic activity and metastasis of tumor cells by a recombinant human tissue inhibitor or metalloproteinases. Journal of the National Cancer Institute 82:589–595.

Arch R, Wirth K, Hofmann M, Ponta H, Matzku S, Herrlich P, Zöller M. 1992. Participation in normal immune responses of a metastasis-inducing splice variant of CD44. Science 257: 682–685.

Arguello F, Furlanetto RW, Baggs RB, Graves BT, Harwell SE, Cohen HJ, Frantz CN. 1992. Incidence and distribution of experimental metastases in mutant mice with defective organ microenvironments (genotypes SI/SId and W/Wv). Cancer Research 52:2304–2309.

Bargatze RF, Wu NW, Weissman IL, Butcher EC. 1986. High endothelial venule binding as a predictor of the dissemination of passaged murine lymphomas. The Journal of Experimental Medicine 166:1125–1131.

Behrend EI, Craig AM, Wilson SM, Denhardt DT, Chambers AF. 1994. Reduced malignancy of ras-transformed NIH 3T3 cells expressing antisense osteopontin RNA. Cancer Research 54:832–837.

Bendre MS, Gaddy-Kurten D, Mon-Foote T, Akel NS, Skinner RA, Nicholas RW, Suva LJ. 2002. Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis in vivo. Cancer Research 62:5571–5579.

Blasco AM, Funk W, Villeponteau B, Greider CW. 1995. Functional characterization and developmental regulation of mouse telomerase RNA. Science 269:1267–1270.

Bonnet D, Dick JE. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates form a primitive hematopoietic cell. Nature Medicine 3:730–737.

Boulanger CA, Smith GH. 2001. Reducing mammary cancer risk through premature stem cell senescence. Oncogene 20:2264–2272.

Buck CA. 1995. Adhesion mechanisms controlling cell–cell and cell–matrix interactions during the metastatic process. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: Saunders, pp. 172–205.

Chambers AF, Groom AC, MacDonald IC. 2002. Dissemination and growth of cancer cells in metastatic sites. Nature Reviews Cancer 2:563–572.

Chen J, Willingham T, Shuford M, Nisen PD. 1996. Tumor suppression and inhibition of aneuploid cell accumulation in human brain tumor cells by ectopic overexpression of the cyclin-dependent kinase inhibitor p27KIP1. The Journal of Clinical Investigation 97:1983–1988.

Cheng T, Rodrigues N, Shen H, Yang Y-g, Dombrowski D, Sykes M, Scadden DT. 2000. Hematopoietic stem cell quiescence maintained by $p21^{cip1/waf1}$. Science 287:1804–1808.

Chenn A, Walsh CA. 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 297: 365–369.

Conboy IM, Conboy MJ, Smythe GM, Rando TA. 2003. Notch-mediated restoration of regenerative potential to aged muscle. Science 302:1575–1577.

Cohnheim J. 1875. Congenitales, quergestreiftes Muskelsarcom der Nieren. Archiv fuer Pathologische Anatomie 65:64–69.

Denhardt DT, Guo X. 1993. Osteopontin: a protein with diverse functions. FASEB Journal 7:1475–1482.

Dukes CE. 1932. The classification of cancer of the rectum. Journal of Pathology 35:323–332.

Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG, Parks WC. 1998. Matrilysin expression and function in airway epithelium. Journal of Clinical Investigation 102:1321–1331.

Effros RB, Pawelec G. 1997. Replicative senescence of T cells: does the Hayflick limit lead to immune exhaustion? Immunology Today 18:450–454.

Fang F, Orend G, Watanabe N, Hunter T, Ruoslahti E. 1996. Dependence of cyclin E-CDK2 kinase activity on cell anchorage. Science 271:499–502.

Fidler IJ. 1975. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Research 35:218–224.

Flemming W. 1885. Studien ueber Regeneration der Gewebe. Aus dem Anatomischen Institut Kiel. Bonn.

Gardner HAR, Berse B, Senger DR. 1994. Specific reduction in osteopontin synthesis by antisense RNA inhibits the tumorigenicity of transformed rat1 fibroblasts. Oncogene 9:2321–2326.

George AA, Franklin J, Kerkof K, Shah AJ, Price M, Tsark E, Bockstoce D, Yao D, Hart N, Carcich S, Parkman R, Crooks GM, Weinberg K. 2001. Detection of leukemic cells in the $CD34^{+}CD38^{-}$ bone marrow progenitor population in children with acute lymphoblastic leukemia. Blood 97:3925–3920.

Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. 1991. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 65:13–24.

Halevy O, Novitch B, Spicer D, Skapek SX, Rhee J, Hannon GJ, Beach D, Lassar AB. 1995. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. Science 267:1018–1021.

Haramis AP, Begthel H, van den Born M, van Es J, Jonkheer S, Offerhaus GJ, Clevers H. 2004. De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. Science 303:1684–1686.

Harper JW. 1997. Cyclin dependent kinase inhibitors. Cancer Surveys 29:91–107.

Hayflick L, Moorehead PS. 1961. The limited in vitro lifetime of human diploid cell strains. Experimental Aging Research 25:585–621.

Houghton JM, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. 2004. Gastric cancer originating from bone marrow-derived cells. Science 306:1568–1571.

Huang S, Ingber DE. 1999. The structural and mechanical complexity of cell-growth control. Nature Cell Biology 1:E131–E138.

Hu-Lieskovan S, Zhang J, Wu L, Shimada H, Schofield DE, Triche TJ. 2005. EWS-FLI1 fusion protein up-regulates critical genes in neural crest development and is responsible for the observed phenotype of Ewing's family of tumors. Cancer Research 65:4633–4644.

Katakura Y, Nakata E, Miura T, Shirahata S. 1999. Transforming growth factor β triggers two independent-senescence programs

in cancer cells. Biochemical and Biophysical Research Communications 255:110–115.

Kim NM, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviellom GM, Wright WE, Weinrich SL, Shay JW. 1994. Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015.

Kunkel EJ, Ley K. 1996. Distinct phenotype of E-selectin-deficient mice. E-selectin is required for slow leukocyte rolling in vivo. Circulation Research 79:1196–1204.

La Rocca SA, Vannucchi S, Pompili M, Pinney DF, Emerson CP jr, Grossi M, Tato F. 2002. Selective repression of myoD transcription by v-Myc prevents terminal differentiation of quail embryo myoblasts transformed by the MC29 strain of avian myelocytomatosis virus. Oncogene 21:4838–4842.

Laprise P, Poirier EM, Vezina A, Rivard N, Vachon PH. 2002. Merosin-integrin promotion of skeletal myofiber cell survival: differentiation state-distinct involvement of $p60^{Fyn}$ tyrosine kinase and p38α stress-activated MAP kinase. Journal of Cell Physiology 191:69–81.

Lawler J, Sunday M, Thibert V, Duquette M, George EL, Rayburn H, Hynes RO. 1998. Thrombospondin-1 is required for normal murine pulmonary homeostasis and its absence causes pneumonia. Journal of Clinical Investigation 101:982–992.

Lessard J, Sauvageau G. 2003. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature 423: 255–260.

Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. 1998. Altered wound healing in mice lacking a functional osteopontin gene (spp1). Journal of Clinical Investigation 101:1468–1478.

Lindberg FP, Bullard DC, Caver TE, Gresham HD, Beaudet AL, Brown EJ. 1996. Decreased resistance to bacterial infection and granulocyte defects in IAP-deficient mice. Science 274:795–798.

Liu C, Adamson E, Mercola D. 1996a. Transcription factor EGR-1 suppresses the growth and transformation of human HT-1080 fibrosarcoma cells by induction of transforming growth factor beta-1. Proceedings of the National Academy of Sciences USA 93:11831–11836.

Liu M, Lee MH, Cohen M, Bommakanti M, Freedman LP. 1996b. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes and Development 10:142–153.

Liu SJ, Cai ZW, Liu YJ, Dong MY, Sun LQ, Hu GF, Wei YY, Lao WD. 2004. Role of nucleostemin in growth regulation of gastric cancer, liver cancer and other malignancies. World Journal of Gastroenterology 10:1246–1249.

Magenta A, Cenciarelli C, De Santa F, Fuschi P, Martelli F, Caruso M, Felsani A. 2003. MyoD stimulates RB promoter activity via the CREB/p300 nuclear transduction pathway. Molecular and Cellular Biology 23:2893–2906.

Mauro MJ, Druker BJ. 2001. Chronic myelogenous leukemia. Current Opinion in Oncology 13:3–7.

May AE, Kanse SM, Lund LR, Gisler RH, Imhof BA, Preissner KT. 1998. Urokinase receptor (CD87) regulates leukocyte recruitment via beta 2 integrins in vivo. The Journal of Experimental Medicine 188:1029–1037.

Milstone DS, Fukumura D, Padgett RC, O'Donnell PE, Davis VM, Benavidez OJ, Monsky WL, Melder RJ, Jain RK, Gimbrone MA jr. 1998. Mice lacking E-selectin show normal numbers of rolling leukocytes but reduced leukocyte stable arrest on cytokine-activated microvascular endothelium. Microcirculation 5:153–171.

Miyamoto T, Weissman IL, Akashi K. 2000. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proceedings of the National Academy of Sciences USA 97:7521–7526.

Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56.

Müller-Tidow C, Steffen B, Cauvet T, Tickenbrock L, Ji P, Diederichs S, Sargin B, Kohler G, Stelljes M, Puccetti E, Ruthardt M, deVos S, Hiebert SW, Koeffler HP, Berdel WE, Serve H. 2004. Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells. Molecular and Cellular Biology 24:2890–2904.

Nollen EA, Morimoto RI. 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing "heat shock" proteins. The Journal of Cell Science 115:2809–2816.

Nusse R. 1992. The *Wnt* gene family in tumorigenesis and in normal development. Journal of Steroid Biochemistry and Molecular Biology 43:9–12.

Ortega S, Ittmann M, Tsang SH, Ehrlich M, Basilico C. 1998. Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. Proceedings of the National Academy of Sciences USA 95:5672–5677.

Puri PL, Bhakta K, Wood LD, Costanzo A, Zhu J, Wang JY. 2002. A myogenic differentiation checkpoint activated by genotoxic stress. Nature Genetics 32:585–593.

Ray JM, Stetler-Stevenson WG. 1994. TIMP-2 expression modulates human melanoma cell adhesion and motility. Annals of the New York Academy of Science 732:233–247.

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL. 2003. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 423:409–414.

Reya T, Morrison SJ, Clarke MF, Weissman IL. 2001. Stem cells, cancer, and cancer stem cells. Nature 414:105–111.

Reynolds TC, Smith SD, Sklar J. 1987. Analysis of DNA surrounding the breakpoints of chromosomal translocations involving the beta T cell receptor gene in human lymphoblastic neoplasms. Cell 50:107–117.

Rogler LE. 1997. Selective bipotential differentiation of mouse embryonic hepatoblasts *in vitro*. American Journal of Pathology 150:591–602.

Sakatani T, Kaneda A, Iacobuzio-Donahue CA, Carter MG, de Boom Witzel S, Okano H, Ko MSH, Ohlsson R, Longo DL, Feinberg AP. 2005. Loss of imprinting of *igf2* alters intestinal maturation and tumorigenesis in mice. Science 307: 1976–1978.

Sandritter W. 1981. Allgemeine Pathologie, 2nd edn. Stuttgart/New York: Schattauer.

Schmits R, Filmus J, Gerwin N, Senaldi G, Kiefer F, Kundig T, Wakeham A, Shahinian A, Catzavelos C, Rak J, Furlonger C, Zakarian A, Simard JJ, Ohashi PS, Paige CJ, Gutierrez-Ramos JC, Mak TW. 1997. CD44 regulates hematopoietic progenitor distribution, granuloma formation, and tumorigenicity. Blood 90:2217–2233.

Seale J, Delva L, Renesto P, Balitrand N, Dombret H, Scrobohaci ML, Degos L, Paul P, Chomienne C. 1996. All-trans retinoic acid rapidly decreases cathepsin G synthesis and mRNA expression in acute promyelocytic leukemia. Leukemia 10:95–101.

Sell S, Pierce GB. 1994. Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Laboratory Investigations 70:6–22.

Shapiro SD. 1997. Mighty mice: transgenic technology "knocks out" questions of matrix metalloproteinase function. Matrix Biology 15:527–533.

Shier P, Otulakowski G, Ngo K, Panakos J, Chourmouzis E, Christjansen L, Lau CY, Fung-Leung WP. 1996. Impaired immune responses toward alloantigens and tumor cells but normal thymic selection in mice deficient in the beta2 integrin leukocyte function-associated antigen-1. Journal of Immunology 157:5375–5386.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. 2003. Identification of a cancer stem cell in human brain tumors. Cancer Research 63:5821–5828.

Sligh JE jr, Ballantyne CM, Rich SS, Hawkins HK, Smith CW, Bradley A, Beaudet AL. 1993. Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. Proceedings of the National Academy of Sciences USA 90:8529–8533.

Steeg PS, Bevilacqua G, Kopper L. 1988. Evidence for a novel gene associated with low tumor metastatic potential. Journal of the National Cancer Institute 80:200–204.

Stevens LC. 1958. Studies on transplantable testicular teratomas of strain 129 mice. Journal of the National Cancer Institute 20:1257–1270.

Stevens LC. 1970. The development of transplantable teratocarcinomas from intratesticular grafts and pre- and postimplantation mouse embryos. Developmental Biology 21:364–382.

Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO, Wagner DD. 1995. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. The Journal of Experimental Medicine 181:2277–2282.

Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Pure E, Henson PM, Noble P. 2002. Resolution of lung inflammation by CD44. Science 296:155–158.

Thomas DM, Carty SA, Piscopo DM, Lee JS, Wang WF, Forrester WC, Hinds PW. 2001. The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. Molecular Cell 8:303–316.

Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, Dunson DB, Rogan EG, Morham SG, Smart RC, Langenbach R. 2002. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Research 62:3395–3401.

Tong Q, Goekhan D, Xu H, Ting C-N, Leiden JM, Hotamisligil GS. 2000. Function of GATA transcription factors in preadipocyte-adipocyte transition. Science 290:134–138.

Tontonoz P, Singer S, Forman BM, Sarraf P, Fletcher JA, Fletcher CD, Brun RP, Mueller E, Altiok S, Oppenheim H, Evans RM, Spiegelman BM. 1997. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. Proceedings of the National Academy of Sciences USA 94:237–241.

Tsai RY, McKay RD. 2002. A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. Genes and Development 16:2991–3003.

van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H. 2002. The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111:241–250.

Watt FM, Hogan BLM. 2000. Out of Eden: Stem cells and their niches. Science 287:1427–1428.

Weber GF, Ashkar S. 2000. Stress response genes – the genes that make cancer metastasize. Journal of Molecular Medicine 78:404–408.

Weber GF, Ashkar S, Glimcher MJ, Cantor H. 1996. Receptor-ligand interaction between CD44 and osteopontin (ETA-1). Science 271:509–512.

Weber GF, Bronson R, Ilagan J, Cantor H, Schmitz R, Mak TW. 2002. Absence of CD44 prevents sarcoma metastasis. Cancer Research 62:2281–2286.

Weinstein IB, Carothers AM, Santella RM, Perera FP. 1995. Molecular mechansims of mutagenesis and multistage carcinogenesis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: Saunders, pp. 59–85.

Weiss L. 1990. Metastatic inefficiency. Advances in Cancer Research 54:159–211.

Weiss L. 1992. Comments on hematogenous metastatic patterns in humans as revealed by autopsy. Clinical and Experimental Metastasis 10:191–199.

Weng AP, Ferrando AA, Lee W, Morris JP IV, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. 2004. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269–271.

Witt O, Schulze S, Kanbach K, Roth C, Pekrun A. 2001. Tumor cell differentiation by butyrate and environmental stress. Cancer Letters 171:173–182.

Wong CW, Lee A, Shientag L, Yu J, Dong Y, Kao G, Al-Mehdi AB, Bernhard EJ, Muschel RJ. 2001. Apoptosis: an early event in metastatic inefficiency. Cancer Research 61:333–338.

Wong CW, Song C, Grimes MM, Fu W, Dewhirst MW, Muschel RJ, Al-Mehdi AB. 2002. Intravascular location of breast cancer cells after spontaneous metastasis to the lung. American Journal of Pathology 161:749–753.

Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR, Nusse R. 2003. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature 423:448–452.

Xu H, Gonzalo JA, St Pierre Y, Williams IR, Kupper TS, Cotran RS, Springer TA, Gutierrez-Ramos JC. 1994. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. The Journal of Experimental Medicine 80:95–109.

Xu J, Grewal IS, Geba GP, Flavell RA. 1996. Impaired primary T cell responses in L-selectin-deficient mice. The Journal of Experimental Medicine 183:589–598.

Xu Q, Wu Z. 2000. The insulin-like growth factor-phosphatidylinositol 3-kinase-Akt signaling pathway regulates myogenin expression in normal myogenic cells but not in rhabdomyosarcoma-derived RD cells. Journal of Biological Chemistry 275:36750–36757.

SECTION II

ALTERATIONS INTRINSIC TO THE TUMOR CELLS

CHAPTER 3

CELL DIVISION AND SURVIVAL

The most prominent characteristic of cancer, uncontrolled cell division, is caused by dysregulation of the cell cycle clock [Weinberg 1996]. Cell cycle progression is promoted externally by growth factors and internally by a series of protein kinases that induce cell division in accord with the needs of the whole organism. The two principal quality control mechanisms in cell proliferation are cell cycle arrest and apoptosis. Proto-oncogenes physiologically induce cell division or survival by operating in the pathways, through which cells recognize and respond to growth factors or prevent programmed cell death. Conversely, tumor suppressor gene products antagonize proto-oncogenes either by activating cell cycle checkpoints or by inducing apoptosis. The abnormal growth rate of cancerous cells is mediated by the activation of genetic programs that promote cell cycle progression and overcome the otherwise dominant control mechanisms of cell cycle arrest and apoptosis. Transforming genetic alterations affect the expression or the function of the gene products involved and may occur in distinct ways:

– Gain-of-function mutations of proto-oncogenes affect signal transduction pathways, which are linked to receptors for growth factors and cause overactivation of the associated processes with consecutively excessive cell divisions.
– Loss of function of tumor suppressor genes eliminates cell cycle checkpoints or prevents the induction of apoptosis.

Division of cancer cells leads to the formation of more cancer cells, indicating that the characteristics of transformation originate in genetic changes that are stably inherited (Table 3.A).

3.1 CELL CYCLE CONTROL

3.1.1 Components of the cell cycle

The cell cycle describes the time period between two consecutive cell divisions and consists of four strictly regulated phases, referred to as gap 1 (G_1) for cell growth, DNA synthesis (S) for duplication of the genetic material, gap 2 (G_2) for preparation for cell division (G_1, S, and G_2 are collectively named interphase), and mitosis (M) for execution of cell division (Figure 3.1.1.A). Quiescent cells that are not growing reside in a resting state called gap 0 (G_0). While the length of the G_1 phase is variable, the duration of S, G_2, and M phases is relatively invariant. The length of G_0 is variable and is determined by the absence of external growth stimuli.

Cyclin Dependent Kinases (CDKs) are serine/threonine kinases. The regulation of their activity constitutes a rate-limiting step in cell cycle progression, so that activation and inhibition of these kinases pace the progression through the cell cycle. While the expression levels of CDKs remain fairly constant, their activity is highly regulated.

– Cyclins serve as regulatory subunits of CDKs. They contain the Cyclin box, a homologous region of about 100 amino acids, through which they bind to their partnering CDKs. A Cyclin can assemble with an appropriate catalytic CDK subunit. The known Cyclins include A_{1-2}, B_{1-3}, C, D_{1-4}, E_{1-2}, F, G_{1-2} (Table 3.1.1.A). The levels of Cyclins fluctuate with the phases of the cell cycle, due to changes in synthesis and degradation. Furthermore, the subcellular localization of Cyclins into the nucleus or the cytoplasm is important in cell cycle control.

Table 3.A. Cancer genes and their functions. Tumors are caused through gains of function of oncogene products or losses of function of tumor suppressor gene products. Oncogenes and tumor suppressor genes can be grouped according to their roles in the control of the cell cycle or apoptosis

(A) Oncogenes		
Growth factors		
sis (*pdgf-B*)	Platelet-Derived Growth Factor expressed on endothelial cells, targets mesenchymal/glial/smooth muscle cells	Sarcoma, glioma, squamous cell carcinoma
int-2 (*fgf-3*)	Member of the Fibroblast Growth Factor family	Ovarian cancer, breast carcinoma,stomach carcinoma,Kaposi sarcoma
hst (*fgf-4*)	Member of the Fibroblast Growth Factor family, targets vascular endothelial cells/fibroblasts	Stomach cancer, Kaposi sarcoma
fgf-5	Member of the Fibroblast Growth Factor family	Renal cancer, breast, cancer, prostate cancer, pancreas carcinoma
igf-1	Insulin-Like Growth Factor, expressed in liver	Prostate cancer
egf	Epidermal Growth Factor, expressed in submaxillary gland, targets epithelial/mesenchymal/glial cells	Breast cancer, lung cancer
tgf-α	Transforming Growth Factor, expressed in platelets targets epithelial/mesenchymal/glial cells	Breast adenocarcinoma, lung cancer
shh	Ligand for PTC, inhibits PTC function	Basal cell carcinoma, medulloblastoma, meningioma
wnt-1	Growth factor, signals through β-Catenin and LEF	Breast cancer
wnt-2	Growth factor	Breast cancer, gastrointestinal cancers
Growth factor receptors		
erbA	Thyroid Hormone Receptor	Acute promyelocytic leukemia, thyroid cancer
erbB	Part of Epidermal Growth Factor Receptor, receptor protein tyrosine kinase	Glioblastoma, breast cancer, bladder cancer, squamous cell lung cancer, lung adenocarcinoma, head and neck cancer, colon cancer
fms	Receptor for Macrophage Colony-Stimulating Factor,	
ret	growth factor receptor for Glial Cell Line-Derived Neurotropic Factor (GDNF), activates I-κB Kinase resulting in NF-κB activation	Thyroid carcinoma, pheochromocytoma, multiple endocrine neoplasia type 2
ar	Androgen Receptor	Prostate cancer
Signal transduction molecules associated with growth factor receptors		
Protein kinases		
ros	Insulin Receptor β-chain, receptor, protein tyrosine kinase	Glioblastoma
met	Hepatocyte Growth Factor Receptor, receptor protein tyrosine kinase	Osteosarcoma, renal cancer
trk	Nerve Growth Factor Receptor, protein tyrosine kinase	Colon carcinoma
erbB2 (*neu*) (*her-2*)	Receptor protein tyrosine kinase	Neuroblastoma, glioma, breast adenocarcinoma, ovarian cancer, lung adenocarcinoma, salivary gland cancer
c-kit	Receptor tyrosine kinase, binds stem cell factor	Gastrointestinal stromal tumors, mast cell tumors
abl	Nonreceptor protein tyrosine kinase	Chronic myelogenous leukemia, acute lymphocytic leukemia
src	Nonreceptor protein tyrosine kinase	Colon cancer
raf-1	Protein serine/threonine kinase	Stomach cancer, laryngeal cancer, parotid gland tumors
mos	Protein serine/threonine kinase, phosphorylation of Tubulin, regulation of meiotic cell cycle	Burkitt lymphoma, acute myeloblastic leukemia
pkc	Protein Kinase C, protein serine/threonine kinase	Colon cancer, pituitary adenoma, thyroid cancer
pik3CA	Catalytic subunit of Phosphatidylinositol 3-Kinase	Cervical cancer, ovarian cancer
pik3R1	Regulatory subunit of Phosphatidylinositol 3-Kinase	Ovarian cancer, colon cancer
pkb (*akt*)	Protein serine/threonine kinase, binds lipids via Pleckstrin homology domain, phosphorylates Histone H2B, phosphorylates Caspase-9 which prevents activation by Cytochrome *c*-induced proteolysis, is a target of Phosphatidylinositol 3-Kinase activity	Glioblastoma, breast cancer, chronic lymphocytic leukemia

(continued)

Table 3.A. (continued)

frat	Frequently rearranged in advanced T-cell lymphomata, binds to and inactivates GSK3β	T-cell lymphomata
c-cbl	Substrate of protein tyrosine kinases, binds GRB-2 and PI 3-Kinase, negative regulator of SYK	Acute myeloid leukemia
GTP-binding proteins		
N-ras	Guanine nucleotide-binding protein GTPase	Bladder cancer, colon cancer, pancreas carcinoma, leukemia
K-ras	Guanine nucleotide-binding protein GTPase	Lung cancer, ovarian cancer, colon cancer, pancreas cancer
gsp	Encodes the α subunit of G_s	Thyroid cancer, pituitary cancer
bcl-2	GTP-binding, antagonism of apoptosis, B-cell antioxidant	Follicular lymphoma
prc17	RAB-GAP	Prostate cancer
Cyclin–CDK activity		
cyclin A	Associates with RB, the RB family member P107, the transcription factor E2F, the oncoprotein E1A, CDC2	Hepatocellular carcinoma
cyclin D_1	Associates with CDKs 2, 4, 5	Parathyroid adenoma
prad1	Cyclin D_1–CDK4 complex phosphorylates RB	B-cell lymphoma
(*bcl-1*)	Activation of cell cycle kinases	Breast cancer, head and neck cancer
cdk4	Phosphorylation of RB causing release of E2F-DP1	Melanoma, pancreas cancer
cdc25A	Phosphatase, activates CDKs, is regulated by Cyclin B	
cdc25B	Phosphatase, activates CDKs, is regulated by Cyclin B	Breast cancer
mdm2	Nuclear zinc finger protein, transcription of S phase genes by binding to and inactivation of P53	Osteosarcoma, soft tissue sarcomas
Transcription factors		
jun	Component of the AP-1 transcription factor	Hodgkin lymphoma, anaplastic large cell lymphoma
fos	Component of the AP-1 transcription factor	Osteosarcoma
ets-1	Transcription factor	Lymphoma
c-myc	Acts together with MAX, sensitizes cells to CD95-mediated apoptosis	Burkitt lymphoma, leukemia, breast cancer, stomach cancer, lung cancer
N-myc	Acts together with MAX	Neuroblastoma, glioblastoma
L-myc	Acts together with MAX	Lung cancer
e2A	Transcription factors E12 and E47 that bind to the *igκ* gene E-box motif	Pre-B-cell ALL
e2F	Dimer with DP1 initiates transcription of S phase genes	Lung cancer, breast cancer
dp1	Prostaglandin D receptor, dimer with E2F initiates transcription of S phase genes	Colorectal cancer, skin cancer
c-maf	Transcription factor, transactivates *il-4* promoter	Multiple myeloma
rel	Nuclear protein	B-cell lymphoma
mll (*htrx*) (*all-1*)	Transcription factor	Acute myeloid leukemia, acute lymphoid leukemia
tcl-5 (*tal-1*)	Helix–loop–helix transcription factor	T-cell leukemia, stem cell leukemia, T-cell acute leukemia
<u>*Function not grouped*</u>		
β-catenin	Key component of cell–cell adhesive junctions, associates with Cadherin, WNT signal transduction, APC plus GSK-3β regulate the level of free β-Catenin, binds to TCF and LEF transcription factors to alter gene expression	Colon cancer, melanoma
hox11 (*tcl-3*)	Homeobox protein	T-cell leukemias
tcl-2 (*rhom 2*) (*atl*)		T-cell leukemias
hpc1	Possibly identical to *ski*, *abl2*, or *trk*	Hereditary prostate cancer
bmi-1	Lymphoma	
pttg	Securin, binds to Separin	Pituitary tumor
bcas-1 (*nabc1*)		Breast cancer

(continued)

Table 3.A. (continued)

(B) Tumor suppressor genes		
<u>*Receptors*</u>		
dcc	Membrane Adhesin-like protein, receptor for Nectrin-1	Colon carcinoma, non-Hodgkin lymphoma, ovarian carcinoma
ptc	Represses transcription of genes encoding *tgf-β*, *wnt* class genes, and *ptc* itself	Basal cell carcinoma, medulloblastoma, meningioma
rarα	Retinoic acid receptor, nuclear hormone receptor, terminal myeloid and granulocytic differentiation, transactivates $p21^{CIP1/WAF1}$	Acute promyelocytic leukemia
<u>*Signal transduction molecules associated with growth factor receptors*</u>		
Inactivation of G-Protein-GTP signal		
nf-1	GTPase-activating protein, inactivation of RAS, inhibition of *ras*	Neurofibromatosis Recklinghausen, pheochromocytoma, myeloid leukemia
tsc2	Tuberin, activation of GTPase	Astrocytoma, rhabdomyosarcoma
rassf1A	RAS association domain family protein	Medulloblastoma, nasopharyngeal cancer, lung cancer
rcc1	RAN-GEF	Mantle cell lymphoma
Cytoskeleton structure		
apc	Communication between cell surface and microtubules, GSK3β substrate, binds to β-Catenin in WNT signaling pathway, blocks progression to S phase of cell cycle	Familial adenomatous polyposis, colon cancer, stomach cancer, medulloblastoma
nf2	Cytoskeletal protein Merlin	Acoustic neuroma, meningioma, glioma, ependymoma, schwannoma
pten (*mmac1*)	Homology to Tensin, tyrosine phosphatase, dephosphorylates Focal Adhesion Kinase, inhibits cell migration and Integrin-mediated cell spreading, is tyrosine phosphorylated upon Integrin-mediated cell adhesion, dephosphorylates phosphatidylinositol-3,4,5-trisphosphate thus preventing the activation of PKB	Endometrial cancer, cancer, glioblastoma, breast cancer, kidney cancer, brain cancer, prostate cancer, thyroid cancer, pancreas cancer
fhit	Possible Diadenosine Triphosphate Hydrolase, binds Tubulins and promotes microtubule assembly lung cancer, breast cancer	Stomach cancer, colon cancer, esophagus cancer, cervical cancer
Blockage of Cyclin–CDK activity		
chk2	Kinase may phosphorylate P53, cell cycle checkpoint	Breast cancer, brain cancer, leukemia
cdkn1A (*waf1*/*cip1*) (*p21*)	Binding to and inhibition of CDK2 and CDK4, activated by P53, inhibits DNA synthesis when complexed with PCNA, transcription induced by STAT1	Leukemia, lung cancer
cdkn1B (*kip1*) (*p27*)	Inhibition of CDKs	Osteosarcoma, prostate cancer
cdkn2A (*mts1*) (*p16*) (*ink4*)	Cyclin-Dependent Kinase Inhibitor 2A Multiple Tumor Suppressor 1 Inhibitor of CDK4 and CDK6	Melanoma, lung cancer, medulloblastoma, pancreas carcinoma, leukemia, lymphoma
cdkn2B (*mts2*) ($p15^{INK4b}$)	Inhibitor of Cyclin-Dependent Kinases	Acute lymphoblastic leukemia, lung cancer, melanoma, glioma
ppp2r1B	β form of the serine/threonine Protein Phosphatase 2A, down-regulates MAP Kinase cascade, inhibits nuclear Telomerase activity	Lung cancer, colon cancer
Transcription factors		
p53	Transcription factor, stimulates transcription of *p21*, cell cycle regulator, is phosphorylated by CDK and Casein Kinase, induces apoptosis via transport of CD95 from the Golgi complex	Osteosarcoma, breast cancer, brain tumor, Li–Fraumeni syndrome, pancreas carcinoma, small cell lung cancer
p73	Transcription factor, cell cycle regulator, apoptosis, only 1 copy active due to imprinting	Neuroblastoma
wt1	Transcription factor	Wilms tumor, nephroblastoma

(continued)

Table 3.A. (continued)

rb1	Negative regulation of transcription factors E2F-DP1, cell cycle regulation, activity regulated by phosphorylation (low in G_0/G_1, high in G_1/S)	Retinoblastoma, osteosarcoma, small cell lung cancer, bladder cancer, breast cancer, prostate cancer, cervical carcinoma
brg1	Component of the SWI–SNF chromatin remodeling complex, inhibition of proliferation through interaction with RB pancreas cancer	prostate cancer, breast cancer, lung cancer
vhl	Negative regulation of transcription factor Elongin	von Hippel-Lindau disease, renal carcinoma pheochromocytoma
dpc4	Signal transduction that inhibits cell division,	Pancreas cancer, brain
(*smad4*)	signal transduction of TGF-β-like ligands, growth inhibitory signals	Cancer, colorectal cancer
ing1	Four splice variants 47 kD, 33 kD, 27 kD, 24 kD, cell cycle arrest in G_1 by transactivation of $p21^{WAF1/CIP1}$ and down regulation of *cyclin* B_1 in the presence of P53, apoptosis, transcriptional coactivator	Esophageal squamous cell cancer, head and neck cancer
klf6	Zinc finger transcription factor, up-regulates $p21^{CIP1/WAF1}$ independently of P53	Prostate cancer
ctcf	Eleven zinc finger transcription factor, represses *myc* promoters, regulates expression of *arf*, *pim1*, *plk*, *igf2*	Breast cancer, prostate cancer, Wilms tumor
dmp1	Transcriptional activation of $p14^{ARF}$	Lymphoma
pml	Nuclear RING finger protein, necessary for the retinoic acid-mediated transactivation of $p21^{CIP1/WAF1}$, contributes to terminal myeloid differentiation	Acute promyelocytic leukemia
mxi1	Competition with MYC for MAX, competition with MYC for consensus DNA-binding sites, recruitment of transcriptional corepressors	Prostate adenocarcinoma, T-cell leukemia, glioblastoma, squamous skin carcinoma
noey2	Down-regulation of *cyclin* D_1 promoter activity, induction of $p21^{CIP1/WAF1}$	Breast cancer, ovarian cancer
Inducers of cell death		
dapk	Death-Associated Protein Kinase, mediator of apoptotic signals	Lymphoma, bladder cancer
wwox (*fox*) (*wox1*)	Down-regulation of *bcl-2 and bcl-*x_L, up-regulation of *p53*, enhancement of TNF cytotoxicity	Esophageal cancer, gastric cancer, ovarian cancer
bax	Promotion of apoptosis, P53-independent tumor suppression	Colon cancer, leukemia
beclin-1	60 kD BCL-2 interacting coiled-coil protein, promotes autophagy	Breast cancer, ovarian cancer
Function not grouped		
riz1	RB-interacting zinc finger, frequent frameshift mutations in cancers with microsatellite instability, contains RB binding motif and nuclear hormone receptor binding motif	Colorectal cancer, gastric cancer, endometrial cancer
tsc1	Hamartin	Facial angiofibroma
men1	Cofactor in Histone Methyl Transferase complex	Multiple endocrine neoplasia type 1, pancreas cancer, leukemia
ext1	Polymerizes heparan sulfate	Chondrosarcoma
syk	Tyrosine kinase	Breast cancer
s100A2	Nuclear calcium-binding protein	Breast cancer, lung cancer
hic-1	Located on chromosome 17p13.3, frequently hypermethylated in cancer	Medulloblastoma, lung cancer, colon cancer

It is controlled by their nuclear localization sequences.

– The Cyclin/CDK complex requires phosphorylation on threonine for activation. The CDKs are phosphorylated, and thus activated by CDK-Activating Kinases (CAK).
– A level of CDK activation is the dephosphorylation of its ATP-binding site by dual specific tyrosine and threonine Phosphatases of the CDC25 family.

Quiescence. In the absence of growth factors, cells cease proliferation in the G_1 phase of the cell cycle and enter G_0 [Cooper 1999]. The exposure of G_0-arrested cells to growth factors re-activates cell cycle progression by inducing transcriptional activity.

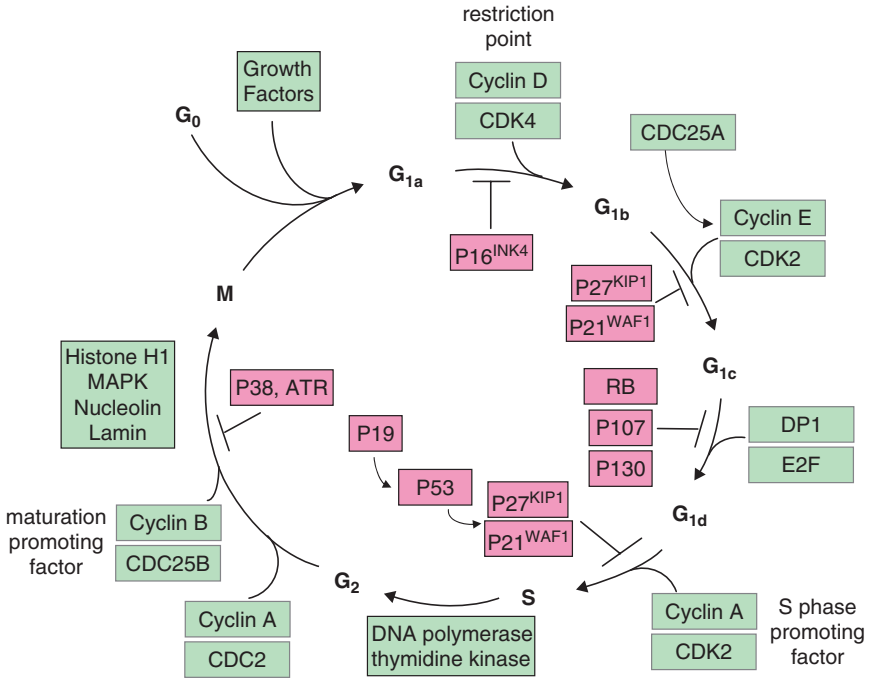


Figure 3.1.1.A. Components of the cell cycle. Promoting factors are depicted on green background outside the cell cycle, while inhibitory factors are shown on red background inside the cell cycle. Cells in G_0 have exited the cell cycle, but can be stimulated to reenter it by growth factors.

The transcription of early response genes is induced within a few minutes and is not dependent on protein synthesis, because the required transcription factors are present in G_0 cells and are activated by posttranslational modifications, such as phosphorylation. Many of the early response gene products, such as c-FOS and c-JUN, stimulate the expression of the delayed response genes. After peaking at about 30 min following the exposure to growth factors, the concentrations of the mRNAs generated in the early response fall to a lower level that is maintained as long as growth factors are present. Most of the immediate early proteins are unstable and, consequently, decrease in concentration as their rate of synthesis declines. After the restriction point in G_1, extracellular factors are usually unnecessary because cells are guided through S, G_2, and M phases by an internal program. If growth factor stimulation ceases before passage through the restriction point, the transcription of the G_1 phase CDKs and Cyclins terminates. Because these proteins and the mRNA messages encoding them are unstable, their concentrations fall precipitously. As a consequence, the cells do not pass the restriction point and do not reduplicate. Components that modulate exit from G_0 and progression through G_1 are critical for determining the growth rate of most cells.

Table 3.1.1.A. Cyclins in the cell cycle. Progression through the cell cycle is tightly controlled by Cyclins, Cyclin-Dependent Kinases (CDKs), and CDK-Activating Kinases (CAKs). They act in a hierarchical order, with CAKs activating CDKs, which in turn activate Cyclins. Individual Cyclins act selectively at specific stages of the cell cycle

Cyclin	CDK	CAK	Stage
D_{1-4}	2,4 (PSK-J3), 6		G_1
C	8 (K35)		G_1
E_{1-2}	2, 4, 5, 6		G_1/S
G_{1-2}	GAK (G-Associated Kinase)		G_1/S
A_{1-2}	1, 2, 3		S
A_{1-2}	1, 2, 3	Cyclin H/CDK7/MAT1	G_2/M
F			G_2/M
B_{1-3}	1		M

Interphase. During interphase, the chromosomes are decondensed and cell growth occurs. It is the longest period of the complete cell cycle, during

which DNA reduplicates, the centrioles divide, and proteins are synthesized. The interphase generally lasts about 12–24 h. It can be divided into three steps: gap 1, synthesis, and gap 2.

– Gap 1 (G_1) is a period of preparation for DNA synthesis. The expression of D-type Cyclins (D_1, D_2, and D_3) continues throughout G_1 as long as growth factors are present. D-type Cyclins are localized in the nucleus during G_1. They complex with either CDK4 or CDK6 catalytic subunits to form a holoenzyme modified by CAK. This releases the cells from G_1 arrest and reflects the passing of the restriction point, which divides the cell cycle into an uncommitted phase and a committed phase. It occurs through three principal mechanisms.
  – Phosphorylation of the RB protein is initiated by CDK4/Cyclin D and CDK6/Cyclin D in mid-G_1 and releases its repressing function, permitting the activation of the genes required for entry into S phase by a small family of E2F transcription factors. E2Fs are normally inhibited by their binding to the RB family of proteins RB, P107, or P130. This converts them from transcriptional activators to repressors, because RB interacts with Histone Deacetylase complexes (HDACs) to suppress gene expression. The release of E2Fs is required for the expression of several genes that contribute to DNA synthesis and the genes that encode Cyclin A, Cyclin E, and CDK2. In addition, E2Fs activate transcription of the genes encoding themselves, thus forming a positive feedback loop. Once the expression of *cdk2* and *cyclin E* is stimulated by E2F, CDK2/Cyclin E further phosphorylates RB in late G_1. RB is then maintained in the phosphorylated state throughout the remaining phases of the cell cycle by CDK2/Cyclin and CDK1/Cyclin complexes.
  – The basic leucine zipper transcription factor C/EBPβ (NF-IL6, LAP) is a downstream target of Cyclin D_1. C/EBPβ is a constitutive repressor of Cyclin D_1 target genes, and Cyclin D_1 acts by antagonizing this repressor function. [Lamb et al. 2003]. C/EBPβ supports differentiation, its inhibition supports proliferation.
  – The sequestration of P27 by Cyclin D_1 facilitates the activation of Cyclin E/CDK2. At this time, the continued passage through the cell cycle is independent of CDK4/Cyclin D or CDK6/Cyclin D activity, so that progression occurs even when mitogens are withdrawn and the Cyclin D levels fall. The positive cross-regulation of E2F and CDK2/Cyclin E produces a rapid rise of both activities as the cell approaches the G_1 to S transition point. Genes encoding many of the proteins involved in DNA and deoxyribonucleotide synthesis are induced as the cells pass through the G_1 to S transition. The phosphatase CDC25A is important during the initiation of S phase. CDC25A dephosphorylates and activates the CDK2/Cyclin E complex. Successful G_1 to S transition requires sustained RAS activity and CDK2/Cyclin E complexes until late G_1. The synthesis of the CDC6 protein in late G_1 promotes the binding of MCM proteins to chromatin and the cell assembles a pre-reduplication complex (prereplication complex) at future origins of DNA reduplication. This is followed by activation of the master coordinator CDC28, which phosphorylates three components of the pre-reduplication complex (ORC, CDC6, and MCM2–7). Through phosphorylation, the initiator protein CDC6 is targeted for degradation and MCM2–7 are eliminated from the nucleus. This assures that only one round of reduplication of DNA takes place per cell cycle. The unidirectional progression of the cell cycle is enforced by the proteolytic degradation of Cyclins and CDKs in a temporally controlled manner. Cullins contribute to ubiquitination of Cyclins and CDKs in G_1. From anaphase through G_1, mitotic Cyclins are proteolyzed by the anaphase-promoting complex (APC), a Ubiquitin Ligase complex that induces the destruction of Cyclins through the proteasome pathway. This is terminated by CDKs as the cells prepare to enter S phase.
– During synthesis (S) phase, the DNA is reduplicated so that, upon its completion, the cell has a tetraploid set of chromosomes. As the DNA reduplication is initiated, Cyclin D_1 translocates from the nucleus to the cytoplasm, where it is targeted for degradation in the proteasome by the Ubiquitin Ligase SCF. Cyclin E colocalizes with CDK2 in Cajal bodies, subcellular organelles associated with Histone gene clusters, at the G_1/S boundary. As the reduplication of DNA continues, Cyclin E is destroyed. The synthesis of Cyclin A begins as cells approach the G_1 to S transition, and the protein is immediately transported into the nucleus. This is a prerequisite for DNA synthesis. Activation of the S phase complex CDK2/Cyclin A triggers the initiation of origins that had previously formed pre-reduplication

complexes, while at the same time it blocks any further assembly of pre-reduplication complexes, ensuring that origin of initiation remains a singular event per cell cycle.

– During Gap 2 (G_2), the period between DNA synthesis and mitosis, the cell continues to grow and to produce new proteins. The cell assembles Cyclin H, CDK7, and MAT1 to form CAK [Tassan et al. 1995], which can phosphorylate the regulatory protein CDK1 ($P34^{CDC2}$, CDC2). CDK1 forms complexes with Cyclin B_1 (CCNB1) to form the maturation promoting factor (MPF) during S and G_2. This results in the phosphorylation of threonine 161 and the dephosphorylation on threonine 14 and tyrosine 15 of CDK1 and activates the transition into M phase. Substrates of CDK1 ($P34^{CDC2}$) are proteins involved in the maintenance of the cell during the G_2 phase. The phosphorylation of these proteins changes their functions and permits the cell to enter M phase:
  – Centrosomal proteins, which are associated with centrioles, the organizing center of the cell for microtubules associated with the cytoskeleton
  – Lamin, a protein whose phosphorylation leads to the breakdown of the nuclear envelope
  – $P60^{SRC}$, whose phosphorylation on specific sites may influence the cytoskeleton and lead to changes in cell shape
  – Histone H1, the phosphorylation of which is important for chromatin condensation to occur
  – Other DNA-binding proteins that need to be released for chromosomal condensation to occur

MPF activity is also controlled by regulation of the nuclear transport of Cyclin B. The activity of CDK1 is regulated positively by the phosphatase CDC25, which dephosphorylates tyrosine 14 and threonine 15, and negatively by the kinases WEE-1 and MYT-1, which phosphorylate these residues. MYT-1 is cytoplasmic and phosphorylates threonine 14, while WEE-1 is nuclear and phosphorylates tyrosine 15. CDC25 is activated at the end of G_2. $P13^{SUC1}$ is a protein of 13 kD, which may be involved in the inactivation of CDK1 ($P34^{CDC2}$) late in mitosis by inhibiting its kinase activity or promoting its rephosphorylation at the end of M phase. CDC34 is a Ubiquitin-conjugating enzyme. It targets the CDK Inhibitor $P40^{SIC1}$ for degradation, which activates Cyclin B/CDC28. At the end of this gap is a control checkpoint, the G_2/M checkpoint, which determines whether the cell can now proceed to enter M (mitosis) and divide.

Mitosis phase (M). Cell growth and protein production stop during M phase and the division into two similar daughter cells commences. Mitosis is much shorter than interphase, lasting perhaps only 1–2 h. The basic events of M phase include chromosome condensation, formation of the mitotic spindle [Bütschli 1876], attachment of the chromosomes to the spindle, sister chromatid separation, and formation of daughter nuclei with chromosome decondensation. During mitosis, Cyclin A is degraded. Cyclin B_1, which is in the cytoplasm during S and G_2, translocates to the nucleus. The maturation promoting factor (MPF), consisting of Cyclin B and CDK1 ($P34^{CDC2}$, CDC2), initiates mitosis [Masui and Markert 1971; Lohka et al. 1988]. It mediates the breakdown of the nuclear envelope, chromatin condensation, spindle formation, and fragmentation of the Golgi complex and endoplasmic reticulum. The activation of M phase CDKs promotes the formation of the Ubiquitin Ligase complex called anaphase-promoting complex/cyclosome (APC/C), which induces the loss of sister chromatid cohesion and the destruction of M phase Cyclins, including Cyclin B, through the Ubiquitin and proteasome pathway [Glotzer et al. 1991; Hershko et al. 1991]. The APC consists of BIM-E, CDC27, CDC16, CDC23, APC2 (related to Cullins), APC4, APC5, and APC7. Activity of the APC is stimulated by the regulatory proteins CDC20 and CDH1 in a cell cycle-dependent manner. CDC20 association with APC is required for APC activity in early mitosis, but CDH1 association is required for APC activity during late mitosis and G_1. Cyclins contain a conserved nine residue region, called the destruction box, which is subject to ubiquitination. From anaphase through G_1, mitotic Cyclins are proteolyzed by the APC. The APC is inactivated by the accumulation of G_1 CDKs [Nasmyth 1996]. The mutual inhibition between APC and CDKs explains how cells suppress mitotic CDK activity during G_1 and then establish a period with elevated kinase activity from S phase until anaphase. Polo-Like Kinases (PLKs), comprising PLK1, PLK2 (SNK), and PLK3 (FNK, PRK), are serine/threonine kinases that are required at several points during mitosis, including activation of the anaphase promoting complex/cyclosome (APC/C), centrosome maturation, and mitotic exit. Their kinase domains are located at the NH_2-terminus. The COOH-terminal

regions of PLKs contain a conserved domain, the Polo-box, which is required for determining their subcellular localization and for the interaction with their substrates. PLKs are localized to the kinetochore of chromosomes. The multiprotein complex Cohesin (SMC1, SMC3, SCC-1/MCD1) creates physical links between sister chromatids [Michaelis et al. 1997; Guacci et al. 1997]. The phosphorylation of Cohesin by PLKs enhances its propensity to be cleaved by Separin (Separase), which then leads to chromatid separation. The inhibition of Separin is released by the degradation of Securin. APC/C acts as a Ubiquitin Protein Ligase, triggering the degradation of the anaphase-inhibiting protein Securin (PDS1) (Figure 3.1.1.B).

– Prophase is the first mitotic stage, during which the nucleolus fades and chromatin (reduplicated DNA and associated proteins) condenses into chromosomes. Each chromosome comprises two chromatids, with corresponding genetic information. The Aurora Kinases phosphorylate Histone proteins, thereby promoting chromosome condensation, and interact with a number of chromosomal passenger proteins, including Inner Centrosome Proteins (INCEPs) and Survivin. Microtubules of the cytoskeleton, responsible for cell shape, motility, and attachment to other cells during interphase, disassemble. The building blocks of these microtubules are used to grow the mitotic spindle from the region of the centrioles.

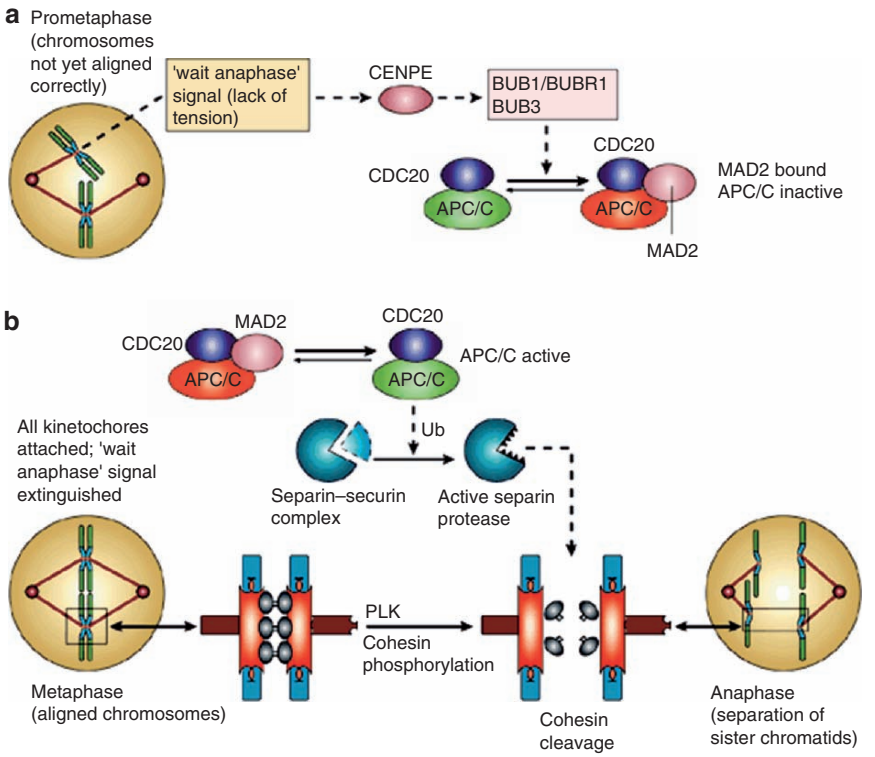


Figure 3.1.1.B. Sister chromatid separation. (a) In prometaphase, cells contain condensed chromosomes that actively establish bipolar attachments to the mitotic spindle. Unattached chromosomes generate a signal that delays progress to anaphase until all sister chromatids are attached to the spindle apparatus. This signal is transduced by a relay of spindle checkpoint proteins that include CENPE and the MAD/BUB proteins. This ultimately results in inhibition of the anaphase promoting complex/cyclosome (APC/C), which is associated with the mitotic cofactor CDC20. (b) Following attachment of the last kinetochore to the mitotic spindle, the "wait anaphase" signal is extinguished. This allows APC/C and CDC20 to become active, resulting in the Ubiquitin-dependent degradation of *S*ecurin and liberation of active *S*eparin. This protease catalyzes the cleavage of Cohesin complexes that bridge the aligned sister chromatids. The newly separated sister chromatids can then migrate to the poles along the spindle axis during anaphase. PLK = Polo-Like Kinases, Ub = Ubiquitin. [Reproduced from Jallepalli and Lengauer 2001. With permission from Macmillan.]

– In the prometaphase stage, the nuclear envelope breaks down, so there is no longer a recognizable nucleus. The mitotic spindle fibers elongate from the centrioles and attach to the kinetochores, protein bundles located on the chromosomes. The growth of microtubules from the centrosomes is mediated by polymerization that is controlled by GTP caps, which can exist if the rate of GTP hydrolysis lags slightly behind that of Tubulin polymerization. Ends without a GTP cap have high Tubulin off rates and shrink, whereas ends with a GTP cap grow [Mitchison and Kirschner 1984a,b]. Tubulin is the protein subunit of the microtubules [Weisenberg et al. 1968].
– Tension applied by the spindle fibers in metaphase aligns all chromosomes in one plane at the center of the cell.
– In anaphase, spindle fibers shorten, the kinetochores separate, and the chromatids (daughter chromosomes) are pulled apart and begin moving to the cell poles. Anaphase begins when the APC destroys Securin. This releases the inhibition of the protease Separin and allows the breakdown of Cohesins, which hold sister chromatids together. Anaphase is triggered by the proteolysis of the Cohesin subunit SCC-1 by a Separase.
– The daughter chromosomes arrive at the poles in telophase. The spindle fibers that have pulled them apart disappear.
– In cytokinesis, the spindle fibers begin to break down. A contractile ring cleaves the cell into two daughter cells. This process involves components of the central spindle, RHO-A and its regulators or effectors, nonmuscle Myosin II, Actin and regulators of filament assembly, and factors required for the fusion of membranes. The central spindle consists of the microtubulin-associated proteins Polycomb Repressive Complex 1 (PRC1) and KIF4, the centralspindlin complex MGC/RAC–GAP (CYK-4) and MKLP1, the RHO-Guanine Nucleotide Exchange Factor (GEF) ECT2, and the Aurora Kinase complex with Aurora B/Incep/Survivin/Dasra. The RHO-A GTPase module plays a central role in contractile ring assembly, with its critical GEF being ECT2 and CYK-4 possibly acting as its GAP. RHO-A-GTP activates pathways that lead to Actin polymerization and Myosin II activation. The microtubules then reorganize into a new cytoskeleton for the return to interphase. The exit from the cytokinetic phase of the cell cycle depends on Ubiquitin-mediated proteolysis of various M phase-selective proteins.

After the cells complete mitosis, the fall in CDK/Cyclin levels leads to the dephosphorylation of RB by phosphatases. As a consequence, hypophosphorylated RB protein is available to inhibit E2F activity during early G_1 of the next cycle.

Consistent with their critical role in facilitating cell divisions, the components of the cell cycle are frequently deregulated in cancer. This may arise through activating mutations or through overexpression of their genes.

- Cyclin D_1/CDK4 kinase activity is elevated in various cancers, including head and neck cancer, hepatocellular carcinoma, and colorectal carcinoma, either through the overproduction of *cyclin D_1* or through mutations in *cdk4*, which make Cyclin D_1 insensitive to the inhibitory effects of $P16^{INK4a}$.
- Overexpression of Cyclin D is associated with about 50% of breast cancers.
- The overexpression of Cyclin D_3 and Cyclin A occur early in pancreatic intraepithelial neoplasia and reach close to 100% prevalence in pancreatic cancer. A common substitution polymorphism A870G in the *ccnd1* gene, which encodes Cyclin D_1, occurs in the conserved splice donor region of exon 4. It results in an altered mRNA transcript that encodes a protein with prolonged half-life and is associated with colorectal cancer.
- PLK-1 is overexpressed in tumors and may be a prognostic indicator in non-small cell lung cancer, squamous cell carcinoma of the head and neck, melanoma, oropharyngeal cancer, ovarian, and endometrial carcinomata.

3.1.2 Cell cycle checkpoints

The gene products mediating cell cycle progression may acquire changes that lead to a gain of function and turn them into oncogenes. Therefore, the cell cycle contains checkpoints, at which arrest may occur if the fidelity of the process is compromised. The proteins mediating cell cycle arrest are products of tumor suppressor genes. Their control is dominant over the action of cell cycle promoters. For cancer to occur, loss-of-function mutations have to affect both alleles of tumor suppressor genes. As in other aspects of cancer, including apoptosis and metastasis formation, the mechanisms restraining carcinogenic dysregulation

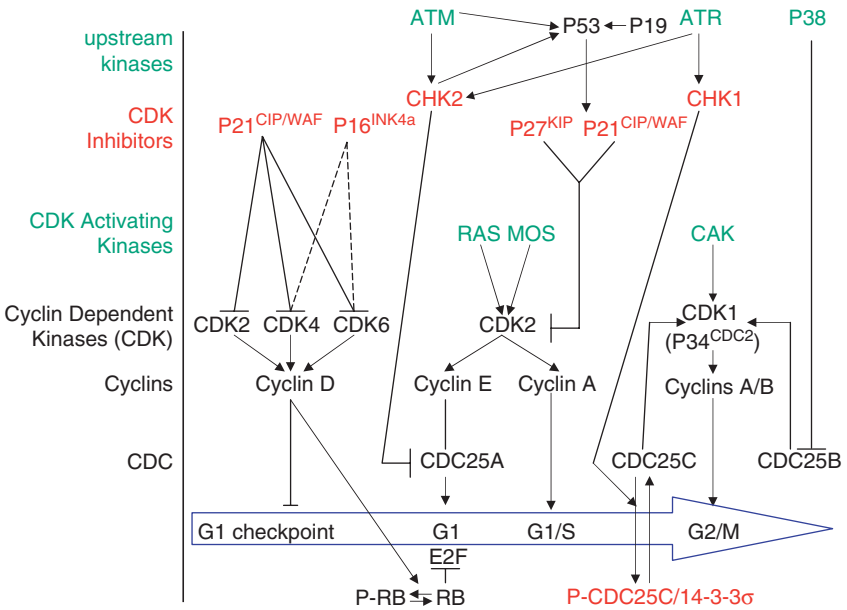


Figure 3.1.2.A. Checkpoints of the cell cycle. The passage past cell cycle checkpoints depends on Cyclin/CDK complexes. Their activity is tightly regulated. Various kinases (shown in green) activate Cyclin/CDK complexes and facilitate progression through the cell cycle. Cyclin-Dependent Kinase Inhibitors (shown in red) prevent this activation and cause cell cycle arrest. In addition, the roles of RB and CDC25C in the cell cycle are also determined by their phosphorylation status.

are dominant over the mechanisms promoting them (Figure 3.1.2.A).

Three essential components of cell cycle control are the Ubiquitin–proteasome pathway, CDK Inhibitors, and checkpoint kinases. Reversal of the cell cycle is prevented by the rapid degradation of Cyclins. This is predominantly accomplished by the APC/C. Components of the APC are required for ubiquitination of substrates and targeting to the proteasome. CDK Inhibitors block the activation of CDKs by CAKs, mostly through blocking of their ATP and Mg^{2+} binding sites. They comprise the CIP/KIP and INK4 families of proteins. Their expression and cell cycle control functions are largely controlled by P53 and RB. Checkpoint kinases act upstream of CDK Inhibitors. They include four conserved upstream kinases, ATM, ATR, CHK1, and CHK2. In an alternative pathway, P38 also acts as upstream kinase.

G_0 arrest. Differentiated mammalian cells often reside in G_0, a phase of no active division. Reentry into the cell cycle is inducible by growth factors, which stimulate Cyclin D synthesis. After the removal of growth factors, the concentration of Cyclin D in the cell falls rapidly.

G_1 checkpoint. A critical checkpoint arises in the late G_1 phase, where a decision is made between entering S phase or reverting to G_0 phase. The prime mechanisms controlling this checkpoint depend on the RB and P53 pathways, and on CDK Inhibitors of the CIP/KIP and INK4 families. The tumor suppressor LATS-2 also plays a role.

Genes of the *retinoblastoma* family are expressed in all normal tissues and act as negative regulators of cell cycle progression. The gene family has three members, *rb1* (encoding P105) {13q14.1–q14.2}, *rb2* (encoding P130) {16q12.2} [Yeung et al. 1993], and *rbl1* (*retinoblastoma-like-1*, encoding P107) {20q11.2} [Ewen et al. 1991]. The *rb1* gene has a complex organization with 27 exons, spanning greater than 200 kb of DNA, and an RNA transcript of about 4.7 kb (Figure 3.1.2.B). The RB protein contains 928 amino acids, which form four domains, the NH_2-terminal domain that may determine the conformational state of the protein, the R motif, the A/B pocket that forms a functional repressor motif, and the COOH-terminal domain

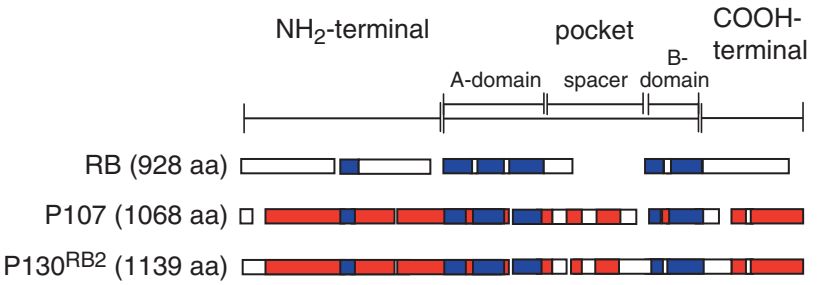


Figure 3.1.2.B. Structure of RB proteins. Sequence homologies among RB protein family members. Blue boxes indicate high homology among the three proteins. They correspond mostly to the A-pocket and B-pocket domains. Red boxes are homologies shared only between P107 and P130.

that regulates the A/B domain and contains a nuclear localization signal (NLS).

The *rb* gene products share the ability to negatively control the cell cycle by inhibiting the transition from G_1 to S.

– Underphosphorylated RB binds the Cyclins D_1 and D_3 and sequesters them.
– Binding and sequestration of the transcription factor E2F by RB prevents the expression of gene products that mediate cell cycle progression. E2F binding sites (TTTCGCGC) exist in the *cyclin E* and *cyclin* D_1 promoters, and both genes are activated by E2F.
– RB may form a complex with E2F and HDAC-1 (Histone Deacetylase-1), acting as a transcriptional repressor.
– The Cyclin D–RB–E2F pathway may have a role in linking protein synthesis and cell growth to proliferation [Adams and Kaelin 1998]. Loss of *rb* leads to a cell cycle that is relatively resistant to inhibitors of protein synthesis, suggesting that RB restrains growth by repressing the transcription of *RNA polymerase I* and *III*.
– During differentiation, hypophosphorylated RB interacts directly with C/EBPβ (NF-IL6, LAP, TCF5), a member of the CAAT/EBP family of transcription factors. RB enhances the binding activity of C/EBPβ to its cognate DNA sequences and the resulting gene expression [Chen et al. 1996]. This activates genetic programs of differentiation.

RB can be phosphorylated by CDK4/Cyclin D on serines 780 and 795 and by CDK2/Cyclin A on threonine 821. The phosphorylation of RB inactivates its cell cycle blocking function, which is a prerequisite for progression from G_1 to S. Progressive dephosphorylation of RB takes place in mid to late G_1 phase.

The P53 protein (Figure 3.1.2.C) is essential for the checkpoint control that arrests cells with damaged DNA in G_1. *p53* {17p13.1} is approximately 20 kb in length. The gene yields a 2.8 kb mRNA transcript and encodes for a 53 kD nuclear phosphoprotein that contains 393 amino acids. Eleven exons are contained within the transcript, with the first exon being noncoding.

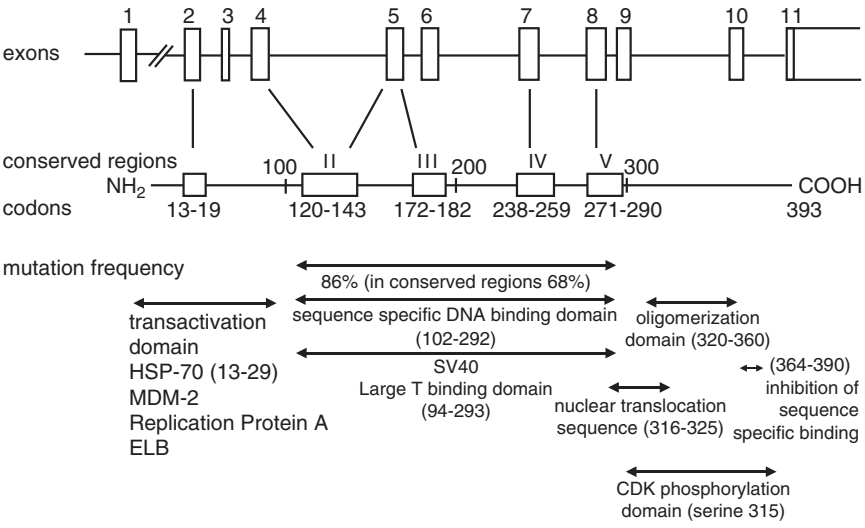


Figure 3.1.2.C. Structure of P53. The top row shows the exon/intron structure of the gene. The middle row depicts conserved regions of the protein. In the lower panel, the functions dependent on individual domains are outlined. [Adapted from Sherbet and Lakshmi 1997.]

– P53 may induce cell cycle arrest in G_1 and it establishes one of the most critical checkpoints in cell cycle control.
– The P53 protein prevents cells from completing the cell cycle by binding to the transcription factor E2F. This prevents E2F from binding to the promoters of such proto-oncogenes as *c-myc* and *c-fos*, whose transcription is needed for mitosis. Therefore, blocking the transcription factors needed to express these genes prevents cell division. P53 induction can also down-regulate the promoters for *hsp-70*, *c-jun*, *mdr1*, and *pcna*.
– P53 induces *p21* expression. P21 inhibits CDKs and it may directly inhibit DNA synthesis by interacting with the DNA Polymerase δ subunit PCNA (Proliferating Cell Nuclear Antigen).
– TBP (TATA-Binding Protein) interacts with P53 and the complex then binds the TATA box, activating the transcription of cell cycle control genes.
– P53 induces the expression of IGF-BP3, a binding protein and negative regulator of IGF-1 (Insulin-Like Growth Factor-1). The inhibition of IGF signaling leads to G_1 arrest.
– Members of the *gadd* (*growth arrest and DNA damage inducible*) gene family are regulated by P53. This is the case for the expression of GADD45, a nuclear protein with high expression in quiescent cells in G_1. GADD45 can interact with PCNA and P21.

Unlike other cell cycle proteins, P53 is expressed at very low levels in normal cells, because it is extremely unstable and rapidly degraded. DNA damage by ultraviolet (UV) or γ-irradiation, heat, or hypoxia leads to the activation of kinases, including ATM and DNA-PK (DNA-Dependent Protein Kinase), which phosphorylate P53, resulting in its stabilization, and thus in a marked increase in its concentration. Enhancement of P53 function may be induced by phosphorylation, by acetylation, by sumoylation, or by stabilization of the P53 tetramer.

The RB and P53 pathways of cell cycle control interact in at least five ways.
– Because P53 induces the transcription of *p21*$^{CIP1/WAF1}$ and P21 negatively regulates the phosphorylation of RB by Cyclin D/CDK4, the P53-dependent and RB-dependent pathways of G_1 checkpoint control cooperate, with P53 acting proximally to RB.
– The P53 pathway protects against deregulation of the RB pathway. Functional inactivation of RB results in excessive E2F activity, which leads to the induction of *p14*^{ARF}. $P14^{ARF}$ then inhibits MDM2 and allows P53-dependent cell cycle arrest.
– P53 contributes to the regulation of *rb* transcription [Sherbet and Lakshmi 1997; Adams and Kaelin 1998].
– A single genetic locus encodes $P14^{ARF}$ and $P16^{INK4a}$, which negatively regulate the P53 and RB pathways, respectively.
– Both P53 and RB are negatively regulated by MDM2.

The CIP/KIP class of CDKIs, which mediates arrest in G_1, comprises $P21^{CIP1/WAF1}$, $P27^{KIP1}$, and $P57^{KIP2}$. The expression of the CIP/KIP family of inhibitors is tissue-specific with little overlap between $P21^{CIP1/WAF1}$ and $P57^{KIP2}$. P21 [Xiong et al. 1993, el-Deiry et al. 1993; Harper et al. 1993] is a potent inhibitor of the G_1 CDKs (CDK2, CDK4, and CDK6). The COOH-terminal domain of P21 contains potential binding sites for other proteins, which may lead to the formation of quaternary complexes with a CDK, a Cyclin, and the PCNA, a molecule that associates with various DNA Polymerases and functions as a sliding clamp in DNA synthesis. In contrast, neither $P27^{KIP1}$ nor $P57^{KIP2}$ bind PCNA. Elevated levels of $P27^{KIP1}$ may constitute a barrier to CDK activation that must be overcome during mitogen-induced cell cycle progression. Thus, a major function of extracellular growth-promoting agents is the elimination of functional $P27^{KIP1}$ [Harper 1997]. Transcriptional induction is a predominant mechanism for regulating $P21^{CIP1/WAF1}$ levels. The promoter region of *cdkn1A* (the gene encoding P21) {6p21.2} contains a binding site for P53 and a sequence that is associated with TGF-β-mediated activation. $P27^{KIP1}$ is frequently induced upon exit from the cell cycle, although *cdkn1B* (encoding $P27^{KIP1}$) {12p13} RNA levels remain fairly constant. This is accomplished by an increase in half-life. Degradation of $P27^{KIP1}$ occurs in the Ubiquitin pathway.

The INK4 (Inhibitor of CDK4) family of CDKIs is composed of $P16^{INK4a}$, $P15^{INK4b}$, $P18^{INK4c}$, and $P14^{ARF/INK4d}$. They are comprised of Ankyrin repeat proteins. The *ink4a* gene locus {9p21} produces two transcripts from distinct promoters that have alternative first exons, but share second and third exons resulting in the expression of $P16^{INK4a}$ and $P14^{ARF}$. $P16^{INK4a}$ is highly selective for CDK4 and CDK6, which associate with Cyclin D and regulate RB phosphorylation. $P14^{ARF}$ supports P53 activity by

binding to and inhibiting MDM2, thus preventing MDM2 from targeting P53 for degradation. Cell cycle progression past the G_1 restriction point is facilitated by MDM2. Forming a tight complex with P53, the MDM2 proto-oncogene product inhibits P53-mediated transactivation and targets P53 for degradation in the proteasome. MDM2 also interacts physically and functionally with the RB protein and can inhibit its growth regulatory capacity. $P14^{ARF}$ effects may account for the stimulation of P53-dependent cell cycle arrest by c-MYC, E2F1, or E1A [Adams and Kaelin 1998]. P15 {9p21} is induced by TGF-β and causes G_1 arrest.

The expression of LATS-2 (KPM) {13q11–q12} inhibits the G_1 to S transition. It down-regulates Cyclin E/CDK2 Kinase activity [Li et al. 2003].

S checkpoint. Cells monitor the presence of Okazaki fragments (100–1,000 base pair fragments of DNA that are joined by DNA Ligases to allow for chain growth) on the lagging DNA strand during reduplication. The cell cycle does not proceed until these have disappeared. Radiation is among the principal insults leading to activation of the S phase checkpoint.

– When exposed to ionizing radiation, cells activate the ATM gene product, which phosphorylates CHK2 (CDS1, RAD53). CHK2 then phosphorylates CDC25A on serine 123, leading to its degradation in the Ubiquitin pathway. The phosphatase CDC25A is important during the initiation and progression of S phase. CDC25A dephosphorylates and activates the CDK2/Cyclin E complex. In the absence of CDC25A, the activation of the CDK2 by dephosphorylation of tyrosine 15 and threonine 14 is prevented and DNA synthesis does not take place. NBS1 is required for S-phase checkpoint activation induced by ionizing radiation. The ATM-mediated activation of CHK2 by ionizing radiation is dependent on NBS1. The ATM substrate BRCA1 also plays a role in the activation of the S phase checkpoint.
– UV light leads to the rapid degradation of CDC25A via tyrosine phosphorylation, ubiquitination, and the proteasome pathway. CHK1 mediates the UV light-induced degradation of CDC25A, as well as the degradation of CDC25A by DNA double-strand breaks caused by ionizing radiation. The checkpoint response to UV treatment occurs in two phases. The CHK1→CDC25A→CDK2 pathway is activated rapidly (within minutes) after UV damage followed, after several hours, by the P53→P21 pathway.
  • Germline mutations in the *nbs1* gene cause the tumor susceptibility disease Nijmegen breakage syndrome, which has increased susceptibility to lymphomata and meningiomata.
  • Ataxia telaangiectasia is caused by germline loss-of-function mutations in the *atm* gene.
  • *brca1* is mutated in hereditary breast and ovarian cancer.

G_2/M checkpoint. Cyclins A and B in association with CDK1 (CDC2, $P34^{CDC2}$) regulate the entry into M phase. Protein Phosphatase 2A synergizes with CDC25C in activating CDK1 and facilitating cell cycle progression at this stage. DNA damage leads to cell cycle arrest either at the G_2/M border or in metaphase [Murakami et al. 1995].

– The NDR (Nuclear DBF-2-Related) family of protein serine/threonine kinases comprises NDR and LATS. The Large Tumor Suppressor (LATS, WTS) is phosphorylated in a cell cycle-dependent manner and complexed with CDK1 in early mitosis. It functions as a negative regulator of Cyclin A/CDK1 [Tao et al. 1999]. LATS1-associated CDK1 does not interact with mitotic Cyclins and has no kinase activity. This leads to cell cycle arrest in G_2/M [Yang et al. 2001]. A combination of LATS-2 and LATS-1 control cell proliferation by negatively regulating multiple cell cycle check points. Somatic cells mutant for *lats* {13q11–12} [Hori et al. 2000a] undergo extensive proliferation and form large tumors. Chromosome 13q11–12 is a hot spot for loss of heterozygosity in non-small cell lung cancer.
– Arrest in this phase may result from activation of the ATR protein. ATR phosphorylates and activates the protein kinase CHK1. Alternatively, ATM activation mediates phosphorylation of CHK2 (CDS1, RAD53). CHK1 or CHK2 activation is followed by phosphorylation on serine 216 and inhibition of the phosphatase CDC25C, which is then sequestered by 14-3-3σ (HME-1, Stratifin). This prevents CDC25C from removing inhibitory phosphates from CDK1. Phosphorylated CDK1 is inactive and leads to G_2/M arrest, whereas dephosphorylated CDK1/Cyclin B is active and mediates entrance into mitosis [Weinert 1997]. While the Ubiquitin Ligase MDM2 is phosphorylated on threonine 216 in S phase, thus weakening its interaction

with P53, P53 is preferentially bound to MDM2 during G_2. CHK2 can phosphorylate P53 on serine 20, which interferes with binding to MDM2 [Hirao et al. 2000]. The 14-3-3σ protein, which is expressed in keratinocytes and epithelial cells, can bind to and sequester CDC25C. Its gene is a transcriptional target for P53. This may explain the role of P53 in G_2/M arrest [Adams and Kaelin 1998]. Sustenance of this arrest furthermore depends on the P53-mediated transcription of *p21* [Bunz et al. 1998]. CHK2 is necessary for the maintenance of irradiation-induced cell cycle arrest in G_2. CHK2 inhibits CDK1 activation through inactivation of CDC25C [Hirao et al. 2000].

– Following cell damage by ionizing radiation, G_2/M accumulation of cells that had been in earlier phases of the cell cycle at the time of exposure may occur in an ATM- and ATR-independent manner [Xu et al. 2002]. During G_2 and M phase, CDC25A may bind to and activate CDK1/Cyclin B. The absence of CDC25A delays entry into mitosis. G_2 arrest caused by DNA damage is accompanied by the rapid degradation of CDC25A.
– An alternative G_2/M checkpoint pathway is induced by UV light. It activates the kinase P38, which binds to and phosphorylates CDC25B at serines 309 and 361. Phosphorylation on these residues is required for binding to 14-3-3 proteins. Sequestration of CDC25B prevents it from activating CDK1 [Bulavin et al. 2001].

Spindle assembly checkpoint. (Spindle checkpoint, mitotic checkpoint, and kinetochore checkpoint) [Hoyt et al. 1991; Li and Murray 1991]. The alignment of the chromosomes on the metaphase spindle is checked before the initiation of anaphase. The mitotic checkpoint detects the orientation of the chromosomes, ensuring that all the pairs of sister chromatids establish bilateral attachments to the mitotic spindle and become aligned on the metaphase plate. Any failure of the spindle fibers to attach to the kinetochores leads to cell cycle arrest in metaphase. A single unattached kinetochore can delay the segregation of the already aligned chromosomes. Consistent with the effect of chromatid separation on genomic stability, many of the spindle checkpoint proteins also play roles in DNA repair (Figure 3.1.2.D).

The APC/C is a multi-subunit Ubiquitin Ligase that, at defined points during mitosis, targets specific proteins for proteasomal degradation. After the proper attachment of all sister chromatids to the mitotic spindle, the spindle checkpoint is satisfied. Securin is then ubiquitinated by the APC. This leads to Separase activation, SCC-1 cleavage, loss of chromosome cohesion, and onset of anaphase. The spindle checkpoint regulates the APC by inactivating its cofactor CDC20. Spindle checkpoint components

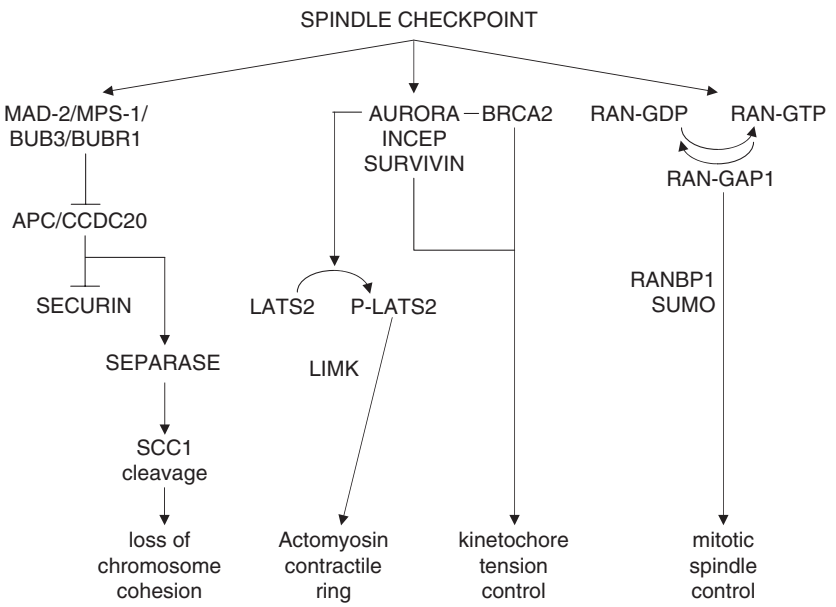


Figure 3.1.2.D. Spindle checkpoint. The mitotic checkpoint detects the orientation of the chromosomes, ensuring that all the pairs of sister chromatids establish bilateral attachments to the mitotic spindle and become aligned on the metaphase plate. The molecular components involved include the mitotic spindle, the kinetochore, the contractile Actomyosin ring that separates the daughter cells, and chromosomal Cohesins. Any failure of the spindle fibers to attach to the kinetochores leads to cell cycle arrest in metaphase.

and CDC20 are recruited to improperly attached kinetochores and the APC then colocalizes with them. The spindle checkpoint components inhibit the Ubiquitin Ligase activity of the APC, thus preventing premature chromosome segregation and ensuring the accurate partition of the genetic material. Inhibition of CDC20 is accomplished by the mitotic checkpoint complex (MCC) that is composed of MAD2, BUBR1, BUB3, and MPS1. BUBR1 and MAD2 directly bind CDC20. Both BUBR1 and MAD3 contain GLEBS motifs that mediate their binding to BUB3, which is constitutive, and is required for the localization of BUBR1 to the kinetochores during mitosis. BUBR1 also binds to the mitotic motor protein CENP-E, which is a prerequisite for the maintenance of stable kinetochore–microtubule interactions and for proper checkpoint signaling. Furthermore, the kinase activity of BUBR1 may be required for the recruitment of CENP-E and MAD2 to the kinetochore.

To prevent errors in chromosome segregation, Cohesin holds replicated chromosomes together until every pair of sister chromatids is bioriented on the mitotic spindle. Biorientation generates tension on the chromosomes, which is monitored in the spindle checkpoint. The chromosome passenger proteins Aurora B, INCEP, and Survivin physically interact and form a complex, which localizes to the kinetochores in prometaphase, to the cell equator during metaphase, and to the midbody during cytokinesis. Aurora B and Survivin are required for destabilizing the kinetochore microtubule attachments in the absence of tension. It is possible that Aurora B directly phosphorylates BUBR1 and MPS1.

LATS2 is phosphorylated on S83 by the kinase Aurora A. LATS2 and Aurora A colocalize at the centrosomes during mitosis. LATS-1 binds to LIMK-1 and colocalizes with LIMK-1 at the Actomyosin contractile ring during cytokinesis. Thus, LATS-1 is a cytoskeleton regulator that affects cytokinesis by regulating Actin polymerization through negative modulation of LIMK1 [Yang et al. 2004].

RAN is an abundant nuclear GTPase that is required for spindle assembly and mitotic regulation. The spindle checkpoint is directly responsive to the levels of RAN-GTP [Arnaoutov and Dasso 2003]. The nucleotide-binding state of RAN is regulated by a GTPase-activating protein, RAN-GAP1, and by a GEF, RCC1 (CHC1) [Kalab et al. 1999]. The association of RAN-GAP1 with the mitotic spindle critically involves its sumoylation, which is enhanced by its interaction with the nuclear pore protein and guanine nucleotide dissociation inhibitor RANBP. RANBP1 levels oscillate during the cell cycle, and increased concentrations of RANBP1 prolong mitosis.

3.1.3 Growth factors and their receptors

Cells need external growth factors for the progression from G_0 or G_1 to S. Growth factors act through binding to receptors on the cell surface or inside the cells. These receptors then transmit signals to additional intracellular targets, resulting in programmed changes in gene expression. A variety of proteins are produced after the cells leave quiescence, including enzymes that expand metabolic functions necessary for the ensuing phases of the cell cycle, those that provide energy, and those that synthesize ribosomes. Cyclin D synthesis is induced in response to growth factor stimulation, and the D-type Cyclins continue to be synthesized as long as growth factors are present. Their concentration, however, declines rapidly after the growth factor signaling subsides. These Cyclins move the cells beyond the restriction point in late G_1, which marks the end of a requirement for stimulation by external growth factors. Growth factor receptors on the cell surface are frequently receptor tyrosine kinases or G-Protein-coupled receptors. Steroid hormones signal through receptors of the nuclear receptor superfamily.

Tyrosine kinase receptors. Tyrosine kinase receptors are directly linked to intracellular enzymes and they phosphorylate their substrate proteins on tyrosine through the transfer of the γ-phosphate in ATP. This family includes the receptors for most polypeptide growth factors, including EGF, NGF, PDGF, and Insulin. The tyrosine kinase receptors are organized in a NH_2-terminal extracellular ligand-binding domain, a single transmembrane α-helix, a cytosolic domain with protein tyrosine kinase activity, and a COOH-terminal tail. The extracellular ligand-binding domain is often glycosylated. The juxtamembrane sequence that separates the transmembrane and cytoplasmic domains is variable among families of receptors, but conserved within individual receptor families. This domain plays a role in modulating receptor responses by heterologous stimuli (receptor transmodulation). Receptor

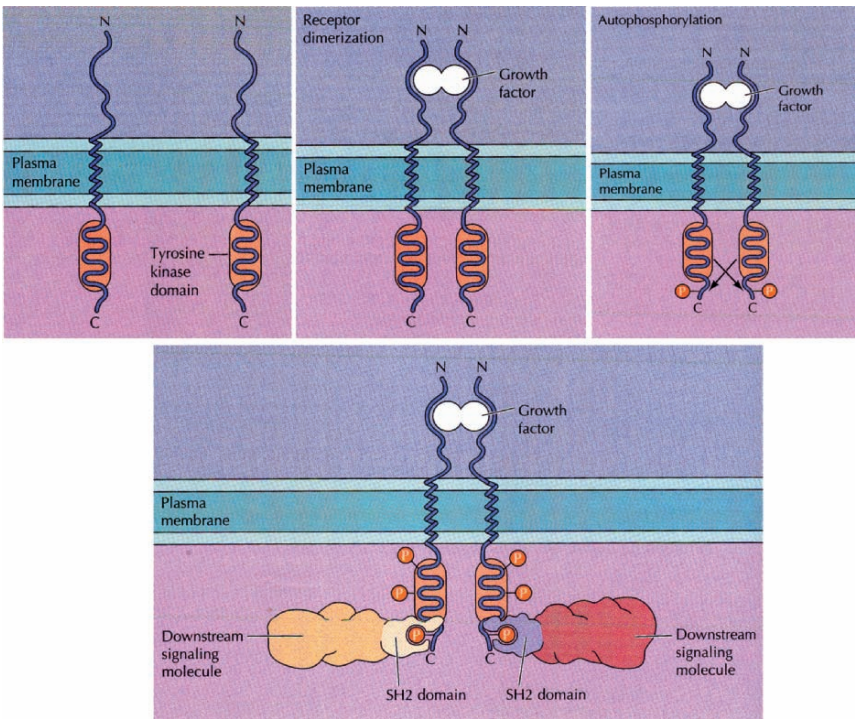


Figure 3.1.3.A. Action of tyrosine kinase receptors. Dimerization and autophosphorylation of tyrosine kinase receptors. Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation. The SH2 domains of intracellular signaling molecules then bind to specific phosphotyrosine-containing motifs of the activated receptors. [Reproduced from Cooper 1997. With permission.]

transmodulation is a structural modification, such as phosphorylation, in the juxtamembrane domain, which is induced by unrelated stimuli and leads to altered receptor responses. (Figure 3.1.3.A)

The growth factor receptor tyrosine kinase family comprises four classes with distinct structural features [Ullrich and Schlessinger 1990].

– A class is represented by the ERBB family.
– A class includes dimeric receptors, such as the Insulin Receptor, as well as the proto-oncogene products TRK, MET, and ROS.
– A class is characterized by the presence of five Immunoglobulin-like domains in the extracellular region and by the interruption of the catalytic domain in two parts by a specific hydrophilic sequence of variable length. This class includes the proto-oncogene product FMS (CSF1 Receptor) and KIT, as well as two PDGF Receptors, and FLT1.
– A class is represented by the FGF Receptors encoded by *flg* (*flt2*) and *bek*. These receptors have strong sequence similarities to the products of the third class, but possess only three Immunoglobulin-like domains in the extracellular region and a short kinase insert in the intracellular domain.

The cytokine receptor superfamily constitutes a variation of tyrosine kinase receptors. Cytokine receptors contain an NH_2-terminal extracellular ligand-binding domain, a single transmembrane α-helix, and a COOH-terminal cytoplasmic domain. These receptors share a common motif in their extracellular portions that includes NH_2-terminally conserved cysteine residues and the downstream pattern WSXWS. While the cytosolic domain is devoid of catalytic activity it associates with nonreceptor tyrosine kinases, which are activated following receptor ligation. The associated nonreceptor tyrosine kinases comprise two major families, the SRC kinases and the JAK kinases.

The first step in the signal transduction initiated by most tyrosine kinase receptors is ligand-induced receptor dimerization, which leads to autophosphorylation and activation of the catalytic domain,

followed by the recruitment and phosphorylation of intracellular signaling molecules.

– PLCγ and STATs are substrates for receptor tyrosine kinases that are activated by tyrosine phosphorylation.
– $P85^{PI\ 3\text{-}K}$ and SH-PTP2 (SYP) are activated by conformational changes when their SH2 domains bind to receptor tyrosine kinases.
– The adapter molecule GRB-2 ,virtually composed of only SH2 and SH3 domains, is recruited to activated receptor tyrosine kinases, and mediates signaling through SOS (Son Of Sevenless, RAS-GEF), RAS, and ERK.
– The multiadapter protein GAB-1 is recruited to receptor tyrosine kinases through its SH2 domains and is tyrosine phosphorylated, which activates its binding to downstream signaling molecules.

The diversity in tyrosine kinase receptor signaling can be expanded by the formation of heterodimers in response to certain stimuli. The best characterized domains for the interactions between receptor tyrosine kinases and associated signal transduction molecules are the SH (SRC homology) domains, SH1, SH2, and SH3. Effector proteins contain the SH2 sequence motif of about 100 amino acids, which has significant homology to a region of the c-SRC protein. Individual SH2 domains interact directly with specific phosphotyrosine residues to form complexes between the activated receptor and cytoplasmic effector proteins. Containing another motif, phospho-tyrosine interaction domains (PID, phospho-tyrosine binding domains, PBD) have the capacity to bind specific peptide sequences containing phosphotyrosines.

CRK-I, CRK-II, and CRK-L are members of the family of adaptor-type signaling molecules that consist mostly of SH2 and SH3 domains. CRK family proteins are involved in a variety of signaling cascades associated with growth factor receptors. The primary function of CRK is to recruit cytoplasmic proteins in the vicinity of tyrosine kinases through SH2-phosphotyrosine interactions. Downstream signals from CRK depend on the SH3-binding proteins, which include the C3G guanine nucleotide exchange protein for RAP1, ABL tyrosine kinase, DOCK180, and the SOS guanine nucleotide exchange protein for RAS. The variety of the CRK-binding proteins is reflective of the pleiotropic function of CRK.

Gain-of-function mutations that mediate constitutive kinase activity lead to uncontrolled activation of growth pathways. They may initiate transformation.

- The *erbB2* gene is activated by a point mutation encoding V664E, which may result in cell transformation in breast cancer, bladder cancer, colon cancer, lung cancer, and gastric cancer.
- Point mutations in the kinase domain convert MET to an oncogenic receptor. Such mutants are catalytically highly active, which leads to more efficient MET autophosphorylation, phosphorylation of substrates, and transforming ability. Point mutations in *met* occur in hereditary and sporadic papillary renal carcinomata, hepatocellular and gastric carcinomata, and head and neck squamous carcinomata.
- Mutations in the tyrosine kinase receptor gene *kit*, either somatic or in some instances germline, can underlie gastrointestinal stromal tumors. c-KIT is also an important regulator of small cell lung cancer growth.

G-Protein-coupled receptors. A very large and diverse number of receptors are associated with heterotrimeric G-Nucleotide-Binding Proteins (G-Proteins). These G-Protein-coupled receptors mediate proliferative responses to many mitogens present in serum, including Thrombin and lysophosphatidic acid (LPA). In addition, Bombesin, Vasopressin, Bradykinin, Substance K, Acetylcholine Receptor agonists, and Angiotensin II stimulate cell proliferation by acting on receptors coupled to G-Proteins. Receptors coupled to G-Proteins are structurally and functionally related cell surface molecules characterized by seven membrane spanning α-helices (Figure 3.1.3.B). Ligand binding induces a conformational change that allows the cytosolic receptor domain to bind a G-Protein associated with the inner cell membrane. This interaction activates the G-Protein, which then induces the exchange of bound GDP for GTP and dissociates from the receptor and further signals through second messengers. The activation of Adenylyl Cyclase by GTP mediates the conversion of ATP to the second messenger cyclic adenine 3′-5′-monophosphate (cAMP).

Heterotrimeric G-Proteins consist of α, β, and γ subunits. In the resting state, α is bound to GDP in a complex with βγ. The activated GTP bound α subunit dissociates from the βγ complex. The α subunit

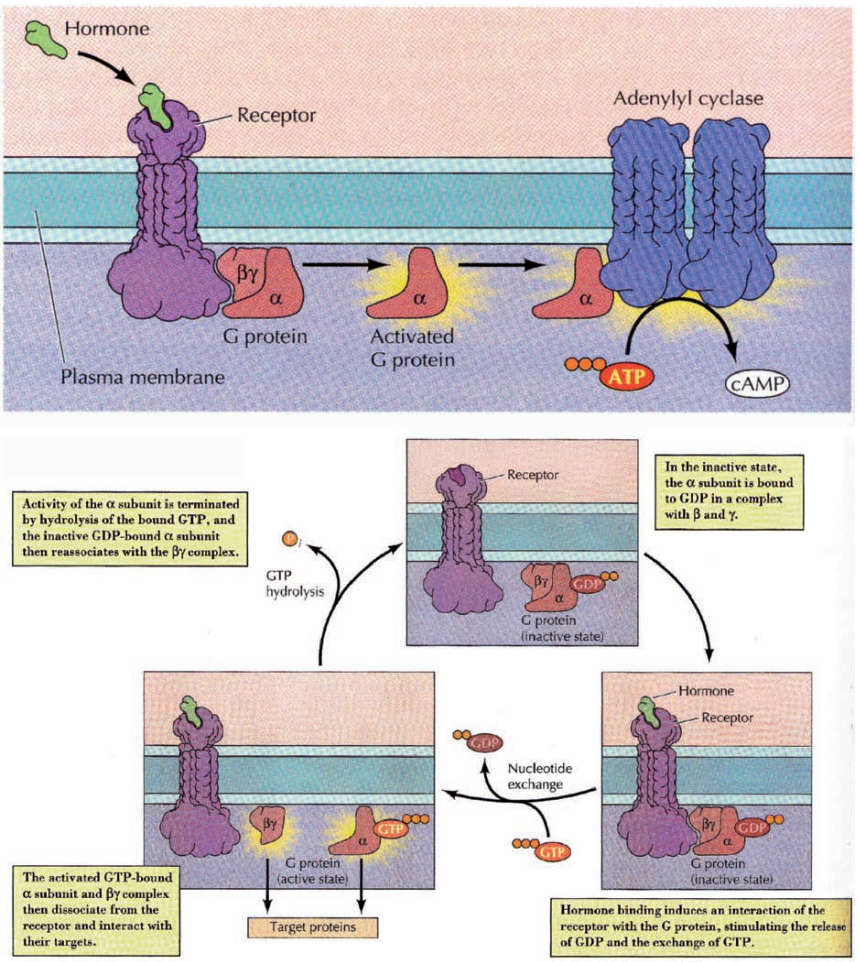


Figure 3.1.3.B. Action of G-Protein-coupled receptors. (*Top panel*) The binding of a cognate ligand, such as a hormone, initiates the interaction of the receptor with a G-Protein. The activated G-Protein α subunit then dissociates from the receptor and stimulates Adenylyl Cyclase, which catalyzes the conversion of ATP to cAMP. (*Bottom panel*) The cycle of activation and restoration of G-Protein-coupled receptors. [Reproduced from Cooper 1997. With permission.]

and the βγ subunits then interact with their targets to elicit an intracellular response. The activity of the α subunit is terminated by hydrolysis of the bound GTP to GDP. This leads to its reassociation with the βγ subunits and completion of the cycle of activation. There are at least 16 α subunits, 6 β subunits, and 12 γ subunits composing the various G-Proteins, some act stimulating and some inhibitory.

Based on their primary sequence similarity, the known G-Protein α subunits fall into four families: G_s, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$. They regulate the activity of various second messenger-generating pathways.

– The members of the G_s family activate Adenylyl Cyclases, whereas G_i family members can inhibit a subset of these enzymes, thereby controlling the intracellular concentrations of cAMP.
– Gα subunits of the G_i family, which includes $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_t$ (Transducin), and $G\alpha_{gust}$ (Gustducin), activate a variety of Phospholipases and Phosphodiesterases, and promote the opening of several ion channels. $G\alpha_i$ and $G\alpha_o$ activate SRC and STAT3.
– The G_q family controls the activity of phosphatidylinositol specific Phospholipases, such as Phospholipase-Cβ, that hydrolyze phosphatidylinositol 4,5-bisphosphate to generate the second messengers inositol 1,4,5-trisphosphate and diacylglycerol, which, in turn, mediate an increase in the intracellular concentrations of free

calcium and the activation of a number of protein kinases, including Protein Kinase C (PKC). The G_q pathway is activated by various hormones.
– Although $G\alpha_{12}$ and $G\alpha_{13}$ belong to the same family, they may produce distinct signaling outputs. $G\alpha_{12}$ directly interacts with a GTPase-activating protein. $G\alpha_{13}$, which is coupled to the LPA Receptor and the Thromboxane A2 Receptor, directly interacts with $P115^{RHO\text{-}GEF}$, and thus activates RHO. Through PYK2, $G\alpha_{13}$ may also activate the Phosphatidylinositol 3-Kinase pathway.

Associating with the many Gα subunits are G-Protein β subunits and G-Protein γ subunits. The α subunit, once bound to guanine nucleotides, dissociates from the Gβγ dimers, which can themselves regulate the activity of various signaling molecules, including ion channels, Phosphatidylinositol 3-Kinases, Phospholipases, Adenylyl Cyclases, and receptor kinases. Gβγ subunits signal mostly through the RAS and PLCβ pathways.

G-Protein-coupled receptor signaling is regulated on various levels.
– Nine distinct Adenylyl Cyclases are known, each of which is distinctly regulated by G_s and G_i, as well as by Gβγ subunits, intracellular calcium, and PKCs. Thus, the impact on the intracellular concentrations of cAMP by agonists acting on G Protein-coupled receptors is highly dependent on the forms of Adenylyl Cyclases expressed in each cell type.
– Activated G-Protein-coupled receptors act as GEFs (guanine nucleotide exchange factors) for heterotrimeric G-Proteins, inducing the replacement of bound GDP with GTP. $G\alpha_s$ and $G\alpha_i$ can link to the small GTP-binding protein RAP1A. $G\alpha_{13}$ interacts with $P115^{RHO\text{-}GEF}$ and with $PDZ^{RHO\text{-}GEF}$ through their RGS (regulator of G-Protein signaling) domains and activates them. This leads to RHO-dependent transcriptional activation of the serum response element. Positive regulators of heterotrimeric G-Protein signaling are GAPs. They may stabilize the active conformation of G-Proteins.
– Following their ligation, G-Protein-coupled receptors are frequently endocytosed. From there, they may be recycled to the cell surface or degraded. β-Arrestins bind to all G-Protein-coupled receptors and stop receptor signaling by mediating receptor endocytosis [Luttrell and Lefkowitz 2002]. The β-Arrestins -1 and -2 bind to G-Protein-coupled receptors in Clathrin-coated pitts. Their dissociation from β-Arrestins before endocytosis favors receptor dephosphorylation and recycling to the cell membrane, whereas persistent binding to β-Arrestins favors routing of the endocytosed receptors to the lysosomes for degradation.

- If persistently activated, some G-Protein-coupled receptors contribute to transformation, and ultimately to cancer [Dhanasekaran et al. 1995]. Certain activated Gα mutants are oncogenes, including those of *gsp* [Landis et al. 1989], *gip2* [Lyons et al. 1990], and *gep* [Xu et al. 1993; Xu et al. 1994]. They induce proliferation through activating ERKs P42 and P44 or via signaling through SRC and STAT3 [Ram et al. 2000]. $G\alpha_S$, associated with the Thyrotropin Receptor, may stimulate cell proliferation through an Adenylyl Cyclase-dependent mechanism.
- Some viruses express gene products with high homology to G-Protein-coupled receptors. This includes HHV-8 ORF74, which is homologous to the IL-8 receptor CXCR2 [Guo et al. 1997], constitutively activates the phosphatidylinositol →inositol trisphosphate→PKC pathway [Arvanitakis et al. 1997], and acts as an oncogene in Kaposi's sarcoma [Bais et al. 1998].

Nuclear receptor superfamily. Due to their hydrophobic nature, steroid hormones are able to diffuse across the plasma membrane and bind to their receptors inside cells. Once ligated, nuclear receptors shuttle directly to the nucleus and execute their functions as activators or repressors of target genes. These receptors contain domains for ligand binding, DNA binding, and transcriptional activation (Figure 3.1.3.C).

The nuclear receptor superfamily comprises some 50 members, including the Glucocorticosteroid Receptor (GR), Estrogen Receptor (ER), Progesterone Receptor (PR), Androgen Receptor (AR, Dihydrotestosterone Receptor, DHTR), Retinoic Acid Receptors (RARs), Retinoid X Receptors (RXRs), and Peroxisome Proliferator-Activated Receptor (PPARs). Nuclear receptors can exist as homodimers or heterodimers, with each partner binding to specific response element sequences that exist as half-sites and are separated by variable length nucleotide spacers between direct or inverted half-site repeats. [Olefsky 2001] (Figure 3.1.3.D).

Nuclear receptors interact extensively with coactivators. The CDC25 family of dual specificity phosphatases activates CDKs to enable progression through the cell cycle. In addition, CDC25B functions as a Steroid Receptor coactivator, which can

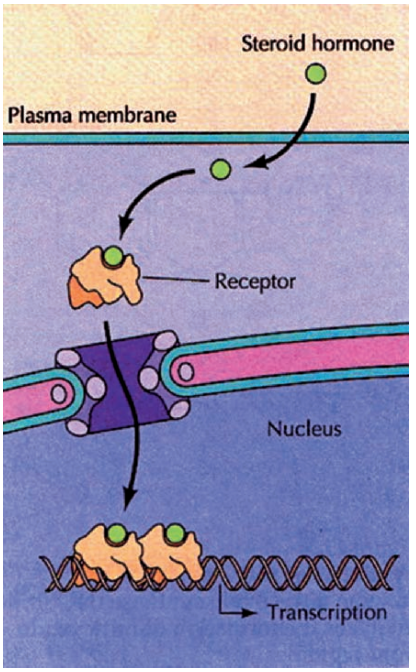


Figure 3.1.3.C. Action of steroid hormones. Steroid hormones diffuse through the plasma membrane and bind to intracellular receptors, which directly stimulate the transcription of their target genes. The Steroid Hormone Receptors bind DNA as dimers. [Reproduced from Cooper 1997. With permission.]

up-regulate the expression of the Estrogen Receptor target genes *cyclin* D_1 and *lactoferrin*. The coactivation of CDC25B extends to the Glucocorticoid Receptor, Progesterone Receptor, and Androgen Receptor [Ma et al. 2001]. The direct interaction between CDC25B and these nuclear receptors recruits and enhances the activity of Histone Acetyl Transferases (HATs). Transcription factors of the FOXO class act as essential cofactors in the transcriptional activation induced by Estrogen Receptor (ERα), Retinoic Acid Receptor (RAR), and Thyroid Receptor (TR).

- Breast cancers may become estrogen independent by Estrogen Receptor mutations that result in increased sensitivity to the ligand. Increased sensitivity to coactivator recruitment may also play a role. Missense mutations that substitute tyrosine 537 in the ligand-binding domain for asparagine occur in metastatic breast cancer.
- Most prostate carcinomata are androgen dependent. Androgen independence may develop consecutive to missense mutations, which cause the Androgen Receptor to loose its ligand specificity. Receptors with the mutations T877A and L701H have increased affinity for glucocorticosteroids. The H874Y mutation influences the binding of coactivator proteins by affecting the conformation of helix 12.

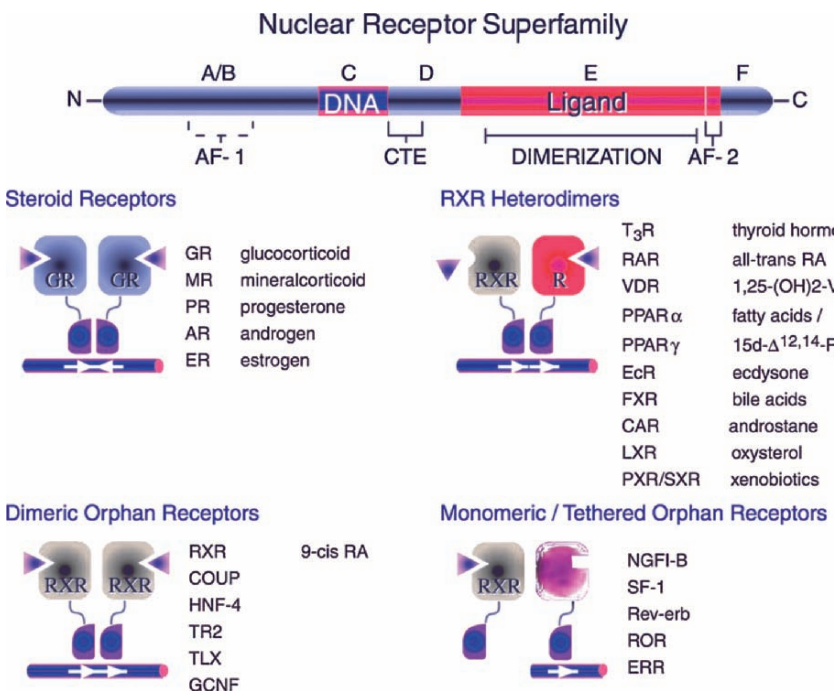


Figure 3.1.3.D. The nuclear receptor superfamily. Nuclear receptors share common structural motifs. A typical nuclear receptor contains a variable NH_2-terminal region (A/B), a conserved DNA-binding domain (C), a variable hinge region (D), a conserved ligand-binding domain (E), and a variable COOH-terminal region (F). Nuclear receptors can be grouped into four classes (steroid receptors, RXR heterodimers, heterodimeric orphan receptors, and monomeric orphan receptors) according to their ligand binding, DNA binding, and dimerization properties. Shown are representative receptors for each group. Question marks refer to orphan receptors for which ligands are unknown. [Adapted from Mangelsdorf et al. 1995. With permission.]

There are multiple families of growth factors. They are characterized by structural homologies and by functional convergence in the induction of cell cycle progression.

The Epidermal Growth Factor family. EGF, Heregulins (Neuregulins), Amphiregulin (AREG, SDGF), Betacellulin, and Transforming Growth Factor-α (TGF-α) are members of the same family of growth factors. All of these molecules share sequence similarity and conservation of the six cysteine residues $X_nCX_7CX_{2-3}GXCX_{10-13}CXCX_3YXGXRCX_4LX_n$ present within the mature sequence of EGF. The mature forms of these family members are generated by proteolysis of much larger transmembrane glycoproteins.

EGF is a 53 amino acid polypeptide constrained by three internal disulfide bonds. It exists in many cells in the form of an inactive high molecular weight precursor. The active form binds to a transmembrane receptor, which then becomes internalized and activated as a tyrosine kinase. EGF is produced predominantly by the salivary gland and gastrointestinal cells. It stimulates many epithelial and mesenchymal cells. EGF possesses two binding sites for its receptor, one site binds monovalently to the receptors while the other site bridges two ligand–receptor complexes. TGF, which is probably a fetal form of EGF, is produced by the placenta and binds to the EGF Receptor. Amphiregulin is a heparin binding, bifunctional growth modulator. It contains a very basic 40 amino acid stretch at its NH_2-terminus, which is also rich in potential *N*-linked and *O*-linked glycosylation sites. Within this region, there are also two putative nuclear localization signals that may allow this protein to shuttle into the nucleus.

The four *erb* genes *erbB1* (*egfr*), *erbB2* (*her-2*/*neu*), *erbB3* (*her-3*), and *erbB4* (*her-4*) form the family of EGF Receptor tyrosine kinases. They are composed of a cysteine-rich extracellular domain, a single transmembrane domain, and an intracellular catalytic domain. Ligands that bind to ERBB1 include EGF, TGF-α, and Amphiregulin. Ligands that bind to ERBB1 and ERBB4 include Betacellulin, HB-EGF, and Epiregulin. All Neuregulins bind to ERBB3 and ERBB4. Heregulins are a group of growth factors for receptors including ERBB3 and ERBB4. Heregulins do not bind directly to ERBB2 even though they activate the ERBB2 kinase. Although no ligand for ERBB2 has been identified, it is the preferred heterodimerization partner of the other ERBB receptors. Ligand-induced ERBB1 activation is driven, in part, by stable receptor dimerization and by rapid extensive phosphorylation of all ERBB1 receptors on the cell, which may occur through lateral signaling to unligated receptors.

– The activated receptors bind to signal transduction proteins that contain SH2 (SRC homology 2) domains. Signaling through SRC may be particularly important in breast cancers, where both SRC and EGFR are often up-regulated. SRC is required for the activation of STATs after ligation of ERBB1 by EGF. The activation of STAT3 and STAT5 occurs via JAK2.
– The ensuing signal transduction proceeds through GRB2, SHC, PLC-γ, Phosphatidylinositol 3-Kinase, and the activation of Protein Kinase B (PKB).
– Furthermore, upon ligation, ERBB1 induces the activation of ERK1 and ERK2 via RAS.

ERBB1 promotes cell survival by down-regulating BAX and up-regulating BCL-2. Following engagement by its ligand, the EGF Receptor is internalized and degraded. The 185 kD ERBB2 transmembrane protein is related to, but distinct from ERBB (EGFR). ERBB2 transduces signals through Phospholipase C, phosphatidylinositol 1,4,5-trisphosphate, and diacylglycerol. One ensuing effect is the cellular influx of calcium ions from the exterior. ERBB2 stimulates the overexpression of BCL-X_L and BCL-2.

TGF-α has about 40% sequence homology with EGF and competes with EGF for binding to the EGF Receptor, stimulating its phosphorylation and producing a mitogenic response. MMPs (Matrix Metalloproteinases) and ADAMs (A Disintegrin and Metalloproteinase-Like Domains) participate in the release of TGF-α from its cell membrane-bound precursor.

Gain of function in EGF Receptor pathways is common in carcinomata.

- Squamous carcinoma cells have a greatly increased number of EGF Receptors on their surfaces as compared with normal keratinocytes. Increased EGFR expression is also common in lung cancers. EGFR molecules are up-regulated in 30% of breast cancers and frequently in ovarian cancer.
- ERBB2 expression in breast cancer is an indicator of poor prognosis. Cells that overexpress ERBB2 have up-regulated PKB kinase activity, which phosphorylates MDM2 on the residues 166 and

186, inactivating P53 and leading to increased resistance to apoptosis.

- The *erbB2* gene is activated by a point mutation encoding V664E, which may result in cell transformation in breast cancer, bladder cancer, colon cancer, lung cancer, and gastric cancer. Point mutations in the transmembrane domain of ERBB2 enhance its transforming properties. They may have a stabilizing effect on the conformation, which results in dimerization and activation. Activating mutations of EGFR frequently occur in glioblastoma. Two classes of somatic mutations of the EGFR kinase domain in lung cancer have been identified, comprising either amino acid substitutions of the P-loop (exon 18) and the activating domain (exon 21) or in-frame deletions within exon 19 that alter the structure of the αC helix.
- A truncated, constitutively active form of the EGF Receptor is encoded by the *v-erbB* oncogene.
- *amphiregulin* (*areg*, *schwannoma-derived growth factor*, *sdgf*) encodes a heparin-binding glycoprotein that regulates the growth of tumor cells through ligation of the EGF Receptor. It is a major transcriptional target of WT1 [Lee et al. 1999a] and may play a role in Wilms tumor.
- For some tumor cells, TGF-α may act in an autocrine or paracrine fashion, maintaining growth.

The Fibroblast Growth Factor family. Consists of around 20 members. Fibroblast Growth Factors share homology in a central core of 140 amino acids, which folds into 12 antiparallel β-strands that form a cylindrical barrel. Because these proteins can bind to and have their biologic activities modulated by heparin, they are also termed Heparin-Binding Growth Factors (HBGFs). FGFs are important in development because they induce division in cells of mesodermal and neuroectodermal origin in the early embryo and in organogenesis. As embryonic inducers, they do not circulate in the blood, but are integrated into the basement membrane of cells that produce them through heparan-like glucosaminoglycans in the extracellular matrix. The release of FGFs occurs after cleavage of extracellular matrix components by proteases or Heparanase, or by FGF binding to a carrier protein, such as FGF-Binding Protein (FGF-BP). This may create a local reservoir of growth factors and could be important in processes such as limb development.

– The *fgf-1* (*acidic fgf*) open reading frame encodes a single protein of 155 amino acids. It does not have a signal peptide, but possesses a nuclear localization signal, which is important for mitogenesis. In the absence of a leader sequence, secretion may occur after binding to Synaptotagmin-1. FGF-1 ligates the FGF Receptors -1, -2, -3, and -4.
– The *fgf-2* (*basic fgf*) gene can generate four proteins of 18, 22.5, 23.1, and 24.2 kD through the use of alternate CTG start codons upstream from the ATG site. FGF-2 does not contain a signal sequence, but the larger forms contain a nuclear localization sequence. FGF-2 is a substrate for PKC and Protein Kinase A. FGF-2 can engage FGF Receptors -1, -2, -3, and -4.
– FGF-3 (INT-2) is expressed primarily during development. It is a 239 amino acid polypeptide with a defined NH_2-terminal signal sequence and a COOH-terminal nuclear localization sequence. FGF-3 can ligate FGFR-1 and FGFR-2.
– The *fgf-4* (*heparin secretory transforming protein-1*, *hst-1*, *Kaposi sarcoma oncogene-3*, *ks3*, *kfgf*) gene codes for a 206 amino acid protein. It contains a signal sequence and an *N*-glycosylation site, and it ligates FGF Receptors -1, -2, -3, and -4.
– FGF-5 contains 267 amino acids and is secreted as a glycoprotein.
– FGF-6 (HST-2) is a 198 amino acid protein with a signal sequence and a glycosylation site.
– FGF-7 (Keratinocyte Growth Factor, KGF) contains 194 amino acids with a signal sequence and an *N*-linked glycosylation site. It is produced by fibroblasts, and is only mitogenic for epithelial cells.
– FGF-8 (Androgen-Induced Growth Factor, AIGF) occurs in up to seven forms, generated by alternative splicing. The variants differ in their NH_2-termini; however, the signal sequence is not altered.
– FGF-9 (Glia Activating Factor, GAF) has 208 amino acids and does not contain a signal sequence. It is mitogenic for glial cells and fibroblasts, but not for endothelial cells.
– FGF-10 is a glycoprotein of 208 amino acids with a signal sequence. It is mitogenic for keratinocytes, and in high concentrations stimulates fibroblasts. FGF-10 is expressed in stromal cells, particularly of muscle origin. It has high affinity to heparin and is associated with the extracellular matrix.
– FGF-11, -12, -13, and -14 (FGF Homology Factors, FHFs) all contain nuclear localization signals, but no signal sequences for secretion. FGF-13 can form two variants by alternative splicing of the first exon.

– *fgf-15* may be a target of the oncogenic transcription factor E2A-PBX1 (pre-B-Cell Leukemia Transcription Factor), which is generated by the t(1;19) translocation in pre-B-cell leukemias. The excess expression of the growth factor accelerates cell division.
– FGF-16, -17, -18, and -19 range in size from 207 to 216 amino acids. They play various roles in development. [Powers et al. 2000].

There are four *fgf receptor* genes. Additional diversity of FGF Receptors is generated through alternative splicing, leading to the expression of truncated receptor forms or to the expression of diverse variants of the IgIII domain. FGF Receptors are members of the Immunoglobulin superfamily of receptors. (Figure 3.1.3.E) They contain three Immunoglobulin-like domains, an acidic region between Immunoglobulin domains I and II, a transmembrane domain, and an intracellular kinase domain. The engagement of FGF Receptors by their cognate ligands results in their dimerization, autophosphorylation, and activation. This interaction is stabilized by binding of FGFs to heparan sulfate proteoglycans on the cell surface and results in mitogenic activity. FGF is a monomeric ligand that needs an accessory molecule to induce receptor dimerization. Signal transduction is initiated by tyrosine phosphorylation of the FGF Receptor cytoplasmic tails. There are seven tyrosyl residues in the cytoplasmic tail of FGFR-1, out of which Y653 and Y654 are important for the catalytic activity of the activated receptor. The phosphorylated tyrosyl residues in the cytoplasmic tails of the dimerized receptors recruit other signal transduction molecules, which initiate four principal pathways.

– CRK is an SH2/SH3-containing adapter protein, which links the FGF Receptor to SHC, C3G, and CAS, leading to cell cycle progression.
– The 90 kD SNT-1 (FRS2) is recruited and phosphorylated by FGF Receptors. It links the FGF Receptors to the MAP Kinase signaling pathway via GRB-2, SOS, and RAS.
– SRC is a nonreceptor tyrosine kinase that can link FGFR signaling to the proto-oncogene product Cortactin (EMS-1). This may alter the anchorage dependence of the affected cells.
– Y766 in the cytoplasmic tail of FGFR-1 binds to the SRC homology 2 (SH2) domain of PLCγ. Activated PLCγ cleaves phosphatidylinositol-4, 5-bisphosphate to inositol trisphosphate and diacylglycerol. This pathway may, however, not contribute critically to FGF-dependent mitogenesis.

FGFs and their receptors can have multiple roles in tumor formation.

- FGFs may be secreted by tumor cells and by stromal cells and act as autocrine or paracrine growth factors. FGF-4 can contribute to transformation in stomach cancer and in Kaposi sarcoma. Kaposi sarcoma releases FGF-2 in response to Tumor Necrosis Factor α (TNF-α), IL-1, or IFN-γ. FGF-2 is often overexpressed in gliomata, where it correlates with the degree of malignancy and vascularity.
- Overexpression of FGF Receptors occurs in brain cancer, breast cancer, prostate cancer, thyroid cancer, and melanoma. Overexpression of FGF Receptor-2 in undifferentiated gastric cancers is a sign of poor prognosis.
- A point mutation S267P, in the extracellular third Immunoglobulin-like domain, and a splice site

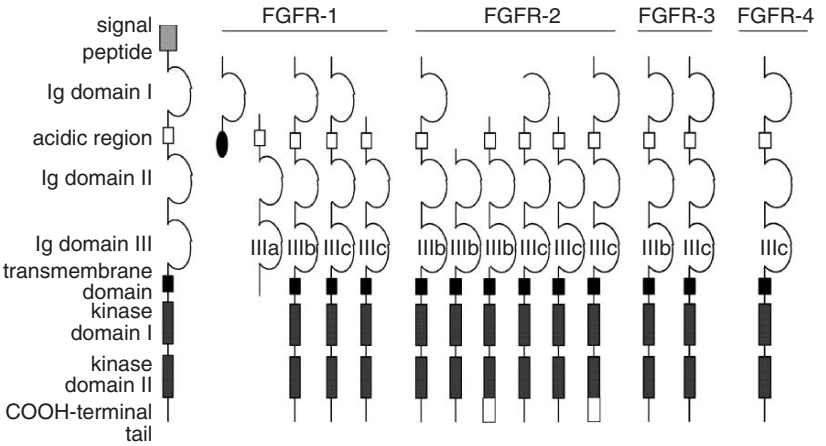


Figure 3.1.3.E. Structures of FGF Receptors. The structures of FGF Receptors are modular. Their variety is possible through the use of alternative splicing. The basic structure is depicted to the left. The solid symbol represents a premature truncation. Note the use of alternative COOH-termini (open boxes).

mutation 940-2A → G of the *fgf receptor-2* gene occur in gastric carcinoma. Another constitutively active form of FGF Receptor-2 may be expressed in osteosarcoma secondary to a chromosomal rearrangement that fuses the NH_2-terminus of FGF Receptor-2 to an unknown protein. Mutations in the *fgfr-3* gene are associated with colorectal cancer [Powers et al. 2000].

- Myeloid cells containing the t(4;14)(q16.3;q32.3) translocation overexpress FGF Receptor-3.

Insulin-Like Growth Factors. Insulin, IGFs -1 and -2, and Relaxin stimulate DNA synthesis and cell growth. All four growth factors are disulfide-linked heterodimers that are generated from precursor forms by proteolytic cleavage.

– Insulin is produced as proinsulin [Steiner and Oyer 1967] by the β cells of the islets of Langerhans in the pancreas.
– IGF-1 is mainly secreted by the liver and smooth muscle cells as a result of stimulation by growth hormone. Most cells are responsive to IGF-1, especially cells in muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs. A large fraction of the circulating IGF-1 is attached to IGF-binding proteins (IGF-BPs).
– IGF-2 is secreted from fetal liver and placenta and from adult brain, kidney, pancreas, and muscle. It is more specific in action than IGF-1.
– Relaxin is a peptide hormone produced by the corpora lutea of ovaries during pregnancy. The secretion of the hormone into the blood stream just before parturition results in softening and lengthening of the pubic symphysis and a softening of the cervix, which facilitates the birth process. By inhibiting uterine contractions, Relaxin may influence the timing of parturition. The factor consists of two peptide chains covalently linked by disulfide bonds.

IGFs (Somatomedins) are expressed in two types, IGF-1 (Somatomedin C) and IGF-2 (Somatomedin A, Multiplication-Stimulating Factor, MSF). They are bound to carrier proteins and are maintained at relatively steady serum levels. While IGF-2 is a primary growth factor required for early development, IGF-1 expression mostly occurs in later life.

The mature Insulin Receptor is derived from precursor polypeptides. It consists of two α subunits (1,370 amino acids or 1,382 amino acids, generated by alternative splicing) and two β subunits (624 amino acids). The α subunits are extracellular, while the β subunits traverse the membrane and possess tyrosine kinase activity. The proto-oncogene *ros* encodes the Insulin Receptor β chain. Although several receptors for IGFs exist, the biological actions of IGF-1 and IGF-2 are predominantly exerted through the type-1 IGF-1R, which binds IGF-1 and IGF-2 with high affinity. Activation of the IGF-1R occurs following IGF-1 binding to the α subunit of the IGF-1R on epithelial cells, leading to autophosphorylation of the β subunit. The IGF-1R displays potent mitogenic, antiapoptotic, and transforming activities, which may be a prerequisite for oncogenesis. IGF-1R is a heterotetrameric tyrosine kinase receptor, closely related to Insulin Receptor in sequence and structure. It is linked to the RAS→RAF→MAPK and to the PI 3-K→PKB signal transduction cascades. In contrast, IGF-2R is a single polypeptide chain with a short cytoplasmic tail that lacks kinase activity. IGF signaling supports cell proliferation in several ways (Figure 3.1.3.F).

– Engagement of the Insulin Receptor by Insulin stimulates the phosphorylation of the Insulin Receptor Substrates (IRS) -1, -2, and -3. These proteins associate with the regulatory subunit, P85, of Phosphatidylinositol 3-Kinase and activate it, which in turn mediates the activation of PKB-2 (AKT-2). PKB plays a central role in glucose uptake and glycogen synthesis.
– IGF-1 may signal to prevent apoptosis through RAS, Phosphatidylinositol 3-Kinase, and PKB (AKT Kinase), ultimately impacting on BAD, a key modulator of the BCL-2 family. mTOR (Mammalian Target of Rapamycin) is a serine/threonine kinase and a substrate for PKB, which plays a critical role in promoting cell survival.
– Receptor ligation by IGFs rapidly activates nuclear PLCβ1, which in conjunction with the phosphatidylinositol pathway contributes to the control of mRNA export from the nucleus and is essential for the mitogenic effect of IGF [York et al. 1999].

The biological actions of IGF are mediated primarily by its association with the type-1 IGF-1R, which is regulated by a group of high affinity IGFBP-1 through IGFBP-6. The IGF-BPs have growth inhibitory effects by competitively binding IGFs and preventing their association with the IGF-1R. All IGF-BPs have 16–18 conserved cysteine residues in the NH_2- and COOH-terminal regions. IGF-BP3 is the most abundant binding protein in the circulation

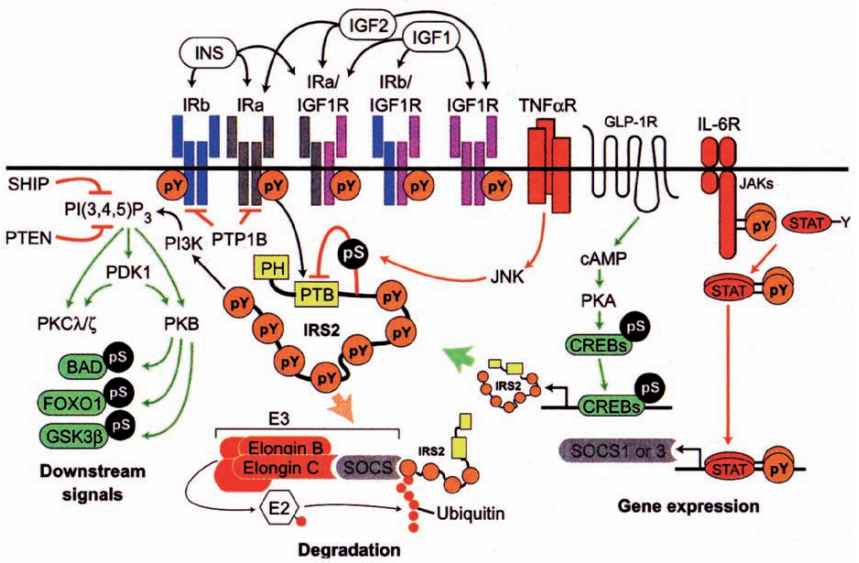


Figure 3.1.3.F. Insulin and IGF signaling. Insulin and IGF-1 (Insulin-Like Growth Factor 1) Receptors form hybrids that modulate the selectivity and affinity for Insulin, IGF-1, and IGF-2. Insulin or IGF binding stimulates tyrosine autophosphorylation in the receptor β subunits, which activates the kinase and recruits the cellular substrates IRS-1 and IRS-2 for tyrosine phosphorylation. This recruitment is regulated by serine phosphorylation of the IRS proteins, which inhibits the interaction between their PTB domains and the phosphorylated receptor. Proinflammatory cytokines increase the synthesis of SOCS-1 or SOCS-3, which promote the ubiquitination and degradation of IRS-1 and IRS-2. cAMP synthesis enhances the expression of IRS-2 through the activity of phosphorylated CREB. Tyrosine phosphorylation of IRS-1 or IRS-2 recruits and activates various SH2 domain-containing proteins, including PI 3-Kinase, which activates the PKB cascade. pY = phosphotyrosine, pS = phosphoserine, PKCλ/ζ = Protein Kinase C λ or ζ, E2 = Ubiquitin conjugating enzymes, TNFαR = Tumor Necrosis Factor α Receptor, GLP-1R = Glucagon-Like Peptide-1 Receptor, IL6R = Interleukin-6 Receptor. [Reproduced from White 2003. With permission.]

and controls the actions of the IGFs by regulating their distribution and bioavailability to target tissues.

- IGF-1 may act as an autocrine growth factor in neuroendocrine tumor cells. In carcinoid tumor cells, IGF-1 stimulates Phosphatidylinositol 3-Kinase, P70 S6 Kinase, and ERK-2. Melanoma cells do not respond to growth stimulation by IGF-1 because of a constitutive activation of the MAP Kinase pathway [Satyamoorthy et al. 2001].
- Stromal–epithelial interactions are vital for full mammary gland development. IGFs are synthesized by stromal cells of the mammary connective tissue. IGF-1 and IGF-2 are potent mitogens and survival factors for breast epithelial cells. They act primarily through the IGF-1R, which is significantly overexpressed and highly activated in breast tumors.
- In sporadic cases of Wilms tumor, the expression of the *igf-2* gene is markedly increased relative to the surrounding tissue or to adult kidneys, but is comparable to the level of expression in several fetal tissues including kidney, liver, adrenal, and striated muscle [Reeve et al. 1985; Scott et al. 1985].
- IGF-2 is a modulator of muscle growth and differentiation. Its gene is widely expressed during prenatal development and is regulated by genomic imprinting (inactivation of the maternal copy). While this leads to monoallelic expression of *igf-2* in normal adult muscle tissue, two or more copies of active *igf-2* alleles are associated with rhabdomyosarcoma, arising either by relaxation of imprinting or by duplication of the active allele. IGF-2 acts as an autocrine growth factor for rhabdomyosarcoma cells, and its elevated expression may be an important step for the initiation or progression of rhabdomyosarcoma. The loss of imprinting (LOI) of *igf-2* is associated with all histologic subtypes of rhabdomyosarcoma, but not with leiomyosarcoma [Pedone et al. 1994].
- Glucocorticosteroids counteract the effects of Insulin and suppress the growth of certain tumors [Osborne et al. 1979].
- The proto-oncogene *ros* (*mcf-3*) encodes the Insulin Receptor β chain, which acts as a protein tyrosine kinase receptor. It is highly expressed in glioblastomata. In some glioblastomata, an

interstitial deletion of 240 kb on 6q21 fuses the *fig* gene to the *ros1* gene. The resulting ROS1–FIG fusion protein is a constitutively activated tyrosine kinase [Charest et al. 2003a,b].

- *ros1* is located on chromosome 6q22. Chromosomal rearrangements in the 6q11-q31 region are associated with acute lymphoblastic leukemia (ALL), malignant melanoma, and ovarian carcinoma. They may reflect a role for *ros1* in transformation.

Hepatocyte Growth Factor. Hepatocyte Growth Factor (HGF, Scatter Factor) is encoded by a single transcript, whose 728 amino acid product is processed by proteolytic cleavage into a 87 kD disulfide-linked heterodimer of a heavy chain and a light chain. HGF contains a serine protease-like domain (with critical histidine and serine residues mutated) and Kringle domains. Under physiologic conditions, HGF is not an autocrine, but rather a paracrine, factor. Mesenchymal cells produce HGF, which is mitogenic for melanocytes, renal tubular cells, and some epithelial cells.

The 145 kD proto-oncogene product and receptor tyrosine kinase c-MET acts as a receptor for HGF. The product of the *met* proto-oncogene {7q31}, MET, is a transmembrane protein, synthesized as a single chain precursor, which undergoes intracellular proteolytic cleavage at a basic amino acid site, yielding a disulfide-linked heterodimer. Its COOH-terminal, intracellular region contains a multifunctional docking site that binds to various signaling molecules. The MET receptor tyrosine kinase family consists of two related proteins, MET and RON. The extracellular regions of both MET and RON display structural similarities with Semaphorins and Plexins (Figure 3.1.3.G). The RON receptor tyrosine kinase recognizes Macrophage-Stimulating Protein (MSP) (Figure 3.1.3.H).

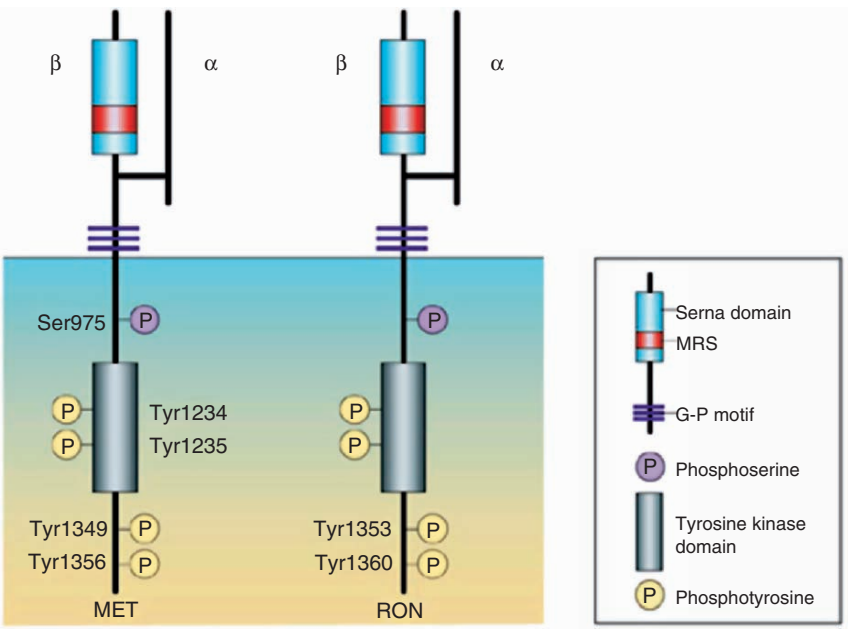


Figure 3.1.3.G. Structural organization of Scatter Factor Receptors. The MET Receptor for Hepatocyte Growth Factor and the RON Receptor for Macrophage-Stimulating Protein are single pass, disulfide-linked αβ heterodimers that form by proteolytic processing of a common precursor in the post-Golgi compartment. In both receptors, which share 63% overall homology, the α chains are completely extracellular, whereas the β chains are transmembrane subunits that contain the tyrosine kinase activity. The extracellular regions of Scatter Factor Receptors display structural analogies with the extracellular domains of Semaphorins and Plexins. They contain a sema domain, which is a conserved sequence of about 500 amino acids, and comprise a peptide module of eight cysteines that is conventionally termed MRS (MET-related sequence), together with three G–P (glycine- and proline-rich) repeats. The intracellular domains include tyrosine kinase catalytic sites that are flanked by distinctive juxtamembrane and COOH-terminal sequences. In the MET protein, phosphorylation of the tyrosines 1234 and 1235 within the catalytic site results in positive modulation of the enzyme activity, whereas phosphorylation of a serine residue in the juxtamembrane domain down-regulates the kinase. The COOH-terminal domains of both receptors include two critical tyrosine residues that, when phosphorylated, together form a docking site for several signal transducers and adaptors. [Reproduced from Trusolino and Comoglio 2002. With permission from Macmillan.]

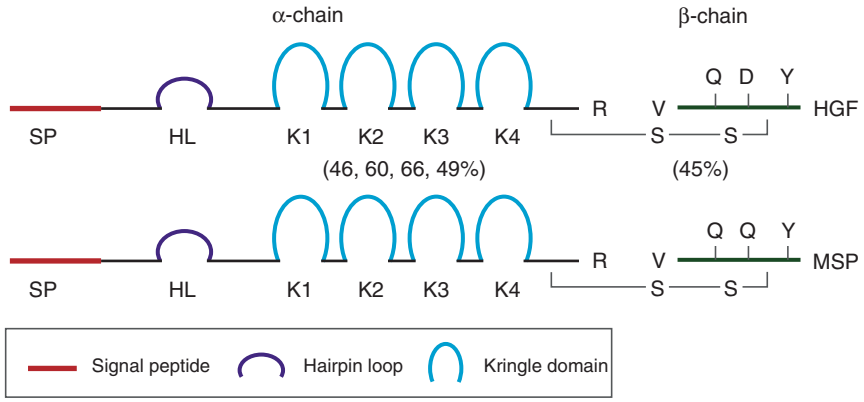


Figure 3.1.3.H. Structural organization of Scatter Factors. Scatter Factors, comprising Hepatocyte Growth Factor (HGF) and Macrophage-Stimulating Protein (MSP), belong to the Plasminogen family of proteins, which are defined by the presence of at least one characteristic kringle domain (an 80 amino acid double-looped structure that is formed by three internal disulfide bridges), a serine protease domain, and an activation segment that is located between the kringle and the protease domains. Among the members of this family, HGF and MSP are unique in that they lack proteolytic activity, owing to the replacement of the histidine and serine residues that are contained within the catalytic site of serine proteases with glutamine and tyrosine, respectively. Scatter factors are secreted as single-chain, biologically inert glycoprotein precursors and are converted into their active forms in the extracellular environment by specific proteases, which break the bond between two positively charged amino acids (the dibasic site R494 to V495). The mature factors are heterodimers that consist of one α-chain and one β-chain linked by a disulfide bond. The α-chain contains a hairpin loop of about 27 amino acids (homologous to the preactivation peptide of Plasminogen) followed by four kringles, whereas the β-chain contains the serine protease-like structure. The percentage of homology between HGF and MSP of individual kringles and the β-chain is specified in the figure. [Reproduced from Trusolino and Comoglio 2002. With permission from Macmillan.]

HGF binding to MET results in receptor autophosphorylation and up-regulation of MET kinase activity. Most receptor tyrosine kinases use distinct tyrosines to bind specific signaling molecules, but the Scatter Factor Receptors contain two crucial tyrosines in a degenerate motif that, when phosphorylated, recruit an array of molecules, including Phosphatidylinositol 3-Kinase, SRC, the adaptor proteins GRB2 and SHC, and the multiadapter protein GAB1. The associated signal transduction proceeds via Phosphatidylinositol 3-Kinase→PKB and RAS pathways.

Gain of function of the MET pathway in cancer may come about in several ways:

- Wild-type MET can engage in a paracrine or autocrine loop in the presence of high levels of HGF. Such HGF-driven positive feedback loops, that sustain MET-induced oncogenic transformation, occur in mesenchymally derived tumors. Although MET is not expressed in adult skeletal muscles, a significant fraction of rhabdomyosarcomata that endogenously secrete HGF also express MET [Ferracini et al. 1996]. In addition, HGF–MET autocrine loops arise in gliomata, osteosarcomata [Ferracini et al. 1995], and mammary [Tuck et al. 1996], prostate, and lung carcinomata. They are often associated with tumor progression and correlate with poor prognosis.
- Wild-type MET may induce cell divisions in the absence of ligand if it is overexpressed due to gene amplification [Takeo et al. 2001; Di Renzo et al. 1995]. Defects of negative regulators of MET signaling, including inactivating cytosolic phosphatases, can also cause excessive activity. *met* overexpression occurs in almost every case of renal differentiated papillary carcinomata. It also arises in thyroid carcinoma [Di Renzo et al. 1992], ovarian carcinoma [Di Renzo et al. 1994], hepatocellular and gastric carcinoma [Ueki et al. 1997]. Increased levels of MET expression are associated with carcinomata of colon, pancreas, and osteogenic sarcomata.
- Ligand independent activation may occur if MET is processed abnormally or truncated (cytoplasmic MET). The subcellular localization of MET contributes to determining its carcinogenic potential. Cytoplasmic MET may be tumorigenic in the breast, but not in the liver, whereas transmembrane MET can cause hepatocellular carcinoma, but not breast carcinoma.

- Certain genetic alterations, including activating mutations, of *met* can render its product independent of the physiologic ligand. The mature MET consists of two subunits, α and β, that arise from proteolytic cleavage of the single chain precursor. As a result of defective posttranslational processing, the precursor fails to be cleaved in some colon carcinoma cells. Consequently, MET is expressed on the cell surface as a single-chain molecule, which is constitutively tyrosine-phosphorylated. Point mutations in *met* occur in hereditary and sporadic papillary renal carcinomata [Schmidt et al. 1997], hepatocellular, and gastric carcinomata [Park et al. 1999; Lee et al. 2000], and head and neck squamous carcinomata [Di Renzo et al. 2000]. Point mutations in the kinase domain convert MET to an oncogenic receptor. Such mutants are catalytically highly active, which correlates with more efficient MET autophosphorylation and phosphorylation of substrates. The constitutive binding of c-SRC to the cytoplasmic domain of the MET M1268T mutant in renal papillary carcinomata, elevates c-SRC phosphorylation and activity. MET M1268T also phosphorylates substrates of the cytosolic kinase c-ABL, whereas wild-type MET does not. The expression of MET M1268T induces β-Catenin tyrosine phosphorylation and accumulation, induces constitutive activation of the transcription factor TCF, which acts in concert with β-Catenin in the nucleus and increases the expression of the β-Catenin/TCF target genes *myc* and *cyclin* D_1.
- *met* may be subject to gene translocations, such as *tpr-met* in osteosarcoma [Cooper et al. 1984]. The TPR–MET fusion protein is constitutively active and potently oncogenic as a result of TPR leucine zipper interactions, which allow MET kinase dimerization, autophosphorylation, and activation.
- MET kinase activity can be regulated through other receptors by HGF-independent mechanisms. The ligation of Integrins can cause MET tyrosine phosphorylation through lateral signaling. CD44 can promote MET activation by two mechanisms, the binding of CD44 to hyaluronate causes HGF-independent MET activation, leading to cell growth and migration, and a heparan sulfate proteoglycan isoform of CD44 binds HGF and presents it to MET in the form of a multivalent complex, inducing a higher level of MET activation than soluble HGF. Because RON belongs to the same family of receptor tyrosine kinases as MET and shares many common structural features, activated RON can trans-phosphorylate MET, and vice versa. Preexisting, ligand-independent heterodimers between MET and RON form on the cell surface and may be able to trans-phosphorylate and to activate one another. Some hepatoma cells, but not normal hepatocytes, are activated by an autocrine loop involving TGF-α and EGF Receptor, which leads to constitutive, ligand-independent tyrosine phosphorylation of MET [Danilkovitch-Miagkova and Zbar 2002].

Platelet-Derived Growth Factor Family. The release of Platelet-Derived Growth Factor (PDGF) during blood clotting initiates its function in connective tissue proliferation associated with inflammation and tissue repair. Quiescent skin fibroblasts can be stimulated to reenter the cell cycle after tissue injury by the release of PDGF from platelets. PDGF has two distinct, but highly homologous subunits. The A chain (PDGF-1) {7p22} induces the proliferation of Leydig cells. The B polypeptide of PDGF (PDGF-2) is encoded by the *c-sis* proto-oncogene {22q12.3–q13.1} [Doolittle et al. 1983]. PDGF molecules exist as AA and BB homodimers, as well as an AB heterodimer. PDGF-D is a specific, protease-activated ligand for the PDGF-β Receptor [Bergsten et al. 2001].

Receptors for PDGF are prominent on mesenchymal cells, including fibroblasts and smooth muscle cells. PDGF Receptor-α (PDGFR-2) {4q12} and PDGF Receptor-β (PDGFR-1) {5q31–q32} are members of the Immunoglobulin superfamily of receptors. PDGF-AA can bind only the PDGF-α receptor dimer, whereas PDGF-BB can interact with α, αβ, or β receptor dimers. The PDGF-AB heterodimer preferentially interacts with α and αβ receptors. It may bind the PDGF-β receptor, but does not induce its dimerization. The ligation of PDGFRs initiates receptor dimerization and activates a Phosphodiesterase in the cell membrane that hydrolyzes phosphatidylinositol bisphosphate to form inositol trisphosphate and diacylglycerol. While inositol trisphosphate causes the release of calcium from intracellular stores, diacyl glycerol activates PKC bound to the internal surface of the plasma membrane. This enzyme then causes the phosphorylation and activation of membraneous ion channels, which leads to the extrusion of H^+ ions and influx of Na^+ ions, resulting in a rise of intracellular pH, which may be significant in supporting cell division.

The pathway PDGF→PDGFR→SRC→STAT3→ c-MYC mediates cell growth [Bowman et al. 2001].

The *fms* proto-oncogene {5q33.2–q33.3} encodes CSF1R (Colony-Stimulating Factor-1 Receptor, Macrophage Colony-Stimulating Factor Receptor). FMS belongs to the same family as PDGFRB; their respective genes are linked physically in a head-to-tail array, with less than 500 base pairs between the polyadenylation signal of *pdgfrB* and the transcription start point of the *csf1r*. The *fms* gene consists of 21 small exons, interrupted by introns that range in size from <0.1 to 6.3 kb. The CSF-1R contains five Immunoglobulin-like loops in the extracellular domain and an insert within the kinase domain. CSF-1 and CSF-1R are essential for normal monocyte development. Significant increases in the levels of CSF-1 and CSF-1R proteins also occur in the epithelial cells of the mammary glands during pregnancy and lactation. The expression in the breast of CSF-1 can be regulated by Prolactin and Insulin, whereas CSF-1R expression is regulated by glucocorticosteroids.

Steel Factor (Stem Cell Factor, Mast Cell Growth Factor, MGF, KIT Ligand, KITLG) {12q22} is a hematopoietic and tissue growth factor. It is a 165 amino acid polypeptide, which is heavily *N*- and *O*-glycosylated and exists as a dimer. Alternative splicing of the gene results in predominantly secreted and predominantly membrane-bound forms. Cell surface Kit Ligand not only stimulates cell proliferation, but also mediates cell–cell adhesion [Flanagan et al. 1991]. The receptor for Steel Factor is a receptor of the PDGF Receptor family, which is encoded by the proto-oncogene *c-kit* {4q12}. Most proliferating cells are programmed to undergo apoptosis, unless specific survival signals are provided. PDGF inhibits apoptosis. Activated dimerized PDGF Receptor associates with the SH2 domain of SRC via binding of specific phosphotyrosine residues in the juxtamembrane region. PDGF activates the RAS→PI 3-Kinase→PKB-1→I-κB Kinase→NF-κB1 pathway. In this pathway, NF-κB1 does not induce *c-myc* and apoptosis, but instead induces putative antiapoptotic genes [Romashkova and Makarov 1999]. KIT also mediates the up-regulation of BCL-2 and phosphorylation of BAD.

Various alterations of PDGF or PDGFR family members are associated with specific forms of malignancies.

- In familial and sporadic meningiomata, a deletion in the fifth intron of the *sis* gene may occur. The intact *sis* gene has an Alu sequence in this region, which includes two perfect 130 nucleotide-repeated sequences, separated by five base pairs. The deleted allele in a fraction of meningioma cases misses one copy of the 130 base pairs repeat and the intervening five base pairs [Bolger et al. 1985].
- The tyrosine kinase receptor c-KIT is an important regulator of small cell lung cancer growth. Furthermore, mutations in the *kit* gene, either somatic or in some instances germline, can underlie gastrointestinal stromal tumors. Gastrointestinal stromal tumors, which are not caused by mutations in *kit*, are often caused by mutations in *pdgfrA*.
- Dermatofibrosarcoma protuberans, an infiltrative skin tumor of intermediate malignancy, presents reciprocal translocations t(17;22)(q22;q13) and supernumerary ring chromosomes derived from t(17;22). These rearrangements fuse the *pdgfB* gene and the *col1A1* gene. The gene fusions delete exon 1 of PDGFB and release this growth factor from its normal regulation [Simon et al. 1997]. Autocrine transforming interactions occur in a number of malignancies. At least one PDGF chain and one of its receptors are expressed in a high fraction of sarcomata and in glial-derived neoplasms [Maxwell et al. 1990; Nister et al. 1988].
- *prlts* (*PDGFR-β-like tumor suppressor*) is physiologically expressed as a 1.6 kb transcript at low levels in colon, lung, and the liver. The 375 amino acid protein derived from this sequence contains an NH_2-terminal signal peptide. It shows about 25% identity with the extracellular domains of PDGFR-β and FLT, respectively. A 600 kb region on chromosome 8p22-p21.3 is commonly deleted in hepatocellular carcinoma, colorectal carcinoma, and non-small cell lung carcinoma. This reflects a loss of function of *prlts*. Somatic missense and frameshift mutations of *prlts* arise in hepatocellular carcinoma and colorectal cancer [Fujiwara et al. 1994; Fujiwara et al. 1995].
- The codons 969 and 301 of *fms* are potentially involved in promoting the transforming activity of the gene product. Mutations at codon 301 lead to neoplastic transformation by ligand independence and constitutive tyrosine kinase activity of the expressed receptor. The tyrosine residue at codon 969 is involved in a negative regulatory activity, which is disrupted by amino acid substitutions.

Mutations of FMS in these places are prevalent at around 20% in chronic myelomonocytic leukemia and acute myeloblastic leukemia-type M4, both of which are malignancies of the monocytic lineage [Ridge et al. 1990].

- A break at chromosome location 5q35 is associated with some cases of malignant histiocytosis, a neoplastic process characterized by fever, progressive wasting, lymphadenopathy, hepatosplenomegaly, and the proliferation of atypical histiocytes at all stages of maturation with frequent phagocytic activity. Activation of the *fms* proto-oncogene may be causative for the condition [Morgan et al. 1986].
- CSF-1 and FMS (CSF-1R) have physiologic roles in mammary gland development during pregnancy and lactation. In breast and ovarian cancers, the elevated expression of CSF-1 and its receptor correlates with tumor cell invasiveness and adverse clinical prognosis [Kacinski et al. 1991; Xu et al. 1991; Scholl et al. 1993].
- PDGF-AA homodimers are produced by osteosarcoma, melanoma, and glioblastoma cells.

Heparin Binding Growth Factors. NEGF-1 (Pleiotrophin, PTN, Neurite Outgrowth Promoting Factor, Heparin-Binding Neurite Growth Promoting Factor 1, Heparin Binding Growth Factor 8, HBGF8, Heparin Affin Regulatory Peptide, HARP, HB-GAM) is a developmentally regulated cytokine with prominent function during early embryogenesis, the expression of which reaches its maximum level around birth. NEGF-1 acts as a growth factor for neuronal and epithelial cells [Wellstein et al. 1992]. The *ptn* (*negf-1*) gene {7q33} [Li et al. 1990] spans more than 65 kb and contains at least seven exons, with the open reading frame being located on four exons. The splice sites in the open reading frame coincide with the boundaries of functional domains in the 18 kD NEGF-1 protein. NEGF-2 (Neurite Growth Promoting Factor 2, Midkine) exhibits neurite outgrowth-promoting activity and may play a role in nervous system development and maintenance. The expression of NEGF-2 is predominant only for a short period in midgestation; before and after that, it is mostly limited to the epidermal–dermal junction in normal skin. The *mdk* (*negf-2*) gene {11p11.2} codes for a 143 amino acid protein precursor, which generates a mature protein of 121 amino acids and is about 45% homologous to NEGF-1. Expression of the *mdk* gene is inducible by retinoic acid. Both proteins, NEGF-1 and NEGF-2, are localized in the radial glial processes of the embryonic brain, along which neural stem cells migrate and differentiate.

CD91 (LDL, Receptor-Related Protein, LRP, Apo-Lipoprotein Receptor) {12q13.1–q13.3} is a receptor for NEGF-1 and NEGF-2 [Muramatsu et al. 2000]. Its ligation leads to signal transduction via the JAK and STAT pathways. NEGF-1 is also a ligand of Protein Tyrosine Phosphatase Receptor ζ(PTPRζ, PTPζ, Receptor-Type Protein Tyrosine Phosphatase β, RPTPβ) {7q31.3}, a 2,307 amino acid transmembrane protein that targets GIT1 (G Protein-Coupled Receptor Kinase Interactor 1). CD23 (Nucleolin) {2q12-qter} binds NEGF-2 through its extreme COOH-terminal end, which contains repeats of the amino acid motif RGG. The ligation by NEGF-2 induces lateral assemblies of Nucleolin with specific membrane components of lipid rafts.

- NEGF-1 is overexpressed in various neuroectodermal tumors, gastrointestinal cancer, and pancreas cancer. NEGF-1 is also expressed at high levels in melanoma and contributes to its formation.
- NEGF-1 is expressed in about 60% of breast cancers. NEGF-1 has the potential to support the growth of breast cancer at its primary site and to enhance the ability of tumor cells to metastasize. Specific endocrine signals interact to regulate the expression of *ptn* in breast cancer [Riegel and Wellstein 1994].
- Testicular cancer secretes NEGF-1, leading to markedly elevated serum levels. This occurs, from early stages on, in seminomatous as well as nonseminatous tumors [Aigner et al. 2003].
- NEGF-1 and its receptor RPTPζ are overexpressed in glioblastomata and in astrocytomata, suggesting the existence of an autocrine loop [Ulbricht et al. 2003].
- NEGF-2 is associated with differentiating teratoma cells [Nurcombe et al. 1992].

Neuroendocrine Peptides. As many as 30 peptides and amines derived from neuroendocrine cells may have similar properties. Secretin, Gastrin, Bombesin, Cholecystokinin, Calcitonin, and Vasoactive Intestinal Peptide promote cell growth. Bombesin is a small peptide of 2 kD, which is normally present in the central nervous system. The Bombesin-Like Peptides comprise a large family of

peptides widely distributed in neural and endocrine cells, including the gastrointestinal tract, where they modulate smooth muscle contraction, exocrine and endocrine processes, and metabolism. Bombesin-Like Peptides include Gastrin-Releasing Peptides, which are highly expressed and secreted by neuroendocrine cells, and the amidated decapeptide Neuromedin B.

Bombesin-Like Peptides bind to G-Protein-coupled receptors on the cell surface to elicit their effects. The receptors for Bombesin-Like Peptide include Gastrin-Releasing Peptide Receptor {Xp 22.3–p21.2}, Neuromedin B Receptor {26q21-qter}, and Bombesin Receptor Subtype 3 (BRS3) {Xq26–q28}. Signal transduction proceeds through the MAP Kinase cascade and leads to the expression of cell cycle-promoting genes.

- Bombesin and Neurotensin are secreted as autocrine factors by small cell carcinoma of the lung, a neuroendocrine tumor likely to be of Kultchitsky cell origin. Bombesin-Like Peptides can function as mitogens for normal bronchial epithelial cells and lung cancer cells.
- Gastrin-Releasing Peptides are secreted by prostate carcinoma. The expression of Gastrin-Releasing Polypeptide and its receptor in tumors suggests that these molecules are part of an autocrine loop for growth.
- Neuroendocrine cells are located in the prostatic ductal and acinar epithelium. Neuroendocrine-secreted products, including Serotonin, Somatostatin, and Bombesin, influence prostate cancer growth, in part, by suppressing apoptosis and, in part, by activating the Androgen Receptor. The prostate cancer cells may be sensitized to neuroendocrine factors through the up-regulation of receptor expression on their surface. Neuroendocrine differentiation appears to be associated with the androgen-independent state.
- Many astrocytic or glial-derived brain tumors express functional Substance P and Bombesin Receptors. Their activation stimulates signaling pathways that regulate transcription and translation leading to the induction of mitogenesis.

Neurotrophins. Neurotrophins and their receptors play important roles in regulating the development of both the central and the peripheral nervous systems. They are small molecules of about 14 kD, which contain three cysteine bonds and are secreted as homodimers. Neurotrophins are generated as proneurotrophin precursor molecules of 240–260 amino acids.

Nerve Growth Factor Receptor (NGFR, $P75^{NTR}$) {17q21–q22} is a 399 amino acid transmembrane glycoprotein belonging to the Tumor Necrosis Factor Receptor (TNFR) family. Its extracellular domain contains four repeated modules of six cysteines. Its intracellular section contains a death domain of about 80 amino acids that form two α-helices. $P75^{NTR}$ can bind at low affinity to NGF, BDNF (Brain-Derived Neurotrophic Factor), Neurotrophin-3 (NTF-3), and Neurotrophin-4/5 (NTF-4/5). Higher affinity binding is achieved by association of NGFR with the TRK-A (NTRK-1), TRK-B (NTRK-2), or TRK-C (NTRK-3). TRK-A is specific for NGF, TRK-B is specific for BDNF and NT-4 or NT-5, and TRK-C is specific for NT-3. The mitogenic effect of NGF requires TRK-A, whereas the proapoptotic effect is mediated by $P75^{NTR}$ through TNFR-associated death domain (TRADD) and the NF-κB pathway.

- The oncogene *trk* {1q22–q23} is a chimeric gene formed through a somatic rearrangement involving the neighboring genes for *ntkr1* (*neurotrophic tyrosine kinase receptor type 1*) and *tpm3* (*α-tropomyosin-3*, *nonmuscle tropomyosin*) [Martin-Zanca et al. 1986]. TRK is a 140 kD transmembrane protein tyrosine kinase that is expressed only in neural tissues. In colon cancer, this chimeric gene is generated by somatic rearrangement. The change leading to the transforming capacity of TRK is a replacement of the extracellular domain with sequences coding for the 221 NH_2-terminal residues of Tropomyosin.
- $P75^{NTR}$ is consistently expressed in malignant peripheral nerve sheath tumors (MPNSTs), ganglioneuromata, Schwann cell tumors, dermatofibrosarcoma protuberans, and embryonal rhabdomyosarcomata.
- $P75^{NTR}$ may have pleiotrophic effects in cancer, leading either to cell cycle progression or to programmed cell death. In prostate carcinoma, $P75^{NTR}$ acts as a tumor suppressor. Its progressive loss is associated with the malignant progression [Krygier and Djakiew 2001].

Glial Cell Line-Derived Neurotropic Factor. GDNF {5p13.1–p12} specifically promotes the survival and differentiation of dopaminergic neurons, and it enhances the high affinity uptake of dopamine in

these cells. Neurturin (NRTN) {19p13.3} is a potent neurotrophic factor. NRTN and GDNF form a distinct TGF-β subfamily, referred to as TRNs (TGF-β-Related Neurotrophins).

The growth factors GDNF and Neurturin ligate a receptor heterodimer of the proto-oncogene product RET {10q11.2} and a glycosyl–phosphatidylinositol-anchored GDNF Receptor α {10q26} surface molecule. This leads to RET tyrosine phosphorylation. The ensuing signal transduction comprises GRB2, SHC, Phosphatidylinositol 3-Kinase. Oncogenic RET also activates RAS, RAF, MEKK1, and I-κB Kinase β. It leads to phosphorylation and degradation of I-κB and to activation of NF-κB, which may promote cell expansion through its antiapoptotic effects [Ludwig et al. 2001].

- Germline activation of the *ret* gene, attributable to specific point mutations, causes medullary thyroid carcinoma, a neoplastic transformation of the Calcitonin-secreting thyroidal C-cells.
- Mutations of *ret* may also cause multiple endocrine neoplasia type 2 (C634R in MEN2A, M918T in MEN2B).
- Constitutive kinase activity may be conferred to RET by chromosomal translocation that forms the RET–PTC fusion and occurs in 30% of papillary thyroid carcinomata [Grieco et al. 1990].

Colony-Stimulating Factors. Colony-Stimulating Factors (CSFs) are necessary for the survival and proliferation of hematopoietic progenitor cells. They are named by the cells they stimulate. CSF-1 (Colony Stimulating Factor-1, Macrophage Colony-Stimulating Factor, M-CSF) is synthesized by activated monocytes and macrophages, as well as by fibroblasts and other mesenchymal cells. Mature CSF-1 is secreted as a disulfide-bonded, heterodimeric proteoglycan with a molecular weight of 80 kD. The primary transcript {1p21–p13} encodes a precursor of 554 amino acids, which contains a transmembrane domain. The receptor for CSF-1 is a member of the PDGFR family. CSF-2 (GM-CSF, Granulocyte-M-CSF) {5q31.1} is a homodimeric glycoprotein of 45 kD. The *csf-3* (*granulocyte colony-stimulating factor*, *g-csf*) gene {7q11.2–7q12} has four introns and two distinct polypeptides are synthesized from the same gene by differential splicing of the message in the second intron. The two polypeptides differ by the presence or absence of three amino acids. G-CSF stimulates the proliferation of promyelocytes and myelocytes.

- Children with type-1 neurofibromatosis (NF1) are predisposed to juvenile myelomonocytic leukemia. GM-CSF (CSF-2) is required to drive the excessive proliferation of the affected myelomonocytic cells.
- CREB (cyclic-AMP Response Element-Binding Protein) is a transcription factor that is a downstream component of the GM-CSF signaling pathway. It is overexpressed in blast cells from patients with acute leukemias. CREB contributes to hematopoiesis, cell proliferation, and acute leukemogenesis [Shankar and Sakamoto 2004].
- G-CSF (CSF-3) is expressed in bladder carcinoma and in glioblastoma multiforme.

Interleukins. Interleukins (ILs) are a group of cytokines that are expressed predominantly by white blood cells as a means of communication. The functions of the immune system depend heavily on the effects of Interleukins. These cytokines often act as growth factors for their target cells.

– IL-1 is secreted by macrophages, monocytes, and dendritic cells. It increases the expression of adhesion molecules on endothelial cells to enable the transmigration of leukocytes.
– IL-2 contributes importantly to cellular immunity. It facilitates G_1 to S transition in T-lymphocytes by inactivating $P27^{KIP1}$.
– IL-6 may induce the differentiation of myelomonocytic leukemic cells into macrophages.
– IL-10 enhances the humoral (antibody-dependent) arm of the immune response.
– IL-13 is expressed in activated T-lymphocytes. It inhibits inflammatory cytokine production induced by lipopolysaccharide in peripheral blood monocytes.

The biological activities of Interleukins are mediated by specific membrane receptors which can be expressed on a wide range of cell types. In most cases, their expression is inducible and subject to several regulatory mechanisms. Many receptors are multi-subunit structures with ligand-binding domains and domains that function as signal transducers due to their intrinsic tyrosine kinase activity. Interleukin Receptors often share common signal-transducing receptor components in the same family, and are associated with specific signal transduction pathways in the interior of the cell. Several cytokine receptors can be converted into soluble binding proteins that regulate ligand access to the cell by specific proteolytic cleavage of receptor ectodomains.

- Polymorphisms in the *il-1* gene cluster are associated with a predisposition to gastric cancer. A transition of the normal mucosa to gastritis, which eventually leads to adenocarcinoma, is strongly associated with infection by *Helicobacter pylori*. Individuals with the *il-1β* polymorphisms-31 C/C or -511 T/T, or with the *il-1β receptor* penta-allelic 86 bp tandem repeat in intron 2 are at elevated risk. These alleles cause high expression levels of IL-1 and increase the probability of hypochlorhydria, gastric atrophy, and consecutive distal gastric adenocarcinoma.
- A polymorphic site in intron 2 of the *il-1ra* (*IL-1 receptor antagonist*) gene influences the IL-1RA plasma level. Elevated IL-1β levels are associated with homozygosity for *il-1ra* allele 2 (IL-1RN*2). Individuals heterozygous for *il-1ra* have an increased risk of cervical cancer [Sehouli et al. 2002].
- Pancreas cancer cells express receptors for IL-2 at high density.
- IL-2, IL-6, and IL-7 act as proliferative factors for malignant lymphocytes and plasma cells.
- IL-10 is a vital factor for the differentiation and survival of germinal center B-lymphocytes and is also a negative prognostic factor in non-Hodgkin lymphoma. TIMP-1 regulates the IL-10 expression levels in B-cells and mediates specific B-cell differentiation steps. TIMP-1 expression in B-cell non-Hodgkin lymphoma correlates closely and positively with IL-10 expression and with high histologic grade. Through the inhibition of apoptosis, TIMP-1 may be responsible for the negative prognosis associated with IL-10 expression in these tumors. [Guedez et al. 2001].
- IL-13 and IL-13Rα1 are frequently expressed in Reed-Sternberg cells that exist in Hodgkin lymphoma tissues. IL-13 is important for proliferation and antiapoptosis in Hodgkin lymphoma cells. STAT6 is an important mediator of IL-13 function, which is often activated in Reed-Sternberg cells [Skinnider et al. 2002]. In contrast, IL-13 expression in non-Hodgkin lymphoma is uncommon.

Transforming Growth Factor-β Family. Transforming growth factors (TGFs) are secreted polypeptides that may reversibly confer a transformed phenotype on cells. TGF-β is the prototypic member of this family of about 30 polypeptide regulatory molecules that also includes Activins (Inhibins), Bone Morphogenic Proteins, Nodal, and the Müllerian Inhibitory Substance. TGF-β exists in three forms (TGF-β_1, TGF-β_2, TGF-β_3), encoded by distinct genes, that act as modulators of tumor growth. The TGF-β protein is released as a latent complex comprising the TGF-β dimer in association with two prosegments. Plasmin activates latent TGF-β. TGF-β is also released by MMP-2 or MMP-9 from its inactive extracellular complex.

The receptors for this family of growth factors are typically composed of a pair of subunits, type I of 53 kD and type II of 70 kD, each of which has a cytoplasmic portion that functions as a serine–threonine kinase. There are seven distinct type-I receptors, each of which can associate with five distinct type-II receptors to mediate the signals activated by the TGF-β family of ligands. After binding of TGF-β, the kinase TGF-βRII phosphorylates TGF-βRI in the GS sequence, which is located upstream of the kinase domain. This activates the TGF-βRI kinase and allows autophosphorylation as well as the phosphorylation of downstream targets. Signal transduction from three of the TGF-β type-I Receptors proceeds via the receptor associated SMADs -2 and -3, consecutive oligomerization with SMAD-4, and translocation into the nucleus. Signaling from four of the TGF-β type-I Receptors proceeds through the R-SMADS SMAD-1, -5, or -8. TGF-β receptor engagement primarily inhibits epithelial cell proliferation, typically causing cell cycle arrest in G_1

– Through inhibition of *c-myc* expression. This is required for the induction of $p15^{INK4b}$ (*cdkn2B*) and $p21^{CIP1/WAF1}$ (*cdkn1A*) expression. In proliferating cells, c-MYC is tethered to the proximal promoter regions of the *cdkn2B* and *cdkn1A* genes by the zinc finger protein MIZ-1, causing inhibition of transcription. c-MYC down-regulation in response to TGF-β relieves this inhibition.
– Through complex formation of SMADs with FOXO proteins to activate the transcription of $p21^{CIP1/WAF1}$.
– Through activation of the transcription factor SP1, which plays a pivotal role in inducing the $p15^{INK4b}$ and $p21^{CIP1/WAF1}$ genes by TGF-β. In response to TGF-β, $p15^{INK4b}$ is transcribed and its accumulation on CDK4 displaces $P27^{KIP1}$, which then inhibits the Cyclin E/CDK2 complex.
– Through the down-regulation the CDK2 activator CDC25A. TGF-β increases CDK tyrosine phosphorylation by repressing the CDK-activating tyrosine phosphatase CDC25A.
– Through repression of the growth-promoting transcription factors *id-1*, *id-2*, and *id-3*. ID

proteins function as negative regulators of basic helix–loop–helix (bHLH) transcription factors, that are crucial for cell differentiation.
– Through phosphorylation of RB. Increased binding of HDAC-1 to RB-1 and to $P130^{RB2}$ forms a complex that inhibits the gene expression of *cdc25A*.
– Through activation of serine/threonine kinases. Negative control of proliferation can be exerted via serine/threonine kinase pathways.

In certain cell types, TGF-β-dependent signaling may induce apoptosis. This can be accomplished through down-regulation of BCL-X_L or through activation of Caspase-3 and Caspase-8. During development, TGF-β family signals mediate key decisions that specify stem cell maintenance and germ layer differentiation.

Thrombospondin is an extracellular matrix protein with multiple functional domains and pleiomorphic roles in tumorigenesis. At the boundary between the first and second type-1 repeats, Thrombospondin contains a sequence motif, RFK, which binds and activates TGF-β. The interaction between Thrombospondin and TGF-β inhibits tumor growth.

The Activins and Inhibins constitute dimers of the subunits α, encoded by *inhibin* α{2q33–q36}, βA, encoded by *inhibin βA* (*inhibin* β_1, *activin A*, *frp*, *edf*) {7p15–p13}, βB, encoded by *inhibin βB* (*inhibin* β_2, *activin B*) {2cen–q13}, or βC, encoded by *inhibin βC* (*activin C*) {12q13.1}. Activins are homodimers or heterodimers of related β subunits, while inhibins are heterodimers composed of one α subunit and one β subunit. They have roles in reproduction and development. Activins regulate pituitary function, hormone production in gonadal tissues, and differentiation of erythroid and neural cells. Activins interact with the receptors ACVR-I (ALK-2), ACVR-IB (ALK-4), ACVR-IC (ALK-7), and ACVR-II or ACVR-IIB.

Bone Morphogenic Proteins (BMPs, Osteogenic Proteins, Growth and Differentiation Factors, GDFs) are multifunctional growth factors Their expression is often widespread and dynamic as development proceeds. BMPs are frequently localized to areas of epithelial–mesenchymal interactions, including extraskeletal sites. They have importance in cellular functions and in the embryonic development of heart, nervous system, cartilage, and bone. The BMP family has around 30 members. BMPs are synthesized as large precursor proteins. Upon dimerization, they are proteolytically cleaved at a consensus RX_2R site to yield COOH-terminal mature dimers.

BMPs signal through serine/threonine kinase receptors, composed of type-I and type-II subunits. There are three type-I receptors, BMPR-IA (ALK-3), BMPR-IB (ALK-6), and ACVR-IA (ALK-2), and there are three type-II receptors, BMPR-II, ACVR-II, and ACVR-IIB. These receptors are expressed differentially in various tissues. In response to engagement by ligands, the BMP Receptors form a heterotetrameric complex of two type-I and two type-II chains, which initiates signaling. Downstream, SMAD-1, SMAD-5, SMAD-8, and SMAD-9 become phosphorylated. BMP functions are negatively regulated by Chordin and Noggin, which bind BMPs, sequester them in latent complexes, and block their signaling.

The morphogen Nodal {10} is a member of the TGF-β family that is expressed during gastrulation. Nodal has a left-sided expression pattern that plays important roles in left or right axis development. Moreover, Nodal signals from the epiblast pattern the visceral endoderm by activating a SMAD-2-dependent pathway required for the specification of anterior identity in overlying epiblast cells. The transcriptional corepressor DRAP1 has a very specific role in regulation of Nodal activity during embryogenesis. It interacts with and inhibits DNA binding by the winged-helix transcription factor FOXH1, a critical component of a positive feedback loop for Nodal activity.

The gene for Müllerian-Inhibiting Substance (MIS, Anti-Müllerian Hormone, AMH) {19p13.3–p13.2} has five exons and encodes a 560 amino acid polypeptide. MIS causes regression of the Müllerian duct in the testes during fetal development, thus preventing the development of a uterus and fallopian tubes. It is expressed transiently in the ovaries.

- The T29C polymorphism in the *transforming growth factor-*β_1 gene (leading to L10P) is associated with increased TGF-β_1 serum levels and a decreased risk of breast cancer [Ziv et al. 2001].
- In tumor cells, the expression levels of TGF-β Receptors may be reduced by transcriptional silencing of the receptor genes secondary to hypermethylation of CpG islands or promoter mutations. This confers resistance to the growth inhibitory effects of TGF-β.

- A polymorphic allele (*6A*) of *tgf-βrI* has a deletion of three alanines from a stretch of nine alanines. Among patients with colon cancer, there is an elevated number of *tgf-βrI(6A)* homozygotes. *tgf-βrI(6A)* acts as a tumor susceptibility allele that contributes to the development of colon cancer by way of reduced TGF-β-mediated growth inhibition [Pasche et al. 1999]. Mutations in *tgf-β receptor I* arise in chronic lymphocytic leukemia, prostate cancer, gastric cancer, metastatic breast cancer, and glioblastoma. A frameshift mutation in *tgf-β receptor 1* frequently occurs in ovarian cancer. Whereas, retinal cells bear receptors for TGF-β, retinoblastoma cells lack these receptors. In general, tumors acquire TGF-β resistance at a relatively late stage.
- Inactivating mutations of *tgf-βrII* may be present in 20–25% of colon cancers. Somatic mutations in *tgf-β receptor II* occur most frequently in the tumors of hereditary nonpolyposis colorectal cancer. A repeat stretch of adenines in the coding sequence is prone to mutations in these patients. This may result in a truncated receptor protein that is incapable of signaling.
- The EWS-FLI oncoprotein, which incorporates a partial coding sequence for the ETS family transcription factor FLI-1, represses the expression of *tgf-βrII* and may account for the decreased responsiveness of Ewing sarcoma cells to TGF-β.
- Cytoplasmic PML is an essential modulator of TGF-β signaling. The PML-RARα oncoprotein of acute promyelocytic leukemia can antagonize cytoplasmic PML function. Consistently, acute promyelocytic leukemia cells have defects in TGF-β signaling.
- The intestinal epithelium is patterned into crypts and villi, with BMP-4 expression occurring exclusively in the intravillus mesenchyme. The TGF-β family member BMP-4 is overexpressed and secreted by human cancer cells with mutant *apc* (*adenomatous polyposis coli*) gene. The oncogenic allele of *β-catenin* is absolutely required for the expression of BMP-4, whose receptor, *bmpr1A*, is mutated in a fraction of the rare inherited gastrointestinal cancer predisposition syndrome juvenile intestinal polyposis [Howe et al. 2001].
- The osteogenic BMP-6 (VGR-1) is expressed in a high proportion of prostate carcinomata, but not in benign prostate tissue. Its levels increase in higher grade tumors. The gene expression is androgen independent. BMP-6 may contribute to the formation of osteoblastic metastases.
- MIS may inhibit the growth of ovarian and endometrial cancers.

Steroid hormones. The growth of some epithelia is regulated by steroid hormones. These types of cells can give rise to tumors that also depend on steroid hormones for growth. Steroid-related neoplasms include breast cancer, prostate cancer, and liver cancer. During tumor progression, these growths may loose their hormone dependence.

The steroid hormones (Figure 3.1.3.I), with the exception of retinoic acid, are derived from cholesterol. All but vitamin D contain the same cyclopentanophenanthrene ring as cholesterol. The first reaction in converting C_{27} cholesterol to C_{18}, C_{19}, and C_{21} steroids involves the cleavage of a 6-carbon group from cholesterol. It is the principal committing, rate-limiting step in steroid biosynthesis. This cleavage reaction is catalyzed by P450 CYP11A (Desmolase, 450-Linked Side Chain Cleaving Enzyme, P450SCC), which is located in the mitochondria of steroid-producing cells. Various Hydroxylases involved in the synthesis of the individual steroid hormones also are members of the Cytochrome P450 class of enzymes. The adrenal cortex is responsible for the production of glucocorticosteroids that regulate carbohydrate metabolism, mineralocorticosteroids that regulate the levels of sodium and potassium, and androgens that are male sex steroids. The adrenal cortex is responsive to ACTH (Adrenocorticotropic Hormone), the levels of which are controlled by the hypothalamus and pituitary gland. The testes and ovaries produce various steroids, the two most abundant ones are testosterone and estradiol. These compounds are under the control of FSH (Follicle-Stimulating Hormone) and LH (Luteinizing Hormone), secreted by the pituitary and GNRH (Gonadotropin-Releasing Hormone), secreted by the hypothalamus.

Androgen. The cells of the normal prostate depend on androgens for growth and survival. In this setting, androgens protect the prostate cells by blocking apoptosis. Androgens may inhibit the Caspases -7, -8, and -9, and may attenuate BAX expression and cleavage. Testosterone is the main circulating androgen, mostly bound to Albumin or SHBG (Sex Hormone-Binding Globulin).

The Androgen Receptor is composed of an NH_2-terminal activation domain, a COOH-terminal ligand-binding domain, and a DNA-binding domain containing two zinc fingers in the midregion. Like other nuclear receptors, the Androgen

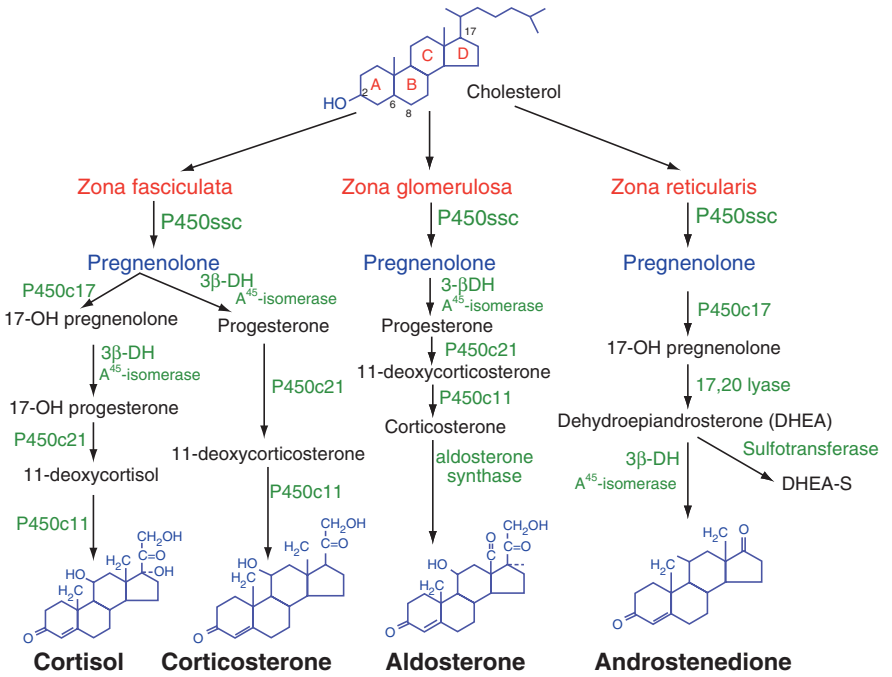


Figure 3.1.3.I. Steroid hormone biosynthesis. The scheme depicts the synthesis of the various adrenal steroid hormones from cholesterol. Only the terminal hormone structures are included. 3β-DH = 3β-Dehydrogenase, P450c11 = 11β-Hydroxylase, P450c17 = 17α-Hydroxylase, P450c21 = 21β-Hydroxylase. [Reproduced from http://isu.indstate.edu/mwking/steroid-hormones.html. With permission from Dr. Michael W. King.]

Receptor in its basal state is bound to heat shock proteins in a conformation that prevents DNA binding. When free testosterone enters prostate cells, most of it is converted to dihydrotestosterone by 5α-Reductase. The affinity to the Androgen Receptor is fivefold higher for dihydrotestosterone than for testosterone. Once the Androgen Receptor binds dihydrotestosterone, it undergoes a conformational change that leads to its dissociation from the heat shock proteins and to receptor phosphorylation. The Androgen Receptor is then translocated to the nucleus, and binds as a homodimer to androgen response elements in the promoter regions of target genes [Feldman and Feldman 2001]. The Androgen Receptor coalesces with two nuclear receptor coactivators: NCOA1 (Nuclear Receptor Coactivator 1, Steroid Receptor Coactivator 1, SRC1) and NCOA2 (TIF2, Transcriptional Intermediary Factor 2). The coactivation of the Androgen Receptor by CDC25B may induce higher expression levels of Steroid Receptor target genes and, in conjunction with the activation of CDKs by CDC25B, may enhance cell cycle progression. Elevated expression of these coactivators increases Androgen Receptor transactivation at physiologic concentrations of androgen (Figure 3.1.3.J).

Most prostate carcinomata are androgen dependent. Their development depends on the Androgen Receptor and its high affinity binding to dihydrotestosterone. Androgen-independent prostate cancer may develop in various ways.

- Hypersensitization to very low levels of androgen can be acquired as a consequence of gene amplification, resulting in elevated numbers of Androgen Receptors on the cell surface. Increased stability and enhanced nuclear localization of the Androgen Receptors can also cause a sensitization. High activity of 5α-Reductase may increase the local concentration of dihydrotestosterone, generate the phenotype of hypersensitization, and thus facilitate tumor progression.
- Consecutive to missense mutations, the Androgen Receptor may loose its ligand specificity and promiscuously respond to a range of steroid hormones and pseudoandrogens. Receptors with the T877A mutation are stimulated by the antagonist flutamide. The double mutant T877A, L701H,

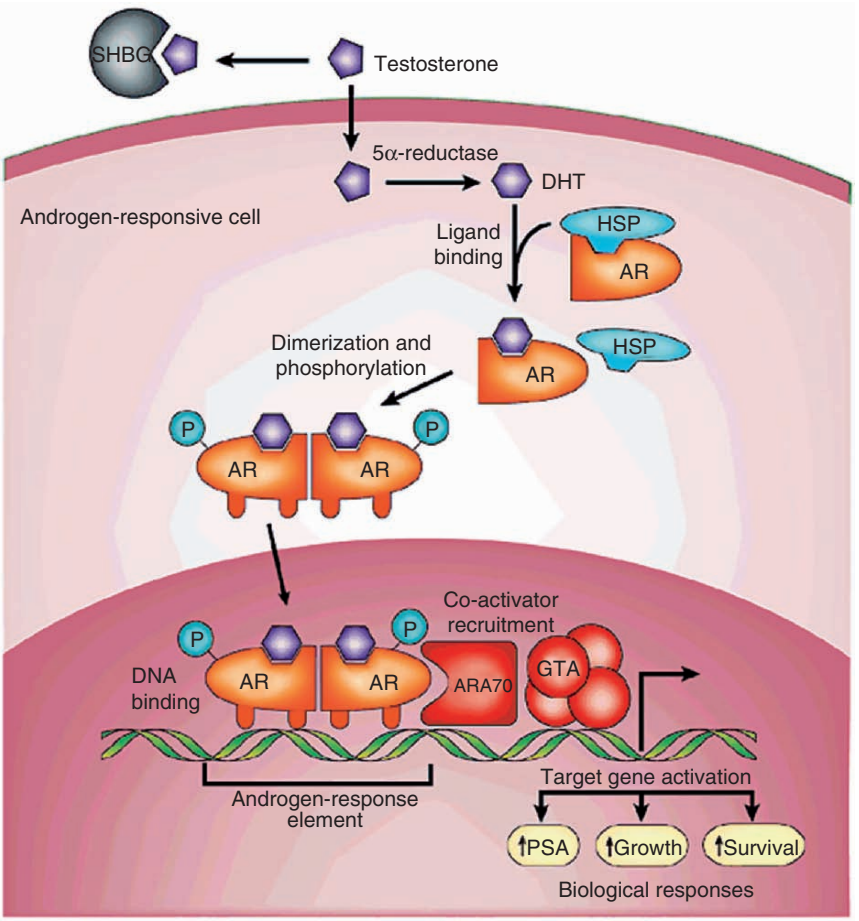


Figure 3.1.3.J. Androgen Receptor signaling. Testosterone circulates in the blood bound to Albumin and Sex Hormone-Binding Globulin (SHBG), and it exchanges with free testosterone. Free testosterone enters prostate cells and is converted to dihydrotestosterone (DHT) by the enzyme 5-Reductase. Binding of dihydrotestosterone to the Androgen Receptor (AR) induces its dissociation from Heat Shock Proteins (HSPs) and Receptor phosphorylation. The AR dimerizes and can bind to androgen response elements in the promoter regions of specific target genes. Coactivators, such as ARA70, and corepressors also bind the AR complex, facilitating or preventing its interaction with the general transcription apparatus (GTA). The activation (or repression) of target genes leads to biological responses including growth, survival, and the production of Prostate-Specific Antigen (PSA). [Reproduced from Feldman and Feldman 2001. With permission from Macmillan.]

called AR^{CCR} (cortisol and cortisone responsive) has increased affinity for glucocorticosteroids. The H874Y mutation influences the binding of coactivator proteins by affecting the conformation of helix 12.

- Androgen Receptor pathways that are activated by ligand-independent mechanisms can support the dysregulated growth of prostate cells. Mutations of signal transduction molecules associated with the Androgen Receptor may underlie this phenomenon. The receptor tyrosine kinase ERBB2 (HER-2/NEU) is consistently overexpressed in androgen-independent prostate cancer cells. ERBB2 can activate Androgen Receptor-dependent genes in the absence of Androgen Receptor ligands, but not in the absence of the Androgen Receptor. This is mediated by MAP Kinase (ERK), which phosphorylates the Androgen Receptor. Alternatively, phosphatase and tensin homolog (PTEN) is frequently inactivated in metastatic prostate cancers. PTEN normally inhibits Protein Kinase B. In its absence, Protein Kinase B can phosphorylate the Androgen Receptor on serines 213 and 791 and activate it.
- The detoxifying enzyme Glutathione S-Transferase π1 is expressed in normal prostatic epithelium,

where it catalyzes the intracellular elimination of electrophilic compounds. It acts as a negative regulator of steroid hormone-associated pathways. The gene for *glutathione S-transferase π1* is often silenced by promoter methylation in steroid hormone-related tumors, including prostate cancer [Lee et al. 1994].

- Overactivation of alternative survival pathways can relieve prostate cells of their dependence on androgen to protect them from apoptosis. BCL-2 is not normally expressed in the secretory epithelial cells of the prostate. Aberrant expression of this gene product in prostate cancer may enhance cell survival.
- LATS1 and LATS2 are tumor suppressors. LATS2 (KPM) is an Androgen Receptor interacting protein. The interaction surface of LATS2 maps to the central region of the protein. On the Androgen Receptor, the ligand-binding domain mediates this interaction. LATS2 functions as a negative modulator of the Androgen Receptor by inhibiting androgen-regulated gene expression. LATS2 expression is lower in prostate tumors than in normal prostate [Powzaniuk et al. 2004].
- Androgen independence of prostate cancer may be the result of an expansion of subpopulations of androgen-independent cells. The rates of proliferation and death of the putative epithelial stem cells among the basal cells of the prostate are independent of androgen and may account for this phenomenon. [Feldman and Feldman 2001].

Estrogen. Estradiol is synthesized primarily by the ovaries, under the regulation by the pituitary Gonadotrophins, FSH (Follicle Stimulating Hormone), and LH (Luteinizing Hormone). Epithelial cells are the main site of estradiol action in the breast.

The biological effects of estrogen are manifested only in cells expressing a specific receptor. The Estrogen Receptors are ligand-dependent transcription factors. There are two structurally related Estrogen Receptors: Estrogen Receptor α (ERα) {6q25.1} and Estrogen Receptor β (ERβ) {14q}. Receptors similar to Estrogen Receptors are Estrogen Receptor-Related α (ERRα) {11q12} and Estrogen Receptor Related β (ERRβ) {14q24.3}. Both , ERα and ERβ, have a ligand- and a DNA-binding domains. The ERβ [Kuiper et al. 1996; Mosselman et al. 1996] is highly homologous to ERα in its DNA- and ligand-binding domains. There are several splice variants of ERβ, some of which may act as dominant negative effectors of ERα. In the normal not lactating breast, about 15–25% of epithelial cells express the ERα. In the absence of ligand, ERα is sequestered in target cell nuclei within a large heat shock protein complex. Upon estrogen binding, the receptor undergoes a conformational change that enables the displacement of the heat shock proteins and facilitates the interaction of a receptor dimer with cognate DNA sequences (Figure 3.1.3.K).

Normal mammary epithelial cells express Estrogen Receptors, but do not proliferate. Therefore, the Estrogen Receptor is not a sufficient link to molecules that induce cell cycle progression. ERα contains two distinct transactivation domains, AF-1 within the NH_2-terminus and AF-2 within the ligand-binding domain. The Estrogen Receptor binds as a dimer through the action of two zinc fingers to small palindromic DNA motifs, known as estrogen response elements (EREs) in gene promoters. Estrogen-induced biologic effects are determined by coregulators (coactivators or corepressors). Transactivation is accomplished in synergy with coactivators that facilitate Histone acetylation, chromatin remodeling, and activation of RNA synthesis.

– PCAF (P300/CBP-Associated Factor, P300/ CREB-Binding Protein-Associated Factor) is a Histone Acetyl Transferase that interacts with the Estrogen Receptor. Three nuclear receptor coactivators of the P160 family, NCOA1 (SRC-1, Steroid Receptor Coactivator-1), NCOA2 (TIF-2, Glucose Receptor Interacting Protein-1, GRIP1), and NCOA3 (AIB1, Activated in Breast Cancer-1, TRAM1), associated with CBP to facilitate Histone acetylation. These proteins use LXXLL motifs to interact with the AF-2 domain of ERα. Among their primary functions is the recruitment of other transcriptional coactivators and Histone Acetyl Transferases. The transcriptional steroid coactivator NCOA3 [Guan et al. 1996] enhances the estrogen-dependent induction of *cyclin* D_1 expression by enhancing the interaction between the Estrogen Receptor and the *cyclin* D_1 promoter [Planas-Silva et al. 2001]. Transcriptional agonism of Estrogen Receptors may be supported by the AF-2-interacting coactivator NCOA1 [Onate et al. 1995], which is present at a higher level in uterine cells than in mammary gland cells. NCOA2 [Voegel et al. 1996] interacts with the AF-2 domain.
– The SWI/SNF complex, active in ATP-dependent chromatin remodeling, promotes transcriptional activation by the Estrogen Receptor. The

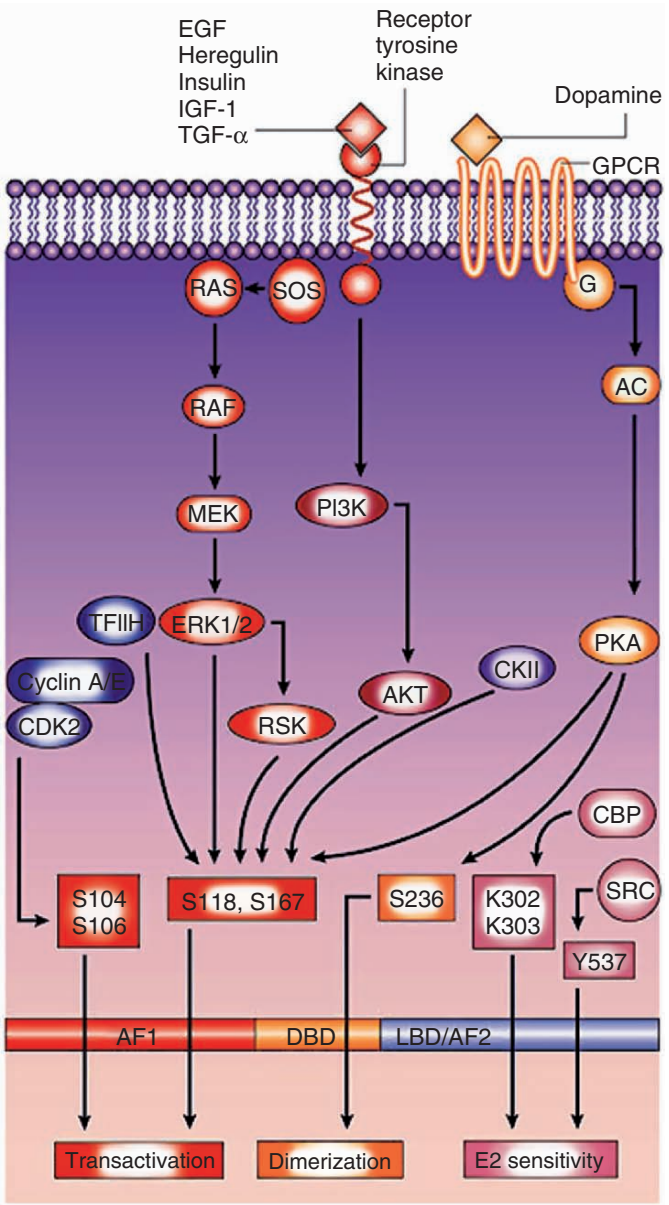


Figure 3.1.3.K. Estrogen Receptor signaling. As well as being regulated by ligand binding, Estrogen Receptor (ER) activity is modulated by posttranslational modifications. Activation of the ER may be caused by the growth factors EGF (Epidermal Growth Factor), Heregulin, Insulin, IGF-1 (Insulin-Like Growth Factor-1), and TGF-α (Transforming Growth Factor α), as well as dopamine, cyclic AMP, or phorbol esters. ER activation by these signal transduction pathways is mediated, in part, by direct phosphorylation, which can stimulate the transcriptional potential of ER, often in a ligand-independent manner. Growth factor receptor tyrosine kinases (RTKs) can lead to ER phosphorylation through two principal pathways, the RAS→RAF→ERK cascade and the PI-3K cascade. In the former, the adaptor proteins GRB2 and SOS activate RAS, which, in turn, activates a serine kinase cascade through RAF, MEK, and the Extracellular Signal-Regulated Kinases ERK-1 and ERK-2. ERKs can phosphorylate ER directly and can activate the kinase RSK, which phosphorylates ER. In the latter pathway, growth factor receptors activate PI-3K, which catalyzes the production of a lipid messenger that activates the serine kinase PKB. The ligands of G-Protein-Coupled Receptors that activate adenylyl cyclase (AC) can lead to an ER phosphorylation event, which causes its dimerization through Protein Kinase A (PKA). Other kinases that can phosphorylate ER include the Cyclin E/CDK2 complex, the general transcription factor TFIIH, and Casein Kinase II (CKII). E2 sensitivity is regulated by acetylation of the lysines 302 and 303 through CBP (CREB-Binding Protein). DBD = DNA-binding domain, GPCR = G-Protein-Coupled Receptor, LBD = ligand-binding domain. [Reproduced from Ali and Coombes 2002. With permission from Macmillan.]

NH_2-terminal regions of SNF2α and SNF2β specifically interact with the AF-2 region of the Estrogen Receptor. These interactions are increased by estrogen.

– TRAP220 (Thyroid Hormone Receptor-Associated Protein, DRIP205) is a component of a large complex. The TRAP/DRIP/SMCC complex is associated with RNA Polymerase II. It interacts with ERα through a LXXLL motif in TRAP220. The RNA coactivator SRA (Steroid Receptor RNA Activator) and the RNA Helicases P68 and P72 interact with and regulate ERα AF-1 function.

ERα is subject to negative regulation, in part, by ERβ. When both receptors are expressed, ERβ functions as an efficient dominant inhibitor of ERα. Several unliganded nuclear receptors, such as the Thyroid Hormone Receptor, repress gene expression through the interaction with the nuclear receptor corepressors, NCR1 or NCOR2, and recruitment of HDACs [Ali and Coombes 2002].

Prolonged exposure to estrogens is associated with an increased breast cancer risk. This includes early menarche, late menopause, and late first full-term pregnancy. Environmental estrogens, including dietary and contraceptive sources, may also contribute to breast cancer risk. Over time, beast cancers may become estrogen independent through several mechanisms.

- Some Estrogen Receptor mutations result in increased sensitivity to the ligand. Increased sensitivity to coactivator recruitment may also play a role. Missense mutations that substitute tyrosine 537 in the ligand-binding domain for asparagine (Y537D) occur in metastatic breast cancer [Zhang et al. 1997]. The lysines 302 and 303 are principal substrates for acetylation by CBP. The missense mutation L303R occurs frequently in premalignant lesions of the breast.
- Aromatase (ARO, Estrogen Synthetase, Cytochrome P450 family 19 subfamily A Polypeptide 1, CYP19A1) synthesizes estrogens from adrenal steroids. Its tissue-specific expression is determined by the alternative use of selective promoters. Breast tumors produce high levels of the enzyme. Constitutional genetic variation at the *cyp19A1* locus is associated with the risk of developing breast cancer. A silent polymorphism (G→A at V80) represents a high-risk allele [Siegelmann-Danieli and Buetow 1999]. A polymorphism in exon 10, reflected in a C→T substitution, is associated with increased RNA levels and occurs more frequently in breast cancer patients than in the healthy population [Kristensen et al. 2000].
- Posttranslational modifications can result in ligand-independent activation of the Estrogen Receptor. These modifications may be triggered by the oncogenic activation of growth factor signaling pathways. PKB phosphorylates the Estrogen Receptor on serine 167. PKB is often elevated due to gene amplification in breast cancer. Furthermore, the negative regulator of PKB, PTEN, is frequently inactivated. Protein Kinase A (PKA) activity stimulates ligand-independent Estrogen Receptor activity and the levels of cyclic AMP-Binding Proteins are often elevated in breast tumors.
- Increased expression of the coactivator proteins that mediate Estrogen Receptor activity leads to estrogen independence. NCOA3 (AIB1) may be overexpressed due to amplification in breast cancer [Planas-Silva et al. 2001]. CBP is mutated in several epithelial cancers, including those of the breast. These mutations cause a loss of function.
- *cyclin* D_1 is overexpressed in about 50% of all breast cancers. *Cyclin* D_1 can interact directly with the Estrogen Receptor and enhance its transcriptional activity [Zwijsen et al. 1997]. In association with CDK4 and CDK6, Cyclin D_1 is required for the recruitment of NCOA1 and PCAF to the Estrogen Receptor [Ali and Coombes 2002].

It is still unclear what role ERβ plays in breast cancer. Loss of ERβ expression is associated with tumor progression in prostate cancer.

Progesterone. Progesterone is a C_{21} steroid hormone involved in the menstrual cycle, establishment and maintenance of pregnancy, and embryogenesis. It is produced in the adrenal glands, the gonads, the brain, and the placenta. Progesterone belongs to a class of hormones called progestagens, and is the only naturally occurring human progestagen. Progesterone is the precursor of aldosterone and, after conversion to 17-hydroxyprogesterone, of cortisol. Further conversion to androstenedione gives rise to testosterone, estrone, and estradiol.

Two structurally and functionally distinct Progesterone Receptors are Progesterone Receptor A (PRA) and Progesterone Receptor B (PRB). PRA inhibits PRB function. The *pgr* gene {11q22} uses separate promoters and translational start sites to produce these two forms, which are identical except for an additional 165 amino acids present only in the NH_2-terminus of PRB. Although PRA and PRB share several structural domains, they are distinct transcription factors that mediate their own response genes and physiologic effects with little overlap. After binding to a member of the progestin class of hormones, the intracellular Progesterone Receptor activates the MAP Kinase signaling cascade. A proline-rich sequence in the Progesterone Receptor binds to the SH3 domain of $P60^{c\text{-}SRC}$, leading to $P60^{c\text{-}SRC}$-dependent activation of MAP Kinase and $P90^{RSK}$, which phosphorylates and inactivates MYT-1. The inhibition of MYT-1 action favors the formation of the activated cell cycle complex Cyclin B/CDC2. A membrane Progestin Receptor is a G-Protein-coupled seven transmembrane spanning molecule. When ligated, this receptor blocks the activity of Adenylyl Cyclase, leading to a decrease in intracellular cAMP. The resulting reduced activity of PKA relieves the inhibition of

CDC25C and indirectly promotes the activation of MAP Kinase signaling. CDC25C then dephosphorylates and activates the Cyclin B/CDC2 complex [Zhu et al. 2003a,b].

- Excessive estrogen stimulation, unopposed by progesterone, strongly predisposes to endometrial cancer. The antiproliferative effect of progesterone requires the Progesterone Receptor. A promoter region polymorphism, +331G–A, creates a unique transcription start site, which increases transcription and favors the expression of PRB. The +331G–A polymorphism is associated with an increased risk of endometrial cancer [De Vivo et al. 2002].
- The nuclear receptor for progesterone is widely expressed in uterine cancer.
- Progestins induce the proliferation of breast cancer cells and are implicated in the development of breast cancer. However, in response to some progestins, cell differentiation and apoptosis may predominate over proliferation.

3.1.4 Proto-oncogenic transcription factors

Growth factor signaling typically leads to the induction of transcription factors that induce the expression of cell cycle-promoting genes. Transcription factors are modular proteins composed of DNA-binding domains, which interact with cognate DNA sequences, and activation domains, which interact with other proteins to stimulate transcription. The binding of a transcription factor to its cognate DNA sequence enables the RNA Polymerase to locate the proper initiation site. The DNA-binding domains contain specific motifs, typically characterized by consensus amino acid sequences, that define the families of homeodomain proteins, zinc finger transcription factors, winged-helix proteins, HLH proteins, leucine zippers, and nuclear receptors. The activation domains induce transcription in conjunction with the DNA-binding domains and exhibit considerable structural diversity (Figure 3.1.4.A). Acidic activation domains exist as unstructured, random-coil regions until they interact with a coactivator protein. This interaction induces the activation domain to fold into an amphipathic α-helix that contacts a complementary surface of the coactivator protein. In contrast to the relatively short, random-coil acidic activation domains, some activation domains are larger and more structured. The ligand-binding domains of some nuclear receptors function as activation domains when they engage their specific ligand. This induces a large conformational change that allows the ligand-binding domain with bound hormone to interact with other proteins. Promoters and enhancers are always on the same strand of DNA as the gene they regulate. Consequently, promoters are referred to as *cis*-acting elements. Transcription factors are sometimes called *trans*-acting factors because they may be encoded by a gene on a DNA molecule other than that containing the gene being regulated.

Some transcription factors, including C_4 zinc finger proteins, basic zipper proteins, HLH proteins, and nuclear receptors, bind to DNA as dimeric units. In some cases, each monomer has a DNA-binding domain with equivalent sequence specificity. In these proteins, heterodimer formation allows the activation domains associated with each monomer to be brought together in a single transcription factor. However, if the monomers have different DNA-binding specificity, the formation of heterodimers increases the range of DNA sequences that a family of factors can bind to. This combinatorial complexity expands both the number of DNA sites, from which these factors can activate transcription, and the ways, in which they can be regulated.

There are three basic routes of activation for proto-oncogenic transcription factors. Some reside in the cytoplasm and shuttle into the nucleus upon binding to a ligand that diffuses into the cells. This is characteristic of nuclear receptors. Others are resident nuclear proteins that are activated by serine kinase cascades. This includes JUN [Vogt et al. 1987], which is activated by phosphorylation on serines 63 and 73. Some transcription factors form latent cytoplasmic complexes that are disrupted in response to certain signaling pathways and cause the transcription factors to translocate into the nucleus. STATs are in this group.

In cancer, aberration in the synthesis or activity of specific transcription factors may suffice to mediate uncontrolled growth.

Homeodomain Proteins. Homeodomain transcription factors contain in their DNA-binding region a conserved structural sequence motif of about 60 amino acids, known as the homeodomain. It is encoded by 180 base pairs, referred to as the homeobox. They target homeotic genes, which specify the differentiation and location of developing

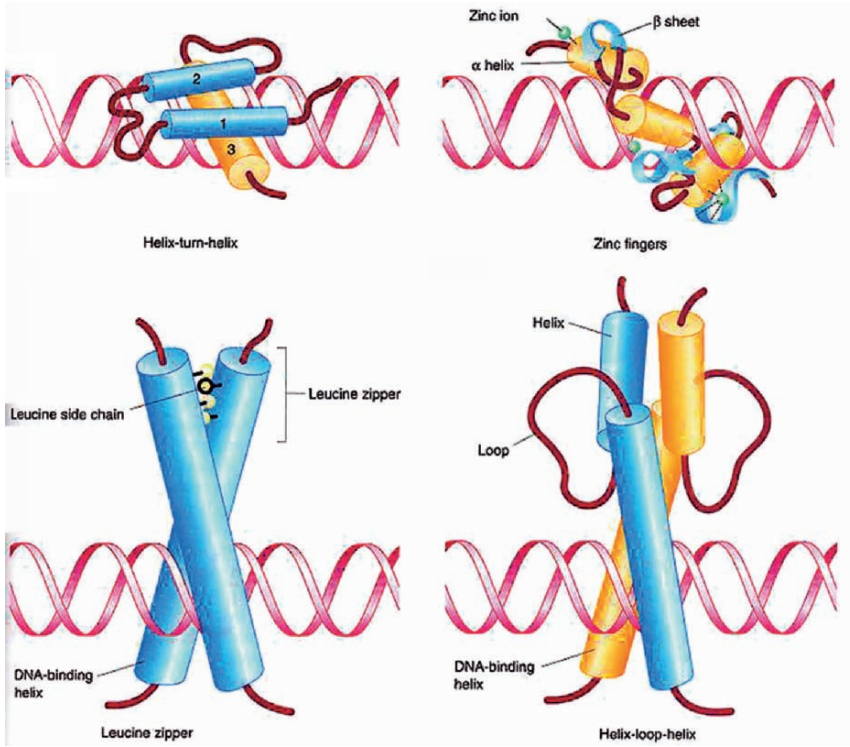


Figure 3.1.4.A. Basic structures of transcription factors. The three-dimensional structures of gene regulatory proteins usually possess axes of symmetry, often accomplished through dimer formation. They contain specific motifs for interacting with DNA, including the helix–turn–helix motif, the zinc finger motif, the leucine zipper motif, and the helix–loop–helix motif. [Reproduced from McKee and McKee 2003. With permission.]

structures in the organism. Mutations in these genes can result in the transformation of one body part into another during development (Figure 3.1.4.B).

Homeobox transcription factors are altered in various malignancies.

- Frequent elevation of *hoxA9* gene expression occurs in acute myeloid leukemia (AML).
- The Histone Methyl Transferase MLL is an upstream regulator of *hox* gene expression through direct promoter binding and Histone modification. Reciprocal rearrangements of the *mll* (*hrx*, *all-1*) gene are most common in infant ALL and secondary AML.
- NUP98–HOX fusion proteins are generated in the chromosome translocation t(7;11)(p15;p15), which is associated primarily with AML (M2 and M4). The chimeric transcription factor acts as an oncogene [Borrow et al. 1996; Nakamura et al. 1996]. The NUP98–HOX fusions consist of the NH_2-terminus of NUP98, containing a region of multiple phenylalanine–glycine repeats (FG repeats) that may act as a transcriptional coactivator through binding to P300/CBP, and the COOH-terminus of HOX, containing the homeodomain.

Zinc Finger Proteins. A number of proteins have regions that fold around a central Zn^{2+} ion, producing a compact domain from a relatively short length of the polypeptide chain, termed a zinc finger. The C_2H_2 zinc finger is one of the most common DNA-binding motifs in transcription factors. Each C_2H_2 finger has the consensus sequence $(Y,F)XCX_{2-4}CX_3(Y,F)X_5LX_2HX_{3-4}H$. The binding of the Zn^{2+} ion by the two cysteine and two histidine residues folds the relatively short polypeptide sequence into a compact domain, which can insert its α-helix into the major groove of DNA. C_2H_2 zinc finger proteins generally contain three or more repeating finger units and bind as monomers. Another type of zinc finger structure, designated the C_4 zinc finger, is a part of more than 100 transcription factors, including the Steroid Hormone Receptor superfamily. The DNA-binding domain of these proteins has the

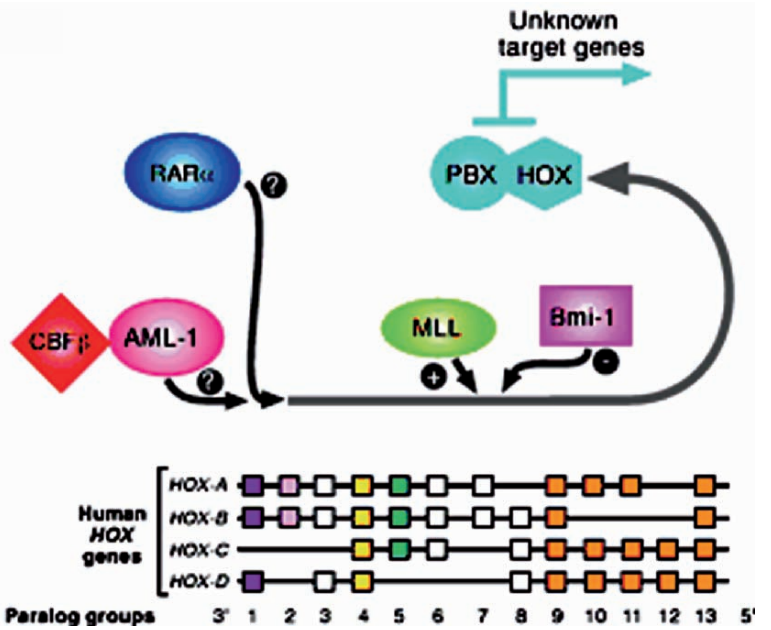


Figure 3.1.4.B. Homeobox genes. Conserved pathways of homeotic gene action in leukemogenesis. The hox genes dictate the body plan during embryogenesis. They are designated hox-A through hox-D and are arranged on four separate chromosomes (four rows of colored squares). Genes within the hox clusters show striking structural and functional conservation, as indicated by the color coding. Most of the hox genes have regulatory roles in normal hematopoiesis. In the model, seven translocation-associated proteins are predicted to regulate hox gene expression. The chimeric oncoproteins involved in leukemogenesis are postulated to act by disrupting the activity of their normal counterparts in hox gene regulation. [Reproduced from Look 1997. With permission.]

consensus sequence $CX_2CX_{13}CX_2CX_{14–15}CX_5CX_9CX_2C$. The four critical cysteines in this region bind two Zn^{2+} ions. C_4 zinc finger proteins generally contain two finger units and bind to DNA as homodimers or heterodimers. Homodimers of C_4 DNA-binding domains have twofold rotational symmetry and therefore bind to consensus DNA sequences that are inverted repeats. Heterodimeric nuclear receptors do not exhibit rotational symmetry. The zinc finger motif is frequent in DNA-binding domains, but it may also occur in proteins that do not bind to DNA.

- The RB-interacting zinc finger protein RIZ1 (PR-Domain-Containing Protein 2, PRDM2) {1p36} is a tumor suppressor and a member of the Histone/Protein Methyl Transferase superfamily. *riz1* inactivation is commonly associated with cancer, specifically with colon cancer and melanoma. It occurs through DNA hypermethylation, frameshift mutations, chromosomal deletion, or missense mutations.
- Krüppel-Like Factor 6 (KLF6) {10p15} is a ubiquitous zinc finger tumor suppressor. Up-regulated expression of *klf6* reduces cell proliferation and increases the levels of $P21^{WAF1}$. It is inactivated by loss or mutation in most sporadic colorectal cancers and most colorectal cancers occurring on the basis of inflammatory bowel disease. Chromosome 10p is deleted in 50–60% of prostate cancers, and loss of heterozygosity of *klf6*, accompanied by mutations in the remaining allele, occur.
- The oncogene *znf217* (*zinc finger protein 217*) encodes alternatively spliced Krüppel-like transcription factors of 1,062 and 1,108 amino acids. Each contains a DNA-binding domain of multiple C_2H_2 zinc fingers, and a proline-rich transcription activation domain. ZNF217 immortalizes mammary epithelial cells. This is associated with initial telomere erosion, followed by an increase in Telomerase activity and telomere length stabilization [Nonet et al. 2001].
- The chimeric *aml 1-evi 1* gene is generated by the t(3;21)(q26;q22) translocation. In AML 1-EVI 1, an NH_2-terminal fragment of AML 1, including a RUNT homology domain, is fused to the entire EVI 1 zinc finger protein. AML 1-EVI 1 plays a pivotal role in progression of hematopoietic stem cell malignancies, such as chronic myelocytic leukemia and myelodysplastic syndrome.

Winged-Helix (Forkhead) Proteins. The forkead box family of genes consists of at least 43 members. Winged-helix proteins generally bind to DNA as monomers. The DNA-binding domains in Histone H5 and several transcription factors that function during early development have the winged-helix motif (forkhead motif). The FOXO subgroup of forkhead transcription factors (Figure 3.1.4.C), comprising FOXO1A (FKHR), FOXO3A

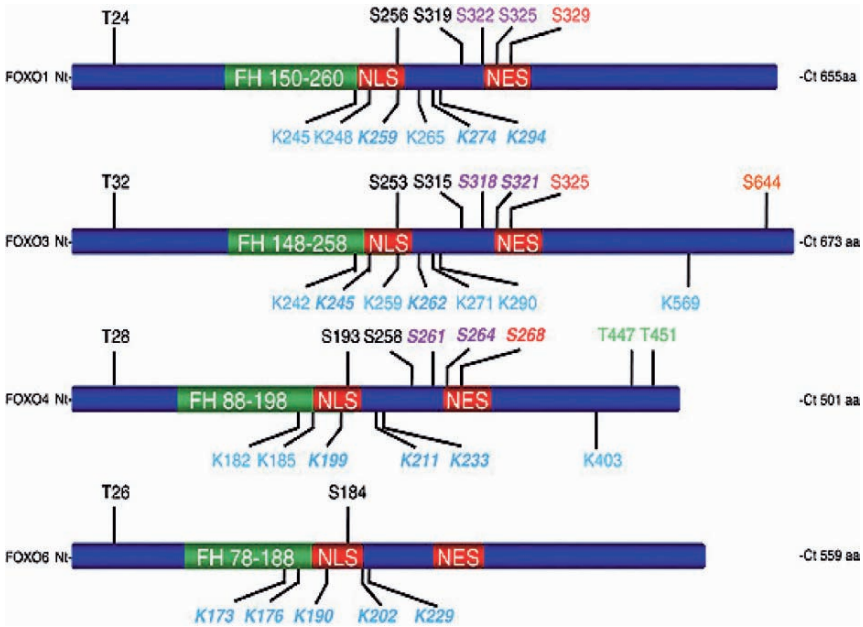


Figure 3.1.4.C. Structure of FOXO transcription factors. FOXO transcription factors are regulated by phosphorylation and acetylation in response to Insulin, growth factors, and stress stimuli. FOXO posttranslational modifications alter the subcellular localization of these transcription factors and affect FOXO degradation, DNA-binding ability, transcriptional activity, or protein–protein interactions. Sites that are conserved in FOXO members but that have not yet been confirmed to be modified in a particular family member are italicized. PKB sites are shown in black, SGK (Serum and Glucocorticoid Inducible Kinase) are black, sites for IKKβ (I-κB Kinase β) are orange, sites for JNK (JUN N-Terminal Kinase) are green, DYRK (Dual Specificity Tyrosine Phosphorylation-Regulated Kinase) sites are red, sites for CK1 (Casein Kinase 1) phosphorylation are purple, and acetylation sites are blue. FH = forkhead domain, NLS = nuclear localization signal, NES = nuclear export sequence. [Reproduced from Greer and Brunet 2005. With permission from Macmillan.]

(FKHRL1), and FOXO4 (AFX), mediates cellular responses that include glucose metabolism, stress responses, cell cycle regulation, and apoptosis. The FOXO factors all function as transcriptional activators and bind as monomers to the consensus DNA sequence TTGTTTAC. FOXO activity is regulated by various growth pathways:

– At rest, FOXO transcription factors are acetylated. Their deacetylation promotes cell cycle arrest and quiescence.
– Activation of the small GTPase RAS regulates FOXO activity through a mechanism that involves the RAL GTPase. Upon mitogenic signaling, RAS associates with and activates several GEFs for RAL (RAL-GEFs). In response to physiologic stimuli, this causes FOXO activation. However, the activation of RAL by oncogenic RAS mediates FOXO4 phosphorylation on threonine 447 and threonine 451, which are located within the COOH-terminal transactivation domain, and suppresses its transcriptional activity.
– FOXO transcription factors are targets of Phosphatidylinositol 3-Kinase→PKB signaling. PKB directly phosphorylates FOXO members on threonine 32, serine 253, and serines 315, leading to their nuclear export and inhibition of FOXO-dependent transcription. This is due to the generation of consensus binding sites for 14-3-3 proteins (RSXpSXP) on FOXO after pho phorylation by PKB. 14-3-3 binding causes the displacement of FOXO from the DNA. FOXO export then proceeds in a manner that requires both 14-3-3 binding and intact FOXO nuclear export sequences. Conversely, the activation of FOXO antagonizes the positive effects of Phosphatidylinositol 3-Kinase on cellular proliferation.
– FOXO family members are substrates for Caseine Kinase-1.
– FOXO3A is a direct target of IKK (I-κB Kinase). Phosphorylation by IKK causes the cellular relocalization of FOXO3A to the cytoplasm, followed by ubiquitination and degradation in the proteasome pathway.

Forkhead transcription factors may play roles in cancer, with FOXO family members acting as tumor suppressors.

- In glioblastoma, deregulation of the Phosphatidylinositol 3-Kinase signaling pathway is common. Activation of PKB may be due to the the loss of *pten*. PKB activity, in turn, is correlated with the phosphorylation of the Forkhead transcription factors FOXO1, FOXO3A, and FOXO4.
- *trail* is a transcriptional target of FOXO3A (FKHRL1), and *cd95l* is a transcriptional target for FOXO1A (FKHR). The decreased activity of FOXO3A and FOXO1A in prostate cancers, resulting from the loss of PTEN, leads to a decrease in TRAIL expression. This may contribute to increased survival of the tumor cells [Modur et al. 2002].
- In chronic myeloid leukemia, BCR-ABL mediates the inhibition of TRAIL and BIM. This occurs through activation of Phosphatidylinositol 3-Kinase and the downstream phosphorylation and inactivation of FOXO3A. FOXO3A activates *bim* transcription through a FOXO binding site (FHRE) located within the promoter. FOXO3A and BCL-6 jointly repress *cyclin* D_2 transcription through a STAT5/BCL6 site located within the *cyclin* D_2 promoter. BCR-ABL signaling abolishes this effect Fernandez de Mattos et al. 2004; Essafi et al. 2005].
- During myoblast differentiation, Phosphatidylinositol 3-Kinase signaling leads to myoblast fusion and activation of the terminal differentiation program. This requires the transient exclusion of FOXO from the nucleus. Unchecked FOXO activity, caused by chromosomal translocations, results in alveolar rhabdomyosarcomata (ARMS). Most of these tumors are caused by the presence of transforming chimeric oncogene products, PAX3-FOXO1 in t(2;13)(q35;q14) or PAX7-FOXO1 in t(1;13)(p36;q14). These chimeric transcription factors activate PAX responsive genes with 10- to 100-fold higher potency than the wild-type PAX proteins.
- In myeloid leukemia, *foxo3* and *foxo4* can participate in chromosomal translocations with the Trithorax-related transcription factor *mll*.
- The Forkhead Box m1b (FOXm1b) transcription factor is essential for the development of hepatocellular carcinoma. In the absence of FOXm1b, resistance to hepatocellular carcinoma development is associated with nuclear accumulation of the cell cycle inhibitor $P27^{KIP1}$ and reduced expression of the CDK1 activator CDC25B [Kalinichenko et al. 2004].

Helix–Loop–Helix Proteins. The helix-loop-helix proteins contain a NH_2-terminal α-helix with basic residues that interact with DNA, a middle loop region, and a COOH-terminal region with hydrophobic amino acids spaced at intervals characteristic of an amphipathic α-helix. Because of the basic amino acids characteristic of this motif, transcription factors containing it are referred to as basic helix-loop-helix (bHLH) proteins. Various helix-loop-helix proteins can form heterodimers, thus extending the range of target sequences. The DNA-binding domain of dimeric helix-loop-helix transcription factors contains a structural motif, in which a nonhelical loop of the polypeptide chain separates two α-helical regions in each monomer.

Notch proteins form transmembrane receptors. After binding to their ligands, two proteolytic cleavages within Notch release the NICD. This fragment translocates to the nucleus where it can interact with inhibitory helix-loop-helix proteins that are bound to DNA. The NICD has a transcriptional activation domain and activates specific genes depending on the cofactor it associates with.

- The *tal1* (*scl*, *tcl5*) gene encodes a basic helix-loop-helix transcription factor required for hematopoiesis and vasculogenesis. Aberrant transcriptional activation of *tal1* is a frequent event in T-cell acute lymphoblastic leukemia (T-ALL). TAL1 can bind the E-boxes in the *p16* and the *pre-tcrα* promoters, and functionally suppress the activity of each promoter. This may account for the influence by TAL1 on T-lymphocyte proliferation and differentiation. The overexpression of *tal1* in hematopoietic progenitor cells promotes cell cycle division [Hansson et al. 2003].
- Constitutive expression of the proto-oncogene *c-myc* results in transformation and contributes to the progression of a wide range of tumors. MYC executes its activities mostly through transcriptional repression of cell cycle inhibitors, including *gas1*, *p15*, *p21*, *p27*, and *gadd-34*, *gadd-45*, and *gadd-153*. This repression occurs through at least two distinct mechanisms.
  - MYC–MAX heterodimers bind to the Inr element in their cognate promoters and inhibit MIZ-1 or other transcriptional activators via the COOH-terminal domain of c-MYC.
  - c-MYC binds to the SP1 transcription factor via the c-MYC central region and inhibits SP1 transcriptional activity.

  The ability of c-MYC to repress the transcription of growth arrest genes may contribute to its potential

to promote proliferation and oncogenesis [Gartel and Schors 2003].

- TFE3 {Xp11.22} binds to the μ-E3 motif of the *immunoglobulin heavy chain* enhancer. It is expressed in many cell types. TFE3 is involved in oncogenic translocations in childhood renal cancers. Common fusion partners include *aspscr1*, *prcc*, *sfpq*, and *cltc*.

Leucine Zipper Proteins. The leucine zipper transcription factors contain the hydrophobic amino acid leucine at every seventh position in the COOH-terminal portion of their DNA-binding domains. These proteins bind to DNA as dimers. The name leucine zipper denotes the existence of two extended α-helices that grip the DNA molecule at two adjacent major grooves, separated by about half a turn of the double helix. The portions of the α-helices contacting the DNA include basic residues that interact with phosphates in the DNA backbone and additional residues that interact with specific bases in the major groove. In other DNA-binding proteins, the leucines are replaced by different hydrophobic amino acids in the critical positions. Like the leucine zipper proteins, they form dimers containing a COOH-terminal coiled-coil dimerization region and NH_2-terminal DNA-binding domain. The term basic zipper (bZip) refers to all proteins with these common structural features. Many basic zipper transcription factors are heterodimers of distinct polypeptide chains, each containing a basic zipper domain. A large number of bZip transcription factors are resident in the nucleus. They include c-JUN, JUN-B, JUN-D, c-FOS, and FRA.

- The translocation t(12;16)(q13;p11) in malignant myxoid liposarcoma causes the fusion of the CHOP-dominant negative transcription factor gene with the nuclear RNA-binding protein TLS (FUS). In TLS-CHOP (Translocation Liposarcoma- CCAAT/Enhancer Binding Protein Homologous Protein), the RNA-binding domain of TLS is replaced by the DNA binding and leucine zipper dimerization domain of CHOP. In myeloid leukemia with the t(16;21)(p11;q22) translocation, ERG is fused with TLS. The NH_2-terminal domain of TLS binds to RNA Polymerase II and this binding is retained by the TLS–ERG fusion protein.
- C/EBPs (CCAAT/EBP) are a family of leucine zipper transcription factors. They regulate cellular proliferation and apoptosis in the mammary gland. Multiple forms of C/EBPβ are generated by variation in translation via proteolytic cleavage. Alterations in the ratio of the C/EBPβ-LIP (Liver-Enriched Inhibitory Protein) form to the C/EBPβ-LAP (Liver-Enriched Activating Protein) form play a role in the development of breast cancer [Zahnow 2002].
- Malignant melanoma of soft parts (MMSP, soft tissue clear cell sarcoma) is a rare and aggressive tumor that mainly develops in tendons and aponeuroses of patients between 15 and 35 years of age, and that may also be derived from neuroectoderm. In malignant melanoma of soft parts, the translocation t(12;22)(q13;q12) fuses the NH_2-terminal domain of EWS {22q12} to the bZIP domain of ATF1 (Activating Transcription Factor 1) {12q13}, a transcription factor that is normally regulated by cAMP [Zucman et al. 1993].

Nuclear receptors. The nuclear receptor superfamily comprises some 50 members, including the Glucocorticosteroid Receptor, Estrogen Receptor, Progesterone Receptor, Testosterone Receptor, Retinoic Acid Receptors, Retinoid Receptors, and PPARs. Nuclear receptors reside in the cytoplasm. Once ligated by steroid hormones that diffuse into the cells, they shuttle to the nucleus and execute their functions as activators or repressors of target genes. These receptors contain domains for ligand binding, for DNA binding, and for transcriptional activation. They can exist as homodimers or heterodimers, with each partner binding to specific response element sequences that exist as half-sites and are separated by variable length nucleotide spacers between direct or inverted half-site repeats.

Enhancers. Enhancers generally range in length from about 50–200 base pairs and include binding sites for multiple transcription factors. The transcription factors that bind to a single enhancer may bind cooperatively, producing a multiprotein complex (enhancosome) on the enhancer DNA. Architectural proteins bind to the minor groove of the DNA, regardless of the sequence and, as a result, bend the DNA molecule sharply. This bending of the enhancer DNA permits the transcription factors to interact properly. The relatively weak interactions among the bound proteins are strengthened by binding of the transcription factors to neighboring sites, which keeps the proteins at very high relative concentration.

Repressors. Transcription is negatively regulated by repressor proteins. There are inhibitory basic zipper and helix-loop-helix proteins that block DNA binding when they dimerize with a partner polypeptide normally capable of binding DNA. When these inhibitory factors are expressed, they repress transcriptional activation by the factors with which they interact. Like activators, many repressors have two functional domains: a DNA-binding domain and a repression domain. A variety of amino acid sequences can function as repression domains. Many of these are relatively short (around 20 amino acids) and contain high proportions of hydrophobic residues. Other repression domains contain a high proportion of basic residues. In some cases, repression domains are larger, well-structured protein domains. The diverse structures of repression domains are probably a reflection of distinct molecular mechanisms for regulating transcription [Lodish et al. 1999].

BTB domains (POZ domains) are protein–protein interaction domains that can form homodimeric or heterodimeric complexes. ZNF145 (PLZF, Promyelocytic Leukemia Zinc Finger Protein) and BCL-6 (ZNF51, LAZ3) are BTB domain containing zinc finger proteins implicated in oncogenesis, as well as in myelopoiesis and lymphopoiesis. ZNF145 may control cell cycle progression by preventing the expression of cell cycle promoters such as *cyclin A*. ZNF145 interacts with CUL-3, a component of Ubiquitin Ligases. This interaction regulates transcription by controlling the stability of ZNF145 [Furukawa et al. 2003]. The transcriptional repressor ZBTB7 (LRF, Pokemon, FBI-1) {19p13.3} binds to BCL-6, but not to ZNF145. This interaction occurs in the nucleus and requires both the BTB and zinc finger domains of the two proteins. ZBTB7 can specifically repress the transcription of the tumor suppressor gene *arf* through direct binding [Maeda et al. 2005]. ZBTB7 is overexpressed in a large number of cancers.

- Retinoids exert their biological functions through the nuclear receptors RAR and RXR. In the absence of ligand, the RXRα ligand-binding site functions as a repression domain. When the same region binds its cognate ligand, 9-cis-retinoic acid, it is converted into an activation domain. In acute promyelocytic leukemia, a chromosomal translocation produces a chimeric protein between RARα and PML. PML–RARα acts as a dominant negative receptor in the leukemic cells, which results in the arrest of cell maturation at the stage of promyelocytes.
- The protein encoded by the *Wilms tumor* (*wt1*) gene is a repressor that is expressed preferentially in the developing kidney. Inheritance of mutations in both the maternal and paternal *wt1* alleles prevents the synthesis of functional WT1 protein and invariably leads to the development of kidney tumors early in life. The WT1 protein, which has a C_2H_2 zinc finger DNA-binding domain, represses the transcription of *egr-1* without inhibiting binding of the two activators that normally stimulate the expression of this gene.
- Loss of imprinting (LOI) is the most common molecular abnormality in Wilms tumor. Loss of imprinting of *igf-2* in Wilms tumor commonly involves altered methylation in the differentially methylated region upstream of the maternal *h19* gene, but not mutations of CTCF or its binding site [Cui et al. 2001].
- Loss of imprinting in cancer involves the loss of the normal silencing of a specific parental allele, and can cause the activation of growth-promoting imprinted genes. Loss of imprinting of *igf-2* occurs concomitantly with microsatellite instability in both, tumor and normal tissue of patients with colorectal cancers. It is linked to increased methylation, specifically at a CpG island that represents a differentially methylated region upstream of the maternal *h19* gene, which regulates the silencing of the *igf-2* gene. The methylated nucleotides include the recognition site for the chromatin insulator CTCF. When it is unmethylated, CTCF binds specifically to is region, separating *igf-2* from its enhancer. In the absence of CTCF binding, the normally silenced allele of *igf-2* can be expressed, initiating a growth-promoting signal [Nakagawa et al. 2001].
- *ctcf* [Lobanenkov et al. 1990] is a single copy gene on chromosome 16q22. The transcription factor CTCF (NeP1) is composed of 11 zinc fingers, ten belonging to the C_2H_2 class and one belonging to the C_2HC class. CTCF binds to a number of important regulatory regions within the 5′ noncoding sequence of *myc* and regulates *myc* expression. CTCF harbors several autonomous repression domains, including a zinc finger cluster, which silences transcription through binding directly to the corepressor SIN3A and recruiting Histone Deacetylases. Insulator elements, which act as a barrier to prevent neighboring cis-acting elements from regulating a distal gene, mediate

their function by CTCF. *ctcf* is located in a small region of overlap for common chromosomal deletions in sporadic breast and prostate tumors, suggesting that CTCF acts as a tumor suppressor [Filippova et al. 1998].

3.2 CONTROL OF CELL DEATH

Endogenous antitumor control mechanisms are physiologically dominant over tumor-promoting functions. As detailed in the preceding section, control of cell proliferation may be accomplished by cell cycle arrest. In addition, programmed cell death [Kerr et al. 1972] can be invoked to prevent uncontrolled growth. In this context

– Death is likely to constitute a default pathway, which is frequently engaged if survival genes are not activated.
– Alternatively, the engagement of death receptors is sufficient for the initiation of apoptosis.

During apoptosis, the cleavage of "death substrates" by proteases of the Caspase family leads to characteristic biochemical and morphological changes

– Cleavage of nuclear Lamins is involved in chromatin condensation, nuclear shrinkage, and breakdown (karyorrhexis).
– Cleavage of ICAD, the inhibitor of the DNAse CAD, causes the release of the endonuclease, which then travels to the nucleus to fragment DNA.
– Cleavage of cytoskeletal proteins such as Actin, Plectin (PCN, PLTN), ROCK1 (RHO Kinase, P160), and Gelsolin leads to cell fragmentation, blebbing, and the formation of apoptotic bodies.
– Phosphatidylserines in the cell membrane invert and facilitate the clearance by phagocytes.

The loss of control over programmed cell death may contribute to tumor growth.

3.2.1 Final common pathways

Mitochondria. Mitochondria constitute general integrators of various proapoptotic signals. They respond to apoptosis inducing events with the release of Cytochrome *c*, AIF, or SMAC (Second Mitochondria-Derived Activator of Caspases, DIABLO, Direct IAP-Binding Protein with Low pI), and converge on common apoptotic pathways consisting of effector molecules (P53, Caspases, Nucleases). These pathways are controlled by regulatory molecules (BCL family members, Inhibitors of Apoptosis, FLIP, FLICE Inhibitory Protein, MORF).

Mitochondria may procure cell death through

– The alteration of cellular reduction–oxidation potential (redox potential), the disruption of electron transport and ATP generation.
– The recruitment of proapoptotic cellular proteins to the mitochondria.
– The release into the cytoplasm of proteins that trigger the activation of Caspases.

In cells that are induced to undergo apoptosis, irrespective of the triggering stimulus, the mitochondrial inner transmembrane potential is disrupted. Common apoptotic features like DNA fragmentation, morphological changes of the nuclei, production of reactive oxygen species, and inversion of phosphatidylserine on the cell surface are invariably preceded by the fall or total disruption of the mitochondrial transmembrane potential, which results from the permeability transition, a sudden increase in permeability of the inner mitochondrial membrane to solutes of less than 15 kD. Inhibitors of the permeability transition suppress the preapoptotic disruption of the transmembrane potential and subsequent apoptosis. Therefore, the preapoptotic collapse of the transmembrane potential, mediated by the permeability transition, constitutes an early and irreversible feature of apoptosis.

During programmed cell death, various cellular proteins translocate to the mitochondria. P53 may mediate its apoptosis-inducing function not only through transcriptional regulation, but also through translocation into the mitochondria. The transcription factor TR3 (NUR77, NGFI-B) of the Steroid/Thyroid Hormone Receptor superfamily can move to the mitochondria, where it triggers membrane permeabilization and Cytochrome *c* release. PKCδ is a proapoptotic kinase that moves to the mitochondria.

In response to apoptotic stimuli, the mitochondria release agents into the cytoplasm that promote the process of programmed cell death.

– The secretion of proapoptotic substances is facilitated by opening of the permeability transition pore, which is normally blocked by BCL-2. Mutant BCL-2, which cannot insert into the mitochondrial membrane, is a less potent inhibitor of apoptosis. The 10 kD protein Cytochrome *c* is one of the released proapoptotic mitochondrial compounds. It is nitrosylated on its heme iron during apoptosis and then rapidly excreted into the

cytoplasm [Schonhoff et al. 2003]. There it acts as a Caspase-3 activator by inducing a cascade that involves the adaptor molecule APAF-1 (Apoptosis Activating Factor-1). APAF-1 induces the dimerization of the Caspase-9 proenzymes. Active Caspase-9 subsequently activates Caspase-3, which cleaves several cellular targets. APAF-1 contains a nucleotide-binding site with ATPase activity. In the absence of ATP, truncated APAF-1 cannot process pro-Caspase-3. The existence of an energy-dependent (ATP hydrolyzing) step may prevent the inadvertent activation of pro-Caspases. The NH_2-terminal 85 amino acids of the 130 kD APAF-1 protein are homologous to the CARD (Caspase Recruitment Domain) segment in the prodomain of several Caspases. CARDs mediate the recruitment of Caspases. The CARD of APAF-1 binds to Caspase-9 in the presence of dATP/ATP and Cytochrome *c*. Furthermore, APAF-1 associates with other Caspases, which contain CARDs within their long prodomain. The COOH-terminal segment of APAF-1 contains 12 WD-40 repeats. WD-40 repeats are minimally conserved sequences of approximately 40 amino acids that typically end in tryptophan-aspartate (WD) and mediate protein–protein interactions.

– In response to stresses, PARP-1 activity in the cell increases substantially. Massive PARP-1 activation can deplete the cell of NAD^+ and ATP. The accumulation of poly(ADP ribose) and the depletion of NAD^+ and ATP induce mitochondrial depolarization and the release of AIF, ultimately leading to energy failure and cell death. AIF is a 57 kD flavoprotein bearing both mitochondrial and nuclear signal sequences. The primary transcript of AIF codes for a 67 kD propeptide, which contains a mitochondrial localization sequence (MLS) within its NH_2-terminal 120 amino acids. This propeptide is processed during import into the mitochondria, resulting in the mature 57 kD AIF. Normally confined to the intermembrane space of the mitochondria, AIF translocates to the nucleus upon apoptotic stimulation and induces large-scale chromatin fragmentation. It may play a critical role in the form of programmed cell death that shapes the early embryo. In contrast to Cytochrome *c*, AIF does not appear to require the presence of further cytosolic factors to induce apoptotic features in nuclei, although it does not possess intrinsic DNAse activity. The AIF pathway is independent of Caspases, and is mediated by an as yet unknown nuclease.
– Mitochondria may release SMAC (DIABLO), a homodimer of two elongated three-helix bundles, which neutralizes the set of Inhibitors of Apoptosis (IAPs) that act as antagonists of Caspases -3, -7, and -9. This causes the deinhibition of these Caspases. The mature, proteolytically cleaved form of SMAC (but not the pro-form) binds to several IAPs and removes their ability to block Caspase-induced apoptosis.

In various cancers, mitochondrial mechanisms of apoptosis induction are impaired.

- BCR-ABL expression in leukemic cells exerts a potent effect against apoptosis by preventing the cytosolic accumulation of Cytochrome *c* and other preapoptotic mitochondrial perturbations, thereby inhibiting the activation of Caspase-3 and the execution of apoptosis [Amarante-Mendes et al. 1998].
- Metastatic melanomata escape mitochondria-dependent apoptosis by failing to express APAF-1, which forms an integral part of the apoptosome [Soengas et al. 2001], and the *apaf-1* locus shows a high rate of allelic loss. The remaining allele is transcriptionally inactivated by gene methylation.

Reactive oxygen species. Various intermediates of the oxidative metabolism may exert distinct effects on cell physiology. The production of hydroxyl radical ($^{\bullet}OH$) typically leads to the induction of programmed cell death. The cellular levels of superoxides and lipid peroxides are increased during apoptosis, induced by a large number of stimuli.

The proapoptotic effects of TNF-α and hypoxia are exerted through increased ceramide formation [Schutze et al. 1992; Obeid et al. 1993]. Sphingolipid ceramide (*N*-acetyl sphingosine) in the cell membrane, once released, acts as a second messenger that disrupts mitochondrial electron transport, activates reactive oxygen species, and leads to apoptosis. The intracellular localization of β-Catenin can be influenced by sphingolipids. Sphingosine reduces cytosolic and nuclear β-Catenin, inhibits growth, and induces cell death.

- The extracellular generation of superoxide anions (O_2^-) is often associated with the transformed state. Nitric oxide (NO) exerts pleiotropic effects on programmed cell death. It requires the

interaction with extracellular superoxide anions and subsequent peroxynitrite formation for selective apoptosis induction in transformed fibroblasts. This process can be inhibited by hydrogen peroxide (H_2O_2) [Haberstroh et al. 2002].

P53. The promoter specificity of the transcription factor P53 for either proapoptotic genes or cell cycle arrest genes is mediated, in part, by coactivators. ASPP and JMY, as well as the P53 family members P63 and P73, function in this role to support P53-induced apoptosis. ASPP proteins act as transcriptional coactivators of P53 by specifically stimulating its apoptotic function, but not its cell cycle-arresting function. Two homologs, ASPP1 and ASPP2, exist. P53BP2 is a COOH-terminal fragment of ASPP2 and may act as a dominant negative mutant of ASPP2 [Samuels-Lev et al. 2001]. In order to function efficiently as an activator of gene expression, P53 forms complexes with other transcriptional regulators, such as P300/CBP. This interaction allows the acetylation of Histones that surround P53 binding sites and open up the chromatin. JMY cooperates with P300/CBP to enhance the ability of P53 to transactivate *bax*, without significantly influencing the induction of *cdkn1A*. Therefore, JMY may be necessary for the induction of apoptosis, but not cell cycle arrest [Shikama et al. 1999]. The induction of cell death by P53 requires the presence of at least one of the other P53 family members, P63 or P73, which facilitate the binding of P53 to promoters of proapoptotic targets.

– In contrast to P53-induced growth arrest, which is frequently accomplished through activation of $p21^{CIP1/WAF1}$ transcription, P53-dependent apoptosis may be mediated by transcription of *bax* and down-regulation of *bcl-2* expression. In P53 mutants, the ability to transactivate these genes is differentially affected [Aurelio et al. 2000].
– P53 induces the expression of *pidd* (*P53-induced protein with a death domain*) {11p15.5} [Lin et al. 2000b], which interacts with RAIDD and participates in Caspase-2-mediated apoptosis [Tinel and Tschopp 2004].
– Transactivation of *pig-3* (*P53 inducible gene-3*) {2p} by P53, through a microsatellite in its promoter region, contributes to apoptosis. This depends on the proline-rich region (amino acids 62 through 91) of P53 and on acetylation of the residues 320 and 373 of P53.
– Transactivation of *igf-bp3* (*insulin like growth factor binding protein-3*) by P53 may contribute to apoptosis [Buckbinder et al. 1995]. IGF-BP3 inhibits IGF-1 from inducing a survival signal. IGF-BP3 may also act in an IGF-independent manner by initiating intracellular signaling from a cell surface receptor, or by direct nuclear action.
– Transcription of *gadd45* (*growth arrest and DNA damage inducible gene 45 kD*, *DNA damage inducible transcript 1*, *ddit1*) may play a role in P53-induced apoptosis [Kastan et al. 1992]. GADD45 is a 165 amino acid nuclear protein that may mediate genotoxic stress or BRCA1-induced apoptosis via activation of JNK or $P38^{MAPK}$.
– During oxidative stress or starvation, P53 activates the transcription of *pac1* {2q11} from a noncanonical, palindromic binding site in the promoter. PAC1 (Phosphatase of Activated Cells 1, Dual Specific Phosphatase 2, DUSP2) is a 32 kD threonine/tyrosine phosphatase that specifically dephosphorylates and inactivates MAPKs. PAC1 is necessary for P53-induced apoptosis [Yin et al. 2003].

P53 may facilitate programmed cell death in transcription-independent modes. P53 induces trafficking of CD95 from the Golgi complex to the cell surface, which may contribute to P53-dependent apoptosis.

P53 effects on the mitochondria contribute to programmed cell death. Upon activation, P53 translocates to the mitochondria, where it interacts with Heat Shock Protein 70. Furthermore, P53 expression mediates the expression of the mitochondrial proapoptotic proteins NOXA (PMAIP-1, APR), P53AIP1 (P53-Regulated Apoptosis-Inducing Protein-1), and PUMA (P53-Upregulated Modulator of Apoptosis). The BH3-only proteins PUMA and NOXA are critical mediators of P53-dependent apoptosis in response to various modes of activation [Villunger et al. 2003]. PUMA binds BCL-2 and BCL-X_L through a BH3 domain (LRRMADDLN) and induces programmed cell death [Yu et al. 2001]. Cytosolic P53 can activate apoptosis independently of transcription. It directly activates BAX to permeabilize the mitochondria. It also releases proapoptotic multidomain proteins and BH3-only proteins that at rest are sequestered by BCL-X_L. In this regard, P53 can function analogously to BID [Chipuk et al. 2004].

RB can induce apoptosis through a pathway that involves P53. E2F released following RB phosphorylation transactivates $p14^{ARF}$. The accumulation of $P14^{ARF}$ blocks MDM2 and hence allows P53 accumulation, leading eventually to programmed cell death.

- About half of all human tumors have mutations in *p53*. Tumorigenesis is favored when mutated P53 does not mediate apoptosis in predisposed cells. Tumor-derived P53 mutants often suffer a selective loss of proapoptotic functions, without impairment in their ability to induce G_1 cell cycle arrest. Alteration of the *aspp2* locus on chromosome 1q42 and down-regulated expression of ASPP frequently occur in breast carcinomata that express wild-type P53 [Samuels-Lev et al. 2001].
- In untransformed cells, proto-oncogene products, such as c-MYC, can induce apoptosis through $P14^{ARF}$ and P53. This depends on the downstream activation of Caspase-9 and APAF-1. Inactivation of this pathway frequently occurs in cancer [Soengas et al. 1999].

Caspases. A variety of death signals to cells proceed in a cascade that converges on the activation of initiator Caspases, which in turn activate effector Caspases as a final common pathway. Caspases (Cysteine-Containing Aspartate-Specific Proteases) are a family of intracellular Cysteine Proteases that play a central role in the initiation and execution of programmed cell death. Caspases share similarity in amino acid sequence, structure, and substrate specificity. Two functional groups of Caspases comprise initiator Caspases (Caspases -2, -8, -9, and -12) and effector Caspases (Caspases -3, -6, and -7). They may respond to the ligation of death receptors ("extrinsic pathway", Caspase-8), cytotoxicity ("intrinsic pathway", Caspases -2 and -9), or endoplasmic reticulum stress ("endoplasmic pathway", Caspase-12). There are at least 14 Caspases, which belong to three different phylogenetic subfamilies (Table 3.2.1.A):

– The ICE (IL-1β Converting Enzyme) subfamily contains Caspases -1, -4, and -5
– The CED-3/CPP32 subfamily of apoptotic executioners comprises Caspases -3, -6, -7, -8, -9, and -10
– In the ICH-1/NEDD2 subfamily is Caspase-2.

Due to the irreversibility of proteolysis, most proteases, including Caspases, are synthesized as catalytically inactive proenzymes (zymogens) and have to be activated by cleavage at internal conserved asparagine residues. Their proenzymes contain three domains, an NH_2-terminal prodomain of variable size is attached to a large

Table 3.2.1.A. Groups of Caspases. Caspases are placed into three groups according to their substrate specificity (recognition motif). Several Caspases have multiple names, which are indicated in parentheses. The Caspase proenzymes are activated through proteolytic cleavage

Name	Molecular mass	Recognition motif	Substrates
Group 1			
Caspase-1 (ICE)	Proenzyme: 45 kD active enzyme: P20 and P10	WEHD YV(A,H)D	Pro-IL-1β, pro-Caspases 1, 3, 4
Caspase-4 (ICErel-II, TX, ICH-2)	Proenzyme: 43 kD	(W,L)EHD	Pro-IL-1β, pro-Caspase-1
Caspase-5 (ICErel-III, TY)	Proenzyme: 48 kD, active enzyme: P20 and P10	(W,L)EHD	
Group 2			
Caspase-3 (CPP32, Yama, Apopain)	Proenzyme: 32 kD, active enzyme: P17 and P12	DEVD DGPD DMQD DEPD	PARP, DNA-PK, SREBP, RHO-GDI, Huntingtin, PKCδ, pro-Caspases 6, 9
Caspase-2 (ICH-1, NEDD2)	Proenzyme: 49 kD, active enzyme: P12/14 and P19	DEVD	PARP
Caspase-7 (MCH3, ICE-LAP3, CMH-1)	Proenzyme: 34 kD, active enzyme: P20 and P11	DEVD	PARP, pro-Caspase-6
Group 3			
Caspase-6 (MCH2)	Proenzyme: 33 kD, active enzyme: P18 and P11	VEID	Lamins A, B1/B2, C, PARP
Caspase-8 (FLICE, MACH, MCH5)	Proenzyme: 55 kD, active enzyme: P18 and P10	LETD	PARP, pro-Caspases
Caspase-9 (ICE-LAP6, MCH6)	Proenzyme: 46 kD, active enzyme: P35 and P10	LEHD	PARP
Caspase-10 (MCH4)	Proenzyme: 59 kD, active enzyme: P23/17 and P12		Pro-Caspases 3, 7

subunit, which contains the active site cysteine, and a small subunit (Figure 3.2.1.A). Activation involves the proteolytic processing of the domains, followed by association between the large and small subunits in a heterodimer. This NH_2-terminal prodomain is separated from the central large Caspase subunit (about 17 or 20 kD, called P17 or P20 subunit) by one or two asparagine cleavage sites. The large Caspase subunit itself is separated from the COOH-terminal small subunit (about 10 kD, called P10 subunit) by one asparagine cleavage site or a linker peptide (two asparagine cleavage sites). Like other proteases, their activity is also tightly controlled by protease inhibitors.

Caspases can activate themselves by autoproteolysis, or can be processed by other active Caspases. The proteolytic cleavage leads to the formation of the active Caspases, which consist of the P10 and P20 subunits. The active proteins are tetramers of 2 P20 subunits surrounding two adjacent P10 subunits. Both, the P20 and P10 subunits are essential for catalytic activity.

The catalytic activity of Caspases depends on a critical cysteine residue within a highly conserved active site pentapeptide QAC(R,G,Q)G. This motif is located in the large subunit (P20 or P17) and forms the primary recognition pocket for the asparagine residue; however, several residues in the P10 and P20 (P17) subunits contribute to the specific binding of the substrate and form secondary recognition pockets. The central cysteine residue in the active site pentapeptide and a histidine residue form a catalytic diad. They directly contribute to the formation of the tetrahedral intermediate state that is formed during the hydrolytic cleavage of substrate peptides by Caspases. While the active site pentapeptide is common to all Caspases, they differ in their recognition sites and their substrate specificity.

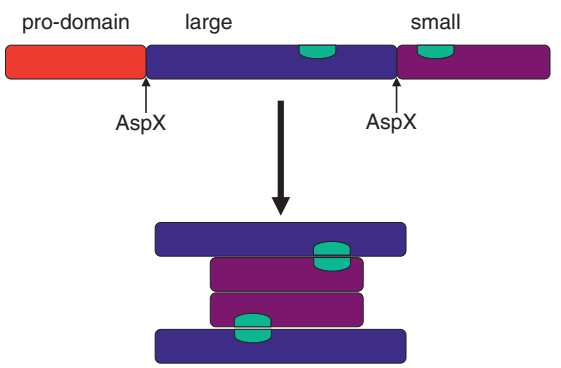


Figure 3.2.1.A. Basic Caspase structure. The scheme depicts the activation of pro-Caspase. The proenzyme is cleaved at two Caspase cleavage sequences (AspX, Asp = asparagine). Two large subunits and two small subunits combine to form the active tetrameric enzyme. The green structures indicate the catalytic domains.

The Caspases specifically cleave their substrates after asparagine residues. The Caspases -3, -7, and -9 recognize the tetrapeptide sequences DEXD, which is present in Poly(ADP-ribose)Polymerase. The motif WEHD is cleaved by Caspases -1, -4, and -5. Caspase-6 is the only Caspase known to cleave Lamin A with the recognition sequence VEID. This cleavage contributes to chromatin condensation and nuclear shrinkage. Caspase-1 [Yuan et al. 1993] cleaves the cytokine pro-IL-1β. In general, the most significant differences in Caspase specificities are found in substrate position P4. In contrast, P3 specificities are similar among Caspases, and in P2 a wide range of amino acids is tolerated. The P4 preferences can be categorized as hydrophobic (Caspases -1, -4, and -6) or aspartate (Caspases -2, -3, and -7).

Several *caspase* genes are expressed as multiple forms by alternative splicing of the primary transcript, including *caspase-1*, *-2*, *-3*, *-6*, *-7* and *-8*. Among those forms are enzymatically inactive variants, which are expressed as modified mRNAs or truncated proteins that may play a crucial role in the negative or positive regulation of Caspase activity. Caspase-8 is expressed in at least seven variants that differ by deletions or sequence variations in the NH_2-terminal prodomain, containing the death effector domains (DEDs), or by loss of the COOH-terminal part that normally encodes the P10 and P20 Caspase subunits. The variant MACH α-3 has a dominant negative effect on the activity of the processed Caspase-8 enzyme and provides effective protection against CD95-mediated apoptosis [Boldin et al. 1996].

The inducer Caspase-8 is associated with apoptosis triggered through death receptors (extrinsic pathway). Upon their ligation, Caspase-8 oligomerization drives its own activation through autocatalytic cleavage. Association with the adaptor protein FADD (FAS-Associated Via Death Domain, MORT-1) through the DED is also required. The Caspase-8/FADD complex is referred to as DISC (death inducing signaling complex). Caspase-8 then activates downstream effector Caspases. It also cleaves and activates BID, the truncated form of

which (tBID) triggers the mitochondrial activation of Caspase-9 by inducing the homooligomerization and allosteric activation of BAK or BAX. Furthermore, Caspase-8 cleaves Poly-(ADP ribose) Polymerase (PARP) [Hopkins-Donaldson et al. 2000]. CD95 mediates the extrinsic, Caspase-8-dependent pathway to apoptosis. FLIP (FADD-Like ICE Inhibitory Protein) is a competitive inhibitor of Caspase-8 that may block death receptor-induced apoptosis by being incorporated into the DISC, but lacking proteolytic enzymatic activity.

The inducer Caspase-9 (APAF-3) is involved in death brought about by cytotoxic agents (intrinsic pathway). Many apoptotic stimuli induce the release of the Caspase activator Cytochrome *c* and the Caspase coactivator SMAC (DIABLO) from mitochondria into the cytosol, where it binds to APAF-1 and induces the interaction with pro-Caspase-9. The Caspase-9/APAF-1 complex is referred to as apoptosome. Binding of pro-Caspase-9 to APAF-1 through the CARD leads to its proteolytic activation by cleavage of the residues 316 through 330 from the Caspase-9 small subunit. The resulting Caspase-9 can no longer be inhibited by XIAP. The activated Caspase-9 cleaves and activates Caspase-3. ARC (Apoptosis Repressor with CARD) is a competitive inhibitor of Caspase-9. Survivin selectively inhibits the intrinsic, Caspase-9-dependent apoptotic pathway.

The inducer Caspase-2 (NEDD-2, ICH-1) of the intrinsic pathway is ubiquitously expressed. It is required for apoptosis in response to genotoxic stress. It induces the cleavage of BID, the translocation of BAX to the mitochondria, and the release of Cytochrome *c* from the mitochondria. Upon activation, Caspase-2 is recruited into a large protein complex, which includes the death domain-containing protein PIDD and the adaptor protein RAIDD [Tinel and Tschopp 2004]. Caspase-2 is required for apoptosis by some cancer cells [Lassus et al. 2002].

The accumulation of unfolded or malfolded proteins causes endoplasmic stress, a process that can lead to apoptosis independently of mitochondria or APAF-1. The relevant inducer Caspase located in the endoplasmic reticulum is Caspase-12. There, it mediates apoptotic responses to stress that affects the endoplasmic reticulum (endoplasmic pathway, unfolded protein response) [Nakagawa et al. 2000]. TRAF2 interacts with pro-Caspase-12 and promotes its clustering with ensuing activation by cleavage. BAX and BAK can also localize to the endoplasmic reticulum. In stress situations, they undergo conformational changes and oligomerization, which leads to Caspase-12 cleavage. Downstream, Caspase-7 is activated and the transcription factor eIF2α (eukaryotic Translation Initiation Factor 2α) is dephosphorylated and inactivated.

Caspase-3 is a prominent effector Caspase that mediates DNA fragmentation, cell rounding, and the formation of apoptotic bodies.

– Caspase-3 cuts the chaperone ICAD (Inhibitor of the Caspase-Activated Deoxyribonuclease). This releases the ICAD partner CAD (Caspase-Activated Deoxyribonuclease), which translocates to the nucleus and degrades DNA. This may account for the DNA laddering in apoptosis.
– Caspase-3 cuts Gelsolin, a protein that normally binds to the Actin filaments that help give a cell its shape. Cells with degraded Gelsolin round up.
– Caspase-3 cuts and activates PAK2 (P21-Activated Kinase-2) that regulates the cytoskeleton and may contribute to the formation of apoptotic bodies.
– Apoptosis is regulated, in part, by phosphorylation of serine 14 in the tail of Histone H2B. This event may trigger the chromatin condensation that is followed by DNA fragmentation. The active kinase in this process is MST1, which is induced by Caspase-3.

RGD containing peptides may enter cells and directly induce autoprocessing and enzymatic activity of pro-Caspase-3 through an interaction with its RGD-binding motif DMM. This mechanism may be activated after the cleavage and internalization of extracellular matrix proteins [Buckley et al. 1999].

Upon induction of apoptosis, pro-Caspase-6 is processed at aspartate residues to yield a large (18 kD) and a small (11 kD) subunit, which associate to form the active enzyme. Caspase-6 catalyzes the proteolysis of Poly-(ADP-ribose)Polymerase (PARP), an enzyme that is involved in DNA repair and genomic maintenance. Caspase-6 is the main effector Caspase in glucocorticosteroid-induced apoptosis.

The effector Caspase-7 (Mch3, ICE-LAP3, CMH-1) cleaves substrates responsible for producing the morphological and biochemical changes associated with apoptosis. Its α form of 303 amino acids is processed upon activation to a 20 and 12 kD subunit. A 253 amino acid β form results from

alternative splicing and lacks the cysteine protease active site (QACRG). The β form may function as a dominant negative regulator of apoptosis. The active Caspase-7 is involved in the proteolysis of Poly(ADP-ribose)Polymerase (PARP).

- Methylation of *caspase-8* occurs in some childhood tumors and in neuroendocrine lung tumors. *caspase-8* expression is silenced by gene methylation in malignant neuroblastoma, and this correlates with resistance to cell death [Hopkins-Donaldson et al. 2000].
- eIF4E (Eukaryotic Translation Initiation Factor 4E) is an mRNA cap-binding protein required for the translation of cellular mRNA. eIF4E is a major target for the regulation of translation by growth factors and hormones. When overexpressed, eIF4E profoundly suppresses proto-oncogene-dependent apoptosis, causing malignant transformation. eIF4E rescues cells from endoplasmic reticulum stressors and functions as a pleiotropic regulator of cell viability. This is accomplished, in part, by preventing the release of Caspase-12 from the endoplasmic reticulum [Li et al. 2004].
- The tumor suppressor gene product DRS (Domain Rich in Serine, Pinin, PNN) activates Caspase-12 and ensuing programmed cell death. The release of Cytochrome *c* from the mitochondria into the cytoplasm is not associated with this form of apoptosis [Tambe et al. 2004]. The expression of *drs* {14q13} is markedly down-regulated in renal cell carcinoma, transitional cell carcinoma, and in cancers of the colon and the prostate. This may be caused by promoter methylation.

Endonucleases. The digestion of the genomic DNA within an apoptotic cell is accomplished by endonucleases. They cut the DNA into fragments of about 180 bp in size, reflecting the length of 1 wrap of DNA around Histone proteins (internucleosomal DNA cleavage).

DFF (DNA Fragmentation Factor) is composed of a heterodimer of a catalytic endonuclease subunit DFF40 (DFFB, CAD, Caspase-Activated Deoxyribonuclease) {1p36.3} and a chaperone/inhibitor subunit DFF45 (45 kD DNA Fragmentation Factor, DFFA, DFF1, ICAD-L) {1p36.2–36.3} or DFF35 (35 kD DNA Fragmentation Factor, ICAD-S). Cleavage of the inhibitor DFF45 causes the release of the DNAse DFF40, which travels to the nucleus to fragment DNA. The cleavage of DFF45 or DFF35 by Caspase-3 is accompanied by DFF40 homooligomer formation, with a tetramer being the smallest unit. Intact DFF45 can inhibit the nuclease activity by associating with these homooligomers, without mediating their disassembly.

CIDE-A and CIDE-B (Cell Death-Inducing DFFA-Like Effectors A and B) [Inohara et al. 1998] activate apoptosis in a manner that is inhibitable by DFF45, but is independent of Caspases. Another family member, CIDE-3, is also competent to induce DNA fragmentation [Liang et al. 2003]. The COOH-terminal region of CIDE-A is necessary and sufficient for killing, whereas a region with homology to DFF45 located in the NH_2-terminus is required for DFF45 to inhibit CIDE-A-induced apoptosis. The expression pattern of CIDE-3 is different from that of CIDE-B, with expression of CIDE-3 mainly in small intestine, heart, colon, and stomach, while CIDE-B is strongly expressed in the liver and small intestine, and at a lower level in colon, kidney, and spleen. CD95-mediated apoptosis can be enhanced by CIDEs.

ENDO-G (Endonuclease G) [Ruiz-Carrillo and Renaud 1987] is an Mg^{2+}-dependent DNA endonuclease that has a strong preference to nick within long tracts of guanine residues. It is located in the mitochondria. In response to apoptotic stimuli, ENDO-G is released simultaneously with Cytochrome *c* into the cytoplasm and travels to the nucleus. Once released from the mitochondria, ENDO-G cleaves chromatin DNA into nucleosomal fragments independently of Caspases [Li et al. 2001].

3.2.2 Apoptosis checkpoints

BCL family. There are at least 15 family members of the BCL family, which are divided into three subfamilies:

1. BCL-2 subfamily (prosurvival): BCL-2, BCL-X_L, BCL-w, MCL-1, A1 (BFL1), BOO (DIVA), and NR-13
2. BAX subfamily (proapoptotic): BAX, BAK, and BOK (MTD)
3. BH3 subfamily (proapoptotic): BAD, BID, BIK (NBK), BLK, HRK, BNIP3, BIML, BMF, NOXA, and PUMA (BBC3, BCL-2-binding component-3)

The BCL family molecules share common homology domains (BH1, BH2, BH3, and BH4) (**Figure 3.2.2.A**).

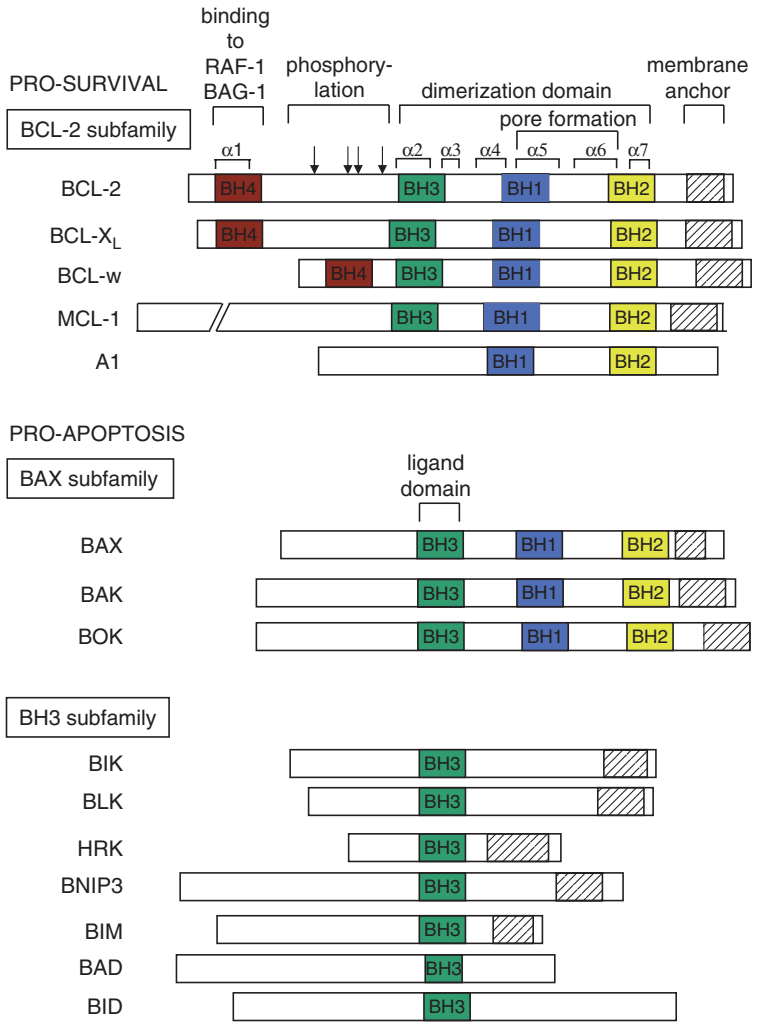


Figure 3.2.2.A. Structures of BCL molecules. The BCL superfamily comprises three families. The BCL-2 group promotes cell survival, whereas the BAX and BH3-only groups facilitate apoptosis. BH1 and BH4 are conserved sequence motifs. The BAX family resembles the BCL-2 family but lacks functional BH4 domains. The BH3-only family is not homologous to the others, except for its signature motif. α1 though α7denote helices in BCL-X_L, in which a core of the two hydrophobic helices α5 and α6 is flanked by five amphipathic helices. A flexible loop connects α1 with α2. The arrows indicate serine and threonine residues that can be phosphorylated.

While BCL-2 and its most similar prosurvival homologs BCL-X_L and BCL-w contain all four BH domains, the other prosurvival members contain at least BH1 and BH2. While the members of the BAX subfamily (BAX, BAK, BOK) contain BH1, BH2, and BH3, and resemble BCL-2 fairly closely, the members of the BH3 subfamily possess only the central short (9–16 residues) BH3 domain and are structurally unique. They are named BH3-only proteins because they carry only the third domain of the four that characterize BCL-2 family members.

– The homology domains 1 through 3 strongly influence homodimerization and heterodimerization by generating an elongated hydrophobic cleft, to which a BH3 amphiphatic α-helix can bind. Proapoptotic and antiapoptotic family members can thus heterodimerize. This BH3 cleft coupling may account for all dimerizations within the family.
– BH4 is conserved in antiapoptotic BCL-2 family members (eg. BCL-X_L) but absent in apoptosis agonists except BCL-X_S. This domain allows the interaction with death regulatory proteins such as RAF-1 and BAD.

Many BCL-2 family proteins, except BAD and BID, reside in the mitochondrial outer membrane, anchored by a hydrophobic stretch of amino acids located within their COOH-termini. BAD and BID are located throughout the cytoplasm.

BCL-2 family members may have several distinct mechanisms of function, which are not mutually exclusive.

– Dimerization regulates the decision to proceed to apoptosis. BCL-X_L homodimers are required to actively suppress apoptosis or to actively promote survival. Conversely, BAX homodimers are

required to actively promote apoptosis or to actively inhibit survival. Thus, BCL-X_L/BAX heterodimerization interferes with the transduction of either proapoptotic or antiapoptotic signals. Activated BAX may form heterodimers with BCL-2 and inhibit its antiapoptotic function. However, it is unclear whether BCL-2 must form homodimers to promote cell survival or whether it must form a heterodimer with a proapoptotic member to block the associated death pathway. In the BH3 subfamily, heterodimerization is not required for prosurvival function, but is essential for the proapoptotic activity. The BAX subfamily members do not depend essentially on heterodimerization, but possibly have an independent cytotoxic impact. In death antagonists, BH1 and BH2 allow the heterodimerization with BAX to repress apoptotic cell death. In death agonists, BH3 allows the heterodimerization with BCL-X_L and BCL-2 to promote apoptosis. BAK oligomerization predisposes to apoptosis. In viable cells, BAK is maintained in an inactive monomeric conformation at the mitochondria. There, it is complexed with the outer membrane protein VDAC2. Death signals activate BH3-only molecules, including tBID, BIM, or BAD, which displace VDAC2 from BAK, enabling homooligomerization of BAK and apoptosis [Cheng et al. 2003].

– The ratio of antiapoptotic BCL-2 members to proapoptotic members is important in determining whether programmed cell death will proceed. Excess death antagonists promote survival, whereas excess death agonists promote apoptosis. A possible reason for this is the availability of binding partners for dimerization.
– Subcellular localization affects function. BCL-2 and BCL-X_L localize predominantly to the outer mitochondrial membrane, but also to the nuclear and endoplasmic reticulum membranes. BCL-2 can cause the translocation of the GTP-binding protein RAF-1 to the mitochondrial membrane, which raises the possibility that BH4 domain containing death antagonists may serve in a second messenger role. Phosphorylation of RAF-1 on serines 338 and 339 by PAK (P21-Activated Protein Kinase) can also mediate the translocation of RAF-1 to the mitochondrial membrane. There, RAF-1 mediates a different signaling pathway than it does at the plasma membrane, where it is involved in the ERK→MAP Kinase pathway. At the mitochondrial membrane, RAF-1 recruits a kinase that phosphorylates BAD on serine 112. Phosphorylation of BAD leads to its inactivation via binding to a 14-3-3 protein and supports survival by allowing BCL-2 and BCL-X_L to create survival-promoting dimers. Activated BAX translocates to the mitochondrial membrane and interacts with the permeability transition pore complex. BAX and the constitutive mitochondrial protein ANT cooperate to form a composite channel, increase the mitochondrial membrane permeability, and to trigger cell death.
– BCL-2 family members regulate pore formation. The mitochondrial permeability transition pore is formed by a complex of the Voltage-Dependent Anion Channel (VDAC), the Adenine Nucleotide Translocase (ANT), and Cyclophilin-D (CYP-D), at contact sites between the mitochondrial outer and inner membranes. Members of the BCL-2 family, including BCL-X_L and BAX, can form selective ion channels in membranes and can regulate the changes of the mitochondrial membrane potential. These pores may explain the membrane permeabilty transition, which occurs in apoptosis. The overexpression of the prosurvival protein BCL-2 prevents the permeability transition and protects from apoptosis. BCL-2 can maintain organelle integrity by preventing pore formation by the death agonist BAX and by preventing the release of Cytochrome *c* from mitochondria [Yang et al. 1997].
– The BCL-2 proteins BAX and BAK regulate the calcium dynamics between mitochondria and endoplasmic reticulum. Distinct categories of death stimuli require BAX and BAK at either mitochondrial control points. The control of endoplasmic reticulum calcium by BAX and BAK is a prerequisite for apoptosis induced by lipid second messengers and oxidative stimuli.

The expression of *bcl-2* family members is regulated on the transcriptional level. The expression of several prosurvival genes, including *bcl-2* family members, is induced by certain cytokines through their cognate receptors. Ligation of TNFRSF13B (TACI) or BMA triggers the expression of BCL-2-related proteins that inhibit apoptosis. The engagement of CD40 on germinal center B-lymphocytes inhibits their entry to apoptosis through the induction of BCL-2 expression. Conversely, DNA alteration to O^6-methylguanine may induce apoptosis. It is preceded by a decrease of BCL-2 protein levels and hypophosphorylation of BAD, and it is executed by activation of the Caspases -3 and -9.

P53 controls *bax* expression while reducing the expression of *bcl-2*. In combination, these effects lead to programmed cell death.

Alterations in gene expression are not always necessary for changes in the activity of BCL-2 family members. Phosphorylation in the variable loop domain can change the activity state of many proteins in this group. Phosphorylation of BAD via a RAF-1-mediated pathway at the mitochondria causes it to be sequestered by the 14-3-3 protein, leading to the inactivation of BAD. In hematopoietic cells stimulated by IL-3, the BH3 subfamily member BAD is phosphorylated and the product is sequestered in the cytosol by 14-3-3 proteins, preventing its inhibition of BCL-X_L [Datta et al. 1997]. In the case of prosurvival members, phosphorylation may either augment or suppress activity. The loop domain influences the phosphorylation status of BCL-2. This may reflect the existence of phosphorylation sites (serines or threonines) within the loop itself or kinase recognition sites on the loop, possibly for JUN Kinase, which then phosphorylates the protein on different sites.

The proto-oncogene *bcl-2* is located on chromosome 18 and codes for a 25 kD protein. In multiple cell types, including lymphocytes, fibroblasts, neurons, and hematopoietic cells, the expression of *bcl-2* is able to delay or even prevent apoptosis. Conversely, the down-regulation of *bcl-2* expression in many of these same systems promotes apoptosis. *bcl-2* mRNA levels are high during pre-B-cell development, but are down-regulated with maturation. *bcl-2* is quiescent in resting B-lymphocytes but up-regulated with B-cell activation. The COOH-terminus encodes a stretch of 21 hydrophobic amino acids that are important in membrane docking. This causes BCL-2 to be an integral membrane protein located in the membranes of the endoplasmic reticulum, nuclear envelope, and the outer membranes of the mitochondria, where it may close pores. BCL-2 prevents most proapoptotic signaling molecules from permeabilizing the mitochondrial membrane. A central checkpoint of apoptosis is the activation of Caspase-9 by APAF-1 upon Cytochrome *c* release from the mitochondria. The BH4 domain of BCL-2 can bind to the COOH-terminal part of APAF-1, thus inhibiting its association with Caspase-9 [Hu et al. 1998; Huang et al. 1998; Pan et al. 1998].

The *beclin-1* gene consists of 12 exons that extend over 12 kb. Beclin-1 is a 450 amino acid-coiled coil protein that interacts with BCL-2. Endogenous Beclin-1 expression is ubiquitous at high levels in normal breast epithelia.

BCL-X_L is composed of seven α-helices joined by flexible loops. It shares similarity to the pore-forming domains of some bacterial toxins and can form an ion channel, which becomes cation selective at physiologic pH.

- BCL-X_L may maintain cell survival by regulating the permeability of the intracellular membranes to which it is distributed.
- BCL-X_L binds APAF-1 through its BH4 region and pro-Caspase-9 through its NH_2-terminal CARD. These interactions prevent the progression of the proapoptotic signaling cascade.
- BCL-X_L sequesters tBID and curtails its ability to activate BAX.

BCL-X_S, a shorter splice variant of BCL-X_L, can counteract the antiapoptotic effect of BCL-X_L. The stress-inducible kinase JNK (Stress-Activated Protein Kinase, SAPK) may phosphorylate and inactivate BCL-X_L. Likewise, BAD inhibits BCL X_L.

Deamidation of asparaginyl and glutamyl residues of proteins results in the introduction of a negative charge and can lead to alterations in their tertiary structure. Such modifications can be important for function. The amidation of BCL-X_L on two specific aspartate residues in the regulatory region of the regulatory domain, converting them to asparagines, confers to BCL-X_L the ability to block the proapoptotic activity of BH3-only proteins. The deamidation of BCL-X_L is facilitated in the absence of functional RB [Deverman et al. 2002].

Apoptosis is regulated, in part, by the calcium dynamics between mitochondria and endoplasmic reticulum. Distinct categories of death stimuli require BAX and BAK at either mitochondrial or endoplasmic reticulum control points. Both multidomain proapoptotic molecules, BAK and BAX, are required to initiate the mitochondrial pathway of apoptosis, mediated by BH3-only proteins. Furthermore, the control of endoplasmic reticulum calcium by BAX and BAK is a prerequisite for apoptosis induced by lipid second messengers and oxidative stimuli. Intrinsic apoptotic signals require both, mitochondrial and endoplasmic reticulum signals [Scorrano et al. 2003].

Hypophosphorylated BAD interacts preferentially with proapoptotic molecules. BAD phosphorylation,

induced by survival factors, leads to its preferential binding to 14-3-3 proteins and suppression of its death-promoting function. PKB (AKT) can phoshorylate and inactivate BAD. Signaling by survival factors through PKB is a prerequisite for the phosphorylation and sequestration of BAD.

Activation of Caspase-8 cleaves the inactive cytosolic form of BID (26 kD), generating a truncated 15 kD COOH-terminal fragment and a 11 kD NH_2-terminal fragment. Following cleavage by Caspase-8, a glycine on the P15 fragment is exposed and *N*-myristoylated. This targets the complex of P15 and P11 BID fragments to the mitochondrial membranes. The structure of BID supports two modes of proapoptotic action.

– BID can interact through its BH3 domain with the antiapoptotic BCL-X_L and thus prevent the formation of the antiapoptotic complex between BCL-X_L and APAF-1. This Heterodimerization with BCL-X_L may be facilitated by the truncation of BID. The 15 kD fragment tBID (truncated BID) contains the BH3 domain and triggers the homooligomerization of BAK or BAX, respectively, activating their proapoptotic effects and resulting in the release of Cytochrome *c* from the mitochondria.
– BID contains the structural motifs for pore formation, and after truncation it is potentially able to form selective ion channels similar to BAX. In this way, BID may promote apoptosis independently of its BH3 domain and without inhibiting BCL-2.

This pathway is essential for apoptosis and mediated by UV radiation, growth factor deprivation, or endoplasmic reticulum stress. BID is cleaved in response to signaling through the death receptors CD95 (FAS), TNFR, or TRAIL.

PUMA (P53-Upregulated Modulator of Apoptosis, BCL-2 Binding Component-3, BBC3) {19q} contains 193 amino acids and belongs to the BH3-only group of BCL family proteins [Yu et al. 2001]. Through the use of alternative initiating exons, two forms, PUMAα and PUMAβ are generated. The PUMA protein is exclusively mitochondrial and binds to BCL-2 and BCL-X_L through its BH3 domain. It may be a direct mediator of P53-induced apoptosis.

- Deregulated *bcl-2* expression promotes the growth of B-cell lymphomata [Vaux et al. 1988]. Its translocation t(14;18)(q32;q21) is causative in follicular lymphomata. This translocation generates heterogeneous 4.2–7.2 kb *bcl-2-immunoglobulin* chimeric mRNAs, resulting from alternative *bcl-2* 5′ exons and varied *ig* 3′ untranslated regions.
- Endogenous Beclin-1 expression is frequently low in breast epithelial carcinoma. This may reflect allelic deletions of *beclin-1*, but not *beclin-1* coding mutations. The *beclin-1* gene is monoallelically deleted in 40–75% of cases of sporadic breast, ovarian, and prostate cancer. Loss of the *beclin-1* gene {17q21} leads to an increase in spontaneous tumors. This may reflect a role for this gene as a tumor suppressor that mediates autophagic cell death.
- BCL-X_L is highly expressed in prostate cancer cells.
- Elevated levels of Casein Kinase II in tumor cells protect from TRAIL-induced apoptosis by inhibiting Caspase-8-mediated cleavage of BID and promoting the NF-κB-dependent expression of BCL-X_L and c-FLIP [Ravi and Bedi 2002].
- BAK is expressed in normal gastrointestinal epithelium. Missense mutations in *bak* may occur in gastric and colorectal cancers. They arise only in advanced stages of the disease [Kondo et al. 2000].
- In certain types of cancer, the tumor suppressor and proapoptotic BCL-2 family member BAX is mutated [Rampino et al. 1997; Meijerink et al. 1998]. *bax* mutations occur in about 50% of colon carcinomata with microsatellite instability. They typically constitute frameshift mutations in a tract of eight deoxyguanosines, spanning the codons 38–41 in the third coding exon. Because these cancers do not normally contain mutations of P53 it is possible that loss-of-function mutations in BAX eliminate the selective pressure in this regard [Rampino et al. 1997].
- Frameshift mutations of *bax* that lead to loss of expression, and mutations in the BH domains that result in loss of functions, are common. Tumor cells with frameshift mutations are more resistant to apoptosis. Reduced BAX expression may be associated with shorter patient survival in breast adenocarcinoma [Krajewski et al. 1995].
- The antiapoptotic effects of the serine/threonine kinase PKB are required for the transformation of hematopoietic cells by the oncogenic tyrosine kinase BCR-ABL. PKB is a downstream target of BCR-ABL that promotes the survival of hematopoietic cells by inducing the activity of mitochondrial RAF-1 in a RAS-independent, but PKC-dependent manner [Majewski et al. 1999]. RAF-1 causes the phosphorylation and sequestration of BAD.

IAP family. IAP counteract programmed cell death through the negative regulation of Caspases. BIR (baculoviral IAP repeat) is an approximately 70 amino acid motif that is a common structural feature of all IAP family members. The BIR motif is present in 1–3 copies, and it may be necessary and sufficient for the antiapoptotic effect of IAPs through the specific inhibition of Caspases [Takahashi et al. 1998]. Nine human IAP members are known: the Neuronal Apoptosis Inhibitory Protein (NAIP, BIRC1, Baculovirus IAP Repeat-Containing Protein-1), cIAP1 (MIHB, HIAP-2, BIRC2), cIAP2 (HIAP-1, MIHC, API2, BIRC3), XIAP (hILP, MIHA, ILP-1, BIRC4), ML-IAP, ILP2, Apollon, Livin (KIAP), and Survivin. Their overexpression leads to diminished Caspase activity and increased resistance to a variety of apoptotic stimuli. A subfamily comprises cIAP1 {11q22–q23}, cIAP2 {11q22–q23}, and XIAP {Xq25}. Members of this subfamily contain three IAP repeat domains in the NH_2-terminal region and a RING finger domain close to the COOH-terminus. The 80 kD IAP1 is recruited to the cytosolic domain of TNFR-2 (Tumor Necrosis Factor Receptor-2) via its interaction with TRAF-1 and -2 (Tumor Necrosis Factor Receptor-Associated Factors -1 and -2). It binds to and inhibits Caspases -3 and -7, and pro-Caspases -8 and -9. XIAP selectively inhibits Caspases -3 and -7. XIAP, cIAP1, and cIAP2 prevent the proteolytic processing of the pro-Caspases -3, -6, and -7 through blockage of the Cytochrome *c*-induced activation of pro-Caspase-9 by binding directly to pro-Caspase-9. These IAPs do not prevent the Caspase-8-induced activation of pro-Caspase-3, however, they subsequently inhibit the processing of Caspase-3 directly, thus blocking downstream apoptotic events such as further activation of Caspases [Deveraux et al 1998].

Survivin (BIRC5, API4) {17q25} inhibits apoptosis via its BIR domain, by either directly or indirectly interfering with the function of Caspases. Survivin is also a chromosomal passenger protein that is required for cell division. It is expressed in embryonic tissues, but is not expressed in most normal adult tissues. Survivin selectively inhibits the intrinsic, Caspase-9-dependent apoptotic pathway. Survivin is phosphorylated on threonine 34 and activated by $P34^{CDC2}$. This phosphorylation stabilizes Survivin and leads to the suppression of apoptosis. Survivin and XIAP protect from BAX- and CD95-induced apoptosis by inhibiting the processing of pro-Caspase-7. Survivin and XIAP bind to active Caspase-3 and Caspase-7, but not their inactive pro-forms. Therefore, Survivin and XIAP act on the level of the effector Caspases, but do not act on the level of initiator Caspases.

SMAC (DIABLO) is a negative regulator of IAPs. It forms homodimers through an extensive hydrophobic surface and its NH_2-terminal amino acids are indispensable for its function. SMAC is a mitochondrial protein that is released into the cytosol when cells undergo apoptosis. Mitochondrial import and cleavage of its signal peptide are required for SMAC to gain its apoptotic activity. SMAC promotes Caspase-9 activation by binding to IAPs and removing their inhibitory activity.

- Survivin is selectively expressed in fetal tissue. While Survivin is rarely present in normal adult tissues, it is commonly expressed in many cancers [Ambrosini et al. 1997], such as lung, colon, pancreas, prostate, breast, and stomach cancer. In neuroblastoma, survivin expression correlates with a more aggressive and unfavorable disease [Adida et al. 1998]. Possibly, the level of expression can be correlated with the malignancy of a tumor.
- In esophageal squamous carcinoma, amplification of 11q21–q23 frequently occurs. This leads to the overexpression of the antiapoptotic gene *ciap1* (*hiap2*, *mihb*, *birc2*) [Imoto et al. 2001]. IAPs are also overexpressed in certain leukemias and lymphomata. cIAP2 is affected by the translocation t(11;18)(q21;q21) that is associated with about 50% of marginal cell lymphomata of the mucosa-associated lymphoid tissue (MALT) [Dierlamm et al. 1999]. The chimeric product of cIAP2 and MALT1 may enhance the resistance to apoptosis. This indicates a pathogenetic role for cIAP2 in the development of MALT lymphoma.
- ML-IAP is expressed at high levels in melanoma cells, but not in primary melanocytes. Melanoma cells that express ML-IAP are significantly more resistant to apoptosis than those that do not express ML-IAP [Vucic et al. 2000].

FLIP family. The cellular protein FLIP [Irmler et al. 1997] (FLICE Inhibitory Protein, CASH, Casper, I-FLICE, CLARP, FLAME-1, MRIT, Usurpin) is expressed as alternatively spliced variants of a single *flip* gene. All of these variants contain a long prodomain that harbors tandem death effector

domains. Following the prodomain, FLIPs (except the short form FLIP) possess a Caspase protease region, which is enzymatically inactive because essential amino acid residues, namely the catalytic diad forming cysteine and histidine residues, are substituted by tyrosine or arginine, respectively. FLIP is an important regulator of TRAIL-induced apoptosis. High expression levels of FLIP correlate with resistance to TRAIL. FLIP interacts with FADD through the death effector domains. FLIP also forms heterodimers with Capase-8, by which it is then cleaved. This may result in an even tighter binding of the cleaved FLIP to Caspase-8, blocking its proteolytic activity.

- Melanomata and some B-cell lymphomata express high levels of FLIP, which interferes with apoptosis induction at the level of the death receptors.
- In some cases of ovarian cancer, TNF-α can induce a signal that leads to the death of these cells. However, many ovarian malignancies are resistant to the effects of TNF-α. In these cases, the extracellular signals transduced by death receptors are extinguished before the cascade of Caspases can be activated. The overexpression of FLIP, a protein that blocks the Caspase activity of FLICE, mediates this resistance.
- Hodgkin lymphoma is characterized by the presence of malignant Hodgkin–Reed–Sternberg cells, which display resistance to certain apoptotic stimuli, including a lack of sensitivity to CD95-mediated cell death. These cells express CD95 Ligand along with the inhibitory c-FLIP protein. Therefore, Hodgkin–Reed–Sternberg cells are protected from autonomous CD95L-mediated cell death through the expression of FLIP, while preserving their ability to evade immunosurveillance [Dutton et al. 2004; Mathas et al. 2004].
- Primary effusion lymphomata, associated with infection by HHV-8 (KSHV) have constitutive NF-κB activity that is essential for their survival. The viral gene product v-FLIP (K13) activates NF-κB more potently than cellular FLIP in B-lymphocytes and is largely responsible for the NF-κB activation in latently infected primary effusion lymphoma cells [Guasparri et al. 2004]. v-FLIP constitutively up-regulates $P100^{NF\text{-}\kappa B2}$ expression and leads to its processing into the P52 subunit. The increased activity of NF-κB induces the expression of $BCL\text{-}X_L$.

3.2.3 Receptors and ligands modulating cell death

Three families of apoptotic signaling motifs, the death domain (DD), the Caspase recruitment domain (CARD), and the death effector domain (DED), share similar three-dimensional folds, and all mediate the signaling pathways of programmed cell death (Table 3.2.3.A). Yet, only members within the same family interact with each other and do so with stringent specificity (Figure 3.2.3.A).

Tumor Necrosis Factor Receptors. Two related cytokines, TNF-α and TNF-β (Lymphotoxin), have cytotoxic effects on a variety of tumor cells. TNF is a homotrimer of 157 amino acids, primarily produced by macrophages. Apoptosis induced by TNF is typically associated with inflammation.

The TNF Receptor superfamily is defined by homologous cysteine-rich extracellular domains and a cytoplasmic death domain (Figure 3.2.3.B). These related surface molecules are important for lymphocyte development and function. A prerequisite for ligand engagement by TNF Receptor-1 (P55/CD120a) is its preassembly into receptor trimers. This is mediated by an extracellular pre-ligand-binding assembly domain (PLAD), which is distinct from the ligand binding site. The death domain of TNF Receptor-1 may associate with Silencer of Death Domains (SODD). The ligation of the receptor leads to the release of SODD, permitting the recruitment of TRADD (TNF Receptor Associated Death Domain) and FADD (FAS-Associated Via Death Domain). This cascade activates Caspase-8, which cleaves the inactive cytosolic form of BID, generating a truncated 15 kD fragment that relocates to the mitochondria, binds and oligomerizes BAK. The signaling also proceeds through RAIDD and Caspase-2.

Several members of the TNF Receptor superfamily do not mediate, but rather prevent cell death because they lack a death domain. A prerequisite for ligand engagement by TNF Receptor-2 (P75, CD120b) is its preassembly into receptor trimers. This is mediated by an extracellular pre-ligand binding assembly domain (PLAD), which is distinct from the ligand binding site.

– The recruitment of a complex of TRAF2 and RIP initiates the binding of the IKK complex and the activation of NF-κB. The induction of the transcription factor NF-κB in response to TNF-α antagonizes apoptosis. This depends on its induction of $P21^{CIP1/WAF1}$ as a mediator [Javelaud et al. 2000]. NF-κB is essential for B-lymphocyte

Table 3.2.3.A. Death receptors and anti-death receptors. Various cell surface receptors regulate the induction of or resistance to programmed cell death. They respond to their cognate ligands with specific signal transduction cascades

Receptor	Ligand	Signal transduction
Death receptors		
TNF Receptor superfamily		
CD95 (Fas) (APO-1)	CD95L (FasL)	FADD/MORT1 → Caspase-8
TNFR-1	TNF	SODD → TRADD → FADD
DR3	APO-3L (TWEAK)	SODD → TRADD → FADD
DR2	TRAIL (APO-2L)	TRADD/RIP/TRAF2 → IKK
DR4 (TRAIL-R1)	TRAIL	TRADD/RIP/TRAF2 → IKK
DR5 (APO-2)	TRAIL	TRADD/RIP/TRAF2 → IKK
(TRAIL-R2) (KILLER)		
DR6		
DR1	TRAIL (APO-2L)	FADD/MORT1 → Caspase-8
CD27		Siva-1
Anti-death receptors		
TNF Receptor superfamily		
TNFR2	TNF	TRAF → JNK → NF-κB
CD30		TRAF → JNK → NF-κB
CD40	CD40L	TRAF → JNK → NF-κB
BCMA TNFSF13		P38 → JNK → NF-κB
TACI	TNFSF13	NF-κB
		NF-AT
Growth factor receptors		
FGF-2R		
IR		
Integrins		
$\alpha_V\beta_3$	Osteopontin	PKC
		PI 3-K → PKB

survival, possibly through the activation of BCL-2 family members, such as the expression of BCL-X_L.
– The recruitment of TRAF2 to the TNF Receptor activates JNK and consecutively c-JUN.

CD95 (FAS, APO-1). CD95 is a 317 amino acid type-I transmembrane glycoprotein with three extracellular cysteine-rich domains, that belongs to the TNFR superfamily. The CD95L (FAS Ligand, FASL) binding site is located on the cysteine-rich domains -2 and -3. CD95 molecules on the cell surface assemble into trimers, mediated by pre-ligand assembly domains (PLADs), before they are engaged by CD95L. CD95L too preassembles into a trimer [Golstein 2000].

CD95 and CD95 Ligands contribute to programmed cell death predominantly in three contexts:
– The deletion of activated peripheral T-lymphocytes during the termination of an immune response.
– The elimination of virus-infected cells by cytolytic T-lymphocytes and natural killer cells.
– The deletion of immune system cells in immunoprivileged sites, such as the eye.

CD95 induces apoptosis via two alternative signaling pathways. Mostly, apoptosis is induced through FADD, but in some cells a pathway mediated by DAXX and JNK is invoked.
– FADD (MORT1) associates with trimeric CD95. FADD binds to the zymogen form of Caspase-8 (FLICE). It is not regulated by the BCL-2 family of proteins. The recruitment of several pro-Caspase-8 molecules to FADD leads to such a high concentration of pro-Caspases that their low protease activity is sufficient to drive intermolecular proteolytic activation. Caspase-3 zymogens are *S*-nitrosylated on their catalytic site cysteine in resting cells and denitrosylated after CD95 activation. CD95 signaling activates Caspase-3 by inducing its cleavage and by stimulating the denitrosylation of its active site thiol [Mannick et al. 1999].
– DAXX recognizes the CD95 death domain. It then interacts with the JNK Kinase Kinase ASK-1 (Apoptosis Signal Regulating Kinase-1). This

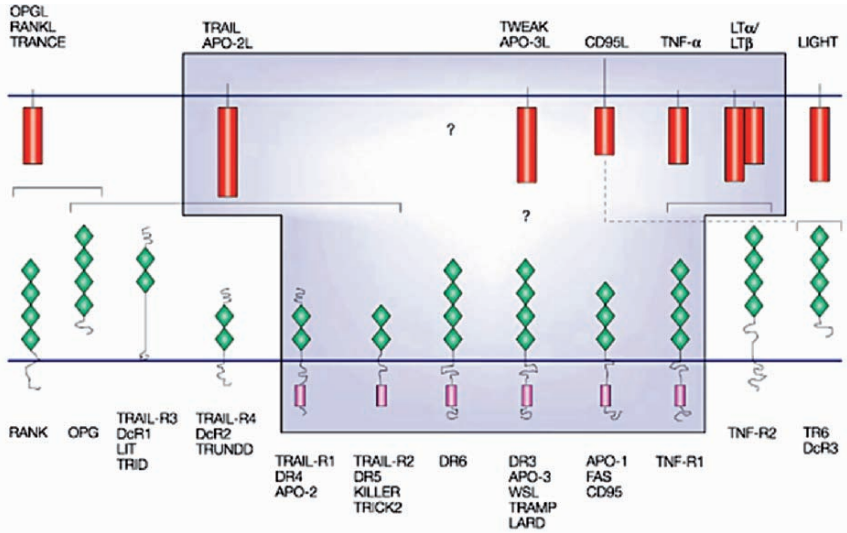


Figure 3.2.3.A. Death receptors and their ligands. Ligands are shown at the top, receptors at the bottom. Death receptors and death ligands are grouped in a box. DcR3 (Decoy Receptor 3) acts as a decoy receptor for CD95L (*dotted line*). The other molecules outside the box can bind to death receptors or ligands as indicated, but have not been shown to transmit an apoptotic signal. The death domain is shown as a pink box. The question marks indicate the unknown ligand for DR6 (Death Receptor 6), and that the interaction between TWEAK (TNF-Related Protein with Weak Ability to Induce Cell Death) and DR3 (Death Receptor 3) is not fully established. APO-2L = APO-2 Ligand, DcR1 = Decoy Receptor 1, DR4 = Death Receptor 4, LARD = Lymphocyte Associated Receptor of Death, LIT = Lymphocyte Inhibitor of TRAIL, LTα,/LTβ = Lymphotoxin α/Lymphotoxin β, OPG = Osteoprotegerin, OPGL = Osteoprotegerin Ligand, RANK = Receptor Activator of NF-κB, RANKL = Receptor Activator of NF-κB Ligand, TNF-α = Tumor Necrosis Factor α, TNFR1 = Tumor Necrosis Factor Receptor 1, TNFR2 = Tumor Necrosis Factor Receptor 2, TRAIL = TNF-Related Apoptosis Inducing Ligand, TRANCE = Tumor Necrosis Factor-Related Activation-Induced Cytokine, TRICK2 = TRAIL Receptor Inducer of Cell Killing 2, TRID = TRAIL Receptor without an Intracellular Domain, TRUNDD = TRAIL Receptor with a Truncated Death Domain. LIGHT is a cytokine that is homologous to Lymphotoxins, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for Herpes Virus Entry Mediator, a receptor expressed by T-cells. [Reproduced from Igney and Krammer 2002. With permission from Macmillan.]

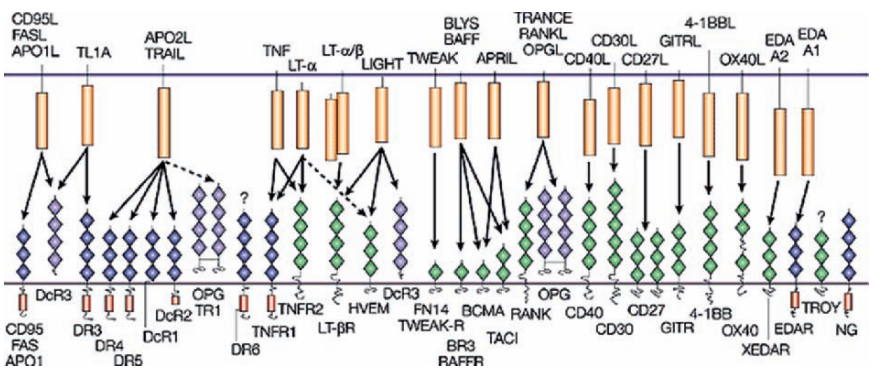


Figure 3.2.3.B. The TNFR superfamily. Ligands are shown in their schematic transmembrane form. Arrows indicate receptor interactions with solid lines for strong binding and dashed lines for low-affinity binding. Question marks indicate that cognate ligands have not yet been identified. Diamonds represent cysteine rich receptor domains and red boxes denote receptor cytoplasmic death domains. [Reproduced from Ashkenazi 2002. With permission from Macmillan.]

relieves an inhibitory intramolecular interaction between the NH_2-terminal and the COOH-terminal domains of ASK-1, activating its kinase activity [Chang et al. 1998], and initiates a JNK-dependent pathway of apoptosis.

- Low expression levels of CD95 and expression of CD95L can arise on malignant melanoma [Hahne et al. 1996a,b,c] and hepatocellular carcinoma [Strand et al. 1996].
- CD95 has a death domain, but splice variants exist that do not contain the death domain and are therefore unable to signal. Mutant CD95 may exert a dominant negative effect by associating with wild-type CD95 in cell surface trimers and preventing their signal transduction.
- A high incidence of *cd95* mutations exists in bladder cancer. The mutation G993A is a hot spot in this malignancy [Lee et al. 1999b].

- Plasmacytomata and non-Hodgkin lymphomata may harbor *cd95* mutations in about 10% of all cases [Landowski et al. 1997; Gronbaek et al. 1998]. Several *cd95* gene mutations occur in myeloma and T-cell leukemia [Cascino et al. 1996]. They include point mutations in the cytoplasmic death domain of CD95 and a deletion that leads to a truncated form of the death receptor. These mutated forms of CD95 might interfere in a dominant negative way with apoptosis induction by CD95. Mutations in the cytoplasmic tail of CD95 (death domain) are frequent in thyroid lymphoma [Takakuwa et al. 2001].
- A soluble form of CD95 (sCD95) can act as a decoy receptor and protect malignancies from cell death. High serum levels of sCD95 are associated with poor prognosis in melanoma patients [Midis et al. 1996; Ugurel et al. 2001].
- The integrity of PTEN function is important for CD95-induced apoptosis, reflecting a negative regulation of CD95 signaling by the Phosphatidylinositol 3-kinase→PKB pathway. This may account, in part, for the tumor suppressor function of PTEN.

Death Receptors. Committed death receptors include DR3, DR4, and DR5. DR3 (TNFRSF25, APO-3, LARD) is expressed mainly in spleen, thymus, and peripheral blood. It has close sequence homology to TNF Receptor-1. TWEAK (TNFSF12, APO-3 Ligand) binds to this receptor, which then triggers apoptosis through the adapter proteins TRADD and FADD (MORT1). FADD recruits the pro-forms of Caspases -8 and -10, thus forming a death inducing signaling complex (DISC). At the DISC, pro-Caspase-8 and pro-Caspase-10 are cleaved and yield active initiator Caspases. Apoptosis induction by TRAIL (TNFSF10, Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand, APO-2 Ligand) requires Caspase activity, but may be independent of FADD (Figure 3.2.3.C). The death domains of DR4 (TNFRSF10A, TRAIL-R1, APO-2) or DR5 (TNFRSF10B, TRAIL-R2, TRICK2) recruit Caspase-8, which cleaves and activates BID. The death domain of Death Receptor-3 may associate with SODD. Receptor ligation leads to the release of SODD from the receptors, permitting the recruitment of TRADD and FADD.

The TNF family member LIGHT is a cytokine that is homologous to Lymphotoxins, exhibits inducible expression, and can compete with herpes simplex virus Glycoprotein D for TNFRSF14 (Herpesvirus Entry Mediator, TR2), a receptor expressed by T-lymphocytes. TNFRSF14 interacts

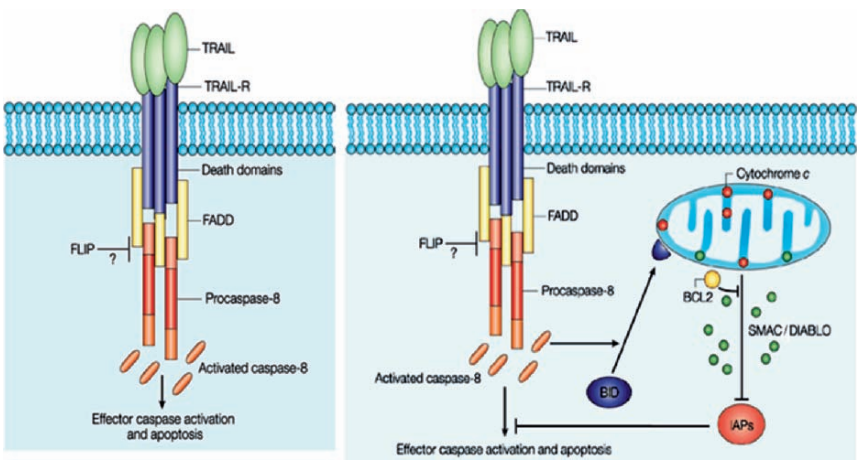


Figure 3.2.3.C. Trail-induced apoptosis. *Left panel*: The classical extrinsic signaling pathway for TRAIL-induced apoptosis. Binding of TRAIL (TNF-elated apoptosis inducing ligand) and trimerization of TRAIL death receptors (TRAIL-R) leads to the recruitment of FADD (FAS-associated death domain), an adapter molecule that recruits and activates Caspase-8. This initiates the Caspase cascade that eventually leads to apoptosis. FLIP (FLICE-Like Inhibitory Protein) has two death effector domains, but an inactive enzymatic site, and interferes with the generation of active Caspase-8. *Right panel*: The mitochondrial pathway to apoptosis mediated by TRAIL. TRAIL-induced truncated BID targets mitochondria, which causes the rapid release of SMAC (DIABLO) into the cytosol, where it binds to IAP (Inhibitor of Apoptosis Protein) family members and reverses their inhibitory effects on activated Caspase-3 and Caspase-9. The release of SMAC from the mitochondria can be inhibited by antiapoptotic BCL-2 family members. FADD = FAS-associated death domain, FLIP = FLICE-Like Inhibitory Proteins, SMAC = Second Mitochondria-Derived Activator of Caspase. [Reproduced from Hersey and Zhang 2001. With permission from Macmillan.]

with TRAF-1, -2, -3, and -5. Its ligation leads to the activation of JNK-1, NF-κB, and AP-1. This represents a signaling pathway of the cellular stress response activation of the immune response.

Decoy receptors bind to proapoptotic ligands without transducing their signals. Therefore, they act in an antiapoptotic fashion. Decoy receptors include DcR1, DcR2, DcR3, and Osteprotegerin. TRAIL can bind to two decoy receptors, DcR1 (TNFRSF10C, TRAIL-R3, TRID) and DcR2 (TNFRSF10D, TRAIL-R4, TRUNDD). DcR1 cannot transmit a TRAIL-dependent signal. DcR2 likewise has a substantially truncated cytoplasmic domain that makes it function as a decoy receptor. DcR3 (TNFRSF6B, TR6) binds to CD95L and LIGHT (TNFSF14) and inhibits CD95L-induced apoptosis. Osteoprotegerin is a decoy receptor for TRAIL, which inhibits TRAIL-induced apoptosis. RANKL specifically binds to Osteoprotegerin and neutralizes its antiapoptotic effect.

- Missense mutations in *dr4* (*trail receptor 1*) and *dr5* (*trail receptor 2*), caused by single nucleotide substitution, may occur in breast cancers [Shin et al. 2001].
- Although the TRAIL Receptors that are competent for signal transduction, DR4 and DR5, are expressed at comparable levels by tumor cells and normal cells, tumor cells are often more susceptible than normal cells to apoptosis induced by TRAIL [Gura 1997]. Untransformed cells, but not cancer cells have a type of receptor for TRAIL, such as DcR1, that is a glycosyl phosphatidylinositol-anchored cell surface protein and lacks the cytoplasmic tail with the intracellular death domain.
- Hormone-insensitive prostate cancer cells may escape programmed cell death by secretion of high amounts of Osteoprotegerin.
- DcR3 is genetically amplified in several lung and colon carcinomata and is overexpressed in several adenocarcinomata, gliomata, and glioblastomata [Pitti et al. 1998; Roth et al. 2001].

CD27. CD27 is a member of the TNFR superfamily, expressed on discrete subpopulations of T- and B-lymphocytes. It is a marker of memory B-lymphocytes and its interaction with its ligand, CD70, is important for the differentiation into plasma cells. CD27 may mediate apoptosis in hematopoietic cells that lack CD95. Although the cytoplasmic tail of CD27 is relatively short and does not contain a death domain, the signaling protein CD27BP (Siva) binds to the CD27 cytoplasmic tail. It has a death domain homology region, a box B-like ring finger, and a zinc finger-like domain. The activation of CD27BP in response to ligation of CD27 induces apoptosis [Prasad et al. 1997].

- In myeloma cells, CD27BP is unable to bind to CD27 consecutive to the engagement by CD70. Therefore, apoptosis may not be induced. During the progression of multiple myeloma, the expression of CD27 is lost. The loss of CD27 correlates with the loss of CD19 and is an indicator of poor prognosis [Katayama et al. 2003; Guikema et al. 2003].
- Primary plasma cell leukemia is a rare plasma cell malignancy, which is related to multiple myeloma and is characterized by a poor prognosis. In contrast to multiple myeloma, primary plasma cell leukemia displays a high expression level of CD27. Upon ligation by CD70, CD27 inhibits apoptosis in these cells. This is associated with the activation of P38 and ERK-1/ERK-2. The ligation of CD27 leads to persistent DNA binding activity of the transcription factor AP-1 [Guikema et al. 2004].
- Glioblastoma cells frequently express CD70. This may induce apoptosis in CD27 expressing immune effector cells, and possibly constitutes a mechanism of immune evasion by glioblastomata [Wischhusen et al. 2002].

Receptor phosphatases. While receptor tyrosine kinases, including the basic FGF Receptor and the receptors for Insulin and IGFs, mediate antiapoptotic signals, receptor tyrosine phosphatases may trigger programmed cell death. Most of the receptor protein tyrosine phosphatases include two phosphatase domains, of which the membrane proximal domain contains all or most of the catalytic activity. The membrane distal phosphatase domain may serve in homodimer and heterodimer formation. As for the transmembrane tyrosine phosphatases RPTPα, the conformation of the distal protein tyrosine phosphatase domain of the transmembrane tyrosine phosphatase PTPRF (Protein Tyrosine Phosphatase Receptor-Type F, Leukocyte Common Antigen Related, LAR) is affected by oxidative stress, leading to their heterodimerization. PTPRF modulates signal transduction from a number of growth factor receptor tyrosine kinases. Its overexpression induces apoptosis without affecting cell adhesion. The death signals are transduced through the Caspase cascade and are independent of P53 [Weng et al. 1998].

- Mutations in the *tyrosine phosphatase* gene superfamily, comprising *ptprF*, *ptprG*, *ptprT*, *ptpN3*, *ptpN13*, and *ptpN14*, affect about 25% of colorectal cancers and a smaller fraction of the lung, breast, and gastric cancers. They may be nonsense, frameshift, or splice site mutations, which result in truncated proteins lacking phosphatase activity. The affected tyrosine phosphatases have the characteristics of tumor suppressor genes [Wang et al. 2004].

3.3 ALTERATIONS OF SIGNALING TO CELL DIVISION AND SURVIVAL IN CANCER

Signal transduction associated with growth factor receptors typically mediates the inactivation of cell cycle checkpoints or activation of cell cycle-promoting gene products. These signals are frequently transduced through G-Protein pathways, kinase pathways, or steroid pathways, are dependent on proto-oncogenic transcription factors, and lead to the expression of Cyclin D. Cyclin D synthesis and degradation thus integrate growth factor signaling with the cell cycle machinery. In accord with the key role of Cyclin D in controlling cell cycle progression, upstream signal transduction pathways associated with growth factor receptors are typically altered by gain-of-function mutations in multiple cancers. Extracellular checkpoint activators inhibit growth in nontransformed cells. They induce signal transduction that mediates either cell cycle arrest or apoptosis. Their loss of function predisposes to transformation.

3.3.1 The RAS pathway and other small G-Proteins

Signals transduced by various proto-oncogenic growth factors proceed through RAS. RAS functions as a membrane-associated biologic switch that relays signals from ligand-stimulated receptors to cytoplasmic MAPK cascades. These receptors include G-Protein-coupled seven transmembrane spanning receptors, tyrosine kinase receptors, and cytokine receptors that cause the stimulation of associated nonreceptor tyrosine kinases. The signal transduction may proceed via growth factor receptor→GRB→ SOS→RAS.

– EGF and NGF activate RAS signaling. This may cause cell cycle progression. While RAS is transiently activated by NGF, this is not sufficient to induce neurite outgrowth.
– Activated TRK-A promotes the conversion of the membrane anchored RAS-GDP into RAS-GTP, which is the active state.
– The members of the c-KIT/c-FMS receptor kinase family are linked with components of the RAS→MAPK signaling pathway.

The proto-oncogene product RAS belongs to a superfamily of small GTP-binding proteins (GTPases) that cycle between an active, GTP-bound, and an inactive, GDP-bound, state. This superfamily includes H-RAS, N-RAS, K-RAS, R-RAS, M-RAS, RAP-1, RAP-2, TC21, RAL, and RHEB. These RAS gene products are involved in the normal control of cell growth. The forms of RAS are mutually identical within their switch I and switch II domains, both of which are essential for appropriate interactions with putative downstream targets. The only substantial differences in their respective amino acid sequences are found in their COOH-terminal hypervariable regions (about 40 amino acids), which also contain the sequences that trigger posttranslational modifications and hence membrane localization.

One function of RAS is to facilitate the localization of its cytosolic effectors to the plasma membrane. RAS is anchored at the plasma membrane via a COOH-terminal farnesyl group. The enzyme Farnesyl Protein Transferase {8p22–q11} catalyzes a key step, the prenylation, in the posttranslational processing of the RAS protein. Farnesyl Protein Transferase attaches a farnesyl group from farnesyl pyrophosphate to cysteine residues at the fourth position from the COOH-terminus of the protein. This modification provides a mechanism for membrane localization of proteins that lack a transmembrane domain. Some RAS proteins (H-RAS1, N-RAS, and K-RAS2) are further lipidated by palmitoylation at one or two cysteines near the farnesylated COOH-terminus. Like farnesylation, H-RAS palmitoylation plays an important role in signaling. Palmitoylated RAS continuously cycles between the cell membrane and the Golgi complex.

The *ras* gene family consists of the *Harvey ras* and *Kirsten ras* genes (*H-ras1* and *K-ras2*), an inactive pseudogene of each (*H-ras2* and *K-ras1*), and the *N-ras* gene. *H-ras1* is located on chromosome 11p15.5, *K-ras2* maps to chromosome 12p12.1, its

pseudogene lies on chromosome 6, and *N-ras* to chromosome 1p13.2. The forms of RAS, H-RAS, K-RAS, and N-RAS, share very high sequence identity, differing only in their COOH-termini that anchor them to cellular membranes.

The *N-ras* gene specifies two main transcripts of 2 and 4.3 kb. The difference between the two transcripts is a simple extension through the termination site of the 2 kb transcript. The *N-ras* gene consists of seven exons (–I, I, II, III, IV, V, VI). The smaller 2 kb transcript contains the exon VIa, and the larger 4.3 kb transcript contains the exon VIb. Both transcipts encode identical proteins as they differ only in the 3′ untranslated region. Most of the N-RAS in the plasma membrane exists in a latent complex with RAF-1 and PKC-ε (PKCε), suggesting that this complex might function to respond to proliferative signals by activating the MAPK (ERK) cascade.

A *K*-RAS gene product binds to microtubules and is localized within the cytoskeletal matrix. This correlates with the possibility that K-RAS2 may be the predominant RAS form to regulate cell migration. The two forms K-RAS4A and K-RAS4B diverge solely in their COOH-terminal 25 amino acids as a consequence of alternate exon utilization. Because of a polybasic stretch of amino acids in the COOH-terminus, K-RAS4B is not palmitoylated and is confined to the plasma membrane.

H-RAS (Harvey Murine Sarcoma Virus Oncogene, RASH1). H-RAS, when inactive, is associated with lipid rafts and caveolae, but when activated, the protein is distributed into the disordered plasma membrane. The *H-ras-1* gene is tightly linked to a mini-satellite, located approximately 1 kb downstream from the gene's coding sequence. This generates multiple alleles [Krontiris et al. 1993].

K-RAS and H-RAS are nearly identical in sequence except for their 20 COOH-terminal amino acid residues. H-RAS and K-RAS differ in their ability to activate RAC-dependent functions. This difference in the ability of H-RAS and K-RAS is mediated by the COOH-terminus of K-RAS, which includes a stretch of lysine residues not existent in H-RAS.

Specific guanine nucleotide exchange factor (GEFs) and GTPase activating protein (GAPs) function as the regulators of the three RAS proteins. GEFs convert RAS into the active GTP-bound state and GAPs terminate the activity by GTP hydrolysis. The RAS proteins bind GDP in the basal state and become activated by exchange proteins of the SOS (Son of Sevenless) or RAS-GRF (RAS GDP Releasing Factor) families. The release of GDP and binding to GTP activates RAS, allowing it to bind to downstream effectors, including the RAF serine/threonine kinase, the RAL-GDS exchange factor, and Phosphatidylinositol 3-Kinase. RAL-GEFs (RAL-GDS, RGL, and RGF) are activated via binding to GTP-RAL. RAL-GEFs in turn activate RAL-GTPases by promoting the GTP-bound state of RAL. As members of the RAS subfamily of RAS-related GTPases, RAL proteins (RAL-A and RAL-B) also cycle between the active GTP-bound states and inactive GDP-bound states. RAL-GTP binds RAL-BP1 (RLIP-1, RIP-1, RALl-Interacting Protein-1), which is a GAP for CDC42 and RAC. These two GTPases are involved in the regulation of the Actin cytoskeleton.

RAS-GAPs catalyze the the hydrolysis of GTP by RAS, which converts active RAS-GTP to RAS-GDP, thereby negatively regulating RAS activity. The *nf1* gene {17q11.2} spans over 350 kb, has 60 exons, and produces a transcript of 11–13 kb. It encodes the tumor suppressor Neurofibromin (NF1, NF1-GAP-Related Protein, NF1GRP), a 2,818 amino acid, 220 kD protein that shares homology with the RAS-GAP family. NF1 is a multidomain molecule with the capacity to regulate several intracellular processes, including the ERK (MAP Kinase) cascade, Adenylyl Cyclase, and cytoskeletal assembly. NF1 negatively regulates RAS-mediated signaling. In its absence, RAS is constitutively active. Growth factor signaling may entail the destruction of Neurofibromin in the Ubiquitin–proteasome pathway, with the subsequent activation of RAS. Shortly after Neurofibromin is degraded, its levels reelevate to attenuate RAS activity and prevent excessive cell proliferation. Four alternatively spliced transcripts of *nf-1* are expressed.

RAS can activate at least three downstream cascades (Figure 3.3.1.A).

– MKKK (RAF or COT)→MEK or JNK→MAPK or JUN
– PI 3-K→PKB
– RAL→CDC42→RAC→RHO (which regulates cell motility rather than cell cycle progression)

RAF is a serine/threonine kinase that is involved in the RAS-dependent transduction of mitogenic signals from the cell membrane to the nucleus. There are three active *raf* genes, two of which have related pseudogenes. The *c-raf-1* {3p25} (the homolog to the viral proto-oncogene *v-raf*) and *A-raf* (*rafA1*,

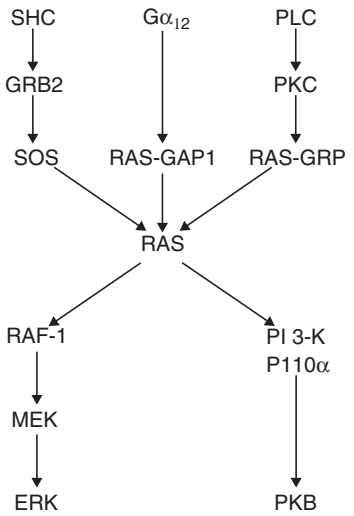


Figure 3.3.1.A. RAS signaling pathways. RAS may mediate signals transduced by G-Protein-coupled receptors or by kinase-associated receptors. The latter may proceed through the cascade SHC→GRB-2→SOS or through PLC→PKC→RAS-GRP. RAS induces cell growth through two principal downstream targets, namely the ERK (MAPK) pathway and the PI3-K pathway.

pks2) {Xp12} genes have 16 coding exons, which span 40 and 20 kb, respectively. The homology between *c-raf-1* and *A-raf* is 71%. *B-raf-1* (*rafB1*) {7q24} is larger and extends over greater than 46 kb. *B-raf-2* is an inactive processed pseudogene located on Xq13. *c-raf-2* is a processed pseudogene on chromosome 4pter-p15. *c-raf-1* RNA is present in many tissues, while *A-raf* and *B-raf* expression is restricted. *A-raf* and *c-raf* encode cytoplasmic serine/threonine protein kinases of 68 and 74 kD, which contain three conserved regions. CR1 and CR2 are in the NH_2-terminal half, CR1 comprises the presumed ligand binding site, and CR3 represents the COOH-terminal kinase domain (Figure 3.3.1.B). Only *c-raf-1* occurs naturally in truncated versions. RAF acts as a MAP Kinase Kinase Kinase (MKKK, MEK Kinase). RAF-1 counteracts apoptosis by suppressing the activation of the 55 kD MST-2 (Mammalian Sterile 20-Like Kinase). RAF-1 prevents dimerization and phosphorylation of the activation loop of MST-2, independently of its protein kinase activity.

COT (MAP3K8, TPL-2, Tumor Progression Locus-2, EST, Ewing Sarcoma Transformant) activates the ERK pathway in parallel to RAF-1, and downstream of RAS. COT belongs to the serine/threonine family of protein kinases in the MAPKKK subfamily. The *cot* proto-oncogene {10p11.2} contains eight exons and is expressed as a 3.2 kb mRNA. Two COT protein forms of 58 kD and 52 kD, generated from alternative translation initiation sites, are located in the cytosol. COT consists of a NH_2-terminal domain of unknown function, which is truncated in some mRNA splice forms, a central catalytic domain (amino acids 139–394), and a COOH-terminal regulatory domain (amino acids 395–467). The COOH-terminal regulatory domain of COT likely exerts a negative effect on its activity. COT is expressed in several normal tissues and in tumor cells. The 58 kD form is activated specifically during the S and G_2/M phases of the cell cycle. The longer form undergoes phosphorylation mainly on serine residues, and the shorter form on both serine and threonine residues [Kyriakis and Avruch 2001]. The expression of COT activates the SAP Kinases and ERKs, specifically MAP2K1 (MEK1, MAPKK1) and MAP2K4

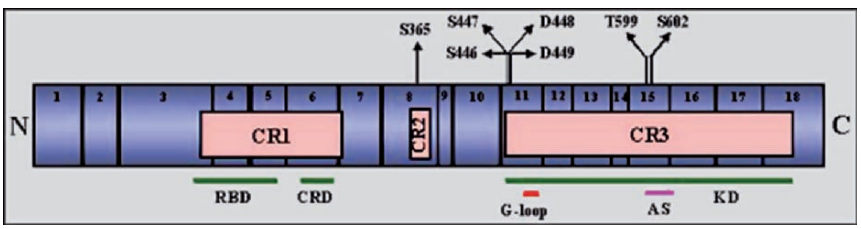


Figure 3.3.1.B. Structure of B-RAF. Diagram of the B-RAF protein in scale. Numbers inside the blue boxes indicate the exon, from which each part of the protein is translated. The three pink boxes inside represent the conserved regions of the protein (CR1, CR2, and CR3) with the A-RAF and RAF-1 proteins. The green bars represent the RAS-binding domain (RBD), the cysteine-rich domain (CRD), and the kinase domain (KD). A conserved glycine motif (G-loop) in exon 11 is indicated with a red bar and the activation segment (AS) in exon 15 with a pink bar. The black arrows reflect the major phosphorylation sites of the protein. C = COOH-terminus, N = NH_2-terminus. [Reprinted from Atlas Genet Cytogenet Oncol Haematol September 2000; Enric Domingo and Simo Schwartz Jr. 2004. http://AtlasGeneticsOncology.org/Genes/BRAFID828.html by permission of the Atlas.]

(SEK1, JNKK1), with equal potency. This leads to signaling through the SAP Kinases, P38, ERK1, ERK2, and ERK5. COT induces c-*jun* transcription, which is dependent on activation of the ERK5→MEF2C pathway as well as on the SAPKs and is inhibitable by MAPK8IP1 (JIP-1, JNK-Interacting Protein-1, IB-1). COT selectively activates P38γ, possibly because sequestration by scaffold proteins isolates COT and MKK6 from all P38 forms other than γ.

Cells have three subfamilies of Mitogen Activated Protein Kinases (ERKs, Microtubule-Associated Protein Kinases, MAPKs). These enzymes are regulated by a characteristic phosphorelay system, in which a series of three protein kinases phosphorylate and activate one another.

– The ERKs function in the control of cell division
– The P38 MAPKs are activated by inflammatory cytokines and environmental stresses
– The JNKs (c-JUN NH_2-Terminal Kinases) are critical regulators of transcription

The effector MAP Kinases (MAPKs, ERKs) are ubiquitous regulators of cell growth and differentiation that have been preserved throughout evolution. All MAP Kinase pathways include central, 3-tiered core signaling modules, in which MAP Kinases are activated by concomitant tyrosine and threonine phosphorylation within a conserved TXY motif in the activation loop of the kinase domain, subdomain VIII. MAP Kinase phosphorylation and activation are catalyzed by a family of dual specificity kinases referred to as MKKs (MAP Kinase Kinases, MAPK/ERK Kinases, MEKs). MKKs, in turn, are regulated by serine/threonine phosphorylation within a conserved motif in kinase domain subdomain VIII, catalyzed by any of several protein kinase families, collectively referred to as MAP Kinase Kinase Kinases (MKKKs, MAP3Ks). The MAP Kinase core signaling modules are themselves regulated by a wide variety of upstream activators and inhibitors.

In response to growth signals,

– ERK controls the synthesis of nucleotides by inducing phosphorylation and activation of Carbamoyl Phosphate Synthetase II (CPS II), which catalyzes the initial, rate-limiting step of pyrimidine nucleotide synthesis.
– ERKs have substrate-docking sites (CD and ED domains). The transcription factor ELK-1 contains a targeting domain that is required for phosphorylation by kinases of the ERK groups. ERK shuttles to the nucleus, where it can phosphorylate ELK-1 and induce transcription factors with the consecutive expression of immediate early genes (rapid response genes), which are essential for growth. ERK may be required for cell growth because it also phosphorylates the transcription factor TCF, resulting in the transcription of *c-fos*. Furthermore, activation of the proto-oncogenic transcription factors of the ETS family (subfamilies ETS, YAN, ELG, PEA3, ERF, and TCF) depends on the RAS→RAF→ERK pathway.
– the ERK pathway increases the transcription of *cyclin D* and thus facilitates the formation of an active Cyclin D/CDK4 complex. This complex is rate limiting for cell growth.
– RSKs (Ribosomal S6 Protein Kinases) are a family of 85–90 kD proteins, comprising RSK-1, RSK-2, and RSK-3, which are substrates for ERKs. RSKs are unusual kinases in that they contain two kinase domains in a single polypeptide. ERK phosphorylates and activates RSK-2 (Ribosomal S6 Protein Kinase 2, MAPKAP Kinase-2), which leads to Histone H3 phosphorylation. The resulting change in chromatin structure may improve the accessibility for transcription factors. RSK also phosphorylates the cell cycle kinase MYT-1 (Membrane Associated and Tyrosine/Threonine Specific 1, CDC2 Inhibitory Kinase).
– ERK enhances the translation of mRNA by phosphorylating MNK1, which increases the ability of eIF4E to recruit ribosomes to mRNA [Whitmarsh and Davis 2000] (Figure 3.3.1.C).

A negative regulator of MEK activity is the phosphatase PP2A. PP2A is an important enzyme that down-regulates the MAPK cascade, relays signals for cell proliferation, and thus may be important for protecting from transformation. The PP2A holoenzyme exists in several trimeric forms, consisting of a 36 kD core catalytic subunit PP2A–C, a 65 kD structural and regulatory component PP2A–A, and a variable regulatory subunit PP2A–B, which confers distinct properties on the holoenzyme.

The SAPK (Stress-Activated Protein Kinase) pathway is activated in response to stress, including radiation, heat shock, reactive oxygen metabolites, and growth factor deprivation. There are three forms

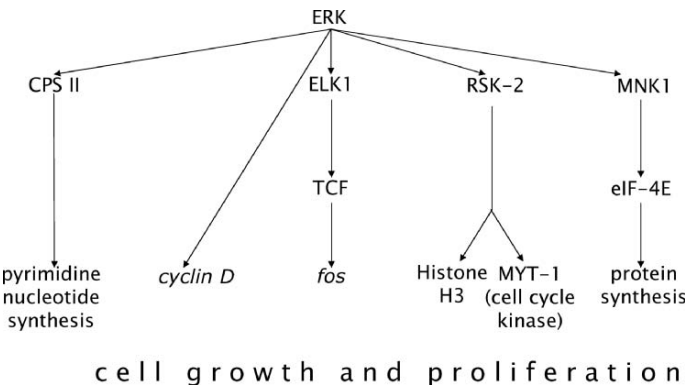


Figure 3.3.1.C. ERK effector pathways. ERK kinase activity phosphorylates and activates multiple downstream targets. This leads to gene expression and to activation of biosynthesis. Commonly, these pathways support cell growth and proliferation.

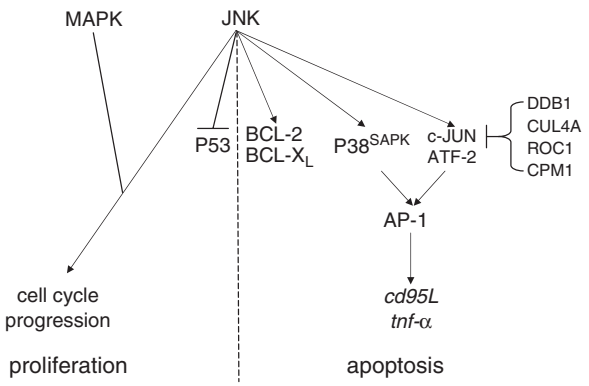


Figure 3.3.1.D. JNK effector pathways. JNK signaling is context dependent. It can lead to the opposing outcomes of either proliferation or apoptosis. Multiple downstream pathways are associated with each outcome. In settings where JNK mediates proliferation, the signaling may converge with ERK (MAPK) targets.

of the stress-responsive kinase JNK, JNK1 (SAPK1, PRKM8, MAPK8), JNK2 (PRKM9, MAPK9) {5q35}, JNK3 (PRKM10, MAPK10) {4q21.32}. Activated JNK binds to the transactivation domain of c-JUN, phosphorylating it on serine 63 and serine 73. Depending on its context, JNK signaling may have two opposing outcomes (Figure 3.3.1.D).

– Signal transduction involving JNK (c-JUN Amino-Terminal Kinase, SAPK) may synergize with the MAP Kinase pathway to mediate cell cycle progression. JUN can also prevent apoptosis by antagonizing P53 activity, a mechanism that may contribute to the early stages of hepatocellular carcinoma.
– Alternatively, JNK may mediate apoptosis through P38 SAP Kinase. P38 SAP Kinase has four isoforms, α, β, γ, and δ. The SAPK pathway induces AP-1, which mediates the expression of the proapoptotic genes *cd95L* and *tnf-α*. JNK is activated when cells are exposed to UV irradiation and is required for UV-induced apoptosis. UV-induced cell death further requires APAF-1, Caspase-9, and Caspase-3, but is independent of Caspase-8. Absence or functional loss of JNK causes a defect in the mitochondrial death signaling pathway, including the failure to release Cytochrome *c*. JNK-induced apoptosis is accomplished through two mechanisms.
  – A major target of JNK signaling is the AP-1 transcription factor. JNK binds to the c-JUN transactivation domain and phosphorylates it on serines 63 and 73. JNK activation leads to phosphorylation and stimulation of the transactivation domains of c-JUN and ATF-2 (CREB-2). Activated c-JUN/ATF-2 heterodimers transcriptionally activate an AP-1-like site in the *c-jun* promoter. AP-1 mediates the expression of the proapoptotic genes *cd95L* and *tnf-α*.
  – JNK mediates the phosphorylation of BCL-2 and BCL-X_L. This may inhibit their antiapoptotic properties and does not depend on gene expression.

DET1 promotes the ubiquitination and degradation of the proto-oncogenic transcription factor c-JUN by assembling a multi-subunit Ubiquitin Ligase-containing DDB1 (DNA Damage Binding Protein 1), CUL4A (Cullin 4A), ROC1 (Regulator of Cullins 1), and CPM1 (Constitutively Photomorphogenic 1). Phosphorylation of the AP-1 transcription factor c-JUN, at multiple sites within its transactivation domain, is required for JNK-induced neuronal apoptosis. The Ubiquitin Ligase SCF^{FBW7} antagonizes apoptotic JNK signaling by ubiquitinating phosphorylated c-JUN and facilitating its degradation [Wertz et al. 2004].

RAS signaling exerts transcriptional and translational control. RAS enhances MYC activity by stabilizing the MYC protein. When produced in the absence of mitogenic signals, MYC has a very short half-life due to degradation by the 26S proteasome, however, the half-life of MYC increases markedly in growth stimulated cells. This stabilization is dependent on the RAS→RAF→ MEK→MAPK pathway and is likely mediated by inhibition of the proteasome-dependent degradation of MYC [Sears et al. 1999].

Translational control has a key role in regulating cell growth. The mRNA-binding translation factors, eIF4s, selectively modulate the translation of various mRNAs based on the extent of inhibitory secondary structure in their 5′ untranslated regions. The mRNA 5′ cap is recognized by eIF4E, which can then recruit other translation factors, including the Helicase cIF4A. Decreased expression of eIF4E leads to reduced rates of cell growth [Flynn and Proud 1996]. eIF4E is subject to phosphorylation, which is increased by agents that stimulate translation. The activity of eIF4E is negatively regulated by the phosphatase PP2A.

The RAS pathway is subject to negative regulation (Figure 3.3.1.E). The RAS-related gene products RAP1A (K-REV-1) {1p13.3}, RAP1B {12q14}, and RAP2 {13q34} share approximately 50% amino acid identity with the classical RAS proteins and have numerous structural features in common. The most striking difference between the RAP and RAS proteins is the replacement of glutamine in RAS by threonine in RAP proteins in amino acid position 61. Hormones that elevate intracellular cAMP often inhibit the physiologic actions of growth factors and may block the transformed phenotype in malignant cells. This reflects the inhibition of cell cycle progression via inhibition of the MAP Kinase cascade. The tumor suppressor gene product RAP1 is activated through cAMP action as well as through phosphorylation by PKA on serine 179 of RAP1B. Active RAP1 associates with RAF-1 and limits the RAS-dependent activation of ERK, resulting in the inhibition of mitogenesis [Schmitt and Stork 2001]. RAP1 (K-REV-1) can suppress the transformed phenotype of K-RAS expressing cells. The Ribosomal Protein S29 (RPS29) can enhance the tumor suppressor function of RAP-1 [Kondoh et al. 1996].

ARHI (RAS Homolog Member I, NOEY-2) {1p31} encodes a 26 kD GTPase with 50–60% amino acid homology to RAS and RAP. ARHI contains a highly conserved GTP-binding domain, a putative effector domain distinct from that of RAS and RAP proteins, and a COOH-terminal membrane localization motif. Also ARHI and RAS proteins share similar GTP/GDP-binding domains, they exert opposite functions. ARHI is a tumor suppressor in the RAS superfamily.

- Mutant forms of RAS that stabilize the GTP bound state contribute to tumor formation. About 30% of cancers have mutations in *ras*. The highest rates occur in adenocarcinomata of the pancreas (90%), the colon (50%), follicular and undifferentiated carcinomata of the thyroid (50%), and the lungs (30%). Mutations that change the amino acid residues 12, 13, or 61 activate the potential of RAS to transform cells. Common mutations include for the N-RAS gene product, Q61R (lung carcinoma) and for the H-RAS gene product Q61R (renal pelvic carcinoma), Q61L (lung carcinoma), Q61K (thyroid cancer), G12V (bladder carcinoma), and G12D (mammary carcinosarcoma). The *ras* mutations in colorectal

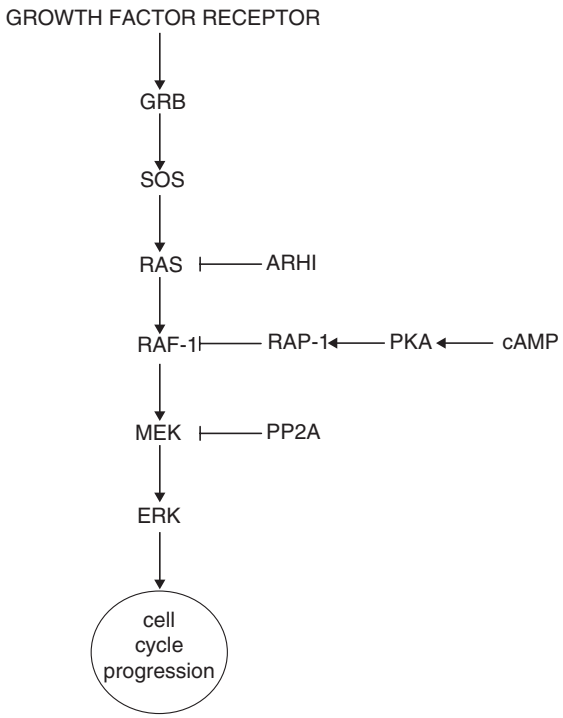


Figure 3.3.1.E. Negative regulation of RAS signaling. The ligation of growth factor receptors often activates the RAS pathway and leads to cell cycle progression through MEK and ERK. This cascade is subject to negative regulation, or inhibition at multiple levels. The negative regulators PP2A, RAP-1, and ARHI have tumor-suppressing properties.

carcinoma are typically acquired activating mutations of *K-ras*. A point mutation in the second exon of the *H-ras-1* gene may occur in melanoma.

- The highly polymorphic *H-ras-1* mini-satellite locus downstream of the proto-oncogene consists of four common progenitor alleles and a multitude of rare alleles, which may derive from mutations of the progenitors. Polymorphisms in this locus have significant associations with carcinomata of the breast, colorectum, and urinary bladder, and with acute leukemia [Krontiris et al. 1993].
- Costello syndrome (Faciocutaneoskeletal syndrome, FCS syndrome) comprises short stature, redundant skin of the neck, palms, soles, and fingers, curly hair, papillomata around the mouth and nares, and mental retardation. The levels of the catecholamine metabolites vanillylmandelic acid and homovanillic acid in the urine of patients with Costello syndrome are elevated. The condition is caused by the germline mutation G12V of H-RAS. Some patients develop rhabdomyosarcoma [Kerr et al. 1998] or bladder carcinoma [Franceschini et al. 1999], suggesting an increased tumor risk associated with the disease.
- Normal cells respond to inappropriate growth signals by inducing tumor suppressor genes. In these cells, the expression of oncogenic RAS induces the expression of $p16^{INK4a}$ and *p53*. Therefore, immortalization, which in epithelial cells is frequently associated with the loss of P16, is a prerequisite for transformation by RAS.
- In the presence of active RAS, TGF-β turns from a tumor suppressor, capable of inducing growth arrest and apoptosis into an inducer of tumor progression. This is due to the induction of MAPK and PI 3-K by RAS. The MAPK pathway cooperates with TGF-β to drive metastasis, while signaling through Phosphatidylinositol 3-Kinase protects from the lethal effects of TGF-β.
- RAS-GAPs are negative regulators of RAS signaling, because they stimulate the hydrolysis of GTP by RAS. However, in cancer cells that express an oncogenic form of RAS, RAS-GAP can no longer inactivate RAS signaling. Instead, it acts as a RAS effector, promoting cell proliferation. An interaction between the SH3 (SRC homology 3) domain of RAS-GAP and the kinase domain of the serine/threonine kinase Aurora may account for this effect. The interaction may directly or indirectly regulate Aurora activity, which is required for mitosis. The antiapoptotic protein Survivin can form a complex with Aurora and RAS-GAP, with the ternary complex regulating cell division and apoptosis in tumor cells [Gigoux et al. 2002].
- *B-raf* is frequently mutated in cancer and becomes oncogenic. Mutations occur in the exons 11 and 15. They include V600, L597, D594, G468, G466, and G464 and increase the kinase activity of B-RAF. *B-raf* is mutated in about two thirds of all melanomata [Davies et al. 2002], in sporadic colorectal tumors with microsatellite instability, in low-grade ovarian serous carcinoma, and in thyroid papillary cancer. 80% of tumor-associated mutations in *B-raf* correspond to the hot spot transversion mutation T1799A that causes the amino acidic substitution V600E. The other 20% accounts for a wide variable range of missense mutations, all of which reside in the glycines of the G-loop in the exon 11 or in the activation segment in exon 15 near the V600. The mutation V600E confers transformant activity to the cells because it mimics the phosphorylations of T599 and S602.
- *c-raf-1* may be involved in mixed parotid gland tumors with the t(3;8)(p25;q21) translocation.
- Neurofibromatosis 1 (NF1) is a autosomal dominant familial cancer syndrome, in which patients develop multiple benign and malignant tumors of the central and peripheral nervous system. Consistent features of this disorder are café au lait spots and fibromatous skin tumors. Type I von Recklinghausen [von Recklinghausen 1882] or classical peripheral neurofibromatosis (NF1) is relatively common with a prevalence of 1 in 3,000 live births in Western countries. A *nf1* microdeletion is the most frequent mutation in individuals with neurofibromatosis 1. The loss of both *nf1* alleles is causative for the fibromata, myeloid leukemias (especially, juvenile myelomonocytic myeloid leukemia, JMML), and pheochromocytomata associated with the syndrome. It also leads to the development of numerous neurofibromata in the Schwann cells of peripheral nerves, which become malignant peripheral nerve sheath tumor (MPNST) in 3–15% of cases. Although the benign tumors of neurofibromatosis are multiclonal, the malignant lesions, neurofibrosarcomata, are monoclonal. Gastrointestinal complications of neurofibromatosis 1 arise during midlife, later than the cutaneous lesions, with a frequency of 12–60% of cases. Pheochromocytoma is not the only cause of hypertension in patients with neurofibromatosis 1, renal artery stenosis due to vascular neurofibromatosis is a relatively common cause.

- Expression of the oncogenic COOH-terminally truncated COT results in substantially increased SAPK and ERK activation, because the negatively regulating activity of the COOH-terminal domain is lacking. Consecutive to a 3′ end mutation, *cot* (*map3k8*) becomes a transforming gene in lung adenocarcinoma. The mutation is localized to exon 8.
- The oncogenic potential of COT is associated with thymomata. In correspondence with the broad effector specificity of COT, COT-dependent transformation requires P38, SAPK, and ERK5.
- In melanoma cells, the signaling cascade ASK1→MKK6→$P38^{HOG}$ silences the *cd95* (*fas*) promoter via a NF-κB/SP-1 site. This occurs through inhibition of I-κBα phosphorylation, thereby limiting NF-κB activity. The lack of CD95 expression renders the melanoma cells resistant to apoptosis [Ivanov and Ronai 2000].
- AP-1 is constitutively activated with robust JUN and JUN-B overexpression in Hodgkin lymphoma and anaplastic large cell lymphoma (ALCL), but not in other lymphoma types. While AP-1 supports proliferation of Hodgkin cells, it suppresses apoptosis in ALCL cells. Furthermore, the BCR–ABL fusion protein activates the JNK signaling pathway in hematopoietic cells and increases transcriptional activity mediated by the transcription factor AP-1. JNK-1 is required for the survival of the transformed cells in the absence of stromal support. Failure to survive is associated with decreased expression of BCL-2.
- Transformed cells express elevated levels of eIF4E. This leads to disordered growth and enhances the translation of *cyclin* D_1 mRNA. Oncogenic RAS induces increased phosphorylation of eIF4E, and the ability of RAS to transform cells is diminished when eIF4E expression is decreased, implying that eIF4E is a key mediator of RAS-induced transformation [Flynn and Proud 1996].
- ARHI is consistently expressed in normal breast epithelial cells but is dramatically down-regulated in more then 70% of breast and ovarian cancers.

3.3.2 Protein Kinase C pathways

Cascades of protein phosphorylation on serine or threonine residues can transduce signals to the nucleus. They may be activated after ligation of protein tyrosine kinase receptors via PLC-γ, or after engagement of G-Protein-coupled receptors via PLC-β. An important pathway in cell cycle progression is constituted by the hydrolysis of phosphatidylinositol 4,5-bisphosphate by Phospholipase C to diacylglycerol and inositol 1,4,5-trisphosphate. While inositol 1,4,5-trisphosphate stimulates calcium mobilization, diacylglycerol activates PKC.

Protein Kinases C constitute a family of 12 distinct serine/threonine kinases that participate in signaling involved in cellular proliferation and differentiation. All forms are composed of a NH_2-terminal regulatory domain and a COOH-terminal catalytic domain (Figure 3.3.2.A). Two characteristic cysteine-rich repeats are conserved among nearly all members of the PKC family. The classical PKCs α, β1, β2, and γ are calcium dependent. The PKCs δ, ε, η, and θ, do not depend on calcium. The atypical PKCs ζ (PKC2) and ι/λ do not bind either calcium or phorbol esters. The PKC β gene codes for two distinct proteins generated by alternative splicing. A distinct subclass of PKCs with unique characteristics is constituted by PKC μ (PKD) and ν, which, unlike other PKCs, contain a Pleckstrin homology domain. PKC μ is a downstream target of the β and γ subunits of heterotrimeric G-Proteins. Gβγ binds to the Plekstrin homology domain, resulting in the activation of PKC μ. This interaction regulates the dynamics of Golgi membranes and protein secretion.

Adapter proteins are key to organizing signaling enzymes near their select substrates and away from others in order to optimize the precision and speed of the response. RACKs (Receptors for Activated C-Kinase) are isoenzyme-selective adapter proteins for individual PKC isoenzymes. In addition to anchoring activated PKC isoenzymes, RACKs anchor other signaling enzymes.

– RACK1, the anchoring protein for activated PKCβII, binds the tyrosine kinase SRC, certain Integrins, and Phosphodiesterase.
– RACK2, the PKCε-specific RACK, is a coated vesicle protein and thus is involved in vesicular release as well as cell–cell communication.

At least some of the proteins that bind to RACKs, including PKC itself, regulate cell growth [Schechtman and Mochly-Rosen 2001].

PKC signaling induces the expression of various genes that drive cell cycle progression.

– PKC may activate the MAP Kinase pathway, resulting in cell proliferation.
– Members of the PKC family of enzymes are capable of translocating to the nucleus or are resident within the nucleus. PKC can alter transcription through the phosphorylation of AP-1 (Activator Protein-1).

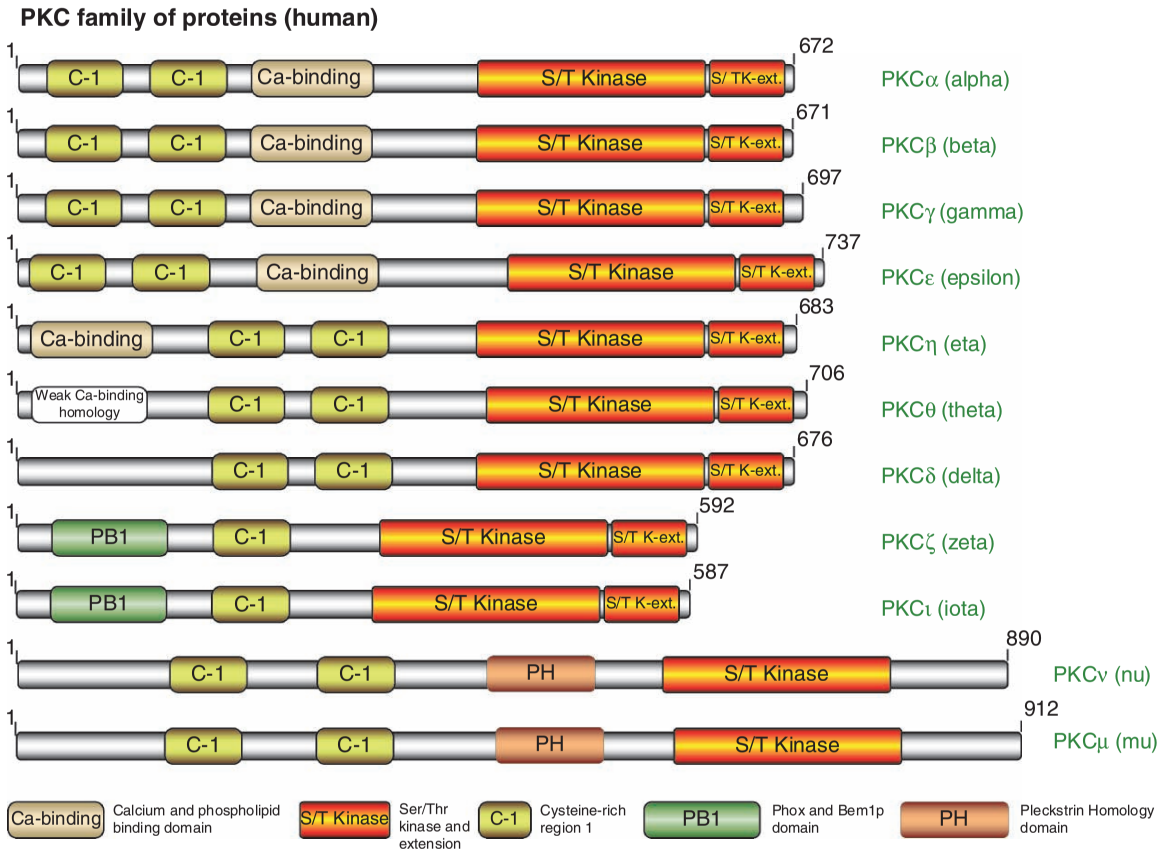


Figure 3.3.2.A. PKC structure. The PKC family contains multiple proteins, which share conserved domains. A cysteine-rich region and the catalytic domain for serine/threonine kinase activity are common to all of them. The various forms are differentially expressed and serve in distinct roles in cell physiology. [Reproduced from http://visiscience.com/scienceslides.php. With permission.]

– PKC can phosphorylate I-κB, targeting it for degradation and allowing NF-κB to shuttle to the nucleus, where it induces the transcription of its target genes. NF-κB may prevent apoptosis and facilitate invasion (Figure 3.3.2.B).

The SRC family of tyrosine kinases consists of SRC (Sarcoma Oncogene) {20q12–q13} and eight closely related proteins (the ubiquitously expressed FYN and YES, and the tissue-specific BLK, YRK, FGR, HCK, LCK and LYN). SRC kinases play an important role in signaling from antigen receptors on B- and T-lymphocytes. There are four SRC homology domains (SH1, SH2, SH3, and SH4). SH1 contains an autophosphorylation site and the kinase domain, SH2 has the capacity to bind to PDGFR, SH3 promotes intramolecular contact with the kinase domain in the inactive state, SH4 contains the myristoylation site that is important for membrane localization. The COOH-terminal tail negatively regulates SRC activity. SRC contains a unique NH_2-terminus of unknown function.

SRC is activated in response to ligation of PDGFR, EGFR, and FGFR. This entails the following changes:

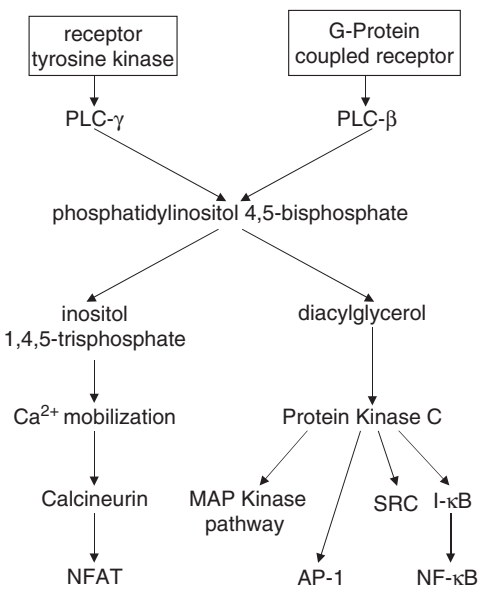


Figure 3.3.2.B. PKC-associated signaling pathways. PKC is linked to growth factor receptors through Phospholipase-C and phosphatidylinositol metabolites. The cleavage of phosphatidylinositol bisphosphate to inositol trisphosphate and diacylglycerol activates two downstream pathways. Inositol trisphosphate mediates calcium mobilization, while diacylglycerol induces PKC kinase activity. PKC has multiple downstream targets that lead to gene activation supporting cell growth and motility.

– SRC autophosphorylates on tyrosine 419, and this is required for optimal activity.
– In the absence of mitogenic stimulation, SRC is maintained in its inactive form by phosphorylation of its tyrosine residue 530. Dephosphorylation of this site turns SRC into an open conformation. This is permissive for the engagement of the SH2 and SH3 domains by other signal transduction molecules. The dephosphorylation reaction can be catalyzed by PTPα, SHP1, SHP2, and PTP1B.
– The family of adapter molecules SIN (EFS1), CAS ($P130^{CAS}$, CRK-Associated Substrate, BCAR1, Breast Cancer Anti-estrogen Resistance 1), and HEF1 (Human Enhancer of Filamentation-1) contain an SH3 domain, proline-rich sequences that bind to SH3 domains, and conserved tyrosine residues that are subject to phosphorylation and mediate interactions with SH2 domain containing substrates. They bind to the SH2 and SH3 domains of SRC and result in the open, activate configuration.

SRC signaling proceeds through CRK and RAP-1. SRC activates promoters under the control of the phorbol ester (TPA, PMA) response element and the serum response element via two distinct intracellular signal transduction mechanisms. The induction of the phorbol ester response element depends on PKC, whereas the induction of the serum response element depends on RAF-1 [Qureshi et al. 1992]. SRC activation leads to gene expression of *hif-1*, *cathepsin L*, and *cyclin* D_1, whereas *marcks*, *fibronectin*, and *drs* transcripts are down-regulated.

SRC signaling is subject to negative regulation.
– The homologous tyrosine kinases CSK and CHK can phosphorylate tyrosine 530 and inactivate SRC.
– SRC is subject to Ubiquitin-dependent degradation. SRC, and probably also FYN, bind to and phosphorylate the proto-oncogene product and E3 Ubiquitin Ligase c-CBL. This activates CBL to recruit other effector molecules.

Alterations in PKC signal transduction contribute to various tumors.

- PKC mediates cell proliferation in response to tumor promoters of the phorbol ester group. The classical PKCs α, β1, β2, and γ can be activated by exposure to phorbol esters.
- A point mutation in PKCα is expressed in a subpopulation of pituitary adenomata, characterized by their invasive phenotype. The same lesion arises in

some thyroid neoplasms. The point mutation is located at position 294 of the protein, in the V3 region, leading to a substitution of a negatively charged aspartic acid by an apolar glycine. In response to exposure to phorbol ester, wild-type PKCα mainly translocates to the plasma membrane, but mutant PKCα translocates mainly to the perinuclear region. The cells that express mutant PKCα display dysregulated growth [Alvaro et al. 1992; Alvaro et al. 1993; Alvaro et al. 1997].

- Amplification of the chromosome locus 2p21 occurs in about 30% of thyroid neoplasms. This causes a rearrangement and amplification of the *pkcε* gene and results in the overexpression of a chimeric truncated *pkcε* mRNA, coding for the NH_2-terminal amino acids 1–116 of the enzyme form, fused to an unrelated sequence. Cells expressing the truncated PKCε are resistant to apoptosis. This is associated with higher BCL-2 levels, a marked impairment in P53 stabilization, and dampened expression of BAX [Knauf et al. 1999].
- In malignant breast tumors, PKC activity is elevated compared with normal breast tissue [Valette et al. 1987]. There is also an inverse relationship between the level and activity of PKC and the level of Estrogen Receptor expression [Borner et al. 1987; Wyss et al. 1987].
- PKC plays a key role in cell differentiation, a process that is impaired in leukemia. Leukemia cells express three major forms of PKC, α, β_{II}, and ι. PKC β_{II} is required for leukemia cell proliferation. This kinase is activated just prior to mitosis and phosphorylates the nuclear envelope protein Lamin B. This triggers the disassembly of mitotic nuclear lamina [Hocevar et al. 1993]. The lack of PKC β_{II} action at this point leads to cell cycle arrest in G_2. PKC ι plays a critical role in the resistance of leukemia cells to apoptosis [Murray and Fields 1997]. PKCθ, which is expressed relatively selectively in T-lymphocytes, plays an important role in proliferation upon its translocation to the plasma membrane. It induces Interleukin-2 gene expression through activation of the transcription factors AP-1 and NF-κB. In some leukemia, PKCθ constitutively localizes to the plasma membrane and protects the leukemic T-lymphocytes from CD95-induced apoptosis.
- Malignant gliomata express higher levels of PKC α and lower levels of PKC δ than low-grade astrocytomata. PKC α induces enhanced proliferation and reduced expression of Glial Fibrillary Acetic Protein (GFAP), conversely PKC δ suppresses proliferation and induces the expression of GFAP. GFAP is a marker of astrocyte differentiation.
- At least some of the proteins that bind to RACKs, including PKC itself, regulate cell growth and may contribute to carcinogenesis [Schechtman and Mochly-Rosen 2001].
- Elevated PKC βII is an early promoter in colon carcinogenesis. Cellular SRC activity is frequently elevated over that in adjacent normal mucosa, with activation being linked to malignant potential. There is some elevation of SRC activity in premalignant lesions and in adenomata, indicating that increased SRC activity can be an early event in transformation. A progressive increase in c-SRC activity occurs as the tumor stage advances. SRC activity is generally highest in malignant polyps. The greatest increases in activity and protein levels are observed in liver metastases [Talamonti et al. 1993]. A subset of advanced metastatic colon cancers harbor an activating mutation within the COOH-terminus of SRC, leading to the production of a transforming protein. A truncating mutation in SRC at codon 531 arises in 12% of cases of advanced colon cancer [Irby et al. 1999]. A C→T transition mutation in *src* occurs in a fraction of colon cancers. It results in the truncation of SRC at tyrosine 530 and elevated kinase activity.
- Elevated levels of SRC in epidermal cells predispose to squamous cell carcinoma [Matsumoto et al. 2003]. SRC expression is also frequently elevated in pancreatic, ovarian, esophageal, gastric, lung, head, and neck cancers [Frame 2002].
- NH_2-terminal myristylation of SRC is required for its association with cellular membranes and is essential for the transforming function of oncogenic SRC mutants.
- A functional loss in the control of SRC signaling can lead to transformation. The kinase CSK, a negative regulator of SRC, is reduced in hepatocellular carcinoma as compared to normal liver tissue, and this altered expression correlates with enhanced SRC activity [Masaki et al. 1999]. The phosphatase PTP1B is elevated in breast cancer cells, where it can dephosphorylate and activate SRC [Bjorge et al. 2000].

3.3.3 The Lipid Kinase pathway

The proto-oncogene product Phosphatidylinositol 3-Kinase (Phosphatidylinositol 3′-Kinase, PI 3-K) is a phosphoinositide kinase specific for the D-3 position of the inositol ring. It catalyzes the synthesis of phos-

phatidylinositol 3,4,5-trisphosphate (PI 3,4,5-P_3) or phosphatidylinositol 3,4-bisphosphate (PI 3,4-P_2), which are ligands for Plekstrin homology domains in various proteins. Phosphatidylinositol 3-Kinase associates with the activated receptors for PDGF, Insulin, MET, and CSF-1, and with the proto-oncogene products PKB, PDK1, SRC, FMS, YES, and CRK. All forms of Phosphatidylinositol 3-Kinase thus couple various classes of receptors to kinases containing Plekstrin homology domains. Downstream targets of this signaling cascade may be calcium mobilization, phosphotyrosine signaling, activation S6 Kinase, ERK-1, and ERK-2.

Phosphatidylinositol 3-Kinases exist as heterodimers consisting of a unique catalytic domain (P110α {3q26.3}, β {3}, γ {7q22}, or δ {1p36.2}) along with a number of shared regulatory subunits (P85α {5q13}, β {19q13.2–q13.4}, or γ {1}). Multiple Phosphatidylinositol 3-Kinase forms are characterized by their subunit composition. Further diversity is generated by alternative splicing of the genes that encode the regulatory domains. The splice variants P55α and P50α retain the SH2 domains and the domain that binds to P110, but lack NH_2-terminal SH3 (SRC homology 3) and BCR (breakpoint cluster region) domains. The splice variants activate P110 more efficiently than P85, presumably because the spliced-out regions have negative regulatory roles. The P85 subunits bind through their SH2 domains to various cytoplasmic and receptor tyrosine kinases and are substrates for them. The P110 subunits contain a domain for interaction with P85 and RAS, a C2 domain that may be important for membrane anchoring, and a kinase domain. The subunits P110α, β, and δ are activated by tyrosine kinase receptors through adapter molecules. P110γ is activated by heterotrimeric G-Proteins.

The proto-oncogenic serine/threonine kinase PKB-1 (Protein Kinase B α, PKBα, AKT-1) {14q32.3} is a downstream effector of Phosphatidylinositol 3-Kinase (Figure 3.3.3.A). Closely related genes encode PKB-2 (PKBβ, AKT-2) {19q13.1–q13.2} and PKB-3 (PKBγ, AKT-3) {1q43–44}. PKB contains a NH_2-terminal Plekstrin homology domain, which functions as a lipid binding module, a central catalytic domain, and a short COOH-terminal regulatory domain. PKB is activated by a dual regulatory mechanism that requires both translocation to the plasma membrane and phosphorylation in the activation loop at threonine 308 and serine 473. Phosphatidylinositol-3,4,5-trisphosphate or, to a lesser extent, phosphatidylinositol-3,4-bisphosphate interact with the Pleckstrin homology domain of PKB and promote its translocation to the plasma membrane. There, the serine/threonine kinase PDK1 (3-Phosphatidylinositol-Dependent Protein Kinase 1) phosphorylates PKB on threonine 308. PKB activation is further enhanced by phosphorylation on serine 473 in the COOH-terminal tail, which occurs through

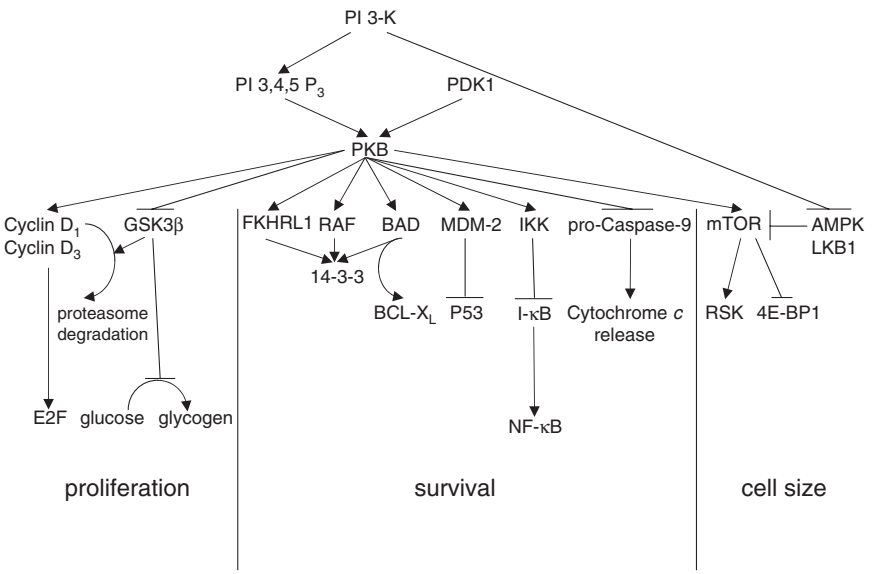


Figure 3.3.3.A. PKB-associated signal transduction pathways. PKB is an essential mediator of the lipid kinase signal transduction pathway. It exerts effects that lead to cell cycle progression, cell survival, and regulation of cell size. In all cases, multiple signaling intermediates synergize to induce the biological effect. PI 3,4,5 P_3 = phosphatidylinositol 3,4,5-trisphosphate.

autophosphorylation or through PDK2 (a complex of Rictor and mTOR). PKB phosphorylation mediates its translocation from the plasma membrane to the cytosol or nucleus, where it exerts its catalytic activity. PKB phosphorylates sites with the consensus sequence RXRXX(S,T).

PKB signaling plays key roles in cell cycle progression, cellular survival, and increased cell mass.

– Cell cycle progression: PKB activation contributes to cell proliferation by enhancing the translation of the mRNAs for *cyclin* D_1 and *cyclin* D_3, which leads to increased E2F activity and progression into S phase. PKB also has an important role in preventing Cyclin D_1 degradation by regulating the activity of Glycogen Synthase Kinase 3β. After phosphorylation by Glycogen Synthetase Kinase 3β (GSK3β), Cyclin D_1 is targeted for degradation by the proteasome. PKB phosphorylates GSK3β and blocks its activity, thereby allowing Cyclin D_1 to accumulate.
– Antiapoptosis: An important antiapoptotic pathway mediated by PKB proceeds predominantly through the phosphorylation of a Forkhead transcription factor, which retains it in the cytoplasm by binding to a 14-3-3 protein. Phosphorylation of FKHRL1 or RAF promotes binding by 14-3-3 proteins to these substrates. In both instances, PKB inhibits the function of its substrates. Similarly, PKB phosphorylates and inactivates BAD. This promotes the binding of 14-3-3 proteins to BAD and releases BAD from its heterodimer with BCL-X_L, which restores the antiapoptotic function of BCL-X_L. Through another mechanism, rescue from P53-induced apoptosis by survival factors may be associated with the activation of PKB. PKB phosphorylates MDM2 on residues 166 and 186. This facilitates the entry of MDM2 into the nucleus, where it binds to P53 and targets it for ubiquitination and degradation. In addition, PKB also may have a role in blocking apoptotic signaling downstream of P53. Furthermore, NF-κB promotes survival in response to various apoptotic stimuli. PKB can exert a positive effect of NF-κB function by phosphorylation and activation of IKK, which induces the degradation of I-κB and facilitates the translocation of NF-κB into the nucleus. PKB activation may increase cell survival through the phosphorylation of pro-Caspase-9 and prevention of its proteolytic activation, through inhibiting Cytochrome *c* release from the mitochondria, and through supporting the overexpression of *bcl-2* or *bcl-*X_L.
– Increase in cell size: The serine/threonine kinase mTOR (FRAP1) is a PKB substrate and a central regulator of cell size. It controls biogenesis on the basis of the availability of nutrients by activating $P70^{Ribosomal\ S6\ Kinase}$ (RSK), which enhances the translation of mRNAs that have 5′ poly-pyrimidine tracts. mTOR also inhibits the translational repressor 4E-BP1 [Vivanco and Sawyers 2002]. The tumor suppressor and erine/threonine kinase LKB1 (Serine/Threonine Protein Kinase 11, STK11) {19p13.3} negatively regulates mTOR signaling [Shaw et al. 2004]. This is accomplished by a protein complex containing AMPK (AMP-Activated Protein Kinase). In response to cellular stresses or increased AMP to ATP ratios, AMPK activates biosynthetic pathways that generate ATP, while suppressing pathways that consume ATP. The activation of AMPK requires phosphorylation on threonine 172 [Hawley et al. 2003]. PI 3-K can prevent this activation.

The serine threonine kinase PKB is a principal target of Insulin signaling. In response to Insulin signaling, it directly phosphorylates GSK3 on serine 9 and inhibits its activity, ultimately leading to the conversion of glucose to glycogen. TRB3 (TRIB3) promotes glucose output from the liver under fasting conditions by binding to and interfering with PKB phosphorylation in response to residual Insulin signaling. This pathway is also involved in IGF signaling.

tcl-1, *tcl-1b*, and *mtcp-1* are members of one gene family. All three proteins expressed from these genes interact with PKBα and PKBβ, but only the 14 kD protein TCL-1 (T-Cell Leukemia-1) interacts with PKBγ. TCL-1 forms trimeric complexes, which interact with three molecules of PKB, resulting in PKB oligomerization. This may promote PKB activation and translocation to the nucleus.

The proto-oncogene product ABL signals through the PI 3-Kinase pathway. Efficient transformation by ABL requires its protein tyrosine kinase activation and membrane association via a NH_2-terminal myristoylation. PI 3-Kinase associates with autophosphorylated, activated protein tyrosine kinase variants of the ABL protein [Varticovski et al. 1991]. The regulatory subunit of PI 3-Kinase, binds directly to the SH3 domain of ABL and to its oncogenic variant BCR-ABL.

Several Phosphatidylinositol Trisphosphate Phosphatases (PIP_3 Phosphatases), including PTEN,

SHIP-1, and SHIP-2, negatively regulate lipid kinase signal transduction. While PTEN limits basal PKB activity, SHIP may regulate PKB activation in response to growth factor stimulation. Protein phosphatases, including CIP and PP2A, can inactivate PKB through dephosphorylation. A unique mechanism of PKB inactivation is exerted by CTMP, which binds to PKB and prevents its downstream signaling.

– Counteracting PKB, the tumor suppressor PTEN (Phosphatase and Tensin Homolog Mutated in Multiple Advanced Cancers, MMAC1) {10q23.31} dephosphorylates the three positions of the inositol ring in phosphatidylinositol 3,4,5-trisphosphate or phosphatidylinositol 3,4-bisphosphate, prevents PKB kinase activation, allows a forkhead transcription factor to shuttle into the nucleus, and leads to apoptosis or to cell cycle arrest in G_1. PTEN consists of a catalytic NH_2-terminal phosphatase domain that acts on both protein and lipid substrates and a COOH-terminal C2 domain that interacts with lipid substrates in a calcium-independent manner. The 403 amino acid sequence of PTEN contains a protein tyrosine phosphatase domain and it suppresses tumor cell growth in part by antagonizing protein tyrosine kinases. The growth suppression activity of PTEN modulates G_1 cell cycle progression through negatively regulating the Phosphatidylinositol 3-Kinase→PKB signaling pathway. One critical target of this signaling process is the CDKI $P27^{KIP1}$. The proto-oncogene product DJ-1 {1p36} negatively regulates PTEN and thus supports the activation of PKB [Nagakubo et al. 1997; Kim et al. 2005].
– The SHIP Phosphatases (Inositol Polyphosphate-5-Phosphatase, INPP5) act on phosphatidylinositol trisphosphate to remove a phosphate from the fifth position of inositol, typically generating phosphatidylinositol 3,4-bisphosphate. SHIP-1 and SHIP-2 contain a NH_2-terminal SH2 domain, a catalytic domain, and several COOH-terminal proline-rich regions, which bind to SH3 domains and NPXY phosphorylation motifs. SHIP-1 is primarily expressed in hematopoietic cells. In contrast, SHIP-2 is expressed ubiquitously.
– Frequently, PKB phosphorylation on serine 473 and threonine 308 is coregulated. However, CIP (Ceramide Induced Phosphatase) inhibits PKB by dephosphorylating serine 473, but not threonine 308. This leads only to partial inhibition.
– PP2A is a heterodimeric phosphatase, consisting of a catalytic subunit and a regulatory subunit, which inactivates PKB. The association of PKB with HSP90 and CDC37 protects it from dephosphorylation by PP2A and from degradation in the proteasome, thereby preventing its inactivation.
– CTMP (Carboxyl-Terminal Modulator Protein) {1q21} is a 240 amino acid protein, which binds PKB and prevents its phosphorylation and downstream signaling. CTMP binds to the COOH-terminal regulatory domain of PKBα at the plasma membrane. The interaction reduces the activation of PKB by inhibiting its phosphorylation and consecutively the phosphorylation of its substrates. CTMP can reverse v-AKT induced tumorigenicity [Maira et al. 2001].

Tuberin, the product of the *tsc-2* (*tuberous sclerosis complex-2*) {16p13.3} tumor suppressor gene of 41 exons is a 1,807 amino acid phosphoprotein that negatively regulates Phosphatidylinositol 3-Kinase signaling downstream of PKB. Phosphorylation of Tuberin on serine 939 by PKB relieves the Tuberin-mediated suppression of Phosphatidylinositol 3-Kinase signaling by facilitating the sequestration of Tuberin by 14-3-3 proteins. All seven forms of 14-3-3 proteins bind to the serine 939 containing domain in a phosphorylation-specific manner.

In various cancers, the lipid kinase signaling pathway is up-regulated (Table 3.3.3.A).

- The gene coding for Phosphatidylinositol 3-Kinase P110α (*pik3ca*) is amplified in a portion of cervical and ovarian tumors. Somatic mutations of *pik3ca* (Figure 3.3.3.B) occur in colon cancer (around 30%), brain cancer (aroung 25%), glioblastomata (around 25%), and gastric cancer (around 30%) [Samuels et al. 2004]. Somatic mutations of the gene for the P85α subunit (*pik3r1*), comprising deletions in the inter-SH2 region proximal to the S608 autoregulatory site, may contribute to colon and ovarian cancers.
- Deregulated signaling through Phosphatidylinositol 3-Kinase is common in glioblastoma. Activation of PKB occurs in high-grade glioblastoma multiforme. This may be due to the expression of the mutant EGF Receptor vIII or to the loss of *pten*. The loss of PTEN is highly correlated with activation of PKB. PKB activity, in turn, is correlated with the phosphorylation of mTOR, of the Forkhead transcription factors (FOXO1, FOXO3a, and FOXO4), and of RSK [Choe et al. 2003].
- Cyclooxygenase-2 expression plays an important role in Ultraviolet B-induced tumor promotion in

Table 3.3.3.A. Deregulation of PI-3K signaling in cancer. Excessive signaling through the lipid kinase pathway may be caused by various modes of overactivation of intermediates or by loss of function of negative regulators. [From Vivanco and Sawyers 2002]

pten mutations	Glioblastoma
	Ovarian carcinoma
	Endometrial carcinoma
	Hepatocellular carcinoma
	Melanoma
	Renal cell carcinoma
	Thyroid carcinoma
	Lymphoid malignancies
PTEN inactivation	Lung cancers
pten silencing	Endometrial carcinoma
	Melanoma
Allelic imbalance of *pten*	Ovarian carcinoma
	Breast carcinoma
Aberrant *pten* transcripts	Cancers of the digestive tract
Elevated PKB1 kinase activity	Breast carcinoma
	Ovarian carcinoma
pkb amplification and overexpression	Ovarian carcinoma
	Breast carcinoma
	Thyroid carcinoma
pi3k p110α amplification	Ovarian carcinoma
pi3k p85α mutation	Ovarian carcinoma
	Cancers of the digestive tract
rsk amplification and overexpression	Breast carcinoma

the skin. Ultraviolet B induces Phosphatidylinositol 3-Kinase activity with the consecutive phosphorylation of PKB on threonine 308 and serine 473 and inhibition of GSK-3β. This leads to the activation of *cox-2* gene expression in keratinocytes [Tang et al. 2001].

- *tcl-1*, *tcl-1b*, and *mtcp-1* are members of a gene family that is involved in the induction of various forms of T-cell leukemia. Physiologically, nonlymphoid cells with the exception of ovarian tissue are negative for TCL-1 expression. The initiation of expression of the *tcl-1* gene {14q32.1} in T-cell chronic lymphocytic/prolymphocytic leukemia, a lymphoproliferative disease derived from postthymic T-cells, leads to an interaction between TCL-1 and PKBα Kinase through the PKB Pleckstrin homology domain. This promotes PKB oligomerization, the translocation of PKB into the nucleus and an increase in its enzymatic activity. In the nucleus, PKB may phosphorylate and inactivate DNA binding by NUR77 (NGFI-B, TR3), which is critical for certain forms of T-Cell Antigen Receptor (TCR)-mediated apoptosis [Pekarsky et al. 2001].
- An alteration that occurs at high frequency in a variety of tumors, is loss of heterozygosity at chromosome 10q23 [Li et al. 1997]. Although rarely seen in low-grade glial tumors and early stage prostate cancers, loss of heterozygosity at 10q23 occurs in approximately 70% of glioblastomata and approximately 60% of advanced prostate cancers. It contains the tumor suppressor gene *pten (phosphatase and tensin homolog deleted on chromosome 10)*. Somatic, genetic, and epigenetic inactivation of PTEN is involved in as high as 93% of sporadic endometrial carcinomata, irrespective of their microsatellite status, and can occur in the earliest precancers. In most cases of endometrial carcinomata and glioblastomata, somatic *pten* mutations result in protein inactivation and, as with germline mutations, recurrent somatic mutations are found in CpG dinucleotides. A mutagenesis by insertion–deletion in repetitive elements is however specifically associated with endometrial carcinomata [Bonneau and Longy 2000]. In acute leukemias and non-Hodgkin lymphomata, about half carry mutations or hemizygous deletions, while one third have low *pten* transcript levels, with more than half of them having low or absent PTEN protein. PTEN and phosphorylated PKB levels are inversely correlated in most tumors, consistent with PTEN-dependent regulation of phosphatidylinositol 3,4,5-triphosphates and apoptosis.
- Germline *pten* mutations cause Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome (Bannayan–Zonana syndrome, BZS), Lhermitte–Duclos disease (LD, cerebelloparenchymal disorder VI), and Proteus syndrome. Hyperplastic–dysplastic changes in the prostate, skin, and colon are characteristic of all four syndromes. Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome share the clinical characteristics of hamartomatous polyps of the gastrointestinal tract and mucocutaneous lesions. The characteristic pathologic features of Lhermitte-Duclos disease are global hypertrophy of the cerebellum, coarse gyri, and the typical "inverted cortex" pattern. Collectively, these are PTEN hamartoma–tumor syndromes [Marsh et al. 1999] with an increased risk of breast, thyroid, and endometrial cancers. Over 100 different germline *pten* mutations underlie these four tumor-predisposing syndromes. The mutations are scattered along the length of the gene, with the exception of exon 9 (no mutation known) and exon 1 (only two mutations known). A mutational hot spot is located

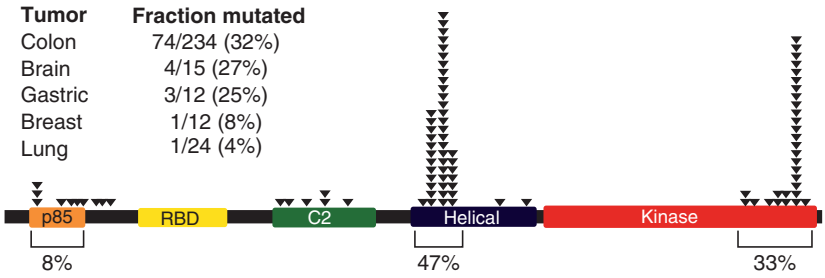


Figure 3.3.3.B. Mutations in PIK3CA. Mutations in the catalytic subunit of Phosphatidylinositol 3-Kinase. The arrowheads indicate the location of missense mutations, and boxes represent functional domains. They include the P85-binding domain, the RAS-binding domain, the C2 domain, the helical domain, and the kinase domain. The percentage of mutations detected within each region is indicated below, and the fraction of tumors with mutations is noted above. [Reproduced from Samuels-Lev et al. 2004. With permission.]

in exon 5, which encodes the phosphatase catalytic core motif, and recurrent mutations also arise at CpG dinucleotides implying mutations induced by deamination. Hot spot mutations on CpG islands are R233X, R235X, and R335X. The G129E mutation in the catalytic domain of PTEN, which disrupts the lipid phosphatase activity, but does not affect PTEN's ability to dephosphorylate protein targets, is specifically associated with Cowden syndrome patients. Hence, the loss of lipid phosphatase activity is sufficient to cause increased susceptibility to cancer. Because the G129E mutant is also defective in mediating G_1 arrest, the lipid phosphatase activity is needed to inhibit cell cycle progression.

- In primary non-small cell lung carcinomata, DJ-1 expression is often increased compared to the surrounding nonneoplastic lung tissue, and correlates positively with relapse incidence [Kim et al. 2005]. DJ-1 is also overexpressed in breast and prostate cancer. This leads to increased activity of the PKB signaling pathway.
- Mutations in the *lkb1* gene cause Peutz–Jeghers syndrome, a disorder associated with multiple gastrointestinal hamartomatous polyps and a 15-fold increased risk of developing cancer. The lack of LKB1 activity allows enhanced signaling through mTOR.
- Tuberous sclerosis occurs based on inherited loss-of-function mutations in the genes *tsc-1* or *tsc-2*. It predisposes to hamartomata in multiple organs, including the brain, skin and kidneys. *tsc-1* (*hamartin*) [van Slegtenhorst et al. 1997] has 23 exons, 21 of which are coding. The transcript is 8.5 kb long and encodes for a 1,164 amino acid transmembrane protein. TSC-1 forms a complex with TSC-2, with both being important in the prevention of hamartomata.

3.3.4 The JAK/STAT pathway

JAK Kinases (Janus Kinases, JAKs) are universally required for signal transduction from cytokine receptors. Because cytokine receptors do not have intrinsic tyrosine kinase activity JAKs serve as the receptor-associated tyrosine kinases. Cytokine receptor ligation and dimerization recruits two molecules of JAKs, which then bind to two molecules of Signal Transducer and Activation of Transcription (STAT) proteins, leading to their dimerization. Interferon signaling is mediated by the JAK/STAT pathway. Similarly, the ligation of receptor tyrosine kinases, such as the EGF Receptor, recruits JAK and SRC, which phosphorylate and activate two molecules of STAT, inducing their dimerization.

There are four JAK proteins, JAK-1 {1p31.3}, JAK-2 {9p24}, JAK-3 {19p13.1}, and TYK-2 {19p13.2}. Upon receptor ligation, JAKs create the STAT docking sites by autophosphorylating and phosphorylating the cytoplasmic receptor domain. The downstream targets of JAK Kinases, the STAT proteins [Schindler et al. 1992; Fu et al. 1992] are critical in mediating virtually all cytokine signaling (Figure 3.3.4.A). This pathway transmits information directly from transmembrane receptors to target gene promoters, without the involvement of second messengers. There are seven known STATs, -1, -2, -3, -4, -5A, -5B, and -6 (Figure 3.3.4.B). In the absence of receptor activation, STATs are localized in the cytoplasm. Upon receptor ligation, they are recruited to the cytoplasmic tail through SH2 domains on STAT and phosphotyrosine moieties on the receptor. These interactions are highly specific and determine the selectivity of receptor mediated STAT activation. The tyrosine phosphorylation of STATs leads to their homodimerization and heterodimerization through

the interaction of their SH2

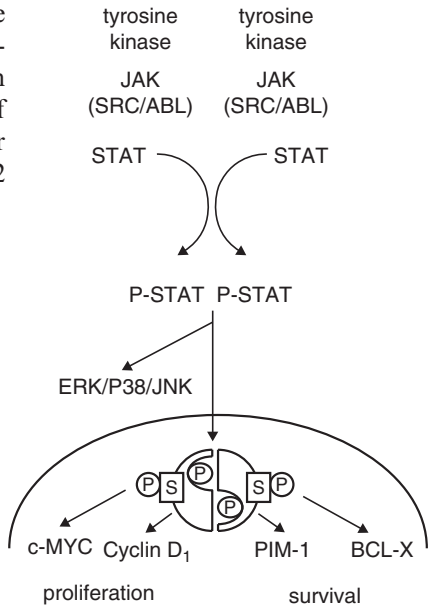


Figure 3.3.4.A. STAT signal transduction pathway. Signaling through JAK and STAT is typically activated in response to ligation of cytokine receptors. Once engaged by their cognate ligand, the receptor tyrosine kinases dimerize and autophosphorylate. This activates JAK and STAT proteins. The activated STATs translocate into the nucleus and initiate gene transcription. The biological outcome of the activation of STATs is often cell proliferation or antiapoptosis.

domains. STAT dimers are rapidly transported to the nucleus. STATs also undergo acetylation of lysine 685 by Histone Acetyl Transferases, which is essential for the formation of stable dimers. Most STATs recognize an inverted repeat element with the consensus sequence $TTX_{4-6}AA$ (*γ-interferon* activating sequence, GAS element). Dimers of STATs induce the transcription of genes for growth promoting or apoptosis preventing effectors, including *bcl-X*, *cyclin* D_1, and *c-myc*. The nonreceptor tyrosine kinases SRC and ABL may cause STAT phosphorylation and activation. JAK-2 or the RAC1 GTPase may bind to and mediate STAT3 activity.

MYC is a target transcription factor in the JAK/STAT pathway. The family of genes that encode the MYC proteins (c-MYC, N-MYC, L-MYC) generates transcription factors of the basic helix-loop-helix zipper family. The 64 kD protein MYC consists of an NH_2-terminal transcriptional activation domain, DNA binding and dimerization domains, and a binding site for the heterologous dimerization partner protein MAX, with which it forms an active complex. Because MAX contains basic helix-loop-helix and leucine zipper domains, but lacks the transactivation domain present in c-MYC, MYC-MAX dimerization is necessary for

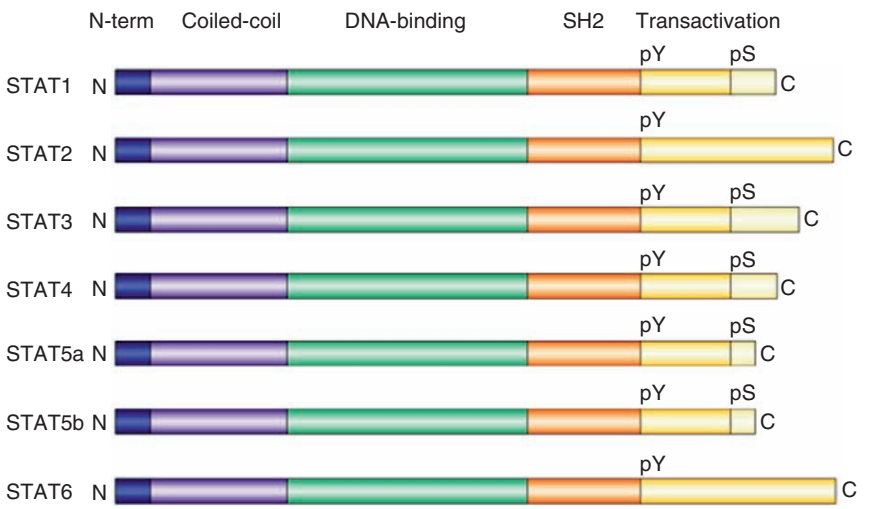


Figure 3.3.4.B. Domain structure of STAT family proteins. The domain structure of the seven STAT family members is shown. The NH_2-terminal domain (N-term) mediates the interaction between two STAT dimers to form a tetramer. This interaction is not essential for STAT function, but it can stabilize the binding of the two STAT dimers to adjacent sites of the DNA. The coiled-coil domain is involved in interactions with regulatory proteins and other transcription factors. The DNA-binding domain makes direct contact with STAT-binding sites in gene promoters, which have the consensus core sequence $TT(N_{4-6})AA$. Reciprocal interactions between the SRC homology 2 (SH2) domain of one STAT monomer and the phosphor-tyrosine (pY) residue of another mediate dimer formation, which is required for the binding of STATs to DNA. The transactivation domain is involved in the transcriptional activation of target genes through interactions with other proteins, such as Histone Acetyl Transferases. This COOH-terminal domain contains a site of serine phosphorylation (pS) that enhances transcriptional activity in some STATs. STAT5a and STAT5b are closely related proteins that are encoded by distinct genes. [Reproduced from Yu and Jove 2004. With permission from Macmillan.]

transcriptional activity. The heterodimer binds to the E box motif CAC(G,A)TG and interacts with TBP (TATA Binding Protein). *cdc25A*, *cyclin A*, and *cyclin E*, as well as *eif-2α* and *eif-4E* are effectors of MYC. A target gene of MYC is *cad*, which is required for DNA synthesis. c-MYC expression can bypass $P16^{INK4a}$-mediated growth arrest. MYC can act as a repressor of genes that contain the initiator element in their promoter. MYC represses the expression of the heavy chain of *ferritin* and stimulates the expression of the *iron regulatory protein 2* (*irp2*). Regulation of the genes controlling intracellular iron concentrations is essential for cell transformation by MYC. Consistently, a reduction in free iron leads to growth arrest and decreased synthesis of the cell cycle regulators $P34^{CDC2}$ and Cyclin A [Wu et al. 1999]. In nontransformed cells, the expression of the *myc* proto-oncogene is tightly linked to mitogenic stimuli and is a prerequisite for cell growth. *myc* is an early response gene, whose expression rises rapidly at the G_0 to G_1 transition and whose functions are largely linked to G_1 and early S progression. Unlike most early response genes, however, *myc* expression is sustained throughout the phases of the cell cycle. In the absence of mitogens, MYC forms heterodimers with MAD, and recruits mSIN3, NCOR, and Histone Deacetylase. IFN-γ and TGF-β lead to the rapid down-regulation of *myc* and these agents also mediate G_1 arrest. CTCF binds to a number of important regulatory regions within the 5′ noncoding sequence of *myc* and regulates *myc* expression. CTCF harbors several autonomous repression domains, including a zinc finger cluster, which silences transcription through binding directly to the corepressor SIN3A and recruiting Histone Deacetylases. Insulator elements, which act as a barrier to prevent neighboring cis-acting elements from regulating a distal gene, mediate their function by CTCF.

Members of the HMG (High Mobility Group) family of non-Histone proteins share a triplicate common DNA-binding domain, the AT hook, that specifically binds to the minor groove of A/T-rich sequences. HMG proteins function as gene transcriptional regulatory units. The HMGA (High Mobility Group A) family includes two alternatively spliced forms, HMGA1a and HMGA1b, of HMGA1 (HMGI-Y) and HMGA2. *hmgA1* is a target gene for MYC. Its promoter contains a MYC/MAX binding site, an E box at nucleotide-1,337. HMGA1 (High Mobility Group AT-Hook 1) {6p21} is important for the expression of *interferon-β*. The architectural transcription factor HMGA2 (HMGIC, BABL, LIPO) is expressed almost exclusively in undifferentiated mesenchymal cells. HMGA2 {12q14.3} plays a critical role in determining body size. The *hmgA2* gene spans more than 60 kb and consists of five exons, the first and last of which include long untranslated regions. The *hmg* genes are abundantly expressed during embryogenesis but not in normal adult tissues.

Negative regulation of STATs operates through tyrosine phosphatases, SOCS (Suppressor of Cytokine Signaling), and PIAS (Protein Inhibitor of Activated STATs). The SOCS family comprises at least eight members, SOCS-1–7 and CIS (Cytokine-Inducible SH2-Containing Protein). SOCSs bind to JAKs and inactivate them. PIAS binds to phosphorylated STAT dimers, preventing DNA recognition.

- Constitutively activated STATs exist in a wide variety of cancers, including almost all head and neck cancers. They are activated by tyrosine phosphorylation through the persistent activity of tyrosine kinases, including SRC, EGF Receptor, JAKs, or BCR-ABL. Such oncogenic tyrosine kinases are often activated as a consequence of permanent ligand–receptor engagement in autocrine or paracrine cytokine and growth factor signaling or represent constitutively active enzymes as a result of genetic alterations. Persistent signaling of specific STATs, in particular STATs -3 and -5, directly contributes to oncogenesis by stimulating cell proliferation and preventing apoptosis. This is accomplished through the up-regulation of gene expression of apoptosis inhibitors and cell cycle regulators, including *bcl-X_L*, *mcl-1*, *cyclins D_1*, *cyclin D_2*, and *c-myc*. The oncogenic potential of STATs (–)3 and (–)5 can be directly activated by point mutations. Hepatocellular carcinoma harbors persistently active STAT3 in association with hypermethylation, and hence suppression, of *socs1*, which encodes a negative regulator of STAT activity.
- In contrast to STAT-3 or STAT-5, STAT-1 plays important roles in growth arrest and apoptosis, and it is implied as a tumor suppressor.
- *ctcf* is located in a small region of overlap for common chromosomal deletions in sporadic breast and prostate tumors, suggesting that CTCF acts as a tumor suppressor. Its absence may lead to overexpression of *myc*.

- Deregulated expression of *myc* genes is frequent in cancer. c-MYC is glycosylated by *O*-linked *N*-acetylglucosamine on threonine 58, a phosphorylation site and a mutational hot spot in lymphomata. Three forms of c-MYC are distinguishable according to no modification, phosphorylation, or glycosylation of T58. Growth factor deprivation may increase T58 glycosylation and correspondingly decrease its phosphorylation, while exposure to growth factors has the opposite effect. A kinase responsible for T58 phosphorylation is the GSK3. The T58 phosphorylated form of c-MYC predominantly accumulates in the cytoplasm rather than the nucleus [Kamemura et al. 2002].
- ID proteins coordinate proliferation and differentiation. ID-2 acts as a dominant antagonist of basic helix-loop-helix transcription factors and proteins of the RB family. ID-2 may be an effector of N-MYC in neuroblastomata. ID-2 is recruited by MYC oncoproteins to bypass the tumor suppressor function of RB [Lasorella et al. 2000].
- *hmg* genes are frequently overexpressed in neoplasias of the thyroid, prostate, cervix, colorectum, and pancreas as well as in pituitary adenomata.
- MYC plays important roles in the pathogenesis of Burkitt lymphoma. MYC induces *hmgA* gene expression. The expression of HMGA1 protein is increased in Burkitt lymphoma cells.
- In translocations associated with lipoma, the 3′ end of the *hmgA2* gene is deleted. Most of the breaks occur within the third intron. Chimeric transcripts are formed, in which HMGIC DNA-binding domains (AT hook motifs) are fused to either a LIM or an acidic transactivator domain [Ashar et al. 1995]. *lhfp* is the fusion partner of *hmgA2* in lipoma with t(12;13). The expressed fusion transcript encodes the three DNA-binding domains of HMGA2, followed by 69 amino acids encoded by frameshifted *lhfp* sequences [Petit et al. 1999]. LPP (Lipoma Preferred Partner) is fused with HMGA2 by a t(3;12) translocation in some forms of benign lipoma. Additional fusions include HMGIC-LHFP, HMGIC-RAD51L1, HMGIC-HEI10, HMGIC-ALDH2, and HMGIC-COX6C.
- Pulmonary chondroid hamartomata are benign tumors of the lungs, characterized by a more or less high degree of mesenchymal metaplasia. In most cases, rearrangements of *hmgA2* underlie this condition [Kazmierczak et al 1996].

3.3.5 The SHH pathway

Signaling by the hedgehog family of secreted glycoproteins is implicated in the determination of embryonic cell fate, in the maintenance of somatic cell fate, in the specification of organ size, and in the patterning of various tissues. They include skin, lung, brain, bone, and blood. There are three known Hedgehog families, Sonic Hedgehog (SHH) {7q36}, Desert Hedgehog (DHH) {12q13.1}, and Indian Hedgehog (IHH) {2q33–q35}. SHH is a receptor that transduces signals, which are instrumental in patterning the early embryo. It is expressed in the Hensen node, the floorplate of the neural tube, the early gut endoderm, the posterior of the limb buds, and throughout the notochord. SHH contributes to the patterning of the ventral neural tube, the anterior–posterior limb axis, and the ventral somites. IHH is expressed in the prehypertrophic chondrocytes of cartilage elements, where it regulates the rate of hypertrophic differentiation. The distribution of DHH is very restricted, limited primarily to the Sertoli cells of developing testes and to the Schwann cells of peripheral nerves.

The products of the *shh* and *patched* genes normally promote organ development in the brain and peripheral nervous system. They convey a key inductive signal in patterning of the ventral neural tube. SHH function is required for the induction of motor neurons by both the notochord and midline neural cells. In the cerebellum, Hedgehog signaling delays neuronal differentiation and induces the proliferation of cerebellar granular neuronal precursors (CGNPs). Activation of the Hedgehog pathway normally requires the inactivation of the 12 transmembrane spanning protein PTC by Hedgehog Ligand, thus releasing the seven transmembrane spanning protein SMO for activation of target genes of the *cubitus interruptus*/*gli* family of transcription factors, which are associated with gliomata.

Hedgehog (HH) signaling promotes the expression of G_1/S Cyclins, including Cyclins D and E, and results in the growth of cells. SHH signaling also opposes epithelial cell cycle arrest by $P21^{CIP1/WAF1}$. After cleavage of the signal sequence, the Hedgehog protein precursor of approximately 45 kD undergoes autocatalytic internal cleavage. This yields an

approximately 20 kD NH_2-terminal domain that has signaling activity and a 25 kD COOH-terminal domain that is active in precursor processing. Hedgehog protein autoprocessing includes peptide bond cleavage and the attachment of a lipophilic adduct to the COOH-terminal region. The lipophilic modification is critical for the spatially restricted tissue localization of the Hedgehog signal domain. Cholesterol is the lipophilic moiety covalently attached to the NH_2-terminal signaling domain during autoprocessing and the COOH-terminal domain acts as an intramolecular Cholesteryl Transferase. Hedgehog proteins bind to PTC or to a PTC/SMO complex and thereby induce SMO activity (Figure 3.3.5.A).

The SHH Receptor and tumor suppressor PTC (Patched) {9q22.3} is a 12-transmembrane spanning member of a class of gene products that are important in controlling early epithelial proliferation. In the absence of HH signaling, PTC represses the genes for *wnt*, *tgf-β*, and *ptc*. PTC suppresses the signaling of SMO (Smoothened) {7q31–7q32} by inhibiting its association with β-Arrestin-2. The ligation of PTC by SHH relieves this inhibition. This leads to phosphorylation of SMO by GRK-2 (G-Protein-Coupled Receptor Kinase-2), interaction with β-Arrestin-2, and endocytosis of SMO in Clathryn-coated pits. PTC induces apoptosis in neuroepithelial cells unless its ligand SHH is present to block the signal. SHH is required for the survival of these cells. The high-affinity binding between PTC and SHH may also provide mitogenic or differentiative signals to basal cells in the skin throughout life. Cell cycle progression following SHH signaling depends on contributions by the PDGF pathway. The transcription of *ptc* is induced by Hedgehog pathway activity, thus serving as a negative feedback loop.

Ligation of the seven transmembrane spanning receptor SMO by the lipid-anchored cell surface ligand SHH activates PTC, which prevents the PKA-dependent phosphorylation and consecutive cleavage of the Krüppel family zinc finger protein GLI. In the nucleus, proteolyzed GLI acts as a repressor of *hedgehog* target genes. In healthy tissues, *gli* gene products are mainly active in precursor cells. The GLI proteins reside in the nucleus and the cytoplasm. In the cytoplasm, they are components of multiprotein complexes that are tethered to the cytoskeleton. In the absence of SHH, GLI is cleaved by the proteasome and COOH-terminally truncated forms translocate to the nucleus. Because the short forms of GLI retain their DNA-binding domain, but have lost their transactivation domain, they act as transcriptional repressors. Following SHH signaling, GLI cleavage is inhibited. There are three GLI proteins. The transcriptional activity of GLI1 {12q13.2–q13.3} is negatively regulated by SUFU (Suppressor of Fused). The activation of GLI2

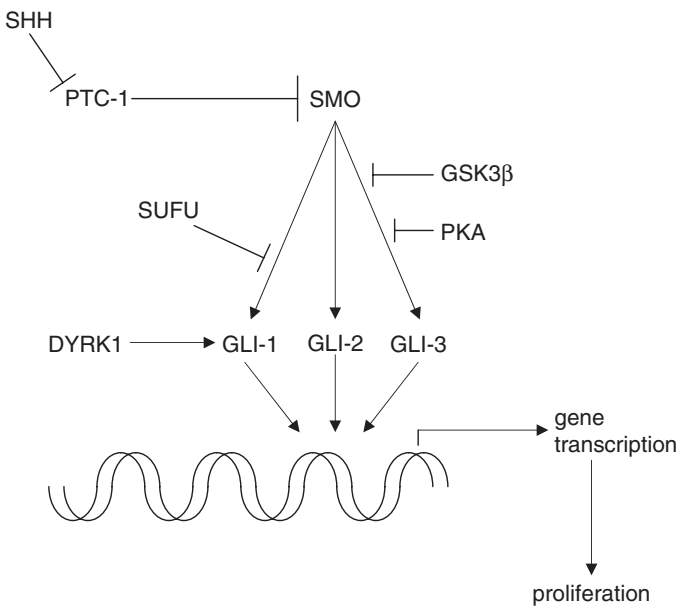


Figure 3.3.5.A. SHH signaling. Once activated, SMO transduces signals that activate the transcription factors GLI-1, GLI-2, and GLI-3. They activate genes that support cell proliferation. Somewhat unique is the two-step negative regulation at the level of the receptors. SHH inhibits PTC-1, which inhibits SMO. Hence, the presence of the ligand SHH relieves the PTC-dependent suppression of SMO signaling and allows the activation of GLI.

{2q14} and GLI3 {7p13} can also be regulated by FGF in the embryonic mesoderm, indicating that GLI may integrate several signaling pathways.

The putative tumor suppressors EXT-1 (Exostosin-1) {8q24}, EXT-2 {11p12–p11}, and EXT-3 {19p} have roles in regulating Hedgehog signaling. EXT-1 is a type-II transmembrane glycoprotein resident in the endoplasmic reticulum,whose expression results in the alteration of the synthesis and display of cell surface heparan sulfate glycosaminoglycans (GAGs). A rate-limiting step in the synthesis of heparan sulfate is the polymerization of alternating GlcA and GlcNAc residues, which is catalyzed by the EXT family of enzymes. EXT-1 and EXT-2 form a heterooligomeric complex that leads to the accumulation of both proteins in the Golgi apparatus. The Golgi localized EXT-1/EXT-2 complex possesses substantially higher Glycosyl Transferase activity than EXT-1 or EXT-2 alone.

The HH pathway is associated with several forms of cancer.

- GLI1 and GLI2 are implicated in tumorigenesis. GLI amplification may be involved in the development of glioma [Kinzler et al. 1987].
- The SHH→GLI pathway is often abnormally activated in medulloblastomata [Oro et al. 1997]. Ligand-independent activation of the Hedgehog pathway in medulloblastoma occurs either through mutations that render SMO independent of PTC or through mutational inactivation of PTC.
- The SHH→GLI pathway is often abnormally activated in basal cell carcinomata [Oro et al. 1997]. Gain-of-function mutations in *smo* occur in sporadic basal cell carcinomata. Mutant SMO, unlike its wild-type counterpart, can cooperate with adenovirus E1A to transform cells. The point mutations W535L in the seventh transmembrane domain of SMO or R562Q in the COOH-terminal cytoplasmic tail of SMO are implicated [Xie et al. 1998]. Genetic mutations leading to a truncated or unstable PTC protein are associated with familial or sporadic basal cell carcinoma. The mutation H133Y constitutes a factor for susceptibility.
- Mutations in the *patched* gene occur in patients with the basal cell nevus syndrome, a hereditary disease characterized by multiple basal cell carcinomata and developmental abnormalities. In this condition, the inhibition of SMO signaling is relieved by the mutational inactivation of PTC [Johnson et al. 1996; Hahn et al. 1996a,b,c].
- Rhabdomyosarcoma is associated with mutations that activate the proto-oncogene *smo* or that inactivate the tumor suppressor *ptc*. This leads to excessive activation of GLI-1.
- Loss of heterozygosity for markers linked to *ext1* and *ext2* arises in chondrosarcomata originating in individuals with multiple exostoses as well as in sporadic chondrosarcomata [Hecht et al. 1997].

3.3.6 The Notch pathway

Genes of the *notch* family [Wharton et al. 1985; Kidd et al. 1986] encode transmembrane receptors that are involved in cell fate decisions during development and postnatal life. There are four *notch* genes, *notch-1* (*tan-1*) {9q34.3}, *notch-2* {1p13–p11}, *notch-3* {19p13.2–p13.1}, *notch-4* (*int-3*) {6p21.3}, that are expressed in overlapping but distinct patterns (Figure 3.3.6.A). The Notch-1 and Notch-2 receptors contain 36 EGF repeats in their ectodomains, whereas the Notch-3 receptor harbors 34 repeats and the Notch-4 receptor harbors 29 repeats. In addition to the EGF-like repeats in the extracellular region of Notch, motifs in the intracellular region of Notch include two nuclear localization signals, a RAM motif, 6 Ankyrin/CDC10 repeats, PEST sequences, and a glutamine rich domain. No transactivation domain is present in Notch-3 or Notch-4. Notch is a receptor with one transmembrane domain. Although synthesized as a single precursor protein, Notch is cleaved during its transport to the cell surface. Proteolytic processing is part of the maturation and activation of Notch-1 [Chan and Jan 1998]. It is mediated by a Furin-like Convertase within the secretory pathway. Cleavage occurs at an extracellular site, called site 1 (S1), downstream of the recognition sequence RQRR. As a consequence, Notch-1 exists as a heterodimeric receptor. The activation of Notch-1 involves its cleavage between G1743 and V1744 (termed site 3 or S3). Site 3 cleavage occurs in response to ligand binding and serves to release NICD from the membrane. Presenilin is required for the release of the intracellular domain of Notch from the plasma membrane. Ligand binding also facilitates the cleavage at another site, named site 2 (S2), within the extracellular juxtamembrane region. This serves to release the ectodomain repression of NICD production. Site 2 cleavage occurs between A1710

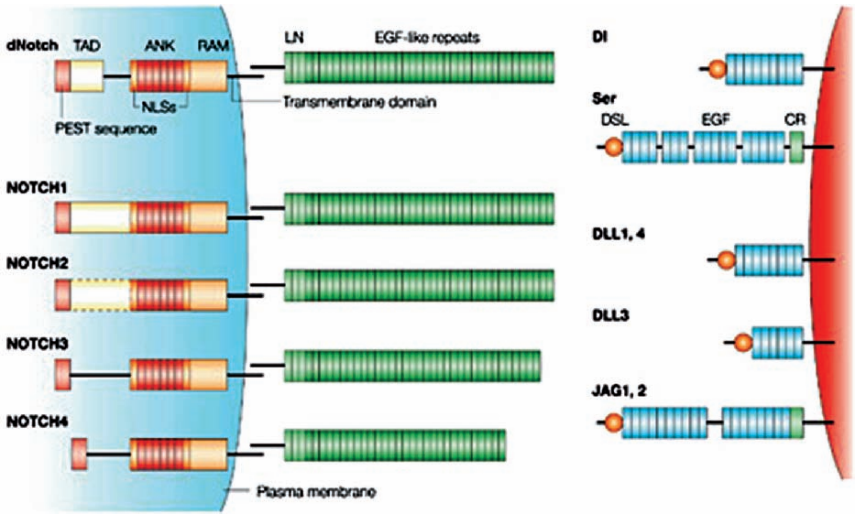


Figure 3.3.6.A. Notch structure. *Left panel*: Structure of Notch proteins and their ligands. Drosophila has one Notch receptor (dNotch) and vertebrates have four (Notch 1–4), which are presented on the cell surface as heterodimers. The ectodomain of Notch receptors contains EGF-like repeats and a cysteine-rich Notch/Lin12 domain (LN), which is followed by a transmembrane domain, the RAM domain and six Ankyrin repeats (ANK, CDC10 repeats), two nuclear localization signals (NLS), followed by the transactivation domain (TAD) and a PEST sequence. Notch-1 contains a strong and Notch-2 a weak transactivation domain in the cytoplasmic part of the receptor. *Right panel*: There are two transmembrane-bound ligands for Notch in Drosophila, delta (Dl), and serrate (Ser). The vertebrates possess three delta homologs, delta-like (DLL)-1, DLL-3, and DLL-4, and two serrate homologs, jagged-1 (JAG-1) and JAG-2. The ligands harbor an NH_2-terminal structure called DSL (Delta, Serrate, and LAG-2), which is common to all family members, followed by EGF-like repeats. Serrate, JAG-1 and JAG-2 harbor a cysteine-rich domain (CR) following the EGF-like repeats. [Reproduced from Radtke and Raj 2003. With permission from Macmillan.]

and V1711, 12 amino acids outside the transmembrane domain, and generates a transient intermediate peptide termed NEXT (Notch Extracellular Truncation). NEXT accumulates when NICD production is blocked by point mutations or γ-Secretase inhibition or by loss of Presenilin-1, and inhibition of NEXT eliminates NICD production. Site 2 cleavage is a ligand-regulated step in the proteolytic cascade leading to Notch-1 activation [Mumm et al. 2000]. The γ-Secretase activity that accounts for the site 2 cleavage is dependent on prior cleavage by the Metalloproteinase TACE (Tumor Necrosis Factor-Converting Enzyme, ADAM17), which plays a prominent role in the activation of the Notch pathway [Brou et al. 2000].

The engagement by the Notch ligands, Delta-Like-1 (DLL-1), DLL-3, DLL-4, Serrate/Jagged-1 (JAG-1), or JAG-2, presented on adjacent cells, initiates a proteolytic cascade that leads to its cleavage and release of the NICD. NICD then translocates to the nucleus, where it associates with HLH transcription factors of the CSL family, specifically with CBF1 (Suppressor of Hairless, LAG1, RBPSUH) {9p13–p12}, and functions as a transcriptional activator. In the absence of Notch, CBF-1 binds to the promoters of its target genes and recruits corepressors and Histone Deacetylases, which inhibit transcription. When Notch is present, it competes with the inhibitory proteins for binding to CBF-1 and then recruits coactivators. In this setting, CBF-1 activates the gene expression of *hes-1* (*hairy/enhancer of split-1*) {3q28–q29}. It may lead to the regulation of programmed cell death by inhibition of NUR77-mediated apoptosis. The promoters for *erbB2* and *nf-κB2* also contain CSL binding sites (Figure 3.3.6.B).

The outcome of Notch signaling is highly context dependent [Radtke and Raj 2003].

– In hematopoietic precursor cells, signaling induced by JAG-1 maintains the precursor pool. Further Notch signaling inhibits B-lymphocyte development and drives the progenitor cells into the T-lymphocyte lineage. Maturation of the T-lymphocytes beyond the $CD4^+CD8^+$ (double positive) stage then requires the discontinuation of Notch-1 signaling.

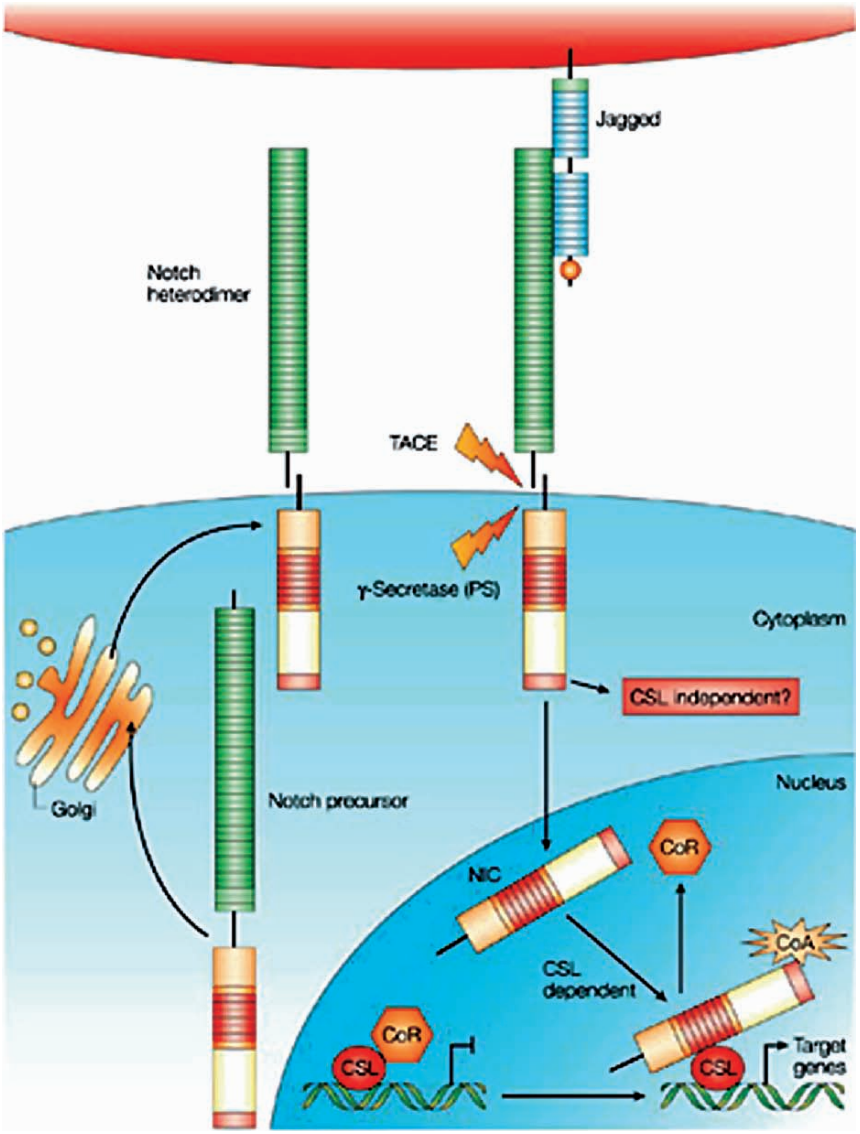


Figure 3.3.6.B. Notch signaling. Notch proteins are synthesized as precursors that are processed by a Furin-Like Convertase in the Golgi organelle before being transported to the cell surface, where they reside as heterodimers. Interaction of Notch receptors with their ligands, such as Delta-Like or Jagged, leads to a cascade of proteolytic cleavages. The first cleavage is mediated by TACE (Tumor necrosis factor-α-converting enzyme/metalloproteinase), followed by a second cleavage mediated by the γ-Secretase activity of Presenilins (PS), which liberates the cytoplasmic domain NIC (Notch Intracellular Domain) of the Notch receptors. The liberated NIC enters the nucleus and binds to the transcription factor CSL, which displaces corepressors (CoR) and recruits coactivators (CoA), leading to transcriptional activation of downstream target genes. This pathway represents the CSL-dependent pathway. Genetic evidence also points to the existence of a CSL-independent pathway. [Reproduced from Radtke and Raj 2003. With permission from Macmillan.]

– Notch signaling participates in cell fate decisions. Notch signaling induces neuronal progenitor cells to retain a stem cell character by preventing them from undergoing neurogenesis. Contact-mediated Notch signaling regulates the capacity of neurons to extend neurites. Up-regulation of Notch activity is concomitant with an increase in the number of interneuronal contacts and cessation of neurite growth. In neurons with low Notch activity, which readily extend neurites, up-regulation of Notch activity either inhibits the extension or causes a retraction of neurites. Conversely, in

more mature neurons that have ceased their growth after establishing numerous connections and display high Notch activity, the inhibition of Notch signaling promotes neurite extension.
– The epithelium of the skin comprises several layers of keratinocytes representing progressive stages of differentiation. Notch-2 is expressed in the basal cell layer; Notch-3 is expressed in the basal cell and the suprabasal cell layers. Notch signaling enhances the differentiation of keratinocytes. NICD directly stimulates the expression of $p21^{CIP1/WAF1}$ and represses the expression of *c-fos*, which causes withdrawal from the cell cycle and facilitates terminal differentiation.
– During lung development, differentiation pathways converge to generate pulmonary epithelial cells or neuroendocrine airway cells. Notch-1 and HES-1 are highly expressed in nonneuroendocrine airway epithelial cells, whereas MASH-1 (Mammalian Achaete Scute Homolog-1, Human Achaete Scute Homolog-1, hASH-1, ASCL1) expression is restricted to neuroendocrine cells. The activation of HES by Notch represses *mash-1* {12q22–q23} and determines the differentiation along the pulmonary epithelial cell lineage.

Notch signaling can be modulated on various levels. The elongation of *O*-linked fucose residues on certain EGF repeats by Glycosyl Transferases of the Fringe family prevents the activation of Notch signaling by Jagged, but not by Data Like ligands.

In various forms of cancer, Notch signaling is altered.
- Transcripts of the gene *notch-1* (*tan1*, *translocation-associated notch homolog 1*) are generated in many normal fetal tissues but are most abundant in lymphoid tissues. The Notch pathway is overactive in some forms of leukemia. Chromosome 7q34–q35, which contains the gene *tcrβ* for the T-Cell Antigen Receptor β chain, is a common site for translocations in T-cell neoplasms. In the translocation t(7;9)(q34;q34.3), which occurs in acute T-cell lymphoblastic leukemia, the locus on chromosome 9 contains a gene of the *notch* family. Breakpoints within 100 bp of an intron in *tan1* result in the truncation of *tan1* transcripts [Ellisen et al. 1991]. In this translocation, only the COOH-terminal portion of Notch-1 is juxtaposed. The expressed truncated Notch-1 protein corresponds to NICD. This maintains the T-lymphocytes in an immature $CD4^+CD8^+$ (double-positive) state and predisposes them to full transformation. Because the overexpression of NICD in hematopoietic cells gives rise exclusively to T-cell neoplasms, Notch-mediated transformation depends on a T-lymphocyte-specific signal transduced through the pre-TCR (pre-T-Lymphocyte Antigen Receptor).
- The Notch pathway is overactive in various epithelial tumors, including mammary adenocarcinoma and colon adenocarcinoma. It is by itself insufficient to cause cancer and needs to partner with other oncoproteins. These partners, RAS, MYC, HPV E6, and HPV E7, share the common property of overriding the G_1/S cell cycle checkpoint. In epithelial cancers, oncogenic RAS activates Notch. Full transformation by Notch then requires active signals from the ERK (MAP Kinase) and PI-3 Kinase pathways downstream of RAS [Fitzgerald et al. 2000].
- In keeping with the context dependence of Notch signaling, Notch-1 and Notch-2 can have tumor-suppressive effects. In small cell lung cancer cells with neuroendocrine differentiation, the proteolytic cleavage products of Notch-1 or Notch-2 can cause cell cycle arrest, in part by repressing *mash-1*.
- Basic helix-loop-helix transcription factors of the MASH family are regulators of development in the central nervous system and neural crest. MASH-1 is highly expressed in two neuroendocrine cancers, medullary thyroid cancer and small cell lung cancer [Ball et al. 1993].

3.3.7 The APC pathway

The WNT family of proteins is involved in embryonic patterning and in the development of the nervous system. There are more than 30 WNT proteins, which are secreted factors that interact with cell surface receptors. WNT factors bind to the seven transmembrane spanning serpentine Frizzled Receptors, which cooperate with LRP6 (Low Density Lipoprotein Receptor Related Protein) to activate G-Proteins. Their signaling critically depends on β-Catenin and regulates cell proliferation (Figure 3.3.7.A).
– WNT–WNT Receptor interaction induces the activity of the cytoplasmic phosphoprotein Dishevelled. Activated Dishevelled inhibits the serine/threonine kinase GSK-3β. When GSK-3β is inhibited, β-Catenin becomes hypophosphorylated. The hypophosphorylated form of β-Catenin migrates to the nucleus and interacts with transcription factors, in particular with LEF-1 (Lymphoid

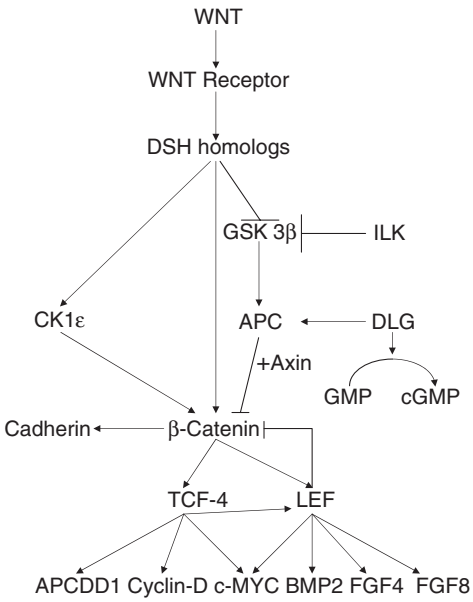


Figure 3.3.7.A. WNT signaling. WNT factors bind to the seven transmembrane spanning serpentine Frizzled Receptors that activate G-Proteins. Receptor ligation induces the activity of the cytoplasmic phosphoprotein Dishevelled (DSH homolog), which inhibits the serine/threonine kinase Glycogen Synthase Kinase-3β (GSK-3β). When GSK-3β is inhibited, β-Catenin becomes hypophosphorylated, the hypophosphorylated form of β-Catenin migrates to the nucleus and interacts with transcription factors, in particular with LEF-1 and TCF-4, thereby inducing gene expression. WNT-dependent signal transduction also antagonizes APC and Axin, which allows the accumulation of γ-Catenin (Plakoglobin) and β-Catenin in the nucleus and drives cell proliferation. Furthermore, WNT signaling activates Casein Kinase 1ε, which can stabilize β-Catenin.

Enhancer-Binding Factor-1, TCF-1α, T-Cell Factor 1α), thereby inducing gene expression.
– WNT-dependent signal transduction antagonizes the APC/Axin effect and allows the accumulation of γ-Catenin (Plakoglobin) and β-Catenin in the nucleus, which then drives cell proliferation.
– WNT signaling activates Casein Kinase 1ε, which can stabilize β-Catenin.

WNT signaling proceeds through inhibition of GSK3β and activation of Protein Phosphatase 2A (PP2A), which leads to the dephosphorylation of Axin. The regulatory subunit of PP2A (B56) binds to APC and targets β-Catenin for degradation in the proteasome. PP2A is an intracellular serine/threonine protein phosphatase that is expressed as a heterotrimeric protein-containing conserved catalytic (C) and structural (A) subunits, and a variable regulatory (B) subunit. PP2A is inhibitory to the WNT signaling process.

GSK3 (Glycogen Synthase Kinase3) is a cytoplasmic serine/threonine kinase that is involved in Insulin signaling and metabolic regulation, as well as in WNT signaling and the specification of cell fates during embryonic development. GSK3 appears in two highly homologous and ubiquitously expressed forms, GSK3α {19q13.1–q13.2} and GSK3β {3q13.3}. The Insulin and WNT signaling pathways differentially regulate GSK3, resulting in distinct downstream events. In the WNT pathway, GSK3 is essential for normal development of the embryo and for regulation of cell proliferation in the adult. WNT signaling inhibits GSK3, resulting in the dephosphorylation of β-Catenin, which then translocates to the nucleus and activates transcription. A multiprotein complex of β-Catenin, Axin, and APC regulates the phosphorylation of β-Catenin by GSK3 and may prevent cross-talk between the Insulin and WNT signaling pathways. Axin binding to GSK3 inhibits GSK3 phosphorylation on serine 9, thus inactivating downstream events of the Insulin pathway [Weston and Davis 2001]. Many of the GSK3 substrates, including Glycogen Synthetase, must be phosphorylated before they can dock with the kinase through a phosphate-binding site on arginine 96. Substrates that are part of the WNT signaling pathway, including β-Catenin and Axin, do not require prephosphorylation.

The *apc* (*adenomatous polyposis coli*) gene {5q21} [Kinzler et al. 1991; Nishisho et al. 1991; Groden et al. 1991; Joslyn et al. 1991] contains 15 exons, spanning approximately 125 kb of DNA and encoding an 8.5 kb coding region in the 10 kb mRNA. An alternative form (9A) splices into the interior of exon 9, removing 101 amino acids from the full-length APC polypeptide. The protein coding region of the *apc* gene is large, encompassing 2,844 amino acids (312 kD). The NH_2-terminal domain conatins a series of repeat sequences (amino acids 6–57), which form α-helical structures capable of homodimerization. The central region (amino acids 453–767) contains binding sites for PP2A and ASEF (Figure 3.3.7.B). Axin-2 (Conductin) binds to to the SAMP repeat motif (serine–alanine–methionine–proline) in APC via its RGS (regulator of G-Protein signaling) domain. APC and Axin are substrates for GSK3β and their ability to bind Catenin is enhanced by phosphorylation. Axin-2 forms a complex with β-Catenin, APC, and GSK3β [Behrens et al. 1998].

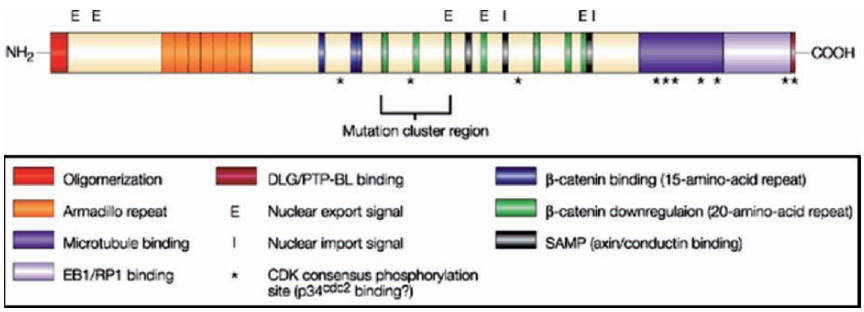


Figure 3.3.7.B. APC structure. Conserved regions, such as the Armadillo repeats, and regions that interact with other proteins, including Tubulin, the microtubule-associated protein EB1, DLG (Discs Large), β-Catenin and Axin/Conductin, are shown. APC also contains several consensus sites for phosphorylation by $P34^{CDC2}$, five nuclear export signals (E) and two nuclear import signals (I). Most somatic mutations occur in the mutation cluster region. Most of these mutations lead to truncated proteins. [Reproduced from Fodde et al. 2001. With permission from Macmillan.]

This multiprotein complex (destruction complex) directs β-Catenin to degradation. After β-Catenin has been phosphorylated on four serine/threonine residues in the NH_2-terminus by the kinase GSK3β in the complex, it is transferred to the SCF complex (SKP/Cullin/F-Box complex), binds to the F-box protein βTrCP, is ubiquitinated and degraded in the proteasome. APC inhibits RB phosphorylation and reduces the levels of Cyclin D_1. This inhibits G_1/S progression [Heinen et al. 2002].

The Catenins are a family of proteins that interact with the cytoplasmic portion of the Cadherin family of cell–cell adhesion proteins, thus linking the Cadherins to the Actin cytoskeleton. Catenins are important in the signaling cascade initiated by the WNT family of proteins. The fate of β-Catenin is a critical determinant for cell proliferation with location of β-Catenin in the nucleus mediating the activation of transcription factors and leading to proliferation, whereas degradation of free β-Catenin in the Ubiquitin pathway prevents cell cycle progression. The intracellular localization of β-Catenin can be influenced by sphingolipids. Cytoplasmic β-Catenin is in equilibrium with β-Catenin in adherens junctions. The fraction of β-Catenin in adherens junctions provides a link between E-Cadherin and α-Catenin, which binds to the Actin cytoskeleton. The growth suppressing activity of E-Cadherin is due, at least in part, to the sequestration of β-Catenin and the resulting inhibition of the β-Catenin→TCF-4 pathway. APC binds to microtubule-associated proteins through a domain in its extreme COOH-terminus. Through these associations, APC and β-Catenin can play a role in mitosis.

The LEF/TCF transcription factors include LEF-1 (Lymphoid Enhancer Binding Factor-1, TCF-1α, T-Cell-Specific Transcription Factor-1α), TCF-1, TCF-3, and TCF-4 (T-Cell Factor-4, ITF-2, SEF-2, E2-2). LEF-1 {4q23–q25} is a 48 kD high mobility group transcription factor. LEF-1 belongs to a family of regulatory proteins that share homology with HMG-1 Lymphoid Enhancer Binding Factor-1. Catenin does not bind to DNA, but it does bind to TCF-4, which itself lacks transactivation activity. Together, they induce the expression of genes that support multiple growth pathways.

– Catenin and TCF-4 induce the expression of *cyclin* D_1 and *c-myc*. Once expressed, MYC binds to SMAD-2 and SMAD-3 and represses the transcription of the $p15^{INK4B}$ gene, thus rendering cells unresponsive to TGF-β-mediated inhibition of cell cyle progression [Feng et al. 2002].
– APCDD1 (Downregulated by APC-1) is a 514 amino acid protein with a molecular mass of about 59 kD that promotes cell growth. Its 2.6 kb transcript is expressed ubiquitously, with abundant levels in the heart, pancreas, prostate, and ovaries. β-Catenin and TCF-4 directly bind to the promoter of *apcdd1* {18p11} and induce its transcription. The expression of *apcdd1* is inhibited by APC and by Axin [Takahashi et al. 2002].
– TCF-4 induces the expression of *ectodermal-neural cortex 1* (*enc1*), in colon epithelial cells. ENC-1 increases the growth rate of colon epithelial cells and prevents their differentiation.
– The growth-promoting gene *af17* is a likely target for transcription by the β-Catenin/TCF/LEF complex.
– *pml* is a target gene of β-Catenin and γ-Catenin (Plakoglobin) independently of TCF (LEF).

PML, P300, and β-Catenin coactivate the transcription of *arf* and *siamois*, but not *cyclin* D_1.

- Allelic loss and point mutations can occur in the *apc* (*adenomatous polyposis coli*) tumor suppressor gene on chromosome 5q21. More than 120 distinct germline and somatic mutations are documented in the *apc* gene. Somatic loss-of-function mutations in *apc* may initiate colorectal cancer development, whereas germline mutations are responsible for familial adenomatous polyposis (FAP). Multiple colonic polyp development characterizes the disease. These polyps arise during the second and third decades of life and become adenomata and malignant carcinomata later in life. The vast majority of these mutations occur in the mutation cluster region (exon 15, codons 1,286–1,513), and lead to COOH-terminal truncations of the APC protein. This results in a lack of Conductin-binding motifs, such as the SAMP repeats, and a lack of the variable number of 20 amino acid repeats that are associated with the down-regulation of intracellular β-Catenin. Normally, the APC/Axin-2/β-Catenin complex stimulates the breakdown of β-Catenin. Therefore, mutations that cause a loss of APC, or a loss of the portion of the APC protein that interacts with β-Catenin, can lead to a constitutive activation of TCF (LEF-1) and unrestricted growth. Cells with mutant *apc*, often in colorectal tumors, have an abundance of spindle microtubules that fail to connect to kinetochores and are characterized by chromosomal instability. Somatic mutations of *apc* also occur in cancers of the stomach, pancreas, thyroid, ovary and breast.
- Methylation in the promoter region of *apc* may lead to inactivation in gastrointestinal tumors. Aberrant methylation occurs early in colorectal carcinogenesis.
- BMP4 is overexpressed and secreted by human cancer cells with mutant *adenomatous polyposis coli* gene. The oncogenic allele of *β-catenin* is absolutely required for the expression of the TGF-β family member BMP-4, whose receptor, *bmpr1A*, is mutated in a fraction of the rare inherited gastrointestinal cancer predisposition syndrome juvenile intestinal polyposis [Howe et al. 2001]. This indicates the presence of regulatory interactions between the WNT and BMP signaling pathways [Kim et al. 2002].
- In normal colonic epithelium, survivin is preferentially expressed in the lower crypt. The expression of *survivin* correlates inversely with the expression of APC, because it is down-regulated by APC→β-Catenin→TCF-4 signaling. The gradual transformation of colorectal epithelium to carcinomata is associated with the progressive inhibition of apoptosis. Survivin is highly expressed in the majority of colorectal carcinomata [Zhang et al. 2001], likely accounting for this phenomenon.
- The expression of *apcdd1* is directly regulated by the β-Catenin/TCF-4 complex. Its expression levels are reduced by APC or Axin-1 activity. Elevated expression of APCDD1 promotes the proliferation of colonic epithelial cells and the molecule is frequently overexpressed in colorectal cancer [Takahashi et al. 2002].
- The PP2A-dependent decrease in β-Catenin is blocked by certain oncogenic mutations in *β-catenin* [Seeling et al. 1999]. In colorectal tumors with intact *apc* gene, gain-of-function mutations of *β-catenin*, that alter functionally significant phosphorylation sites, are frequent. In colorectal cancers with activating *β-catenin* mutations, an inappropriately activated high mobility group transcription factor TCF-4 leads to overexpression of the target genes *c-myc* and *tcf-1*, which then promote neoplastic growth [Roose et al. 1999]. Oncogenic mutants of β-Catenin that lack GSK3β phosphorylation sites do not bind β-TrCP. This protects β-Catenin from Ubiquitin-mediated degradation.
- The aberrant accumulation of β-Catenin in tumors is often associated with mutational inactivation of the P53 tumor suppressor. High-level expression of transcriptionally active P53 down-regulates β-Catenin. This inhibitory effect is likely mediated by the Ubiquitin–proteasome system and requires active GSK3β. These processes imply that there may be a selective pressure for the loss of wild-type *p53* expression in cancers that are driven by excessive accumulation of β-Catenin [Sadot et al. 2001].
- In medulloblastoma, while mutations of *apc* are rare, a hot spot region of *β-catenin* (*ctnnb1*) mutations occurs in a subset of tumors. Point mutations and deletions in *axin-1* may also arise in medulloblastoma.
- The WNT signaling pathway is often up-regulated in epidermal cancers. The gene for the APC homolog *apc-2* (*apcl*) is located on chromosome 19p13.3, a region that is commonly lost in ovarian cancer. High frequency *apc-2* allelic imbalance in ovarian cancers implies that APC-2 may act as a tumor suppressor in this type of malignancy.

3.3.8 The SMAD pathway

SMADs (SMA- and MAD-Related Proteins) mediate signals from members of the TGF-β superfamily of cytokines. There are three classes of SMADs (Figure 3.3.8.A),

– Receptor-regulated SMADs (R-SMADs, comprising SMADs -1, -2, -3, -5, and -8)
– Common mediator SMADs (co-SMADs, comprising SMADs -4 and -10)
– Inhibitory SMADs (I-SMADs, comprising SMADs -6 and -7)

Receptor ligation leads to serine phosphorylation of R-SMADs in the COOH-terminal domain, their dissociation and assembly into complexes with the co-SMADs, SMAD-4 (MADH4, DPC4) and SMAD-10 (SMAD-4β) followed by a translocation of the complexes into the nucleus where the SMADs act as transcriptional comodulators (Figure 3.3.8.B). In the nucleus, activated SMAD complexes recognize DNA stretches with several copies of the SMAD target sequence CAGAC. However, most SMAD responsive promoter elements contain only one copy of this motif, which has only a low affinity for SMAD complexes. For their activation, the core SMAD complex must associate with other DNA-binding factors. SMADs may positively regulate the transcriptional activity at the target elements by recruiting transcriptional coactivators, such as CBP (cAMP Response Element Binding Protein), P300, MSG-1 (Melanocyte-Specific Gene-1), and SMIF (SMAD-4 Interacting Transcription Factor). SMAD-2 binds the forkhead family member FOXH-1. SMADs -3 and -4 bind the transcription factor ATF-2, whereas SMAD-3 binds the basic helix-loop-helix transcription factor TFE-3. SMADs -3 and -4 also bind JUN and FOS and strongly activate promoters containing AP-1 sites. The c-FOS proto-oncoprotein is highly unstable, which is crucial for rapid gene expression shutoff and control of its intrinsic oncogenic potential. FOS is degraded by the proteasome after ubiquitination. Alternatively, binding partners of nuclear SMAD complexes may be corepressors, including P107. SMAD proteins remain in the nucleus only for the duration of the TGF-β stimulus. SMAD-2 (MADH2, MADR2, JV18-1) and SMAD-3 (MADH3) become phosphorylated in response to the Activin/Nodal branch of the TGF-β pathway. Their dephosphorylation initiates their shuttling back to the cytoplasm.

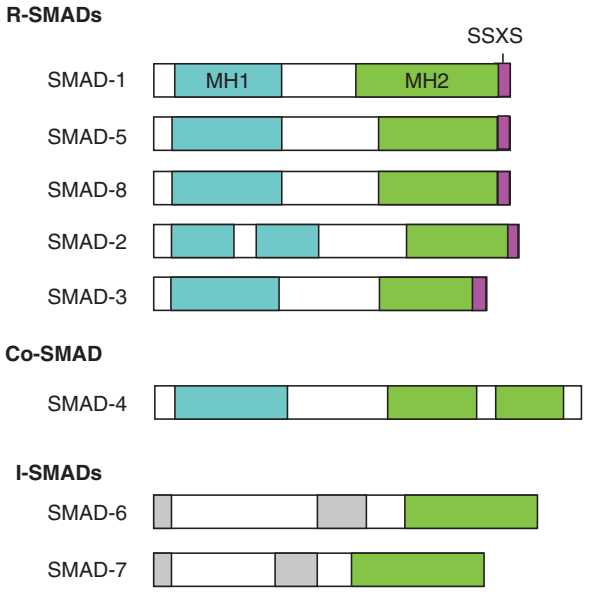


Figure 3.3.8.A. SMAD structure. The SMAD family comprises receptor-regulated SMADs (R-SMADs), common partner SMADs (CO-SMADs) and inhibitory SMADs (I-SMADs). The NH_2-terminal MH1 domain (MAD homology 1 domain) (shown in turquoise) is highly conserved in all R-SMADs and SMAD-4, but not in SMADs 6 and 7. The MH1 domain is a DNA-binding module stabilized by a tightly bound zinc atom. The linker region is quite divergent among the various subgroups, whereas the COOH-terminal MH2 domain (depicted in green) is conserved in all SMAD proteins. The MH2 domain is a protein-binding module in signal transduction. The R-SMADs contain a specific phosphorylation motif, SSXS, at the COOH-terminal region. It is a substrate site for activated receptor kinases.

PEBP2/CBF (Polyomavirus Enhancer Binding Protein 2/Core Binding Factor) is a transcription factor complex composed of α and β subunits. It is crucial for the regulation of hematopoietic differentiation. Members of this class of transcription factors form heterodimers composed of the DNA binding CBFα (RUNX) and a common non-DNA-binding CBFβ subunit. The β subunit increases the affinity of CBFα proteins to DNA, protects the CBFs from proteolysis, and recruits other proteins to the complex. RUNX family gene products comprise RUNX1 (PEBP2αB, CBFA2, AML1), RUNX2 (PEBP2αA, CBFA1, AML3), and RUNX3 (PEBP2αC, CBFA3, AML2). While RUNX1 is essential for mature hematopoiesis and RUNX2 for osteochondrogenesis, RUNX3 has functions in the nervous system. RUNX-mediated intranuclear

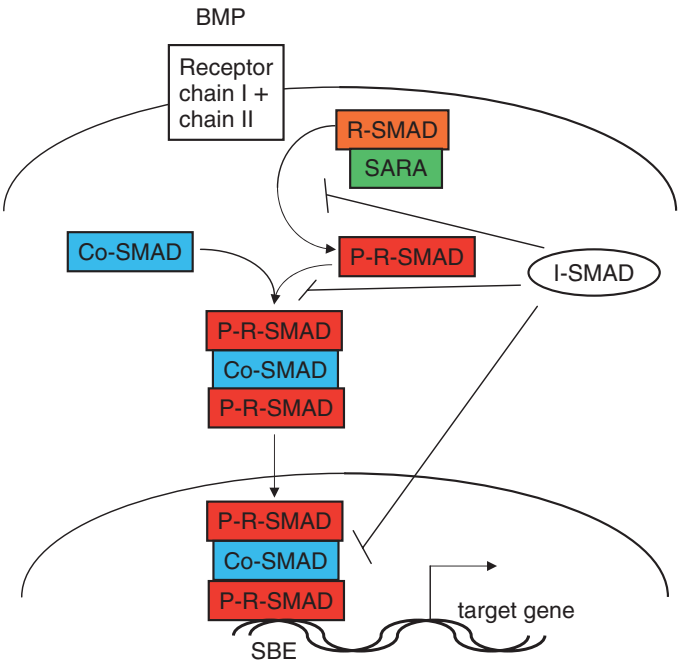


Figure 3.3.8.B. SMAD signaling. In response to receptor ligation, R-SMAD (Receptor-Regulated SMAD) is phosphorylated. This allows the formation of a hetero-complex with CO-SMAD (Common SMAD). Subsequently, this complex translocates into the nucleus, where it regulates the transcription of target genes. I-SMAD (Inhibitory SMAD) negatively regulates SMAD signaling by blocking the binding of R-SMAD to the receptor, the heterocomplex formation between R-SMAD and CO-SMAD, and the transcriptional regulation by R-SMAD in the nucleus.

targeting of SMADs is critical for the integration of distinct pathways that are essential for fetal development. The integration of RUNX and SMAD signals is mediated by interactions at specific foci within the nucleus. Activated SMADs are directed to these subnuclear foci only in the presence of RUNX proteins. SMAD/RUNX complexes associate with the nuclear matrix, and this association requires the intranuclear targeting signal of RUNX factors. The convergence of SMAD and RUNX proteins at these sites supports transcription and functional cooperativity between the proteins [Zaidi et al. 2002]. CBFs can function as activators or repressors of transcription, depending on target gene and cell lineage. The α subunits of PEBP2/CBF, which contain the highly conserved Runt domain, play essential roles in hematopoiesis and osteogenesis. They form complexes with R-SMADs that act in TGF-β/Activin pathways as well as in BMP pathways. Among them, RUNX3 (PEBP2αC, CBFA3, AML2) forms a complex with SMAD-3. PEBP2 may thus be a nuclear target of TGF-β/BMP signaling [Hanai et al. 1999].

The activities of R-SMADs and SMAD-4 can be modulated by adapter proteins in the cytosol, such as Filamin (FLN) and SARA (SMAD Anchor for Receptor Activation). In the basal state, SMAD-2 and SMAD-3 can bind to SARA. This retains these SMADs in the cytoplasm. However, SARA presents SMADs as substrates to the activated TGF-β Receptor complex and their phosphorylation decreases the affinity of SMADs for SARA. The adapter protein ELF, a β-Spectrin, is essential for the transduction of TGF-β signals to SMADs -3 and -4.

The inhibitory SMADs, SMAD-6 and SMAD-7, negatively regulate the R-SMAD pathway by blocking SMAD-4 binding. SMAD-7 acts as an intracellular antagonist of the TGF-β RI kinase domain. The inhibitory SMAD-7 binds to receptor complexes for TGF-β or BMPs to prevent access to and phosphorylation of the respective R-SMADs. SMAD-6 preferentially inhibits BMP signaling by binding to the type I BMP Receptor and preventing SMAD-1 and SMAD-8 from being activated. Elevated SMAD-6 activity delays chondrocyte maturation and differentiation.

The abundance of SMAD proteins is regulated by the Ubiquitin–proteasome pathway. SMADs can associate with E3 Ubiquitin Ligases, such as JAB-1, ROC-1, or SMAD Ubiquitination Regulatory Factors (SMURFs). SMURFs belong to the HECT domain containing E3 enzymes, which interact through their WW domains with a specific proline–tyrosine motif in certain SMADs. SMURF-1 {7q21.1–31.1} [Zhu et al. 1999] is a HECT domain E3 Ubiquitin Ligase. It binds to SMAD-1 or

SMAD-5 via their proline–tyrosine motifs and mediates the ubiquitination and degradation of these SMAD proteins. Consistently, SMURF-1 is a potent antagonist of BMP signaling. SMURF-1 can form a complex with SMAD-6. In this state, it may be exported from the nucleus and targeted to type-I BMP Receptors to induce their degradation. In a negative feedback loop, the activated TGF-β Receptor complex can induce the mobilization of a SMAD-7/SMURF-1 protein complex from the nucleus to the cytoplasm, which recognizes the activated receptor and mediates its ubiquitination and internalization via Caveolin-rich vesicles. This terminates the TGF-β signal. SMURF also recognizes RUNX2 and initiates its degradation.

SKI (Sloan-Kettering Institute Oncogene) {1p36.3} and SNO (SKI-Related Novel Gene, SKIL) {3q26} are negative regulators of TGF-β signaling. SMAD-3 may associate with the nuclear proto-oncogene protein SKI. Binding to SKI prevents the formation of a functional complex between SMAD-3 and the co-SMADs. This association also represses transcriptional activation by SMAD-3 and renders cells resistant to the growth inhibitory effects of TGF-β_1. Two distinct mRNAs are generated from the *sno* gene; *sno-N* does not contain Alu sequences and encodes a 684 amino acid protein, while *sno-A* does contain Alu sequences and encodes a 415 amino acid protein. SNO-N maintains the repressed state of TGF-β target genes in the absence of ligand by binding to the nuclear SMAD-4. This represses TGF-β responsive promoter activity through the recruitment of a nuclear repressor complex. Activation by TGF-β induces the translocation of SMAD-2, SMAD-3, and SMAD-4. SMAD-3 causes the degradation of SNO-N, allowing a SMAD-2/SMAD-4 complex to activate the relevant target genes.

- High levels of TGF-β_1 may be protective against early tumor development, whereas a loss of the antiproliferative responsiveness to TGF-β may be a pivotal step in tumor progression. A loss of responsiveness can be caused by inactivation of the genes for *p15^{INK4b}*, *smad-4*, *smad-2*, or *tgf-β receptors*. The amplification of *c-myc* in cancer cells represses the induction of *p15^{INK4b}* and *p21* by TGF-β. This is mediated by a physical interaction of c-MYC with SMAD-2 and SMAD-3, through which c-MYC inhibits the transcriptional activity of the SP-1/SMAD complex [Feng et al. 2002].
- *smad-2*, located on chromosome 18q21, is a tumor suppressor gene that may be mutated in colorectal cancer and lung cancer. These mutations occur most frequently in the MH2 domain, which mediates heteromeric complex formation and transcriptional activation. COOH-terminal deletions or mutations often inactivate the SMAD, but also mediate dominant negative interference with wild-type SMAD function.
- SMAD-4 contributes to mediating the tumor-suppressive functions of TGF-β. A loss of SMAD-4 (DPC-4) function by loss of heterozygocity, deletions, or point mutations occurs in pancreas adenocarcinoma [Hahn et al. 1996a,b,c]. Biallelic inactivation of *smad-4* is associated with about 50% of sporadic pancreatic adenocarcinomata, but not with familial pancreas cancers.
- A loss of SMAD-4 function by loss of heterozygosity, deletions, or point mutations occurs in colorectal carcinoma. Here, haploinsufficiency can contribute to the progression of cancer.
- Germline mutations in the *smad-4* gene, located on chromosome 18q21.1, result in juvenile intestinal polyposis, which is a gastrointestinal cancer predisposition syndrome [Howe et al. 1998]. Juvenile polyps may be isolated or multiple, even very numerous. The histology and natural history of these polyps suggest that they are hamartomata. In some patients, colon cancer ensues.
- RUNX1 (AML-1) is involved in leukemogenesis. Translocations affecting the *runx1* gene are among the most frequent ones in leukemias. More than 10 different translocation fusion events, mostly occuring in myeloid leukemias, involve this gene. The translocations result in protein fusions that involve a loss of the COOH-terminal transactivation domain of RUNX1. In contrast, the characteristic TEL–RUNX1 fusion of childhood pre-B-cell leukemia involves a NH_2-terminal fusion, in which the RUNX1 moiety is virtually intact. The oligomerization motif and a central repression domain in TEL recruit the nuclear corepressor complex NCOR. Some leukemias have amplification of the *runx1* locus.
- RUNX3 is a major growth regulator of gastric epithelial cells. In its absence, the gastric mucosa exhibits hyperplasias, due to stimulated proliferation and suppressed apoptosis in its epithelial cells. These cells are resistant to the growth inhibitory and apoptosis inducing actions of TGF-β. Between 45% and 60% of gastric cancer cells do

not significantly express RUNX3 due to hemizygous deletion and hypermethylation of the *runx3* promoter region [Li et al. 2003b].
- The viral oncoprotein E1A binds to a region of CBP adjacent to the SMAD binding site, blocks the SMAD–CBP interaction, and suppresses TGF-β signaling.
- The oncogenic protein SKI associates with SMAD proteins and counteracts their activation of gene expression and growth inhibition in response to TGF-β. Levels of SKI are frequently increased in melanoma.

3.3.9 The VHL pathway

The Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor responsible for the oxygen-dependent regulation of genes that respond to hypoxic challenge and are involved in blood vessel branching (Figure 3.3.9.A). The HIF family of transcription factors consists of heterodimeric α {14q21–q24} and β (Arylhydrocarbon Nuclear Translocator, ARNT) {1q21} subunits that bind to the pentanucleotide (G/A)CGTG (hypoxia response element, HRE). While the β subunit is universally expressed, the α subunit is detectable only following hypoxic challenge. In normoxia, newly synthesized HIF α is modified on proline 564 by Prolyl Hydroxylases. Proline hydroxylation is necessary and sufficient for HIF-1α binding to VHL (von Hippel-Lindau). This mediates the assembly of a complex that activates the Ubiquitin E3 Ligase and leads to the degradation of HIF α. In hypoxic conditions, prolyl hydroxylation of HIF α does not take place and heterodimer formation between the α and β chains leads to their translocation into the nucleus, followed by the induction of gene transcription. The tetrameric Prolyl Hydroxylases require oxygen, ferrous iron, and 2-oxoglutarate for activity and thus constitute a mechanism for sensing the normoxic state of a cell.

The RING-H2 finger protein RBX1 (Ring-Box 1, Regulator of Cullins 1, ROC1) is an essential component of two E3 Ubiquitin Ligase complexes, the SCF (SKP1/CUL1/F-box protein) family and the VCB (VHL/Elongin C/Elongin B) families. VHL forms a complex with Elongin B and Elongin C, as well as with Cullin and RBX1. The VHL Ubiquitin Ligase is directly responsible for the ubiquitination of hypoxia inducible transcription factors. The interaction of VHL with HIF-1 is necessary for the oxygen-dependent degradation of HIF α subunits. These interactions by VHL also affect the regulation of transcriptional elongation. The Elongin (SIII) complex is a heterotrimer of the 110 kD Elongin A, the 18 kD Elongin B, and the 15 kD Elongin C. Elongin A (Transcription-Elongation Factor B Polypeptide 3, TCEB3A) {1p36.1} functions as the transcriptionally active component of the SIII complex, whereas Elongin B (TCEB2) and Elongin C (TCEB1) are regulatory subunits. The tumor suppression activity of the VHL gene product is a function of its ability to bind to Elongin B and Elongin C and thereby inhibit transcriptional elongation.

HIF-1 regulates the hypoxia-induced expression of BNIP3 (BCL2/Adenovirus EIB-Interacting Protein 3) and NIX (NIP-Like Protein X). NIP3 is a member of the BBL-3 family that localizes to the mitochondria. NIP3-mediated cell death is independent of APAF-1, Caspase activation, or Cytochrome *c* release. HIF-1α promotes P53-dependent apoptosis, which is mediated by APAF-1 and Caspase-9. The dephosphorylated form of HIF-1 binds to P53 and induces apoptosis. P53 blocks the ability of HIF-1α to activate gene transcription. However, certain mutations in *p53* remove this block.

- The von Hippel-Lindau syndrome (VHL) is characterized by highly vascularized tumors, including benign hemangioblastomata of the cerebellum, spine, brain stem, and retina. Clear cell renal cell carcinoma is a frequent cause of death, occurring in up to 70% of patients with this disease. Von Hippel-Lindau syndrome is an autosomal dominant disorder caused by deletions or mutations in the *vhl* tumor suppressor gene on chromosome 3p25. The VHL gene coding sequence contains three exons and yields two splice forms of mRNA, reflecting the presence or absence of exon 2. Tumors arise following the loss or inactivation of the wild-type allele in a cell. Approximately 20% of patients have large germline mutations, 27% have missense mutations, and 27% have nonsense or frameshift mutations. Pheochromocytomata occur in association with specific alleles. Families may be characterized by the absence (type 1) or presence (type 2) of pheochromocytomata. Most type-2 families are affected by missense mutations, whereas most type-1 families have deletions or premature termination mutations [Friedrich 2001]. Loss-of-function mutations of the VHL gene product prevent it from binding to HIF α.

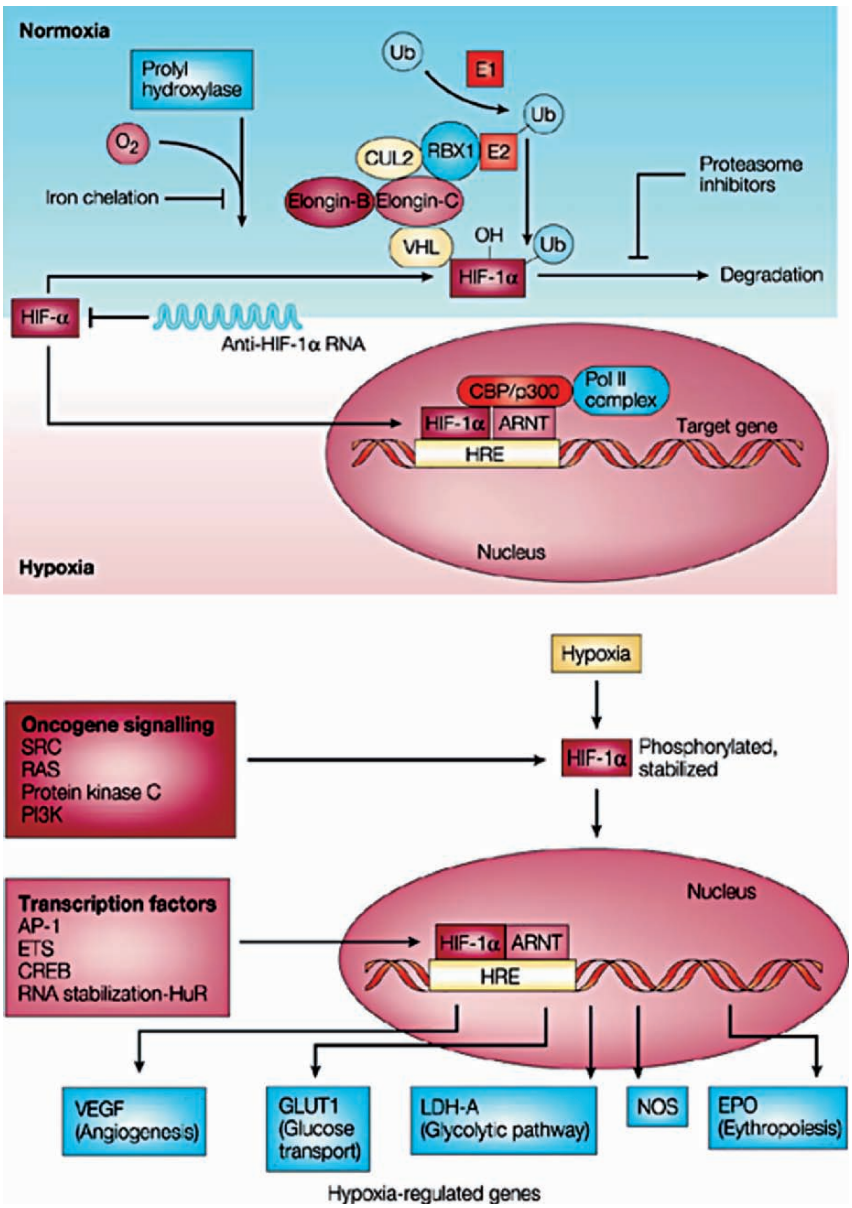


Figure 3.3.9.A. HIF-1 pathway. *Top panel*: In the presence of oxygen (O_2), prolyl hydroxylase posttranslationally modifies HIF-1 (Hypoxia-Inducible Transcription Factor-1), allowing it to interact with the VHL (von Hippel-Lindau) complex. Prolyl Hydroxylase contains an iron moiety, and the chelation of iron inhibits enzyme activity. VHL is part of a larger complex that includes Elongin-B, Elongin-C, CUL2, RBX1, and a Ubiquitin-conjugating enzyme (E2). This complex, together with a Ubiquitin-activating enzyme (E1), mediates the ubiquitination (Ub) of HIF-1, which targets HIF-1 for degradation in the proteasome. In the absence of oxygen, Prolyl Hydroxylase cannot modify HIF-1, and the protein remains stable. Stabilized HIF-1 is translocated to the nucleus, where it interacts with cofactors such as ARNT (Aryl Hydrocarbon Receptor Nuclear Translocator), P300/CBP, and the DNA Polymerase II (Pol II) complex to bind to hypoxia responsive element (HREs) and activate transcription of target genes. ARNT2 and MOP3 are other proteins that heterodimerize with HIF-1 (not shown). *Bottom panel*: Under hypoxic conditions, HIF-1 is phosphorylated and stabilized through oncogenic signaling pathways that involve SRC, RAS, PKC, and PI3-K. In the nucleus, HIF-1 can also interact with transcription factors such as AP-1, ETS, CREB to activate transcription. RNA-binding proteins, such as HU-R, help to stabilize mRNA. HIF-1-activated genes include *vegf*, which promotes angiogenesis, *glut1*, which activates glucose transport, *ldh-A*, which is involved in the glycolytic pathway, and *epo*, which induces erythropoiesis. HIF-1 also activates the transcription of *nitric oxide synthase*, which promotes angiogenesis and vasodilation. [Reproduced from Harris 2002. With permission from Macmillan.]

In VHL-defective cells, HIF α subunits are stabilized, HIF-1 is constitutively active, and certain hypoxia-regulated genes are constitutively expressed. They include *vascular endothelial growth factor*, *glucose transporter 1*, and *carbonic anhydrase-9*.

- The expression of HIF-1α is associated with cancer progression in head and neck cancer, ovarian carcinoma, and esophageal carcinoma. HIF-2α is increased in renal cancer, and cerebellar hemangioblastomata.
- In hepatoma cells, HIF-1α represses the transcriptional activity of the *α-fetoprotein* regulatory region. An overlapping region contains a c-MYC stimulatory element and c-MYC can induce the transcription of *α-fetoprotein*.

3.3.10 Pathways of arachidonic acid metabolites

Arachidonic acid metabolism leads to the generation of biologically active products that regulate cell growth and proliferation as well as survival and apoptosis.

– Cyclooxygenases (Prostaglandin H Synthases) catalyze the conversion of arachidonic acid to prostaglandin G_2, a rate-limiting step in the synthesis of prostaglandins. The overexpression of COX-2 reduces the rate of apoptosis, possibly by induction of *bcl-2* expression, whereas a deficiency of *cox-1* or *cox-2* mediates the premature onset of terminal differentiation in keratinocytes [Tiano et al. 2002]. COX-1 and COX-2 are encoded by distinct genes. *cox-1* {9q32–q33.3} is constitutively expressed while *cox-2* is inducible by various growth factors, cytokines, and tumor promoters. Conversely, glucocorticosteroids inhibit *cox-2* expression. *cox-2* {1q25.2–q25.3} is an immediate early gene that encodes a 71 kD protein, which is up-regulated at sites of inflammation. The COX-2 protein may be localized to the perinuclear envelope [Tiano et al. 2002].
– Lipoxygenases (LOX) constitute a family of lipid peroxidizing enzymes that metabolize arachidonic acid. They are divided into four subtypes (5-, 8-, 12-, and 15-LOX) according to their tissue distribution. 12-LOX is expressed in two main isoforms, a platelet type and a leukocyte type.

The diverse classes of receptors for prostaglandins (Table 3.3.10.A) exert their effects by binding to G-Protein-coupled cell surface receptors. These interactions cause changes in intracellular cAMP and calcium. Ligation of the Prostaglandin E_2 (PGE2) Receptor subtype EP4 activates the Phosphatidylinositol 3-Kinase pathway.

- COX-2 contributes to esophageal, gastrointestinal, pancreatic, lung, head and neck, and prostatic carcinogenesis. Overexpression of COX-2 also occurs in gliomata. Furthermore, in the absence of *cox-1* or *cox-2* gene products, skin or intestinal carcinogenesis are substantially reduced [Tiano et al. 2002]. COX-2 may also be a mediator in other oncogenic pathways. Cells transformed with *v-src* have elevated expression of COX-2. In colon carcinoma, activation of the HER2 and HER3 pathways by Heregulin induces *cox-2* gene expression.
- Ultraviolet B-induced Cyclooxygenase-2 expression is important in skin tumor promotion. $P38^{MAPK}$ is a downstream target of prostaglandin signaling. It phosphorylates CREB/ATF-1 (cAMP Response Element Binding Protein/Activating Transcription Factor-1) and plays a major role in this process. Similarly, the activation of Phosphatidylinositol 3-Kinase and PKB, which mediates the inhibition of GSK-3β, contributes substantially.
- COX-2 overexpression in the mammary gland induces mammary cancer. Prostaglandin E_2, the product of COX-2 catalysis, exerts tumorigenic effects on the mammary gland via its receptor EP2. In the absence of EP_2 signaling, total cellular cAMP levels are decreased and Amphiregulin, a potent mammary epithelial cell growth factor and EGF Receptor ligand, is down-regulated in mammary glands [Chang et al. 2005]. Elevated levels of prostaglandin E_2, caused by high expression of COX-2, occur in invasive breast cancer. They are associated with negative hormone receptor status and increased metastatic potential [Half et al. 2002].
- Normal prostate epithelial cells express low levels of COX-2. In prostate tumor cells, COX-2 may prevent Caspase-3 activation and ensuing apoptosis by inducing the phosphorylation and activation of PKB. This effect does not depend on BCL-2.
- Platelet-Type 12-LOX (Arachidonate 12-Oxidoreductase, ALOX12) {17p13.1} regulates the growth and survival of various cancer cells. In breast cancer cells, the expression and activity of 12-LOX are higher than in the surrounding normal cells. An *alox5* haplotype, containing A instead of G at positions –1,752 and –1,699 within a negative regulatory region of the promoter, may influence colon cancer risk in Caucasians [Goodman et al. 2004].

Table 3.3.10.A. Prostaglandin receptors. Diverse prostaglandin receptors are differentially expressed on various cell types. Their engagement by their cognate ligands induces specific biological responses

Receptor	Size	Ligand	Expression	Signaling	Biology
DP_1	359 aa	PGD_2	Platelets, vascular smooth muscle, nervous tissue, retina, intestines, lungs, stomach, uterus	Elevation of intracellular cAMP through activation of Adenylate Cyclase	Inhibition of platelet aggregation, relaxation of smooth muscle
DP_2	395 aa	PGD_2	Eosinophils, basophils, TH_2 cells	Mobilization of intracellular calcium	Chemotaxis, eosinophil degranulation
EP_1	402 aa	PGE_2	Kidney, lungs, spleen, muscle, testis, uterus	Increase in phosphatidylinositol turnover	Contraction of smooth muscle
EP_2	358 aa	PGE_2, PGE_1	Lung, placenta	Elevation of intracellular cAMP through activation of Adenylate Cyclase	Relaxation of smooth muscle
EP_3	365–425 aa	PGE_2, PGE_1	Kidney, stomach, uterus, pancrease, adrenals, testis, ovaries, intestines	Decrease of intracellular cAMP through inhibition of Adenylate Cyclase	Inhibition of water absorption, inhibition of gastric acid secretion, uterine contraction
EP_4	488 aa	PGE_2, PGE_1	Small intestine, lung, thymus, kidney, uterus, pancreas, spleen, heart, stomach, brain	Elevation of intracellular cAMP through activation of Adenylate Cyclase	Relaxation of smooth muscle
FP	359 aa	PGF_{2a}	Corpus luteum, uterus, stomach, kidney, heart, lung, eye, liver	Increase in phosphatidylinositol turnover	Myometrial contraction, bronchoconstriction
IP	386 aa	PGI_2	Platelets, vascular smooth muscle, kidney, thymus, liver, lung, spleen	Elevation of intracellular cAMP through activation of Adenylate Cyclase	Relaxation of arterial smooth muscle, inhibition of platelet aggregation
TP	343–369 aa	PGD_2 PGE_2 PGF_{2a}	Platelets, vascular smooth muscle, thymus, spleen, lung, kidney, heart, uterus	Increase in phosphatidylinositol turnover	Platelet aggregation, contraction of airway smooth muscle

- Prostaglandin E_2 may promote tumorigenesis in part by engagement of the EP1 receptor subtype, which is localized to the nuclear envelope in various cell types. The EP2 receptor is also linked to carcinogenesis.

3.3.11 Homeobox pathways

Homeobox transcription factors contain a 61 amino acid helix–turn–helix DNA-binding moiety, called homeodomain. In addition, sequences flanking the homeodomain also affect their binding specificity by coordinating the interaction with cofactor proteins. There are at least 39 major *hox* genes, grouped in four clusters, A (comprising A1–A7, A9–A11, A13), B (B1–B9), C (C4–C6, C8–C13), and D (D1, D3, D4, D8–D13), that lie on four distinct chromosomes (Figure 3.3.11.A). They are further divided into 13 paralog groups by structural and functional criteria. The expression of *hox* genes in blood cell progenitors is stage and lineage specific and is universally down-regulated upon differentiation into mature blood cells. *hoxA* and *hoxB* genes are expressed in $CD34^+$ cells, and are down-regulated as the cells leave the $CD34^+$ compartment. HOXB4 induces the selective expansion of primitive hematopoietic stem cells. HOXA10 leads to a selective expansion of the megakaryocytic cell component with diminished numbers of monocytic and B-lymphoid progenitors. Endoderm development depends on the homeobox containing gene *mixer*, which regulates the expression of the two endodermal determinants *sox17*α and *sox17*β [Henry and Melton 1998].

Genes are silenced through the compaction of chromatin. The polycomb group of gene repressors and the trithorax group of gene activators are two antagonistic classes of proteins that act through the modulation of chromatin structure. Together, they maintain the gene expression patterns of key

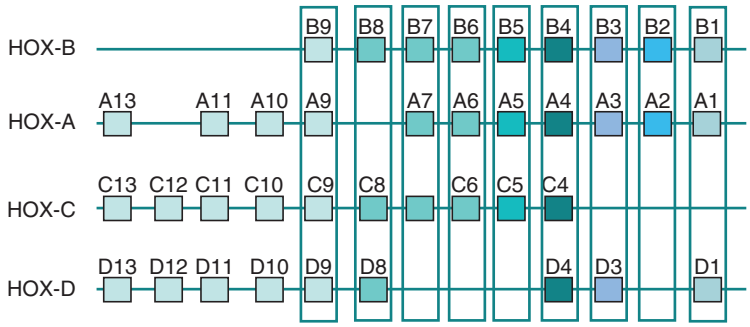


Figure 3.3.11.A. Homeobox gene clusters. The human counterparts of the Drosophila hom-C genes, located on a single chromosome in the fly, are designated hox-A through hox-D and are arranged on four distinct chromosomes. Genes within the hox clusters show striking structural and functional conservation, as indicated by the color coding.

Table 3.3.11.A. Polycomb gene products and their association with cancer. Many Polycomb proteins are members of distinct classes of multimeric complexes, termed Polycomb repressive complexes (PRCs). The more diverse PRC1 complex can recognize trimethylated lysine 27 on Histone H3, which may target PRC1 to appropriate genomic sites. The PRC2 complex is involved in the initiation of gene repression.

Protein	Characteristics	Cancer
Polycomb repressive complex 1		
CBX2	Chromodomain, binds to methyllysine	
CBX4	Recruits the corepressor CTBP, repression of *c-myc* expression	
CBX8	Chromodomain, Pc box, repression of *cdkn2A*	
EDR1	Zinc finger SPM domain	Acute lymphoblastic lymphoma
RING1	RING finger domain	
BMI1	RING finger domain	Lymphoma, leukemia, medulloblastoma, neuroblastoma, lung cancer
ZNF134	Zinc finger domain	Insulinoma, thyroid adenoma
PHF1	Tudor domain, 2 PHD fingers, PCL homology domain	
PCL3	Tudor domain, 2 PHD fingers, PCL homology domain, two nuclear localization domains	Colorectal cancer, lung cancer, cervical cancer, uterine cancer, skin cancer
Polycomb repressive complex 2		
EED	Five WD40 repeats, PEST sequence	Colorectal adenocarcinoma, chronic myeloid leukemia, osteosarcoma
EZH1	SET domain, Histone, Methyl Transferase	
EZH2	Histone Methyl Transferase	Breast cancer, bladder cancer, colon cancer, prostate cancer, lymphoma, melanoma
SUZ12	Zinc finger domain	Breast cancer, liver cancer, colon cancer, endometrial stromal tumors

developmental regulators and hence are crucial players in cellular differentiation.

– The trithorax group contains members of the SWI/SNF family of ATP-dependent chromatin remodeling factors.
– The polycomb group proteins preserve body patterning through development by maintaining transcriptional silencing of homeotic genes (*hox* genes) (Table 3.3.11.A). The proto-oncogenic function of *polycomb* gene products may reflect their role in the maintenance of stem cells.

The Polycomb Repressive Complex1 (PRC1) induces the compaction of defined nucleosomal arrays. Gene silencing factors, such as the PCC complex, HP-1, and H1 stabilize higher order chromatin folding. In contrast, gene activators, such as the SWI/SNF remodeling complexes and Histone Acetyl Transferases, initiate chromatin unfolding.

PBX (Pre-B-Cell Leukemia Transcription Factor) proteins act at the far end of *hox* gene activation. PBX1 {1q23} forms complexes with specific HOX proteins. HIPK2 (Homeodomain Interacting Protein Kinase 2) is a nuclear serine/threonine kinase, which represses the gene expression of homeodomain containing transcription factors. HIP2K localizes with P53 and PML into nuclear bodies and is activated by UV irradiation. The inhibition of expression of homeodomain transcription factors may be a part of the cell cycle control functions of HIPK2.

- The overexpression of *homeobox* genes may lead to leukemia. Regulators of *hox* gene transcription are aberrantly expressed in leukemias more frequently than the *hox* genes themselves. BMI-1 is a member of the polycomb complex of proteins, which act as silencers of *hox* target genes. The polycomb group of gene products comprises transcription factors that may influence tumorigenesis.
- Pre-B-cell ALLs are frequently associated with a t(1;19)(q23;p13.3) chromosomal rearrangement. The recombination mechanism is site specific, frequently occurring within a range of five base pairs. In the resulting chimeric transcription factor, the DNA-binding domain of E2A is replaced by a putative DNA-binding domain of PBX1 [Kamps et al. 1990; Nourse et al. 1990].
- *hox11* does not lie within the major *hox* gene clusters. Its product interacts with phosphatases that normally function at the G_2 checkpoint. Its dysregulation by chromosomal rearrangements leads to T-cell ALL [Look 1997].
- AML1 (RUNX1) and its physiologic binding partner CBFβ may act as upstream regulators that participate in the initiation of specific patterns of *hox* gene expression. Retinoic acid has profound effects on *hox* gene expression during embryogenesis. Oncogenic fusion proteins involving RARα and CBF can act upstream of *hox* genes in acute promyelocytic leukemogenesis.
- An amplicon on chromosome 17q21.3 leads to overexpression of the *hoxB7* gene in a fraction of breast cancers and is associated with poor prognosis.
- The polycomb gene product EZH2 is associated with breast and prostate cancer metastasis. Its expression increases with tumor progression and may reflect the maintenance of the cells at a low level of differentiation.

3.3.12 The RB pathway

The RB pathway regulates the ability of cells to exit from the resting phases (G_0 and G_1) of the cell cycle. Underphosphorylated RB binds the Cyclins D_1 and D_3 and sequesters them. Binding and sequestration of the transcription factor E2F by RB prevents the expression of gene products that mediate cell cycle progression. Phosphorylation of RB inhibits its binding to transcriptional repressor proteins of the E2F family. RB can be phosphorylated by CDK4/Cyclin D on serines 780 and 795 and by CDK2/Cyclin A on threonine 821. This phosphorylation of RB by CDKs relieves the repression of cell cycle progression genes and is a prerequisite for the transition from G_1 to S. $P16^{INK4a}$ is a specific inhibitor of these CDKs. It supports the hypophosphorylation of RB, which leads to the sequestration of E2F transcription factors and to cell cycle arrest (Figure 3.3.12.A).

There are six genes encoding E2F transcription factors. The transcription factor E2F is expressed in a wide variety of cell types and tissues. It is essential for E1A-dependent activation of the adenovirus E2 promoter. Like the other members of the E2F family, the E2F1 protein contains a NH_2-terminal DNA-binding domain and a COOH-terminal acidic amino acid transactivation domain. E2F binding sites are located in the promoters of many genes, whose products are involved in DNA synthesis and cell proliferation. The *e2f1* gene {20q11.2} is composed of seven exons and spans approximately 11 kb. Intron 4 does not have consensus 5′ and 3′ splice sites.

HBP1 is a transcriptional repressor and a cell cycle inhibitor, which can induce cell differentiation and is targeted by the Retinoblastoma family. HBP1 is a

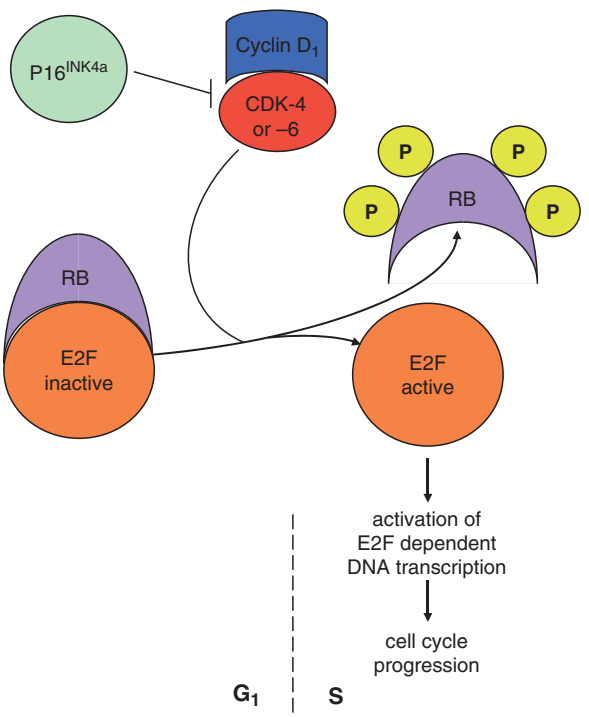


Figure 3.3.12.A. RB pathway. While RB exists in the underphosphorylated state, it can block cell cycle progression in G_1. Consecutive to its phosphorylation in mid-G_1 by Cyclin D_1/CDK 4 or Cyclin D_1/CDK6, progress into S phase can commence and E2F is released and activated. $P16^{INK4a}$ is an inhibitor of Cyclin D_1/CDK complexes and thus can prevent the exit from G_1.

sequence-specific high mobility group (HMG) transcription factor, which contains the two consensus RB interaction motifs LXCXE and IXCXE, and binds selectively to RB and P130, but not to P107. HBP1 can induce G_1 cell cycle arrest through transcriptional repression of the promoters for *cyclin* D_1 and *N-myc* [Tevosian et al. 1997].

The extracellular inhibitor of cell proliferation, TGF-β, induces the CDK Inhibitors $P16^{INK4}$, P15, $P21^{CIP1/WAF1}$, and $P27^{KIP1}$, which bind to Cyclin D/CDK4 complexes, thus preventing RB phosphorylation and arresting the cell cycle in G_1. Conversely, the RB protein is a target for down-regulation by the oncogene proteins of several DNA tumor viruses, including adenoviruses and human papilloma viruses. Viral oncogenes, including human papilloma virus E7, may bind to RB through a LXCXE motif. The RB binding site for E2F lies at the interface between the subdomains A and B of RB.

SWI/SNF is a heterogeneous multi-subunit chromatin remodeling complex, which utilizes the energy of ATP to remodel chromatin structure and contains either SMARCA4 (BRG-1) or BRM, both homologs of the SWI-2 subunit, as the central ATPase. The activity of the core ATPase is required by the SWI/SNF complex to regulate gene transcription. Members of the SWI/SNF complex can activate the RB pathway, while their loss compromises the RB-mediated transcriptional repression [Strobeck et al. 2000].

– SMARCA4 stimulates RB activity and is involved in chromatin remodeling
– SNF5 mediates G_1 arrest associated with the induction of $p16^{INK4a}$ and activation of RB

The *abl* gene has a size of about 225 kb and is expressed as either a 6 kb or a 7 kb mRNA transcript, with alternatively spliced first exons, 1b and 1a, respectively, added to the common exons 2–11. Encoding of the NH_2-terminal region of the ABL protein by exon 1a favors its localization in the nucleus, while encoding by exon 1b places a myristoylation site at the NH_2-terminal glycine and directs that protein to the plasma membrane. The NH_2-terminal domain contains the tyrosine kinase site, a SH2 domain, and a SH3 domain (Figure 3.3.12.B). The activity of the ABL protein is negatively regulated by its SH3 domain, the loss of which turns it into an oncoprotein. Oncogenic forms of ABL can autophosphorylate on tyrosine 412. The COOH-terminal domain contains three distinct nuclear localization sequences, one nuclear export sequence, three HMG-like boxes that bind cooperatively to A/T-rich DNA, a G-Actin binding domain, and an F-Actin binding domain. P73 is a target of regulation by c-ABL in DNA damage responses. c-ABL stabilizes P73, thus activating its proapoptotic functions. ATM and DNA-PK activate c-ABL in response to DNA damage by ionizing radiation if the insult occurs at the G_1/S transition. Preceding this stage, the binding of ABL to RB prevents its activation in the damage response pathway. RB binds to the tyrosine kinase domain of c-ABL and inhibits it. RB can also recruit c-ABL to E2F and form a DNA-binding complex, without being a kinase substrate for ABL. Nuclear ABL becomes active at the time of S phase entry. The phosphorylation of RB on serines 807 and 811 releases it from ABL, which leads to the induction of ABL kinase activity. Nuclear c-ABL phosphorylates RNA Polymerase II. This phosphorylation correlates with the transition from transcription initiation to elongation. Adhesion to the extracellular matrix causes the translocation of c-ABL from the nucleus to the cytoplasm, in a manner that depends on the nuclear export sequence of ABL. As cells spread on the extracellular matrix, c-ABL is imported back into the nucleus. Both tyrosine kinases, c-ABL1 (Abelson Murine Leukemia Viral Oncogene Homolog 1) {9q24.1} and c-ABL2 (Abelson-Related Gene, ARG) {1q24–q25}, shuttle between nucleus and cytoplasm.

- A number of inactivating point mutations in cancer map to the subdomain B and to the interface between the subdomains A and B of RB. The majority of *rb1* mutations result in truncated or unstable proteins, which leave E2F-1 free to continually initiate the transcription of cell cycle promoting genes. RB is often defective in RB and in osteosarcoma.
- Alterations in the *rb2* gene are associated with various cancers. Loss of heterozygozity of chromosome 16q12.2 occurs in breast carcinoma, ovarian carcinoma, and prostate carcinoma. Down-regulation of *rb2* gene expression may arise in small cell lung cancer, endometrial carcinoma, vulvar carcinoma, choroidal melanoma, and non-Hodgkin lymphoma. Point mutations in *rb2* are associated with small cell lung cancer, non-small cell lung cancer, nasopharyngeal carcinoma, and EBV^+ Burkitt lymphoma [Paggi and Giordano 2001].

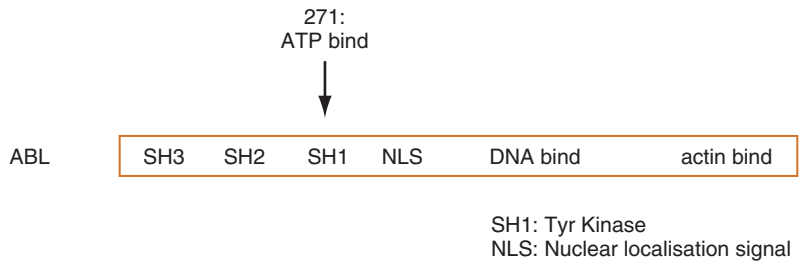


Figure 3.3.12.B. Structure of ABL. The protein comprises 1130–1143 amino acids. Functional domains include SH3 (binds BP1 to inhibit SH1 activation), SH2, SH1 (with a tyrosine that is subject to autophosphorylation), a nuclear localization domain, a DNA-binding domain, and an Actin-binding domain. Note that the form 1b (but not the alternatively spliced form 1a) is myristylable allowing anchorage to the membrane. [Reprinted from Atlas Genet Cytogenet Oncol Haematol. October 1997. Huret JL. ABL1 (v-abl Abelson murine leukemia viral oncogene homolog 1). http://AtlasGeneticsOncology.org/Genes/ABL.html. With permission from Atlas.]

- Cyclin D_1/CDK4 kinase activity is elevated in various cancers, including breast cancer, head and neck cancer, hepatocellular carcinoma, and colorectal carcinoma, either through the overproduction of Cyclin D_1 or through mutations in *cdk4*, which makes Cyclin D_1/CDK4 insensitive to the inhibitory effects of $P16^{INK4a}$. Furthermore, the tumor suppressor protein $P16^{INK4a}$ is itself commonly mutated in certain cancers. It is the only INK4 homolog strongly implicated in carcinogenesis.
- In squamous cell carcinoma, CDK6, normally a target of P16 may be affected. This leads to unchecked inactivation of the RB tumor suppressor. The overexpression of CDK6 mediates accelerated progression through G_1, dependent on its NH_2-terminal INK4 interaction domain.
- Most tumors harbor dysregulations of the E2F family of transcription factors, resulting in abnormal cell cycle progression. This may occur as a consequence of the loss of the CDKI $P16^{INK4a}$, the overexpression of Cyclin D, or the loss of RB. The apoptotic activity of E2F1 reflects, at least in part, its ability to transactivate the promoters of *p73* and *p53*.
- RIZ-1 (Retinoblastoma-Interacting Zinc Finger Protein) is a tumor suppressor and a member of the Histone/Protein Methyl Transferase superfamily. *riz1* inactivation commonly arises in diffuse large B-cell lymphomata, breast cancers, and liver cancers. It occurs through DNA hypermethylation, frameshift mutations, chromosomal deletion, or missense mutations.
- Truncating mutations and homozygous deletions in the SNF5 (INI1, BAF47) subunit of SWI/SNF complexes occur in most malignant rhabdoid tumors [Biegel et al. 2002].
- The concomitant loss of the SWI/SNF ATPase subunits SMARCA4 (BRG-1) and BRM occurs in approximately 10% of non-small lung cancers. This is associated with a reduced life expectancy.
- A common chromosome translocation in CML leads to the formation of the oncogenic fusion protein BCR–ABL. The BCR–ABL oncoprotein strongly associates with F-Actin. The formation of BCR–ABL oligomers is permissive for the simultaneous binding of multiple F-Actin filaments (Figure 3.3.12.C).

3.3.13 The P53 pathway

P53 has a domain structure with a transactivation domain (amino acids 1–101), a sequence specific DNA-binding domain (amino acids 102–292), an oligomerization domain (amino acids 320–360), and a region for inhibition of sequence-specific binding (amino acids 363–393) (Figure 3.3.13.A).

– P53 recruits the basal transcriptional machinery through the transactivation domain (amino acids 1–101). It interacts with TBP (TATA Box Binding Protein) and TAF (TBP-Associated Factor, a component of TFII-D). P53 contains a nuclear export signal in amino acids 11 through 27. Phosphorylation in this region correlates with reduced nuclear export of P53. The oncoprotein MDM2 (an E3 Ubiquitin Ligase with a RING finger motif) binds to the NH_2-terminal region of P53 and thus inhibits its transactivating activity.
– P53 binds to the consensus nucleotide sequence (A/G)(A/G)(A/G)C(A/T)(A/T)G(C/T)(C/T)(C/T) (P53 responsive element) as a tetramer. The DNA-binding domain of P53, from amino acid

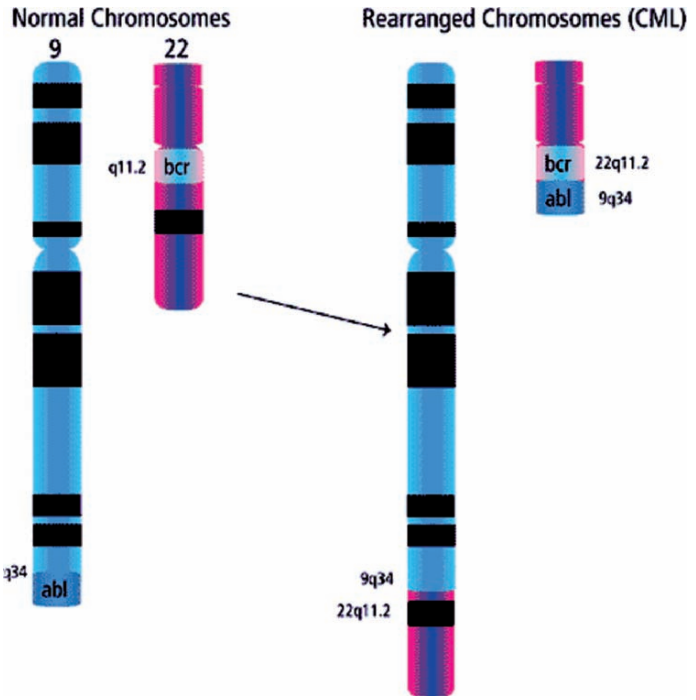


Figure 3.3.12.C. Philadelphia chromosome. Generation of the Philadelphia chromosome is associated with more than 95% of chronic myelogenous leukemias (CML). Normal representations of chromosome 9 and 22 are displayed on the left. The right side shows the rearranged chromosomes, where the *c-abl* proto-oncogene from the distal tip of chromosome 9q34 has been translocated into the *bcr (breakpoint cluster region)* locus on chromosome 22q11.2. This t(9;22) translocation generates a chimeric gene that expresses a chimeric *bcr–abl* mRNA, yielding a fusion protein. [Reproduced from http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cancer_Research/Key_Resources/Overview_of_Cancer_Biology.html. There are instances where we have been unable to trace or contact the copyright holder. If notified the publisher will be pleased to rectify any errors or omissions at the earliest opportunity.]

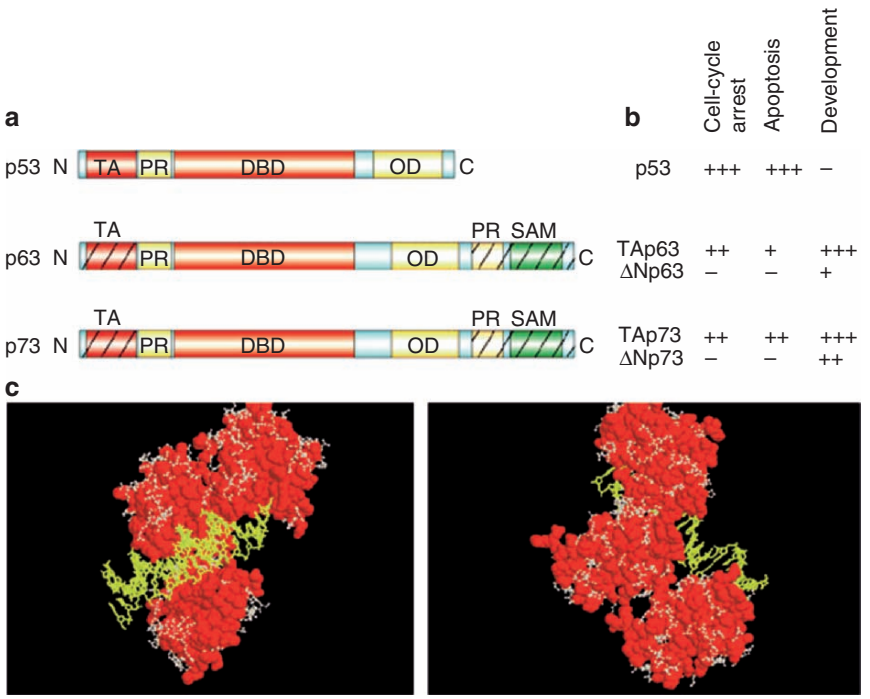


Figure 3.3.13.A. The P53 family. (a) The p53 family includes threegenes that encode P53, P63, and P73. The overall domain structure of P53, P63, and P73 is conserved. In contrast to P53, P63, and P73 have many different isoforms with distinct NH_2- and COOH-termini. Full length-isoforms contain the transactivation domain, and are designated TA. NH_2-terminally deleted isoforms are designated N. Dashed lines indicate different isoforms. (b) The functions of the TA and N isoforms are summarized. (c) Structure of the P53 DNA-binding domain. Even though the DNA-binding domains of P73 and P53 display only 63% identity in amino acid sequence, the three-dimensional structure of the region of interaction with DNA is almost identical (*left*), indicating recognition of the same transcriptional targets. Most of the differences are located on the outside surface (right), indicating differential protein–protein interactions and regulation. Residues that are identical in P53 and P73 are shown in red, amino acids that are changed in P73 are in white. DNA is shown in yellow. DBD = DNA-binding domain, OD = oligomerization domain, PR = proline-rich domain, SAM = sterile α motif. [Reproduced from Melino et al. 2002. With permission from Macmillan.]

102 to 292, contains four conserved regions (II–V) and forms a zinc finger structure with four-stranded and five-stranded antiparallel β-sheets. Most frequently, tumor-associated mutations occur in this area.
– A central region (residues 300–360) includes a flexible linker region (amino acids 300–318), connecting the central core domain and a tetramerization domain from amino acids 323–356. A nuclear localization signal (NLS) is present in this moiety of P53. Mutations in NLS1 (amino acids 316–325) retain P53 in the cytoplasm. Also in this region, there is a nuclear export signal between amino acids 320 and 355. The nuclear export signal of P53 is masked by tetramer formation, but functional, when P53 is either a monomer or a dimer.
– COOH-terminal to the oligomerization domain, there is a basic region (amino acids 363–393), which is also known as an apoptopic domain, a transcriptional regulatory domain, or a DNA damage recognition domain. This extreme COOH-terminal domain acts as a negative regulator of P53 sequence-specific binding. Two nuclear localization signals are present in the COOH-terminal region. Mutations of NLS2 (amino acids 369–375) or NLS3 (amino acids 379–384) lead to partially cytoplasmic and partially nuclear localization.

The transcriptional activity for specific genes exerted by P53 is most relevant to its tumor suppressing function (Table 3.3.13.A). Specificity in the transcriptional activity of P53 is achieved by its association with diverse coactivators.
– P53 activation leads to cell cycle arrest. The most important protein relative to cell cycle control, whose transcription is induced by P53, is the Cyclin-Dependent Kinase inhibitor P21, which binds to and inhibits G_1 CDK/Cyclin complexes. As a result, cells with damaged DNA are arrested in G_1 until the damage is repaired and the levels of P53 and P21 fall. The cells then can progress to S phase. This depends, in part, on coactivators, including P300/CBP, TAFII31, or ADA3. Interactions with c-ABL stabilize the tetrameric conformation of P53 and, as a consequence, stimulate P53 DNA binding to four-quarter binding sites (perfect binding sequences). Such sequences are present in the promoter for *p21*, but not in the promoter for *bax*. c-ABL selectively activates P53-mediated growth arrest [Wei et al. 2005].
– Alternatively, accumulation of P53 also leads to the expression of proteins that promote apoptosis. The transcriptional coactivators ASPP, JMY, ZAC-1, P63, and P73 support P53-induced apoptosis. ASPP proteins act as transcriptional coactivators of P53 by specifically stimulating its apoptotic function, but not its cell cycle arresting function. P53-dependent apoptosis may be mediated by the transcription of *bax* and the down-regulation of *bcl-2* expression. This prevents the proliferation of cells that are likely to accumulate multiple mutations.

Table 3.3.13.A. Gene products, the expression of which is induced by P53. P53 regulates cell apoptosis and cycle progression. It also activates autoregulatory mechanisms that assure the temporal limitation of P53 activity

Death receptors	FAS
	PIDD
	DR5 (KILLER)
Proapoptotic pathways	APAF-1
	BAX
	FDXR
	NOXA
	P53AIP1
	P53DINP1
	PERP
	PIG3
	PIG8 (EI24)
	PUMA
	WIP1
Inhibition of survival signals	IGF-BP3
	PTEN
Cell cycle arrest and DNA repair	BTG2
	P21 (CDKN1A)
	14-3-3σ
	GADD45
	P53R2
Autoregulation	MDM2
	P73
	CCNG1

Stress in the form of DNA damage, malfunction of the mitotic spindle, ribonucleotide depletion, hypoxia, heat shock, and reactive oxygen species leads to the rapid induction of P53 activity. There are several mechanisms that elevate the cellular levels or activity of P53.
– Induction occurs largely through the stabilization of the P53 protein. Induction of $P14^{ARF}$ (through translation of an alternative reading frame of the *ink4a* tumor suppressor gene) leads to binding of $P14^{ARF}$ to MDM2 and prevents MDM2-dependent P53 proteolysis.
– Activation of P53 is achieved, in part, through phosphorylation of the NH_2-terminal transactivation domain, specifically on serines 15 and 20 and on threonine 18. ATM (Ataxia Teleangiectasia Mutated)

may mediate the nuclear accumulation of P53 after phosphorylating it on serine 15, which induces the transcription of the CDK2 Inhibitor *p21*$^{CIP1/WAF1}$ and leads to cell cycle arrest in G_1 [Weinstein et al. 1995]. ATM also phosphorylates and activates the checkpoint kinase CHK2 (CDS1), which then phosphorylates P53 on serine 20. The phosphorylation of serines 15 and 20 prevents the binding to MDM2 and stabilizes P53, which would otherwise have a half-life of a few minutes. Mechanisms dependent on CHK2 and P53 are activated in response to DNA double strand breaks, but not single strand breaks. This pathway is also active after γ-irradiation, but not after UV irradiation [Hirao et al. 2000]. Checkpoint activation consecutive to UV irradiation is independent of ATM and CHK2 but may involve the ATR and CHK1 pathway. CHK1 phosphorylates multiple residues on the NH_2-terminus of P53 to activate it. It can also phosphorylate and inactivate CDC25. In response to ionizing radiation, DNA-PK phosphorylates and activates the protein threonine kinase c-ABL. This is facilitated by binding of the proline-rich region of ATM to the SH2 domain of c-ABL. c-ABL binds to P53 and stabilizes the P53 tetramer. This leads to the elevated expression of *p21*. When P53 is phosphorylated on serines 15 and 293, following UV irradiation, it cannot leave the nucleus. DNA-PK can phosphorylate MDM2 as well as the NH_2-terminal serines 15 and 37 in P53. This prevents the two proteins from interacting, thus stabilizing and activating P53. This mode of stabilizing P53 may depend on binding to DNA by P53 and DNA-PK proximal to each other.

– The COOH-terminal regulatory domain of P53 is a target for phosphorylation by Casein Kinase 2 (CK2) and CDKs. PKC phosphorylates P53 on serine 360, serine 370, and threonine 377. This is, however, dependent on the accessibility of the phosphorylation sites. In addition to regulating DNA binding, phosphorylation in the COOH-terminal domain regulates the half-life of P53.
– Nuclear bodies are important functional entities. Upon activation by UV irradiation, HIPK2 (Homeodomain Interacting Kinase 2, PKM) interacts with P53 and CBP (CREB Binding Protein) in PML nuclear bodies. HIPK2 phosphorylates P53 on serine 46, thus facilitating the acetylation of P53 on lysine 382 by CBP. This promotes the transcriptional activation of proapoptotic genes by P53 [Hofmann et al. 2002; D'Orazi et al. 2002].
– Acetylation of P53 occurs in the COOH-terminal part of the molecule. Acetylation of P53 is induced in response to cellular stress, such as DNA damaging agents or γ-irradiation. It is induced by the Histone Acetyl Transferases P300/CBP [Gu and Roeder 1997] on lysine 372, lysine 373, and lysine 381 or by P/CAF [Sakaguchi et al. 1998] on lysine 320. This increases the affinity of P53 for DNA. After induction by RAS, the ring finger containing protein PML localizes to discrete nuclear bodies and colocalizes with P53 and P300/CBP. This is accompanied by acetylation of P53 at lysines 320 and 382. Even though acetylation of P53 may lead to increased DNA binding, it is more important for the recruitment of coactivators. It is unclear whether the subcellular localization of P53 is affected by acetylation [Prives and Manley 2001]. Whereas P53 phosphorylation facilitates the recruitment of Histone Acetyl Transferases, MDM2 binding blocks this interaction. Histone Deacetylase complexes, including HDAC1 or SIR2, silence P53-activated gene expression. This results in part from the deacetylation of nucleosomes in the vicinity of target promoters and in part from the deacetylation of P53. The NAD-dependent Deacetylase SIRT1 (SIR2α) deacetylates P53 and abolishes its transcriptional activity as well as its apoptotic function, whereas inhibition of SIR2 potentiates the effects of P53 [Luo et al. 2001; Vaziri et al. 2001].
– SUMO is a small Ubiquitin-related protein that is conjugated to target proteins through enzyme cascades similar to those responsible for ubiquitination. P53 is SUMO modified at one of the lysine residues that is also ubiquitinated. Although SUMO modification does not regulate P53 stability, this modification enhances the transcriptional activity of P53.
– While changes in the rate of *p53* transcription play a minor role, there is regulation on this level. C-JUN negatively regulates an AP-1-like site in the *p53* promoter and cell cycle progression from G_1 to S depends on the down-regulation of *p53* by c-JUN.

The proto-oncogene *mdm2* (*murine double minute 2*) maps to 12q14.3–q15, distal to *cdk4*, and its 12 exons encode a 90 kD transcription factor. More than 40 splice variants of *mdm2* are expressed in various cell types. Often, splicing occurs at cryptic splice donor and acceptor sites in regions with high sequence homology that are present four times within the coding region of the *mdm2* mRNA. There are two different promoters, one of which is responsive to P53. These promoters generate distinct proteins, the full length P90^{MDM2} and

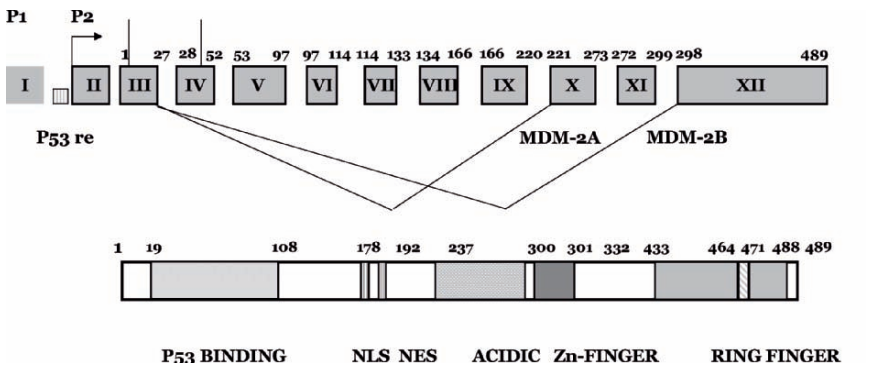


Figure 3.3.13.B. MDM-2 structure. The proto-oncoprotein MDM-2 acts as a cellular inhibitor of P53 by binding to the transactivation domain of P53 and suppressing its ability to activate transcription. The upper panel depicts the gene and the lower panel shows the protein structure. Exon 1 is untranslated. P53 re is the P53 response element. Two messages can be generated through the use of alternative promoters. The protein has multiple functional domains as indicated in the lower panel. NLS = nuclear localization sequence, NES = nuclear export sequence.

a shorter protein $P76^{MDM2}$ that initiates at an internal ATG at positions 19–21. $P76^{MDM2}$ is missing part of the P53 binding domain and can act as a dominant negative inhibitor of P90 with regard to P53 activation. Because the *mdm2* gene is itself transcriptionally activated by P53, MDM2 functions in an autoregulatory feedback loop with P53, perhaps normally preventing excess P53 function.

Under normal conditions, MDM2 (Figure 3.3.13.B) binds to the transactivation domain in the NH_2-terminus of P53, both repressing the ability of P53 to activate gene transcription and mediating P53 degradation. The amino acids 22 and 23 of P53 are crucial for the interaction with MDM2, while G58 and C77 on MDM2 are crucial for binding to P53.

– Forming a tight complex with the P53 gene product, the MDM2 oncogene product can inhibit P53-mediated transactivation.
– MDM2 is an E3 Ubiquitin Protein Ligase specific for P53. Ubiquitination of P53 by MDM2 may unmask the nuclear export signal, which is otherwise masked by tetramer formation, enabling P53 to move to the cytoplasm and be degraded. The ratio of the Ubiquitin Ligase MDM2 to its substrate P53 determines whether poly- or monoubiquitination occurs. Low levels of MDM2 induce monoubiquitination and nuclear export of P53, whereas high levels of MDM2 promote the polyubiquitination of P53 and intranuclear degradation. Therefore, monoubiquitination is important for P53 trafficking [Li et al. 2003a]. Shuttling of MDM2 between the nucleus and the cytoplasm is required for P53 degradation. The MDM2 ring domain is necessary for the nuclear export of P53.

To regulate P53, MDM2 must gain nuclear entry. Mitogen-induced activation of Phosphatidylinositol 3-Kinase and its downstream target, the PKB (AKT) serine/threonine kinase, results in phosphorylation of MDM2 on serine 166 and serine 186. Phosphorylation on these sites is necessary for the translocation of MDM2 from the cytoplasm into the nucleus. A lack of Phosphatidylinositol 3-Kinase →PKB signaling inhibits the nuclear entry of MDM2, increases the cellular levels of P53, and augments P53 transcriptional activity [Mayo and Donner 2001].

MDM2 is subjected to either ubiquitination or sumoylation. MDM2 can be conjugated with SUMO1 within its RING finger domain that plays a critical role in MDM2 self-ubiquitination. When self-ubiquitinated, the Ubiquitin Ligase activity of MDM2 for P53 is impaired. Upon SUMO1 conjugation, MDM2 is protected from ubiquitination and elicits increased Ubiquitin Ligase activity, as reflected in increased ubiquitination and degradation of P53. The phosphorylation of P53 on serine 15 and serine 37 by DNA-PK impairs the ability of MDM2 to inhibit P53-dependent transactivation.

Cyclin G_1, together with MDM2, constitutes a part of a negative feedback system that attenuates the activity of P53. *cyclin* G_1 is a transcriptional target of P53, and its expression is increased after DNA damage. Cyclin G_1 can regulate the levels of P53 by binding to PP2A and inducing it to dephosphorylate MDM2. This inhibits the ability of MDM2 to target P53 for degradation [Okamoto et al. 2002]. PP2A is a trimeric serine/threonine phosphatase with a catalytic A subunit, a C subunit with scaffolding function, and three forms of a regulatory B subunit

(B, B′, or B″). P53 drives a low level negative feedback loop. Increasing the levels of MDM2 terminates the P53 response, but only if MDM2 remains in a hypophosphorylated state.

The *mdm2* gene is regulated in a P53-independent manner by the RAS-driven RAF→MEK→MAP Kinase pathway. MDM2, induced by activated RAF, degrades P53 in the absence of the MDM2 inhibitor $P14^{ARF}$. Because of this regulatory pathway, cells transformed by oncogenic *ras* are more resistant to P53-dependent apoptosis following exposure to DNA damage. Activation of the RAS-induced RAF→MEK→MAP Kinase cascade may therefore play a key role in suppressing P53 during tumor development. RAF also activates the MDM2 inhibitor $P14^{ARF}$. The cellular level of P53 is therefore determined by opposing effects of RAF-induced $P14^{ARF}$ and RAF-induced MDM2. The *mdm2* gene itself gets activated by P53, which provides a basis for negative feedback control of P53 activity.

In addition to the regulation of P53 levels and activity, MDM2 actions are exerted through other pathways. MDM2 also interacts physically and functionally with the RB protein and can inhibit its growth regulatory capacity. MDM2 interacts with transcription factors of the E2F family and with TBP.

The *tp73* gene {1p36} is closely related to *p53* [Kaghad et al. 1997; Jost et al. 1997]. It gives rise to several distinct RNAs, both by alternative splicing and by the use of alternative promoters (Figure 3.3.13.C). At least seven distinct P73 proteins (α–η) are generated

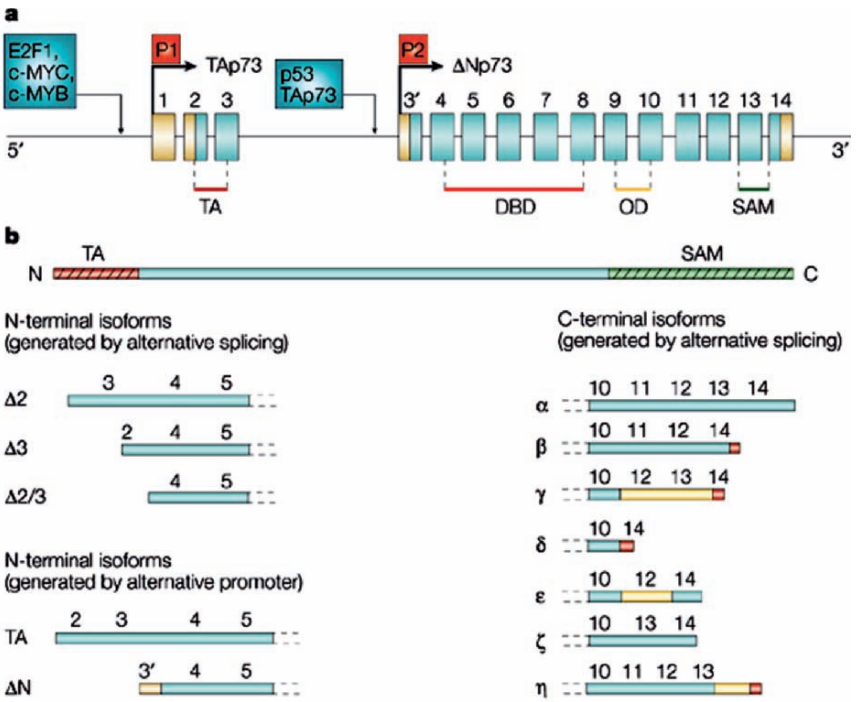


Figure 3.3.13.C. P73 structure. (a) NH_2-terminal isoforms are due predominantly to the activity of two distinct promoters (P1 and P2), the second of which is located in the large (20 kb) intron 3, and is coupled to an ATG on exon 3′, from which transcription proceeds in frame from exon 4–14, in keeping with the other COOH-terminal isoforms. As the first three exons encode for the transactivation (TA) domain, the protein that is encoded by promoter P1 is termed TA, whereas the protein that is coded from promoter P2 is termed ΔN. These two proteins have different upstream regulation. Some of the transcription factors that are active on the promoters are also indicated. Intron/exon size is not proportional; blue boxes indicate coding sequences, whereas yellow boxes represent 5′ or 3′ untranslated regions. (b) NH_2-terminal isoforms can be generated by either alternative splicing or alternative promoter use. COOH-terminal isoforms are due to alternative splicing of exons 11, 12, and 13, which code for the sterile α motif (SAM) domain. The β, γ, δ, and ε isoforms are truncated forms of full-length P73, in which the alternative reading frame of exon 14 (created by splicing) generates a stop codon after a few base pairs (shown by a red bar). The δ isoform lacks most of the COOH-terminal region, and most closely resembles P53. The ζ isoform has an internal deletion, which lacks residues 400–496 of the α isoform. The γ isoform contains a long alternative reading frame (shown by a yellow bar) that leads to the formation of a different, 75 residue COOH-terminus. The ε isoform has a COOH-terminal region that is composed of parts of the γ and α reading frames (alternative reading is shown by a yellow bar). The exon number that encodes for the corresponding residues is shown above. DBD = DNA-binding domain, OD = oligomerization domain. [Reproduced from Melino et al. 2002. With permission from Macmillan.]

by alternative splicing of COOH-terminal domains in normal cells. At least three distinct NH_2-terminal splices (Δ2, Δ3, Δ2/3) exist. The transcriptionally active, full-length P73 (TAP73) induces cell cycle arrest and apoptosis. The NH_2-terminally truncated P73 protein ΔNP73 may have dominant negative function by inhibiting apoptosis-induced by both P53 and full-length P73. The expression of the truncated P73 protein is induced by full-length P73 and P53, creating a negative feedback loop of P53 and P73 function. The P73 protein levels increase after DNA damage due to protein stabilization via a c-ABL-dependent pathway. c-MYC and E1A activate P73. The proteins MDM2, MDMX, and SUMO1 are components of degradation pathways and interact with P73 [Melino et al. 2002].

When the P53 checkpoint control does not operate properly, damaged DNA can reduplicate, producing mutations and DNA rearrangements that contribute to the development of highly transformed cells. There are several ways in which P53 function may be inactivated in cancer. Virtually all P53 mutations in malignancies abolish its ability to bind to particular DNA sequences and activate gene expression.

- Loss-of-function mutations of P53 are associated with about 50% of all human cancers. This may be rooted in the prominent role this molecule plays in G_1 arrest and in sustaining G_2 arrest. While damage to other checkpoint gene products can be compensated for by P53, the converse compensation of damage to P53 by other checkpoint molecules is substantially less efficient.
- 75% of *p53* mutations occur as single missense mutations. The majority of *p53* mutations in cancer are missense mutations occurring throughout the central domain of the coding region (exons 5–9). They affect the ability of the P53 protein to bind to its cognate DNA recognition sequence. Among them, six mutation hot spots (codons 248, 273, 175, 245, 249, and 282) cluster to the DNA-binding surface. Furthermore, the spectra of *p53* mutations in various types of tumors reflect the mechanisms of carcinogenesis. In colorectal cancer, most *p53* mutations arise as a result of deamination of methylated cytosine bases, leading to C→T transition mutations. By contrast, many of the *p53* mutations in lung cancers are transversion mutations, such as G→T, that may be caused by the direct action of carcinogens contained in tobacco smoke on *p53* gene sequences. In squamous cell cancers arising in skin exposed to UV light, a sizeable fraction of the *p53* mutations presumably is caused by the generation of pyrimidine dimer formations. Missense mutations, deletions, or nonsense mutations of the gene prevent the protein from oligomerizing and forming tetrameric complexes that can bind to specific DNA sequences. Mutants from the hot spot regions lose the ability to recognize P53-binding sites and cannot activate gene expression. Other mutations are structural insofar as they alter the protein conformation, disrupting the local structure or causing denaturation. Some mutations alter the conformation of the P53 protein, altering certain functional properties, and can also affect the wild-type molecules complexed with the mutant form in tetramers, thus preventing DNA binding and gene activation [Hainaut and Hollstein 2000]. The active form of P53 is a tetramer of identical subunits. A missense point mutation in one of the two *p53* alleles in a cell can abrogate almost all P53 activity, because virtually all the oligomers will contain at least one defective subunit and such oligomers cannot function as transcription factors. These oncogenic *p53* mutations thus act in a dominant negative fashion. Mutations of distinct NH_2-terminal serine residues, which are kinase targets, result in a significant decrease in suppressor function and reduce transactivation.
- A germline mutation in just one of the *p53* alleles abrogates P53 function and causes Li–Fraumeni syndrome, which predisposes to cancer. The binding as a tetrameric complex is the reason why mutant P53 proteins act in a dominant manner. The mutant proteins are present in complexes with wild-type P53 and alter the function of the normal tetramer. Dominant negative effects may also occur with mutations in the DNA-binding domain. Li-Fraumeni Syndrome may also be caused by mutations in *chk2*. They include a truncating mutation, 1100delC, and a missense mutation, R145W [Lee et al. 2001].
- CDK2 is needed for DNA synthesis. CHK2 mutations that prevent the phosphorylation of CDC25A occur in colon cancer and in Li-Fraumeni Syndrome. Through a related mechanism, the overexpression of CDC25A in human cancers may overcome this DNA damage checkpoint [Mailand et al. 2000]. ATM defects occur in ataxia teleangiectasia. [Falck et al. 2001].
- Proteins that interact with and regulate P53 are altered in many tumors. Phosphorylation of P53

by ATM, as occurs after γ-irradiation, leads to the displacement of bound MDM2 and thus to stabilization of P53. P53 activates the transcription of *mdm2*, initiating a negative feedback loop that keeps P53 function tightly controlled.

- P53 function is down-regulated by scavenging after binding to other proteins followed by degradation through the Ubiquitin pathway. The oncogene product MDM2 binds an NH_2-terminal region of P53 and targets it for degradation. The sequestration and accelerated degradation of P53 after MDM2 binding may contribute to the oncogenicity of MDM2. Enhanced MDM2 levels in tumor cells can cause a decrease in the concentration of functional P53 and abolish the ability of P53 to arrest a cell in response to damage. MDM2 levels are high in many sarcomata that maintain functional P53. A striking amplification of *mdm2* sequences arises in about one third of sarcomata, including common bone and soft tissue forms. Overexpression of the *mdm2* oncogene occurs in leukemias.
- The single nucleotide polymorphism T309G in intron 1 of the *mdm2* promoter (SNP309) is present at a relatively high frequency in both the heterozygous state (T/G, 40%) and the homozygous state (G/G, 12%). It increases the affinity of the promoter segment to the transcriptional activator SP-1, resulting in higher levels of *mdm2* RNA and protein, and the subsequent attenuation of the P53 pathway. SNP309 is associated with accelerated tumor formation in both hereditary and sporadic cancers. In patients with LFS, those carrying the high affinity allele develop tumors on average 7 years earlier than those lacking it. In individuals without hereditary cancer predisposition, sporadic adult soft tissue sarcomata arise on average 12 years earlier in carriers of the SNP309 allele, and the frequency of the SNP309G allele is increased in those who develop soft tissue sarcomata at a young age [Bond et al. 2004].
- Point mutations in the zinc finger-encoding region of *mdm2* are associated with non-Hodgkin lymphomata, leukemias, and hepatocellular carcinomata. Point mutations in other domains occur in liposarcomata. However, the mutation frequency of the *mdm2* gene in general is rather low, as mutations are not typically found in other tumor types.
- In cervical cancers, the expression of the human papilloma virus oncoprotein E6 mediates the degradation of P53.
- In the absence of Cyclin G_1, the cellular level of P53 is about doubled. This leads to a significant decrease in tumor incidence, mass, and malignancy in response to carcinogens [Jensen et al. 2003].
- The expression of ΔNP73 in neuroblastomata is an adverse prognostic marker. The *tp73* gene {1p36.33} maps to a region that is frequently deleted in neuroblastoma. Loss of heterozygosity of *p73* is also associated with breast and ovarian cancer, hepatocellular carcinoma, gastric and esophageal carcinomata, and in lung cancer.

3.3.14 Retinoic acid-dependent pathways

Retinoids (formed from dietary vitamin A or the provitamin β-carotene) induce cell differentiation, inhibit cell proliferation, and may mediate apoptosis. The pool of progenitor cells, which are potentially susceptible to malignant transformation, is restricted by retinoic acid signaling. Retinoid and rexinoid (9-cis-retinoic acid) action is usually associated with a cell cycle block in G_1. This is due to the increased expression of $p21^{CIP1/WAF1}$, a direct target gene of ligated Retinoid Receptors.

Retinoic acid binds to RARs and to RXRs. There are three types of RARs (all-trans-RARs), RAR-α, RAR-β, and RAR-γ, and three types of Retinoid Receptors (9-cis-RARs, RXRs, Rexinoid Receptors), RXR-α, RXR-β, and RXR-γ. The various subtypes of receptors are encoded by distinct genes and are expressed in distinct spatiotemporal expression patterns during development. RAR and RXR may dimerize and act cooperatively. The ligated RXR is transcriptionally inactive, unless its partner RAR is also ligated. This lack of transcriptional activation by RXR alone may be due to allosteric inhibition. The ligand-mediated activation of RAR is dependent on a COOH-terminal α-helical region (activating function 2-AD) located in the ligand-binding domain.

The receptor heterodimers of RAR and RXR bind DNA at retinoic acid responsive elements (RAREs) in gene promoter sequences. RAREs are paired and separated by variable spacers. DR5-type RAREs have five nucleotides between the RAR- and RXR-binding sites. The RAR/RXR heterodimers modulate the frequency of transcription initiation of their target genes. The receptor TR (Thyroid Hormone Receptor) heteromultimerizes with RAR, RXR, and TRAP (Triiodothyronine Receptor Auxiliary Protein) on hormone response elements within these gene promoters. The TR/RXR heterodimer is a stable receptor

complex that remains bound to response elements in the presence of ligand and therefore may be a receptor complex involved in T_3-regulated transcription. Under conditions where RAR, RXR, and TR bind poorly as homodimers to various response elements, strongly cooperative RAR/RXR and TR/RXR binding occurs. The binding efficiency is dependent on the sequence, relative orientation, and spacing of the repeated motifs of the response elements.

Gene expression mediated by RAR/RXR is regulated by chromatin structure. Following receptor ligation, a conformational change generates an interaction surface for coactivators. Coactivators of the P160 family (SRC1, TIF2, AIB1) recruit Histone Acetyl Transferase. A second complex, TRAP (DRIP, SMCC) binds and establishes contact with the basal transcription machinery. The recruitment of Histone Acetyl Transferases leads to chromatin decondensation over the target gene promoter. The acetylation or removal of Histone tails also increases the RAR/RXR affinity to RARE sequences. Upon engagement by ligand, the activating function 2-AD is folded against the ligand-binding domain, creating a new interface suitable for coactivator binding. In addition, ligand binding to RAR leads to the release of the nuclear receptor corepressors NCOR1 (RIP13) and NCOR2 (SMRT, TRAC2). In the absence of ligand, Histone Deacetylase-containing complexes are tethered through SIN3 and the corepressors NCOR1 or NCOR2 to the nonliganded RAR/RXR heterodimer. Deacetylation causes DNA condensation and silencing of the relevant target genes (Figure 3.3.14.A).

The expression of *rarβ* is activated by heterodimers of RARs and RXRs. The orphan receptor COUP-TF is required for the induction of RARβ expression, growth inhibition, and apoptosis by retinoic acid. The effect of COUP-TF requires its binding to a DR-8 element in the *rarβ* promoter. This increases the retinoic acid-dependent transactivation by RARα with its coactivator CBP [Lin et al. 2000a]. Besides transactivating their own target genes, some nuclear receptors, including RAR/RXR heterodimers, interfere with other signal transduction pathways. RARβ constitutively represses AP-1.

- Acute promyelocytic leukemia can be caused by translocations that generate fusion proteins containing RARα, often t(15;17)(q22;q11.2–12) fusing the *pml* and *rarα* genes. Retinoic acid bound to the RAR alters the gene expression in immature white blood cells causing them to mature and stop dividing. By contrast, in acute promyelocytic leukemia the oncogenic transcription factor PML-RAR blocks the maturation of white blood cells. It attracts multiple Histone Deacetylase and DNA Methyl Transferase complexes, leading to methylation of the *rarβ2* promoter and to superrepression. The newly methylated CpG islands then become docking sites for methyl-binding proteins, which in turn interact with the Histone Deacetylase Complex and DNA Methyl Transferases, stabilizing their interaction [Di Croce et al. 2002]. The consequence is a block of differentiation at the promyelocytic stage [Altucci and Gronemeyer 2001].
- The expression of RARβ exerts antiproliferative effects in certain cancers [Hoffman et al. 1996; Liu et al. 1996]. The activation of expression of *rarβ* by heterodimers of RARs and RXRs is often lost in cancer cells, despite the expression of RARs and RXRs. Loss of RARβ expression is associated with the progression of various solid tumors. In breast cancer cells, the *rarβ* promoter may be silenced by DNA methylation or Histone Deacetylase inhibition. During skin squamous cell carcinogenesis, progressive decreases in nuclear Retinoid Receptors occur [Xu et al. 2001].
- Skin carcinogenesis induced by Ultraviolet B radiation is associated with the induction of the AP-1 components FOS and JUN. Similarly, chemical carcinogenesis in the skin may involve the activation of AP-1 through PKC or RAS. The repression of AP-1 by retinoids can reverse this mode of tumor promotion [Huang et al. 1997].
- The metabolism of vitamin A (retinal) to retinyl esters, carried out primarily by Lethicin:Retinal Acyltransferase (LRAT), is greatly reduced in human carcinoma cells of the oral cavity, skin, breast, prostate, and kidney as compared with their normal epithelial counterparts. Immortalization is associated with a reduction, and full transformation with a loss of expression of LRAT [Guo et al. 2002].
- Retinoids induce apoptosis and differentiation of hepatocellular carcinoma. Hepato-carcinogenesis is accompanied by an accumulation of RXRα. Phosphorylation of RXRα at serine 260, a phosphorylation site for MAP Kinase, renders RARα resistant to degradation in the Ubiquitin–proteasome pathway. It also is linked to low transactivating activity by RXRα. This phosphorylation impairs the metabolism and function of RXRα in hepatocellular carcinoma and may lead to growth promotion [Matsushima-Nishiwaki et al. 2001].

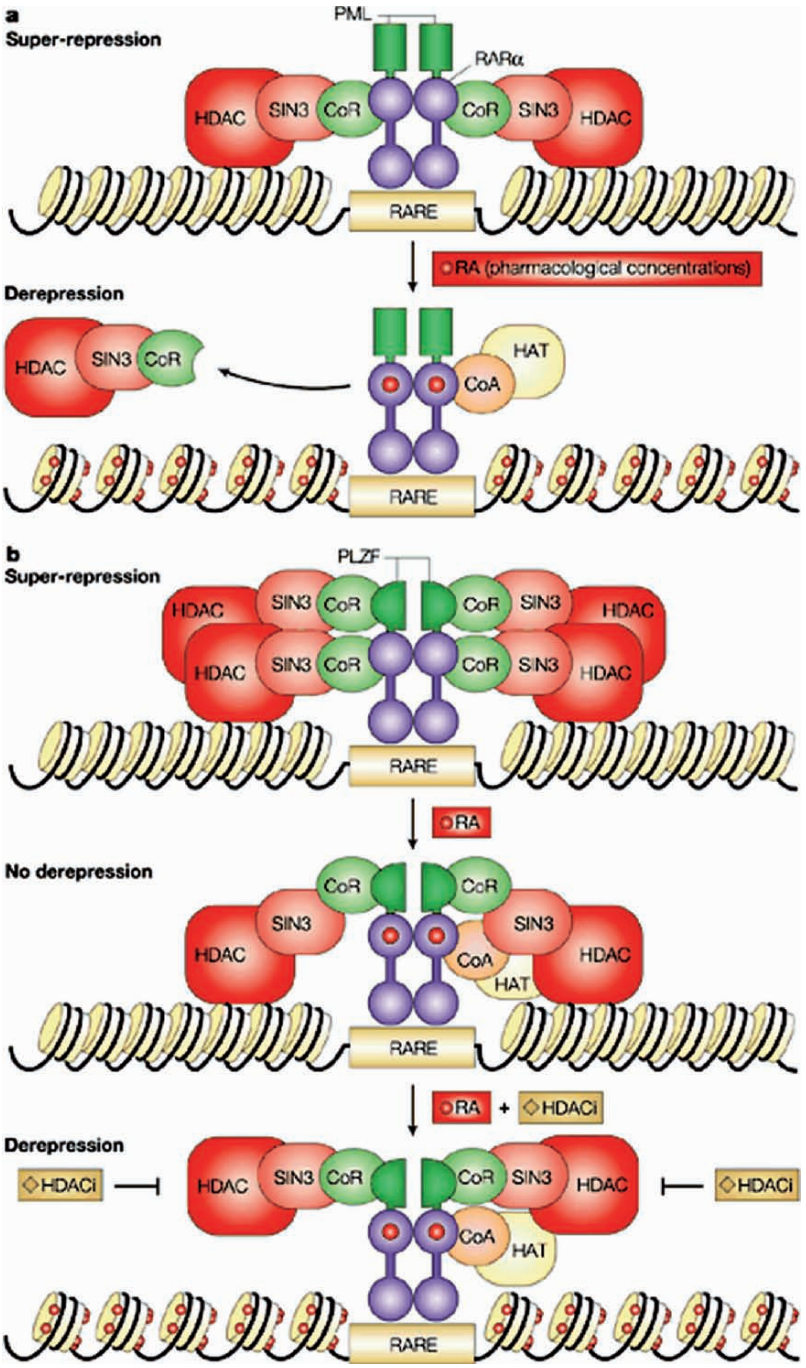


Figure 3.3.14.A. Retinoid responsitivity. Molecular basis of retinoid responsivity and nonresponsivity. apo-RAR/apo-RXR heterodimers can bind to retinoic acid response elements (RAREs) and recruit Histone Deacetylases (HDACs). This leads to gene repression, which can be relieved by retinoids. (a) The t(15;17) chromosomal translocation generates the PML–RAR fusion protein that, in contrast to RAR, can efficiently dimerize or oligomerize, thereby recruiting multiple HDAC complexes and leading to superrepression of target genes. To disrupt this complex, pharmacological concentrations of retinoids are required; this leads to the recruitment of coactivator (CoA)–HAT complexes and gene expression. (b) The t(11;17) translocation generates the PLZF–RAR fusion protein, which contains two CoR interaction surfaces. The one in the PLZF portion of the molecule cannot be dissociated by retinoids, accounting for the insensitivity of this acute promyelocytic leukemia (APL) type to retinoids. Exposure to retinoid agonists allows CoA/HAT recruitment, chromatin derepression and gene activation. CoR = corepressor, HAT = Histone Acetyltransferase, RAR = retinoic acid receptor, RXR = rexinoid receptor. [Reproduced from Altucci and Gronemeyer 2001. With permission from Macmillan.]

3.3.15 Vitamin D-dependent pathways

The active form of vitamin D, $1,25(OH)_2$ vitamin D_3, has prodifferentiative and growth inhibitory effects. Vitamin D binds to the Vitamin D Receptor (VDR, 1,25-α-Dihydroxy-Vitamin D_3 Receptor, Vitamin D Hormone Receptor), which activates the transcription of $p21^{CIP1/WAF1}$. In the prostate, vitamin D induces the expression of IGF-BP, so that IGF-induced proliferation may be inhibited. The synthesis of Osteocalcin, the most abundant non-collagenous protein in bone, is induced by calcitriol, the active hormonal form of vitamin D, through the VDR and a specific vitamin D responsive element in the *osteocalcin* gene promoter.

The VDR belongs to the superfamily of *trans*-acting transcriptional regulatory factors, which also includes the Steroid and Thyroid Hormone Receptors. The *vdr* gene {12q12–q14} contains 11 exons and spans approximately 75 kb. The translated gene product is encoded by exons 2 through 9. The exons 2 and 3 are involved in DNA binding and the exons 7, 8, and 9 in binding to vitamin D. The 5′ non-coding region includes the exons 1A, 1B, and 1C, and three unique forms of mRNA are synthesized as a result of the differential splicing of exons 1B and 1C. The DNA sequence upstream to exon 1A is GC-rich and does not contain a TATA box. The promoter contains several binding sites for the transcription factor SP-1. An intron fragment downstream of exon 1C confers retinoic acid responsiveness, possibly accounting for the ability of retinoic acid to induce *vdr* expression [Miyamoto et al. 1997].

- The VDR functions as a receptor for the secondary bile acid lithocholic acid (LCA). Lithocholic acid is a potential enteric carcinogen, which induces DNA strand breaks, forms DNA adducts, and inhibits DNA repair enzymes. The activation of VDR by lithocholic acid induces the expression of the detoxifying Cytochrome P450 3A (CYP3A). This mechanism may underly the protective effect of vitamin D and its receptor against colon cancer [Makishima et al. 2002].
- In colorectal cancer, high VDR expression is associated with a good prognosis. However, the expression is lost during tumor progression. The transcription factor Snail is associated with epithelial–mesenchymal transition. Snail represses *vdr* gene expression, and high expression of Snail correlates with a down-regulation of *vdr* and *E-cadherin*. The balance between VDR and Snail expression is critical for E-Cadherin expression, which influences cell fate during colon cancer progression [Palmer et al. 2004].
- Low expression alleles of *vdr* are represented with elevated frequencies in parathyroid adenomata. The tumors of patients homozygous for the b, a, or T alleles have lower *vdr* and higher *pth* mRNA levels than those from patients with BB, AA, or tt genotypes, whereas those from heterozygotes have intermediate values. The lower *vdr* mRNA levels associated with the b, a, and T alleles may affect the calcitriol-mediated control of parathyroid function and thereby contribute to the development of sporadic primary hyperparathyroidism [Carling et al. 1998].

3.3.16 The WT pathway

WT1 is a zinc finger transcription factor. The *wt1* (*Wilms tumor*) gene [Bonetta et al. 1990; Call et al. 1990; Gessler et al. 1990] {11p13} comprises ten exons. It contains a GC-rich region that maps to the 5′ end of the gene. The *wt1* promoter lacks a TATA box or CCAAT motif and has a GC content of around 70%. GC boxes are present at nucleotide positions –413, –160, +84, and +158. Four transcriptional start sites are clustered within a 32 bp region. The transcript, which spans approximately 50 kb and encodes an mRNA approximately 3 kb long, is expressed predominantly in the kidneys and in a subset of hematopoietic cells. Four distinct transcripts are expressed, reflecting the presence or absence of two alternative splices that involve the exons 5 and 9. The WT1 transcription factor comprises four zinc finger domains and a region rich in proline and glutamine, and shows significant homology to EGR1 and EGR2. The control of cellular proliferation and differentiation exerted by the *wt1* gene products may involve interactions among the four polypeptides with distinct targets and functions. WT1 is implicated in kidney differentiation. It represses transcription from GC- and TC-rich promoters, but it activates the transcription of *amphiregulin*.

PAX (Paired-Like Homeobox, Paired Box) transcription factors are invovled in regulating the expression of *wt1*. *pax-2* is a member of the family of genes that share a common protein coding domain, the paired box. The *pax* genes are expressed during embryogenesis in a tissue-restricted manner. PAX-2 activates *wt1* expression.

- There are high levels of *pax-2* expression in the epithelial cells of Wilms tumors. Expression of the

pax-2 gene is localized to the nuclei of condensing mesenchymal cells and their epithelial derivatives in the developing kidney, suggesting that PAX-2 is a transcription factor that is active during the mesenchyme-to-epithelium transition in early kidney development and in Wilms tumor.

- In a sporadic, unilateral Wilms tumor, one allele of the *wt1* gene contained a 25 base pair deletion, spanning an exon–intron junction and leading to aberrant mRNA splicing with loss of one of the four zinc finger consensus domains in the protein. The mutation was absent from the germline of the affected patient, consistent with the somatic inactivation of a tumor suppressor gene. In addition to the intragenic deletion affecting one allele, loss of heterozygosity at loci along the entire chromosome 11 indicated an earlier chromosomal nondisjunction and reduplication. The product of this mutation of the *wt1* gene behaves as a dominant negative, suppressing the function of the wild-type protein by a trans-dominant mechanism. The mutant *wt1* gene cooperates with the adenoviral E1A gene in transforming kidney cells. The wild-type WT1 gene product in all of its alternatively spliced forms neither suppresses E1A-induced focus formation nor cooperates with E1A [Haber et al. 1992].
- Frasier syndrome is a rare disorder characterized by male pseudohermaphroditism, glomerulopathy, and the development of genitourinary tumors (gonadoblastoma, and to a lesser extent Wilms tumor). Frasier syndrome is associated with a point mutation in the intron 9 donor splice site of one *wt1* allele, resulting in the loss of expression of the splice variant with incorporation of lysine, threonine, and serine between zinc fingers 3 and 4. Hence, the loss or reduction of one splice variant cannot be fully compensated for by the activity of the others [Frasier et al. 1964; Barbaux et al. 1997].
- Denys–Drash syndrome is the result of a mutation in *wt1* that affect the zinc finger domain and DNA binding of the gene product. It is a rare condition, entailing severe urogenital aberrations resulting in renal failure, pseudohermaphroditism, and Wilms tumor [Denys et al. 1967; Drash et al. 1970].

3.4 CANCER-ASSOCIATED GENE MUTATIONS

Proto-oncogenes encode proteins that participate in signal transduction linked to the promotion of cell cycle progression through G_1 or prevention of programmed cell death. Those include growth factors, their receptors, GTP-binding proteins, protein kinases, Cyclins, and transcription factors. Undue increase in their activity (gain-of-function mutations) can lead to uncontrolled proliferation due to high abundance, increased stability, or loss of regulation of the affected transcripts or proteins. The negative regulators, encoded by tumor suppressor genes, comprise receptors, signal transduction molecules, and transcription factors that activate cell cycle checkpoints or induce apoptosis. Their loss of function can cause uncontrolled cell cycle progression.

There may be more than 25,000 recombination hot spots in the genome [Myers et al. 2005]. Oxidative metabolism at body temperature inevitably leads to DNA damage by direct oxidation of bases, by induction of DNA strand breaks, or by mediation of frameshift mutations in microsatellite DNA. Spontaneous depurination results in miscoding by the residual apurinic site. The deamination of 5-methylcytosine to thymine, which occurs at a rate of $5.8 \times 10^{-17}\ s^{-1}$ at each CpG site, is among the most frequent causes for point mutations. Furthermore, the reduplication of DNA during cell division introduces the possibility of errors at an estimated rate of 1.4×10^{-10} nucleotides/cell/division. When these forms of DNA damage afflict proto-oncogenes or tumor suppressor genes they predispose to cancer.

3.4.1 Gene amplification or loss

Gene amplification repeatedly reduplicates the DNA of a particular locus, generating tandem arrays of the amplified DNA. Those can be excised and form double minutes or integrate into other chromosomes. In either event, gene amplification causes overexpression due to increased numbers of templates [Cooper 1999] (Table 3.4.1.A).

- In breast cancer, various chromosomal regions may be amplified, including 17q22–23, 11q13 (containing *ccnd1* and *cortactin*), and 8q24 (containing *c-myc*). *erbB2* (*her-2/neu*) {17q12} gene amplification occurs at a low frequency in ductal carcinoma in situ (DCIS) of the breast (<10%) and is linked to an adverse outcome. The frequency of *erbB2* amplification appears to be strongly correlated with tumor grade and ductal versus lobular status.

Table 3.4.1.A. Gene amplification in cancer. The amplification of proto-oncogenes can lead to their excessive expression and cause transformation. [From Cooper 1990]

C-myc	Leukemias, breast carcinoma, stomach carcinoma, lung carcinoma, colon carcinoma, neuroblastoma, glioblastoma
N-myc	Neuroblastoma, retinoblastoma, lung carcinoma
L-myc	Lung carcinoma
erbB	Glioblastoma, squamous cell carcinoma
erbB-2	Breast carcinoma, salivary gland carcinoma, ovarian carcinoma
int-2	Breast carcinoma, bladder carcinoma, stomach carcinoma, squamous cell carcinoma
hst	Breast carcinoma, bladder carcinoma, stomach carcinoma
abl	Chronic myelogenous leukemia
myb	Colon carcinoma, leukemia
ets-1	Lymphoma
H-ras	Bladder carcinoma
K-ras	Lung carcinoma, ovarian carcinoma, bladder carcinoma
N-ras	Breast carcinoma

- The gene *pik3CA*, which codes for Phosphatidylinositol 3-Kinase P110α, is amplified in a portion of cervical and ovarian tumors.
- The copy number of the *urokinase plasminogen activator* (*upa*) gene is often increased in hormone refractory prostate cancer.
- Amplification of the chromosome locus 2p21 occurs in about 30% of thyroid neoplasms. This causes a rearrangement and amplification of the *pkcε* gene and results in the overexpression of a chimeric truncated *pkcε* mRNA, coding for the NH_2-terminal amino acids 1–116 of the enzyme form, fused to an unrelated sequence. Cells expressing the truncated PKCε are resistant to apoptosis. This is associated with higher BCL-2 levels, a marked impairment in P53 stabilization, and dampened expression of BAX [Knauf et al. 1999].
- In esophageal squamous carcinoma, amplification of 11q21–q23 frequently occurs. This leads to overexpression of the antiapoptotic gene *ciap1* [Imoto et al. 2001].

The deletion of one allele of a tumor suppressor gene (loss of heterozygosity) [Cavenee et al. 1983] leads to symptoms if the remaining allele is mutated. Losses of heterozygosity, i.e., losses of a maternal or paternal allele in a tumor, are widespread and are often accompanied by a gain of the opposite allele. On average, cancers of the colon, breast, pancreas, or prostate may loose 25% of their alleles. It is not unusual for a tumor to have lost over half of its alleles. Haplo-insufficiency (allelic insufficiency) describes a phenotype resulting from the loss of one functional allele of a given gene in diploid cells.

- Deregulated signaling through Phosphatidylinositol 3-Kinase is common in glioblastoma and in advanced prostate carcinoma. This may be due to loss of heterozygosity at 10q23. It causes the loss of *pten*, which is highly correlated with activation of PKB (AKT). The elevated PKB activity leads to phosphorylation of mTOR and of the Forkhead transcription factors (FOXO1, FOXO3a, and FOXO4). The loss of one copy of *pten* is also sufficient to promote tumor progression in germ cell, gonadostromal, thyroid, and colon tumors.
- The WNT signaling pathway is often up-regulated in epidermal cancers. The gene for the APC homolog *apc-2* (*apcl*) is located on chromosome 19p13.3, a region that is commonly lost in ovarian cancer. High frequency *apc-2* allelic imbalance in ovarian cancers implies that APC-2 may act as a tumor suppressor in this type of malignancy.
- A 600 kb region on chromosome 8p22-p21.3 is commonly deleted in hepatocellular carcinoma, colorectal carcinoma, and non-small cell lung carcinoma. This reflects a loss of *prlts* (*PDGFR-β-like tumor suppressor*) [Fujiwara et al. 1995].
- The endonuclease MUS81 (EME1, MMS4) {11q13} plays a role in processing stalled DNA reduplication forks. Monoallelic loss of the gene encoding MUS81 increases the susceptibility to chromosomal damage and constitutes a profound predisposition to lymphomata [McPherson et al. 2004].
- The transcriptional activator DMP1 regulates the expression of $p14^{ARF}$. Its down-regulation through haplo-insufficiency increases the susceptibility to lymphoma [Inoue et al. 2001].
- *cdkn1B*, the gene encoding $P27^{KIP1}$, is haplo-insufficient for tumor suppression [Fero et al. 1998] in pituitary adenomata and various epithelial cancers.

3.4.2 Deletion of functional domains

The most common mode of activation of receptor tyrosine kinase proto-oncogene products is the deletion of the NH_2-terminal ligand-binding domain, which forms a constitutively active kinase (*erbB*, *erbB2*, *ros*, *met*, *ret*, *trk*). The deletion of sequences at the extreme COOH-terminus may also activate kinase activity (*erbB*, *fms*, *kit*, *ret*).

The activity of the SRC family nonreceptor tyrosine kinases (SRC, YES, FGR, LCK, FYN, LYN, HCK) is negatively regulated by tyrosine phsophorylation in the COOH-terminal portion of the molecule, the deletion of which can be a transforming event. Likewise, deletion or mutation of the NH_2-terminal SH3 region may activate transforming potential. Deletion of the SH3 region of ABL is sufficient to confer the potential for transformation [van der Plas et al. 1991]. Deletion of the NH_2-terminal regulatory domain in the serine/threonine kinase RAF results in constitutively up-regulated activity in the COOH-terminal catalytic domain [Cooper 1999].

Deletions may occur outside the coding regions of genes, including introns or promoters. While these do not affect the structure of the encoded proteins, they may alter gene expression or RNA stability.

- Homozygous deletion may occur in the *nf1* gene in neurofibrosarcoma, pheochromocytoma, and astrocytoma with the consequence of dysregulated $P21^{RAS}$ and unrestricted cell cycle progression.
- Deletion of the NH_2-terminal ligand-binding domain of KIT occurs in gastrointestinal stromal tumors and in mast cell tumors.
- Ligand-independent activation may occur if MET is truncated. This results in its cytoplasmic, rather than transmembrane localization. The subcellular localization of MET contributes to determining its carcinogenic potential. Cytoplasmic MET may be tumorigenic in the breast.
- Several *cd95* gene mutations occur in myeloma and T-cell leukemia. They include deletions that lead to truncated forms of the death receptor. These mutated forms of CD95 might interfere in a dominant negative way with apoptosis induction by wild-type CD95.
- A truncating mutation in SRC at codon 531 arises in about 12% of cases of advanced colon cancer [Irby et al. 1999].
- Genetic mutations leading to a truncated or unstable PTC protein are associated with familial or sporadic basal cell carcinoma.
- In familial and sporadic meningiomata, a deletion in the fifth intron of the *sis* gene may occur. The intact *sis* gene has an Alu sequence in this region, which includes two perfect 130 nucleotide-repeated sequences, separated by five base pairs. The deleted allele in a fraction of meningioma cases misses one copy of the 130 base pair repeats and the intervening five base pairs [Bolger et al. 1985; Smidt et al. 1990].

3.4.3 Point mutations

Spontaneous point mutations may lead to the continuous activation of proto-oncogene products or inactivation of tumor supressor gene products [Cooper 1999]. Point mutations in proto-oncogenic receptor tyrosine kinase genes may lead to conformational changes in the encoded protein that mimic an activated state, without deletion or loss of ligand binding (*fms*, *erbB2*). The constitutive activation leads to ligand-independent signal transduction and results in excessive cell cycle progression.

Mutations of the *p53* gene occur in tumors of the brain, breast, lung, colon, and mesenchyme [Nigro 1989]. Tumor-associated mutations in *p53* are predominantly point mutations that result in single amino acid substitutions (Figure 3.4.3.A). This is distinct from many other tumor suppressor genes, in which large deletions or frameshift mutations tend to result in the complete loss of protein expression. Several types of mutant *p53* exist, according to the sites of mutation and the resulting phenotypes [Michalovitz et al. 1991]. Certain *p53* codons, encoding the residues 175, 245, 248, 249, 273, and 282, have a dysproportionately high mutation frequency. Point mutations in *p53* frequently occur in the DNA-binding region. More than 95% of alterations in the *p53* gene are point mutations that produce a mutant protein, which has lost its transactivational activity,

– Null mutations with totally inactive P53 that do not directly intervene in transformation.
– Dominant negative mutations with a totally inactive P53 that is still able to interfere with wild-type P53 expressed from the wild-type allele; many tumor cells retain the ability to express mutant P53 proteins that may be more stable than wild-type P53 and may act as dominant negative inhibitors.
– Dominant positive mutations, where the normal function of P53 is altered, but in this case the mutant P53 acquires an oncogenic activity that is directly involved in transformation. In paticular, some P53 mutants exhibit a trans-dominant phenotype and are able to associate with wild-type P53, expressed by the remaining wild-type allele, to induce the formation of an inactive heterooligomer [Halevy et al. 1990; Milner and Medcalf 1991].

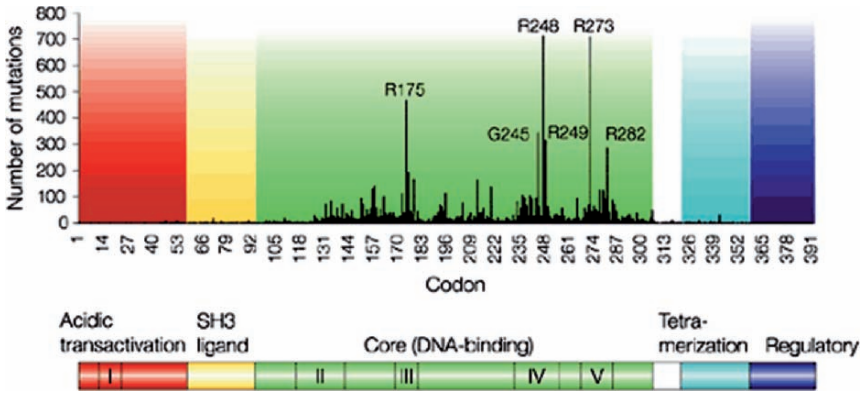


Figure 3.4.3.A. P53 mutations in cancer. P53 is a 393 residue protein that contains a NH_2-terminal transactivation domain, a proline-rich SH3 ligand, a core DNA-binding domain (also a noncanonical SH3 ligand), a tetramerization domain and a COOH-terminal regulatory domain. Five boxes displaying the regions of greatest sequence conservation are shown, four map to the core domain. A histogram of P53 missense mutations shows that 95% of mutations occur in the core domain, six labeled residues are hot spots for mutation. [Reproduced from Bullock and Fersht 2001. With permission from Macmillan.]

In loss of heterozygosity, one allele is deleted, which typically leads to symptoms if the affected gene is a tumor suppressor genes and the remaining allele is mutated. Point mutations of *p53* in cancer are frequently accompanied by heterozygous loss of the short arm of chromosome 17. The majority of colorectal carcinomata have mutations in *p53*. Frequently, mutations in one allele of *p53* are associated with deletions of chromosome 17p [Baker et al. 1989].

- Constitutively activated versions of the CSF-1 Receptor tyrosine kinase c-FMS contain single point mutations (codons S301L and F969Y in 10–20% of AML or myelodysplastic syndrome). Like the v-FMS oncogene product, receptors bearing the activating mutations retain high affinity binding sites for CSF-1, but are retarded in their transport to the cell surface and are phosphorylated on tyrosine in the absence of ligand [Roussel et al. 1988]. Furthermore, transforming point mutations of FMS are often associated with reduced receptor degradation [Morley et al. 1999]. c-CBL binds to a phosphotyrosine at the COOH-terminal end of c-FMS. This leads to ubiquitination of FMS [Mancini et al. 2002].
- Constitutively activated versions of a normal receptor tyrosine kinase, the c-KIT Receptor, are generated by single point mutations. Gain-of-function mutations in *c-kit*, leading to constitutive activation of the KIT Receptor, specifically the D814Y mutation in the cytoplasmic kinase domain, are associated with myeloproliferative disorders (mastocytomata) [Piao and Bernstein 1996]. A point mutation affecting the catalytic region in the kinase domain of the KIT protein leads to spontaneous transformation of melanocytes [Larue et al. 1992].
- In neuroblastomata, the *erbB2* (*her-2/neu*) oncogene may have a point mutation resulting in the alteration of a single amino acid in the transmembrane region of the receptor. This causes its constitutive activation. The *erbB2* gene is also activated by a point mutation encoding V664E, which may result in cell transformation in breast cancer, bladder cancer, colon cancer, lung cancer, and gastric cancer. Point mutations in the transmembrane domain of ERBB2 enhance its transforming properties. They may have a stabilizing effect on the conformation, which results in receptor dimerization and activation.
- A point mutation S267P, in the extracellular third Immunoglobulin-like domain, and a splice site mutation 940-2A→G of the *fgf receptor-2* gene occur in gastric carcinoma.
- Point mutations in *met* occur in hereditary and sporadic papillary renal carcinomata [Schmidt et al. 1997], hepatocellular and gastric carcinomata [Park et al. 1999; Lee et al. 2000], and head and neck squamous carcinomata [Di Renzo et al. 2000]. Point mutations in the kinase domain convert MET to an oncogenic receptor. Such mutants are catalytically highly active, which correlates with more efficient MET autophosphorylation and phosphorylation of substrates. The constitutive binding of c-SRC to the cytoplasmic domain of the MET M1268T mutant in

renal papillary carcinomata, elevates c-SRC phosphorylation and activity. MET M1268T also phosphorylates substrates of the cytosolic kinase c-ABL, whereas wild-type MET does not. The expression of MET M1268T induces β-Catenin tyrosine phosphorylation and accumulation, induces constitutive activation of the transcription factor TCF, which acts in concert with β-Catenin in the nucleus to increase the expression of the target genes *myc* and *cyclin* D_1.

- Germline activation of the *ret* gene, attributable to specific point mutations, causes medullary thyroid carcinoma, a neoplastic transformation of the Calcitonin secreting thyroidal C-cells. Mutations of *ret* may also cause multiple endocrine neoplasia type 2 (C634R in MEN2A, M918T in MEN2B).
- Consecutive to missense mutations, the Androgen Receptor may loose its ligand specificity and promiscuously respond to a range of steroid hormones and pseudo-androgens. Receptors with the T877A mutation are stimulated by the antagonist flutamide. The double mutant T877A, L701H, called AR^{CCR} (cortisol and cortisone responsive), has increased affinity for glucocorticosteroids. The H874Y mutation influences the binding of coactivator proteins by affecting the conformation of helix 12.
- Missense mutations of the Estrogen Receptor that substitute tyrosine 537 in the ligand-binding domain for asparagine occur in metastatic breast cancer [Zhang et al. 1997]. The lysines 302 and 303 are principal substrates for acetylation by CBP. The missense mutation L303R occurs frequently in premalignant lesions of the breast.
- Point mutations leading to *ras* oncogene activation are frequently induced by chemical carcinogens. Point mutations that convey transforming potential to RAS occur in the guanine nucleotide-binding pocket and either decrease the GTPase activity or increase the exchange rate of bound GDP for free GTP.
- ARMET (Arginine-Rich Mutated in Early Stage Tumors, Arginine-Rich Protein, ARP) [Shridhar et al. 1996] is a 234 amino acid arginine-rich protein. The gene is located in 3p21.1 and spans about 600 kb. The multiple arginine-encoding area of the gene is subject to a high frequency of genetic variation. At the cytogenetic level, the region containing *armet* is frequently deleted in a variety of solid tumors, although not in pancreatic cancer. A specific mutation of *armet*, changing codon 50 from ATG to AGG (M50R) or deletion of codon 50 of the *armet* gene occurs in various tumor types [Shridhar et al. 1997a], including renal carcinomata, as do mutations involving codon 50 in pancreatic cancers [Shridhar et al. 1997b]. Either of the changes abolishes a methionine residue and gives rise to an uninterrupted string of AGG trinucleotides, encoding arginines in its predicted protein product. Four other nucleotide substitutions in codon 50 that replace methionine with four different amino acids, other than arginine, may occur, suggesting that loss of this methionine residue is critical to a carcinogenic role of ARMET. Furthermore, a mutation AGG to AAG (arginine to lysine) in the adjacent codon 51 can occur in tumors, reflecting the importance of this region to transformation. Only a single copy of the *armet* gene is mutated in the cancer cells, indicating its possible causal role as an oncogene.
- A single base change in the tumor suppressor gene *apc*, due to a polymorphism, replaces a thymine with an adenine, generating a stretch of eight adenines. Such sequences are often misread by polymerases, causing consecutive somatic frameshift mutations, which then pose a predisposition for malignant transformation to colorectal cancer [Laken et al. 1997].
- Li-Fraumeni syndrome may be caused by a R145W missense mutation in CHK2 that destabilizes the encoded protein. The half-life of the mutated CHK2 is reduced due to degradation in the proteasome pathway. [Lee et al. 2001]. In the absence of CHK2, the S phase checkpoint is impaired and cell cycle progression is facilitated.
- Inactivating germline point mutations in *cdkn2A*, encoding the tumor suppressors $P16^{INK4a}$ and $P14^{ARF}$, predispose to the familial atypical multiple mole melanoma (FAMMM) syndrome, but also occur in rare families with clustering incidence of cutaneous malignant melanoma (CMM). They include M53V, M53I, G101W, G122V, and V126D. The M53V mutation occurs in exon 2, where *p16* and the alternative reading frame for $p14^{ARF}$ both share transcript sequences, and is coupled to a D67G alteration in $P14^{ARF}$. In contrast, the M53I mutation is coupled to a distinct D68H alteration in $p14^{ARF}$ [Yang et al. 2004]. The G122V variant retains some capacity to interact with CDKs, yet it is significantly impaired in its ability to cause G_1 cell cycle arrest. In hereditary melanoma, a G→T transversion mutation in the last nucleotide of exon 2 affects the aspartate residue at position 153 of $P16^{INK4a}$. This mutation, D153spl(c.457G > T), and a related mutation at the next nucleotide, IVS2 + 1G > T, result in identical aberrant splicing. The alternate splice products for $p16^{INK4a}$ and $p14^{ARF}$ include

a 74 bp deletion in exon 2, revealing a cryptic splice site, and completely skip exon 2 [Rutter et al. 2003].
- A high incidence of *cd95* mutations exists in bladder cancer. In codon 251, the mutation G993A is a hot spot in this malignancy [Lee et al. 1999b]. Several *cd95* gene mutations occur in myeloma and T-cell leukemia [Cascino et al. 1996]. They include point mutations in the cytoplasmic death domain of CD95. These mutated forms of CD95 may interfere in a dominant negative way with apoptosis induction by CD95.
- Missense mutations in *bak* may occur in gastric and colorectal cancers. They arise only in advanced stages of the disease [Kondo et al. 2000].

3.4.4 Frameshifts

Frameshift mutations are genetic alterations that insert into a DNA sequence or delete from it a number of nucleotides that is not evenly divisible by 3. Due to the triplet nature of gene expression by codons, the insertion or deletion can disrupt the grouping of the codons, resulting in a completely different translation from the original.

Frameshift mutations occur at simple repeat sequences in tumors of the microsatellite mutator phenotype. Microsatellites are short repetitive sequences, which are often copied incorrectly by DNA polymerases because the template and daughter strands in these regions are particularly prone to misalignment. These replication-dependent events create loops of extrahelical bases, which would produce frameshift mutations unless reversed by mismatch repair. Germline mutations in mismatch repair genes are associated with hereditary nonpolyposis colon cancer. Microsatellite instability and the associated frameshift mutations in genes also arise in sporadic colon, gastric, endometrial, and ovarian tumors.

- A large portion of lobular breast carcinomata and gastric carcinomata contain *E-cadherin* frameshift mutations. This leaves the E-Cadherin protein truncated and unable to mediate adhesion. The truncation mutants may exert a dominant negative effect on other Cadherins.
- In colorectal tumors with microsatellite instability approximately 40% exhibit one base pair deletion, resulting a frameshift mutation in a tract of nine adenosines within the coding region of the *tcf-4* gene, a crucial member of the WNT→APC pathway [Duval et al. 1999].
- Genes containing repetitive sequences within their coding regions can be targets for microsatellite instability tumorigenesis. Frameshift mutations occur in *bax* in about 50% of colon carcinomata with microsatellite instability. They typically affect a tract of eight deoxyguanosines, spanning the codons 38–41 in the third coding exon [Rampino et al. 1997].
- Frameshift mutations of *bax* that lead to a loss of its expression are common. Tumor cells with these frameshift mutations are more resistant to apoptosis. Reduced BAX expression may be associated with shorter survival in breast adenocarcinoma [Krajewski et al. 1995].
- *prlts* (*PDGFR-β-like tumor suppressor*) is a tumor suppressor gene located on chromosome 8p22-p21.3. Frameshift mutations of *prlts* arise in hepatocellular carcinoma and colorectal cancer [Fujiwara et al. 1995].
- A frameshift mutation in *tgf-β receptor 1* frequently occurs in ovarian cancer. Frameshift mutations also arise in *type II TGF-β receptor* in colorectal cancer. They affect a tract of ten adenosines in the coding sequence. A high rate of mutations occurs in tumors at Dukes B stage, showing a great extent of vascular invasion. The frameshift mutations in the TGF-β Receptors inactivate their growth controlling functions.

3.4.5 Chromosome translocations

Translocations involve the exchange of material between chromosomes. Such structural rearrangements at the molecular level can juxtapose segments of DNA that are not normally adjacent to one another. Frequently, these juxtapositions are very precise, with the exchange point in one or both participating chromosomes being positioned within a few base pairs. Balanced translocations generate derivative chromosomes with no apparent loss or gain of sequences from either chromosome. Unbalanced translocations are associated with the loss of sequences from the involved chromosomes. Nonreciprocal translocations are transpositions of two segments between nonhomologous chromosomes with loss or gain of genetic material as the result. Inversions involve only one chromosome, in which two breaks occur and the intervening segment is rejoined in an inverted manner. There is no loss or gain of chromosomal material. An inversion is paracentric if the inverted segment is on the long or short arm of the chromosome and does not include the centromere. The inversion is pericentric if breaks occur on both, the short arm and the long arm, and the inverted segment contains the centromere (Table 3.4.5.A).

Table 3.4.5.A. Chromosome translocations in hematologic malignancies. Chromosome translocations are a common cause for the transformation of blood cells or their precursors. Typically, they either form chimeric proteins or place proto-oncogenes under the control of highly active, lineage specific promoters. ALCL = anaplastic large cell lymphoma, AML = acute myeloid leukemia, ABL = acute basophilic leukemia (a rare type of acute myeloid leukemia), ANLL = acute nonlymphocytic leukemia, ALL = acute lymphocytic leukemia, CLL = chronic lymphocytic leukemia, CML = chronic myeloid leukemia, MALT = mucosa-associated lymphoid tissue

Oncogene	Fusion partner	Consequence	Malignancy
c-myc {8q24}	*igκ* {2p12}	Fusion protein	Burkitt lymphoma
c-myc {8q24}	*igλ* {22q11}	Fusion protein	Burkitt lymphoma
c-myc {8q24}	*igh* {14q32}	Fusion protein	Burkitt lymphoma
pax5 {9p13}	*igh* {14q32}		B-cell non-Hodgkin lymphoma
bcl-1 {1q13}	*igh* {14q32}		CLL
bcl-2 {18q21}	*igh* {14q32}		Follicular B-cell lymphoma
bcl-10 {1p22}	*igh* {14q32}		MALT non-Hodgkin lymphoma
bcl-6 {3q27}	*igh* {14q32}	Promoter exchange Excessive *bcl-6* expression	Non-Hodgkin lymphoma
bcl-6 {3q27}	*igκ* {2p12}		Non-Hodgkin lymphoma
bcl-6 {3q27}	*igλ* {22q11}		Non-Hodgkin lymphoma
bcl-6 {3q27}	*rhoH* {4p13}	Promoter exchange	Non-Hodgkin lymphoma
bcl-6 {3q27}	*obf1* {11}		
bcl-6 {3q27}	*lcp1* {13q14}		Non-Hodgkin lymphoma
bcl-11a {2q13–15}	*igh* {14q32}		CLL, ALL, non-Hodgkin lymphoma
abl {9q34}	*bcr* {22q11}	"Philadelphia chromosome translocation" Oncogenic Tyrosine Kinase	CML
abl-2 {1q25}	*etv6* {12p13}	Fusion protein containing ABL-2 Kinase plus ETV6 HLH Oligomerization Domain	ANLL
chic2 {4q11–12}	*etv6* {12p13}		ANLL
c-myc {8q24}	*tcrα/δ* {14q11}		T-cell neoplasias
tcl1 {14q32.1}	*tcrα/δ* {14q11}	Constitutive PKB activation	Mature T-cell leukemia
mtcp1 {Xq28}	*tcrα/δ* {14q11}		Mature T-cell leukemia
lmo1 (*rhom1*, *ttg1*) {11p13}	*tcrα/δ* {14q11}		T-cell ALL
lmo2 (*rhom2*, *ttg2*) {11p13}	*tcrα/δ* {14q11}		T-cell ALL
hox11 {10q24}	*tcrα/δ* {14q11}		T-cell neoplasms
lyl1, *hox11*, *tal1*, *tal2*, or *lmo2*	*tcrα/δ* or *tcrβ*		T-cell neoplasms
tcl1 {14q32.1}	*tcrβ* {7q25}	Constitutive PKB activation	Mature T-cell leukemia
tal1 {1p32}	*tcrβ* {7q25}	Fusion Protein	T-cell ALL
tal1 {1p32}	*tcrα* {3p21}		T-cell ALL
e2A (*ig* enhancer binding factor) {9p13}	*prl* (homeobox gene) {1q23}		T-cell ALL
e2A {9p13}	*pbx1* {1q23}	Oncogenic transcription, specific binding to *ig* enhancer	B-cell ALL
e2A {9p13}	*hlf* {17q22}	Oncogenic transcription	B-cell ALL
Bcma {16p13.1}	*il-2* {4q26–q27}		T-cell neoplasms
aml1 {21q22}	*tel* (*etv6*, ETS family transcription factor) {12p13}		Pro-B-cell ALL
jak2 {9p24}	*tel* {12p13}	Oncogenic Tyrosine Kinase	T-cell ALL
abl {9q24.1}	*tel* {12p13}	Oncogenic Tyrosine Kinase	Myeloid leukemias
pdgfr {5q31–q32}	*tel* {12p13}	Oncogenic Tyrosine Kinase	Myeloid leukemias
pax5 {9p13}	*tel* {12p13}		Acute lymphoblastic leukemia
rarα {17q11.2–12}	*pml* {15}	Transcriptional repressor	AML
rarα {17q11.2–12}	*plzf* {11}	Transcriptional repressor	AML
rarα {17q11.2–12}	*npm* {5}		AML
rarα {17q11.2–12}	*numa* {11q13}		
rarα {17q11.2–12}	*stat5B* {17q11.2}		

Table 3.4.5.A. continued

mlf1 {3q25}	*npm1* {5q34}		AML, ANLL
hoxA9 {7p15}	*nup98* {11p15}		AML
ddx10 {11q22}	*nup98* {11p15}		AML
hoxD13 {2q13}	*nup98* {11p15}		AML
pmx1 {1q23}	*nup98* {11p15}		AML
rap1gds1 {4q22.3}	*nup98* {11p15}	Chimeric Protein	AML
top1 {20q11}	*nup98* {11p15}		AML
ledgf {9p22}	*nup98* {11p15}		AML
hoxD11 {2q13}	*nup98* {11p15}		AML
erg {21q22.3}	*fus* {16p11.2}		AML
nup214 {9q34.1}	*dek* {6p23}		AML, ANLL
nup214 {9q34.1}	*abl* {9q24.1}	Oncogenic Tyrosine Kinase	T-cell ALL
eto {8q22}	*aml1* {21q22}		AML
evi {3q26}	*aml1* {21q22}	Chimeric Transcription Factor	CML
myh11 {16p13}	*cbfβ* {16q22}		AML
ott {1p13}	*mal* {22q13}		ANLL
gmps {3q24}	*mll* (*all-1*, *hrx*, *trx*) {11q23}		ANLL
lpp {3q28}	*mll* {11q23}		ANLL
septin6 {Xq24}	*mll* {11q23}		AML
septin5 {22q11.2}	*mll* {11q23}		AML
afx1 {Xq13}	*mll* {11q23}	DNA-Binding Protein	AML
af1q {1q21}	*mll* {11q23}		ANLL
af3p21 {3p21}	*mll* {11q23}		ANLL
af4 {4q21}	*mll* {11q23}	Fusion transcript	ANLL, B-cell ALL
af9 {9}	*mll* {11q23}		AML
af17 {17q21}	*mll* {11q23}		Acute leukemias
enl {19p23}	*mll* {11q23}	Fusion transcript	Pre-B-cell ALL
alk {2p23}	*npm* (nucleolar phosphoprotein Nucleophospmin) {5q35}	Constitutively Active Receptor Tyrosine Kinase	ALCL
alk {2p23}	*msn* {Xq11}	Fusion protein with ALK Tyrosine Kinase Domain	ALCL
alk {2p23}	*tpm3* {1q25}	Constitutively Active Tyrosine Kinase	ALCL
alk {2p23}	*tfg* {3q21}		ALCL
alk {2p23}	*cltcl1* {22q11.2}		ALCL
{Xp11}	{6q23}		ABL
{Yq12}	{1q21}		Acute leukemia
{13q14}	{1q32}		Diffuse large B-cell lymphoma
{19p13.1}	{1p13}		Myeloid malignancies
	idic(X)(q13)	Breakpoint within a 450 bp region proximal to XIST, containing an inverted repeat	ANLL
	inv(2)(p23q35)	ATIC–ALK fusion protein Tyrosine Kinase	ALCL

– Chromosomal breakpoints can occur within introns and hybrid genes are formed that produce aberrant proteins with oncogenic activity. The coding exons of the affected genes that are disrupted by a reciprocal translocation form a fusion gene, which generates an oncogenic chimeric protein. The regulatory sequences that drive the expression of the hybrid gene are typically derived from the gene that contributes the upstream sequences. Oncogenic fusions involving plasma membrane receptor tyrosine kinases typically subtract the receptor portion and add a dimerization inducing domain.
– Chromosome translocations may activate proto-oncogenes if they place them in loci of high transcriptional activity. The products of these aberrant genes are most often nuclear proteins active in transcription. Such genes are frequently mobilized into the vicinity of genes encoding discrete chains

of the *T-cell antigen receptor* in T-lymphoid precursors or *immunoglobulin* chain genes in B-lymphoid precursors. These cells are prone to this form of transformation, because recombination is physiologically active in these cells, and *tcr* or *immunoglobulin* genes are expressed at high levels. The translocation results in inappropriate expression of the affected oncogene.
– Chromosome translocations can switch off gene transcription or unmask suppressor sequences. They predispose to transformation if they affect tumor suppressor genes.

Translocations in leukemias. Somatically acquired chromosomal translocations or inversions occur in up to 65% of the acute leukemias [Look 1997]. These phenomena are typical of B- and T-cell malignancies because the physiologic occurrence of recombination to generate the diversity of the antigen receptors confers a genetic predisposition for translocations [Gauwerky and Croce 1995]. Reciprocal chromosomal translocations involving an *ig* locus and a proto-oncogene are hallmarks of many types of B-cell lymphoma. Three types of breakpoints can arise in the *ig* loci,
– Some translocations have breakpoints that are directly adjacent to an *ig heavy chain* J-region (JH) gene segments or that are adjacent to regions where the *ig heavy chain* D-region (DH) joins the J-region (DHJH). These translocations likely reflect errors during V(D)J recombination in early B-lymphocyte development in the bone marrow.
– In some translocations, the breakpoints arise within or adjacent to rearranged V(D)J segments, and these V-regions are always somatically mutated. These translocations occur during the somatic hypermutation process, which is associated with DNA strand breaks.
– Some translocations are characterized by breakpoints in the *igH* constant region switch regions, in which DNA breaks are introduced during class switching. This indicates that these events occur during class switch recombination [Küppers 2005].

Certain translocations in leukemias and lymphomata may form oncogenic kinases. *abl* (Figure 3.4.5.A) forms a fusion protein with *bcr* in chronic myelogenous leukemia ("Philadelphia chromosome translocation") [Cooper 1999]. This generates a chimeric kinase with constitutive activity. JAK plays an essential role in coupling cytokine receptors to downstream intracellular signaling events. A t(9;12)(p24;p13) chromosomal translocation in childhood acute T-cell lymphoblastic leukemia fuses the 3′ portion of *jak-2* to the 5′ region of *tel* (*etv6*), a gene of the *ets* transcription factor family. The resulting fusion protein includes the catalytic domain of JAK-2 and the TEL-specific oligomerization domain. Its oligomerization results in the constitutive activation of its tyrosine kinase activity and cytokine-independent T-cell

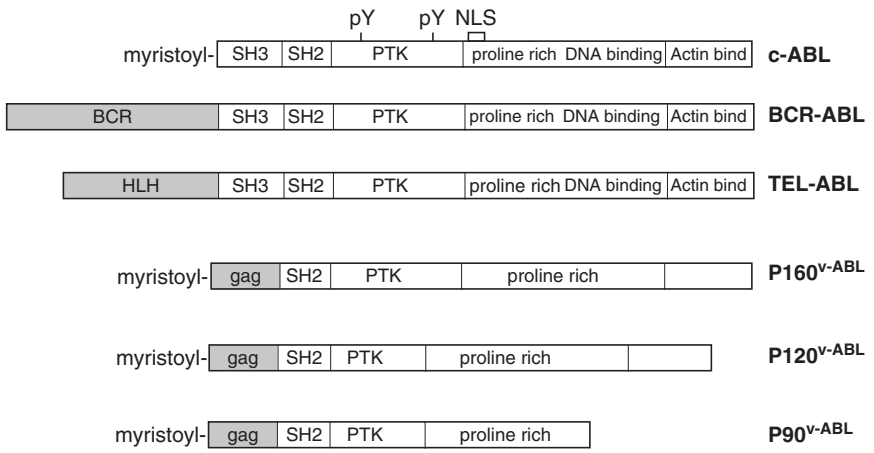


Figure 3.4.5.A. Translocations of *abl*. The proto-oncoprotein ABL has a domain structure. Common translocations place additional functional domains at the NH_2-terminal end. SH = SRC homology domain, PTK = protein tyrosine kinase domain, MLS = nuclear localization signal, BCR = breakpoint cluster region, HLH = helix–loop–helix domain. For comparison, viral ABL sequences are shown in the lower portion.

proliferation [Lacronique et al. 1997]. A unique form of translocation is the inversion of chromosome fragments. This occurs in mature T-cell leukemia, where the fragment 14(q11;q32.1) may link a J plus small D segment of the *tcr* α/δ locus with *tcl-1*. The *tcr* α enhancer may induce the expression of genes at the *tcl-1* locus. TCL-1 is a PKB coactivator that promotes PKB-induced cell survival and proliferation. TCL-1 forms trimers that bind to the PKB pleckstrin homology domain, facilitate the oligomerization of PKB, and increase its kinase activity. TCL-1 also stabilizes the mitochondrial transmembrane potential, thus enhancing cell survival.

Uncontrolled transcriptional activation may be a consequence of translocations, and may underlie some leukemias and lymphomata. Burkitt lymphoma results from chromosomal translocations that involve the *myc* gene and either the λ or the κ light chain *immunoglobulin* genes. Most Burkitt lymphoma cells harbor a specific translocation, involving chromosome 8 (with the breakpoint at 8q24) and either 2, or 14, or 22. The type of Immunoglobulins produced by this B-cell tumor correlates with the underlying translocation. Those cells with the 8;2 translocation produce predominantly κ light chains, those with the 8;22 translocation produce λ light chains, and those with the 8;14 translocation produce Immunoglobulins with both types of light chains. In Burkitt lymphoma of the t(8;22) type, the breakpoint in chromosome 22 is proximal to the λ *immunoglobulin* constant gene cluster, whereas in the CML t(9;22) it is distal. *bcl-2* may also translocate to *immunoglobulin* chains in Burkitt lymphoma. NUP96 and NUP98 are alternatively spliced products of the same gene. NUP98 is a 98 kD component of the nuclear pore complex. NUP98 interacts with intranuclear proteins and transport factors. It participates in the transport of RNA and protein between nucleus and cytoplasm. *nup98* is fused to various *homeobox* genes in AML. The expression of *hox* genes in hematopoietic cells determines lineage commitment and maturation, and their overexpression, particularly *hoxD11* and *hoxD13*, is associated with the development of leukemia. *mll* (*mixed lineage leukemia*) encodes a Histone Methyl Transferase of the trithorax family. It acts as a carcinogen detoxifying gene that is implicated in fusions with more than 25 other genes in various leukemias. Leukemogenic MLL fusion proteins disrupt critical patterns of *hox* gene expression in hematopoietic precursor cells [Look 1997]. *af4* translocates to *mll* in lymphoid leukemias.

Translocations in sarcomata. Soft tissue sarcomata represent a heterogeneous group of tumors, which includes over 50 histologic types. Some of these are characterized by specific chromosomal translocations, whereas others show complex genetic aberrations.

– The Ewing family of tumors is characterized by recurrent reciprocal translocations that generate chimeric proteins. These proteins are potent transcriptional activators and are responsible for maintaining oncogenic properties [Javelaud et al. 2000]. In these malignancies, EWS {22q12} can be fused with one of three distinct ETS transcription factors: FLI1 {11q24}, ERG {21q22.3}, and ETV1 {7p22}. EWS possesses a conserved RNA recognition motif and can bind RNA, suggesting that it may be involved in RNA metabolism. In all known EWS fusion proteins, the RNA recognition motif of EWS is replaced by the DNA-binding domain of the corresponding transcription factor. Thus, the oncogenic conversion of EWS follows a common scheme of activation, exchanging its RNA-binding domain with various DNA-binding domains that are tumor specific.
– CHOP is a member of the C/EBP transcription factor family that comprises part of the adipocyte differentiation machinery. TLS-CHOP is a fusion oncoprotein that arises specifically in a malignant tumor of adipose tissue and results from a t(12;16) translocation that fuses the NH_2-terminal part of TLS to the entire coding region of CHOP. TLS-CHOP blocks adipocyte differentiation by directly preventing C/EBPβ from binding to and transactivating its target genes. Hence, the blockade to normal differentiation is an important aspect of this mode of carcinogenesis [Adelmant et al. 1998].
– Congenital fibrosarcoma is caused by a translocation fusion protein, TEL-NTRK3. The oncogene product TEL (Translocation ETS Leukemia, ETV6) is a transcription factor of the ETS family.
– PLAG1 is a developmentally regulated zinc finger gene product with tumorigenic properties in salivary gland cells and adipocytes. Chromosome 8q12 rearrangements in lipoblastoma bring about promoter swapping events in the *plag1* oncogene. Gene promoter regions from *hyaluronic acid synthetase* or *collagen 1α2* are fused to the entire *plag1* coding region [Hibbard et al. 2000].

– Synovial sarcomata consistently harbor a translocation between segments of Xp11 (*ssx1* or *ssx2*) and 18q11 (*syt*) resulting in either SYT–SSX1 or SYT–SSX2 fusions. Rarely, *syt* fusions with *ssx4*, also located on chromosome Xq11, occur. The fused proteins encode putative transcriptional regulatory proteins that do not contain DNA-binding domains but may act through protein–protein interactions [Crew et al. 1995].

Translocations in carcinomata. Chromosomal translocations encoding fusion oncoproteins are common in leukemias, lymphomata, and sarcomata, but not in carcinomata. Three types of carcinomata with underlying translocations have been described.

– Chromosomal rearrangements arise in thyroid carcinoma [Grieco et al. 1990; Fusco et al. 1987]. The *ret* proto-oncogene encodes a transmembrane receptor of the tyrosine kinase family and is frequently activated in thyroid papillary carcinomata. The RET-PTC1 rearrangement is the result of an inversion of chromosome 10 inv(10)(q11.2;q21), which causes the fusion of the tyrosine kinase domain of the RET gene product {10q11.2} with a section of the H4 gene product {10q21}. In a substantial fraction of normal thyroid cells, *ret* and *h4* genes are in close proximity to one another. The association of these regions may be important in thyroid cell differentiation [Nikiforova et al. 2000]. The chromosomal translocation t(10;17)(q11.2;q23) juxtaposes the tyrosine kinase domain of the *ret* proto-oncogene to a 5′ portion of *pkar1α* (*regulatory subunit of c-AMP-dependent protein kinase*, *tse1*, *tissue-specific extinguisher-1*) on chromosome 17, leading to the formation of the chimeric transforming gene product RET-PTC2 [Sozzi et al. 1994]. One form of rearrangement fuses the *ret* proto-oncogene with a portion of the *ele1* gene, generating RET-PTC3 [Bongarzone et al. 1994]. Rearrangements of the tyrosine kinase receptor TRK (NTRK1, High Affinity NGFR) occur with low frequency in thyroid papillary cancer. TRK-T1 is created by an intrachromosomal rearrangement that juxtaposes the 5′ end of the *tpr* gene to the *trk* tyrosine kinase domain. The resulting hybrid mRNA contains 598 nucleotides of *tpr* (*translocated promoter region*) and 1,148 nucleotides of *trk* [Greco et al. 1992]. An illegitimate recombination that inverts a part of the long arm of chromosome one places a 611 base pair intron upstream of the transmembrane domain of *ntrk1* to the intron between exons 7 and 8 of *tpm3* (*tropomyosin 3*). The resulting gene encodes a chimeric protein of 70 kD, which is constitutively phosphorylated on tyrosine [Butti et al. 1995]. Reciprocal *ntrk1-tpm3* transcripts exist in these tumors, reflecting an intrachromosomal balanced reciprocal inversion. *pax* genes encode a family of transcription factors that are essentially required for the formation of several tissues from all germ layers in the embryo. In the thyroid gland, PAX8 is important for the formation of Thyroxine-producing follicular cells, which are of endodermal origin. A t(2;3)(q13;p25) translocation in thyroid follicular carcinomata results in a fusion of the DNA-binding domains of the thyroid transcription factor PAX8 to domains A to F of the PPARγ1 [Kroll et al. 2000]. The fusion protein PAX8–PPARγ1 inhibits transactivation by PPARγ1 in a dominant negative manner.

– The MITF/TFE subfamily of basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors consists of TFE-3, TFE-B, TFE-C, and MITF. They can form both homodimers and heterodimers. The translocation t(X;1)(p11.2;q21.2) results in the fusion of *prcc* (*papillary renal cell carcinoma*) at 1q21.2 to the *tfe-3* gene at Xp11.2. Through this fusion, reciprocal translocation products are formed, which are both expressed in the nuclei of papillary renal cell carcinomata [Sidhar et al. 1996]. The resulting gene fusion generates excessively active transcriptional activators [Weterman et al. 2000], which are sufficient to cause transformation [Weterman et al. 2001]. A translocation t(X;1)(p11.2;p34) leads to the expression of a PSF–TFE3 fusion protein. Unlike wild-type TFE3 or PSF, which are nuclear proteins, PSF–TFE3 is targeted to the endosomal compartment. PSF–TFE3 acts through exporting TFE3 and P53 from the nucleus to the cytoplasm for degradation, leading to the transformed phenotype [Mathur et al. 2003]. A *cltc–tfe-3* fusion results from t(X;17)(p11.2;q23) in a form of renal carcinoma. The fusion transcript joins the 5′ exons of *cltc* (*clathrin heavy chain*) {17q23} to the 3′ exons of *tfe-3*. The resulting CLTC–TFE3 product retains the nuclear localization and DNA-binding domains of TFE-3, but lacks the multimerization domain of CLTC [Argani et al. 2003]. A subset of childhood renal cell carcinomata displays the

recurrent translocation t(X;17)(p11.2;q25.3) as the sole cytogenetic abnormality. This results in the fusion of the NH_2-terminal part of RCC17 (ASPL, TUG) to the COOH-terminal region of the transcription factor TFE3 [Heimann et al 2001]. The fusion protein is an oncogenic transcription factor, which contains the basic helix-loop-helix DNA-binding domain and the leucine zipper dimerization domain of TFE3. Renal carcinomata share this defect with a subset of alveolar soft tissue sarcomata, with the distinction that the translocation is balanced in renal cancers [Argani et al. 2001]. An X chromosome inversion, inv(X)(p11.2;q12), results in the fusion of the *nono* (*p54^{nrb}*) gene to *tfe-3* in renal tumors. The RNA-binding protein NONO is involved in RNA splicing. In t(6;11)(p21;q13) containing renal cell carcinomata, the *tfe-B* gene on chromosome 6 is fused to the *alpha* gene on chromosome 11. The *alpha–tfe-B* fusion gene contains all *tfe-B* coding exons linked to 5′ upstream regulatory sequences of *alpha* (*pro1073*). This effects a promoter substitution and results in a dramatic up-regulation of TFE-B protein levels, thereby severely unbalancing the nuclear ratios of the MITF/TFE subfamily members [Kuiper et al. 2003]. A reciprocal translocation, t(14;15)(q11;q24), may be the sole cytogenetic aberration in classic congenital mesoblastic nephroma. The cellular variant of congenital mesoblastic nephroma, but not the classic variant, bears the same chromosome aberration and fusion protein, t(12;15)(p13;q25) and ETV6-NTRK3, as infantile fibrosarcoma, a tumor with which it shares morphologic and clinical features. Even in patients with nonhereditary renal cell carcinomata, loss of alleles at loci on the short arm of chromosome 3 is common. An inherited chromosomal translocation, t(3;8)(p21;q24), predisposes to renal cancer [Cohen et al. 1979]. It may cause the activation of the *myc* proto-oncogene on chromosome 8q24. The translocation t(3;8)(p14.2;q24.1) involves the fragile site locus FRA3B in some renal cancers.

– A large proportion of prostate cancers carry fusions of the 5′ untranslated region of *tmprss2* {21q22.3} to a gene for either of two ETS transcription factors, *erg* {21q22.3} or *etv-1* {7p21.2}. ERG and ETV-1 are components of a growth control pathway. The fusion places the regulatory sequence of the androgen responsive *tmprss2* upstream of *erg* or *etv-2*. This leads to overexpression of ERG or ETV-1 [Tomlins et al. 2005].

Translocations in other tumors:

– ROS is a receptor tyrosine kinase. The fusion protein FIG–ROS may cause glioblastoma. A microdeletion on 6q21 results in the fusion of *fig*, a gene coding for a protein associated with the Golgi apparatus, to the kinase domain of the proto-oncogene *c-ros*. FIG acts to localize the tyrosine kinase portion of the plasma membrane protein ROS to the Golgi apparatus, where it exerts its transforming potential [Charest et al. 2003a,b].
– EWS-WT1 is a chimeric transcription factor resulting from fusion of the NH_2-terminal domain of the Ewing sarcoma gene product EWS to the three COOH-terminal zinc fingers of the Wilms tumor suppressor WT1 {11p13}. The expression of EWS-WT1 leads to the induction of growth-associated genes, including the *β chain* of the *IL-2/15 receptor* (*il-2/15rβ*) in desmoplastic small round cell tumors. These tumors are characterized by an abundance of reactive stroma surrounding islets of tumor cells, which is indicative of paracrine signals that contribute to tumor cell proliferation. The high levels of IL-2/15Rβ within the tumor cells, along with the expression of IL-2 and IL-15 by the abundant hyperplastic endothelial cells within the reactive stroma suggest that the transcriptional induction of a cytokine receptor by a tumor-associated translocation product enables a proliferative response of epithelial cancer cells to ligands secreted by the surrounding stroma [Wong et al. 2002].
– Malignant Melanoma of soft parts (MMSP, soft tissue clear cell sarcoma) is a rare and aggressive tumor that mainly develops in tendons and aponeuroses of patients between 15 and 35 years of age. It may be derived from the neuroectoderm. In malignant melanoma of soft parts, the translocation t(12;22)(q13;q12) fuses the NH_2-terminal domain of EWS {22q12} to the bZIP domain of ATF1 {12q13}, a transcription factor that is normally regulated by cAMP [Zucman et al. 1993].
– Parotid adenomata are often caused by structural rearrangements of chromosome 8 [Bullerdiek et al. 1987]. They include the occurrence of trisomy, cases with structural rearrangements involving a breakpoint in 8q11–8q13, and a breakpoint in 8q21. Chromosome 12q breakpoints are frequent in solid tumors. In familial pleomorphic adenoma of the parotid gland, the

translocation t(3;12)(p21;q15) may be causative [Ahn et al. 1999].
– A cytogenetic abnormality associated with cystadenolymphoma of the parotid gland (Warthin tumor) is a reciprocal balanced translocation t(11;19)(q21;p13.1) [Bullerdiek et al. 1988].

3.4.6 Change in methylation status

Methylation is generally targeted to cytosine residues that precede guanines (5′-CG-3′, CpG dinucleotides, Cytosine-phospho-Guanosine dinucleotides). Methylation of CpG islands leads to gene silencing by packaging into a chromatin formation that is less accessible for transcription. Therefore, loss or gain of methylation may cause altered gene expression. The contexts, in which CpGs are located, are
– Large islands of more than 500 bp, frequently associated with promoters
– Small islands of 200–500 bp, characteristically associated with transposons (usually SINEs, short interspersed nuclear elements)
– Nonisland CpGs

About half of all CpG islands correspond to promoters and transcriptional start sites of genes. Gene hypermethylation is often an early event in transformation. Affected genes typically possess CpG islands in their 5′ region, which are not methylated in normal tissues. Many tumor suppressor genes exhibit CpG island hypermethylation in noninherited cancers. As a consequence, hypermethylation of negative modulators in early S phase may facilitate cell cycle progression. DNA repair genes may be inactivated by gene methylation. Silencing of metastasis suppressor genes in some cases also occurs by hypermethylation (Table 3.4.6.A). 5-methylcytosine can be deaminated to produce thymine, which the cell does not recognize as the result of a mutation.

Gene methylation is accomplished by DNA Methyl Transferases (DNMTs). *DNA* (*cytosine-5*) *methyl transferase* genes include *dnmt1* {19p13.3–p13.2}, *dnmt2* {10p15.1}, *dnmt3A* {2p23}, and *dnmt3B* {20q11.2}. The *dnmt1* gene is induced by RAS→JUN signaling [Rouleau et al. 1995]. The abundance of its message is posttranscriptionally suppressed during G_0 and induced upon entrance into S phase. The signal to methylate a sequence in the genome is principally provided by a preexisting methylation of that sequence. After reduplication to daughter chromatids, DNMTs recognize these sequences and restore their symmetric methylation on both DNA strands [Bird 1999].

Table 3.4.6.A. Gene silencing by hypermethylation in cancer. Examples of cancers that are caused, in part, by the hypermethylation of protective genes.

Genes	Tumors
Tumor suppressor genes	
$p16^{INK4}$	Lymphoma, colon cancer, lung cancer, pancreas cancer, upper gastrointestinal cancer, brain cancer, melanoma, bladder cancer
$p15^{INK4b}$	Leukemia, lymphoma, colon cancer
$p14^{ARF}$	Colon cancer, stomach cancer, uterus cancer
p73	Leukemia, lymphoma
apc	Colon cancer, stomach cancer, pancreas cancer, liver cancer
dapk	Lymphoma, bladder cancer, colon cancer, lung cancer, head and neck cancer
hic1	Brain cancer, breast cancer, colon cancer, renal cancer, lung cancer
rb1	Retinoblastoma
riz1	Liver cancer, breast cancer
syk	Breast cancer
crbp1	Lymphoma, gastrointestinal carcinoma, liver carcinoma, breast cancer
casp8	Rhabdomyosarcoma, medulloblastoma, retinoblastoma, neuroblastoma
rassf1A	Medulloblastoma, nasopharyngeal cancer, lung cancer, breast cancer
DNA repair genes	
brca1	Breast cancer, ovary cancer
mlh1	Colon cancer, uterus cancer, stomach cancer, endometrial cancer
gstπ1	Breast cancer, kidney cancer, liver cancer, prostate cancer
mgmt	Colon cancer, brain cancer, head and neck cancer, lymphoma, esophagus cancer
Metastasis suppressor genes	
cdh1	Breast cancer, leukemia, esophagus cancer, bladder cancer
thbs1	Colon cancer, glioblastoma
timp3	Kidney cancer, colon cancer, breast cancer, brain cancer

Consistently, DNMTs are part of the reduplication complex. DNMT1 is associated with the complex for part of the S phase, after which DNMT3A and DNMT3B replace it to methylate late reduplicating DNA. DNMT1 is targeted to the reduplication fork through a NH_2-terminal domain and through its association with PCNA (Proliferating Cell Nuclear Antigen). This interaction with PCNA regulates the activity of DNA (cytosine-5) Methyl Transferase in methylating newly reduplicated DNA. Binding of PCNA requires the amino acids 163–174 of DNMT1, occurs in intact cells at foci of newly reduplicated DNA, and does not alter DNMT1 activity. $P21^{CIP1/WAF1}$ blocks the access of DNMT to PCNA [Chuang et al. 1997]. DNMT binds with higher affinity to DNA strand breaks, gaps, abasic sites,

and uracil than it does to its cognate hemi-methylated CpG sites, consistent with its ancestral function as a DNA repair enzyme. The high affinity binding of DNMT to unrepaired lesions in DNA could sequester available enzyme away from the reduplication fork and promote reduplication-dependent demethylation. Hence, DNA lesions may be a prerequisite for the disruption of normal DNA methylation patterns in preneoplastic and neoplastic cells [James et al. 2003].

Changes in the pattern of DNA methylation are common in tumors. In most transformed cells, several genes are hypermethylated, the profile for promoter hypermethylation being tumor-type specific [Esteller et al. 2001]. Gastrointestinal tumors hypermethylate $p16^{INK4a}$, $p14^{ARF}$, *mgmt*, *apc*, and *mlh1*. Lung cancers and head and neck cancers have hypermethylated *dapk*, *mgmt*, and $p16^{INK4a}$, but not *mlh1* or $p14^{ARF}$. Breast and ovarian cancer cells tend to methylate *brca1*, *gstp1*, and $p16^{INK4a}$. Hematologic malignancies have a high frequency in hypermethylation of *p73* and $p15^{INK4b}$, which are not typically altered in epithelial tumors [Esteller et al. 2001]. Esophageal adenocarcinoma is associated with hypermethylation of *cdkn2A*, *esr1*, *myo-D1*, *calca*, *mgmt*, *timp3*, and *apc* [Eads et al. 2001]. Bladder cancer consistently hypermethylates $p16^{INK4a}$, $p15^{INK4b}$, and *pax6*.

Like hypermethylation, hypomethylation can be important in the initiation or progression of tumors. Severe DNA hypomethylation is sufficient to induce the formation of T-cell lymphomata. The rate of loss of heterozygosity is increased in the context of DNA hypomethylation [Chen et al. 1998]. The loss of DNA Methyl Transferase activity results in the demethylation of repeated sequences, loss of imprinting of the *igf II*, abrogation of the silencing of $p16^{INK4a}$, and growth suppression.

- Overexpression of DNMTs occurs in cancer [el-Deiry et al. 1991; Robertson et al. 1999]. DNMT1 {19p13.3–p13.2} is up-regulated at an early stage in lung cancer [Belinsky et al. 1996]. Its inhibition can be sufficient to prevent tumor formation [Trasler et al. 2003] or to reverse the transformed phenotype of cancer cells [MacLeod and Szyf 1995].
- P21 inhibits DNA synthesis when complexed with PCNA, and it acts as a competitor to DNMT for binding to this molecule. Loss of P21 function allows DNMT more access to DNA replication foci via PCNA [Chuang et al. 1997]. This facilitates hypermethylation and may predispose to transformation.
- The tumor suppressors $p16^{INK4a}$, $p15^{INK4b}$, and $p14^{ARF}$ may be silenced in cancer by hypermethylation. Hypermethylation of $p14^{ARF}$ occurs in colorectal cancer. Hypermethylation of $p15^{INK4b}$ is associated with leukemia and with head and neck cancer. $p16^{INK4a}$ hypermethylation arises in lymphoma, colon cancer, and lung cancer among others.
- In cancers of stomach, ovaries, and colon, the DNA repair gene *mlh1* is frequently hypermethylated. Hypermethylation of *gstp1* is selectively connected to liver cancer.
- 14-3-3σ is regulated by P53 and plays a role in the G_2/M checkpoint of the cell cycle. Inactivation of the *14-3-3σ* gene by 5′ CpG island hypermethylation occurs frequently in gastric cancers and is associated with an impairment of the G_2/M checkpoint [Suzuki et al. 2000].
- A loss of expression of the tumor suppressor FHIT (Fragile Histidine Triad) may occur through tumor acquired methylation of promoter region CpG islands in non-small cell lung cancer and in breast cancer.
- In head and neck squamous cell carcinomata and in non-small cell lung cancers, loss of heterozygosity on chromosome 6q23–q24 is often accompanied by methylation of the tumor suppressor gene *tcf21*. TCF21 contributes to the differentiation of epithelial cells, a process that is frequently deficient in carcinomata [Smith et al. 2006].
- The potential tumor suppressor gene *ras association domain family protein 1* (*rassf1*) {3p21.3} encodes an RAS effector. Hypermethylation of the CpG island promoter region of a major alternative transcript of this gene, *rassf1A*, can play a role in transformation. Methylation of the promoter of *rassf1A* (*ras association domain protein 1A*) on chromosome 3p21.3 occurs in lung, breast, ovarian, and bladder cancers, gastric adenocarcinomata, and in nasopharyngeal cancer.

Genomic imprinting is an epigenetic modification of a specific parental chromosome in the gamete or zygote that leads to differential expression of the two alleles of the affected genes in somatic cells of the offspring. Imprinting typically occurs by DNA methylation on cytosines. Genes subject to imprinting frequently cluster together on chromosomes. Several genes that are important in cancer are imprinted. They include *igf-2*, *igf-2r*, $p57^{KIP2}$, and *arh1*. The maternally expressed imprinted genes $p57^{KIP2}$ (*cdkn1C*) {11p15.5} and *igf-2r* {6q26} retard proliferation and reduce the long-term growth of cells.

In contrast, the paternally expressed growth factor IGF-2 {11p15.5} is essential for long-term proliferation [Hernandez et al. 2003]. The paternally imprinted 2.7 kb gene *h19* {11q15.5} contains five exons. It generates untranslated RNA that has regulatory functions in development and oncogenesis. Loss of Imprinting (LOI) may occur in cancer and lead to activation of the silent copy of growth promoting genes or the silencing of the active copy of growth inhibitory genes.

The embryonal tumors hepatoblastoma, Wilms tumor, and rhabdomyosarcoma have a common pathogenetic mechanism. In these tumors, a loss of constitutive heterozygosity on chromosome 11q15.5 affects *igf-2* and *h19*. Both genes are reciprocally imprinted, with expression of the maternal *h19* and paternal *igf-2* alleles, and are normally characterized by monoallelic expression. Loss of Imprinting in Wilms tumor involves the activation of the normally silent maternal allele of the *igf 2* gene and silencing of the normally active maternal allele of the downstream *h19* gene (Figure 3.4.6.A). This is due to methylation of binding sites for the transcription factor CTCF in the promoter regions of both genes [Cui et al. 2001]. CTCF forms a boundary, that, when engaged with DNA, isolates the sequences upstream of the binding site from the ones downstream. CTCF binding blocks the access of an enhancer to the *igf-2* promoter, leading to silencing. The enhancer can still interact with the *h19* promoter, which coincides with the CpG

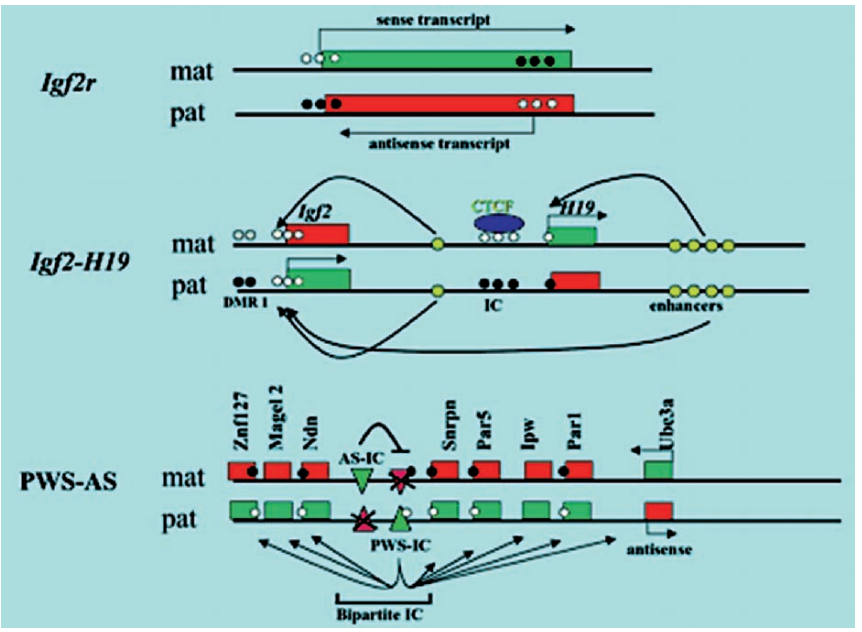


Figure 3.4.6.A. Imprinting of genes. Schematic representation of epigenetic regulation at three different imprinted loci. The *igf2r* locus contains a single imprinted gene regulated by an antisense transcript, which itself is regulated by a differentially methylated germ line imprint located in an intron. The *igf2-h19* locus contains a pair of reciprocally imprinted and coordinately regulated imprinted genes controlled by an intergenic imprinting center (IC), which binds CTCF when unmethylated and insulates the *igf2* fetal promoters from common downstream enhancers in endoderm. In mesoderm, tissue-specific silencers, such as DMR1, play a role in *igf2* regulation. Tissue specific enhancers upstream of the insulator regulate biallelic activity of *igf2* in parts of the brain. A larger gene cluster containing multiple imprinted genes is regulated by a bipartite imprinting center associated with the Prader–Willi/Angelman Syndrome locus (PWS-AS) on chromosome 15q. Here, a bipartite cis-acting imprinting center confers long-range imprinting control on the two parental alleles. Female germline transmission of an AS intergenic imprinting center is required for methylation and repression of the maternal alleles of the paternally active imprinted genes through inactivation of the PWS intergenic imprinting center. On the paternal chromosome, this AS intergenic imprinting center is nonfunctional, allowing the PWS intergenic imprinting center to confer paternal allelic expression on upstream and downstream genes. UBE3a is expressed from the maternal allele in the brain and may be associated with an antisense transcript on the paternal allele in a manner similar to the imprinting of *igf2r*. White circles denote absence of methylation at a differentially methylated region, black circles represent methylated regions, and gray circles indicate partial methylation, while larger yellow circles denote enhancers. Active alleles of genes are noted in green with silent alleles in red. The arrows indicate interactions between cis-elements on the two parental chromosomes. The drawings are not to scale. [Reproduced from Ferguson-Smith and Surani 2001. With permission.]

island that constitutes the CTCF binding site. Loss of Imprinting of the *igf-2* gene may also be a marker for susceptibility to colorectal cancer [Cui et al. 2003]. Loss of Imprinting of *h19* occurs in lung cancer.

Loss of $p57^{KIP2}$ (*cdkn1C*) [Lee et al. 1995; Matsuoka et al. 1995] underlies a fraction of cases of Beckwith–Wiedemann syndrome (BWS syndrome, Exomphalos–Macroglossia–Gigantism syndrome, EMG syndrome) [Wiedemann 1964; Beckwith 1969], which is characterized by organ overgrowth and predisposition to cancer. The principal symptoms of this disorder are exomphalos, macroglossia, and gigantism in the neonate. $p57^{KIP2}$ is genomically imprinted, the maternal allele being preferentially expressed while the paternally inherited allele is methylated and transcriptionally repressed. A microdeletion involving the entire *lit1* gene {11p15} causes silencing of $p57^{KIP2}$ when inherited maternally, and results in Beckwith–Wiedemann syndrome. Adrenal carcinoma, nephroblastoma, hepatoblastoma, and rhabdomyosarcoma occur with increased frequency. Loss of $p57^{KIP2}$ is also associated with Wilms tumor II.

3.4.7 Other dysregulations

Tumor development may be driven, beside genetic defects, by epigenetic events. They include alterations in RNA splicing, RNA stabilization, and RNA editing. Transformation can also be induced by recombination of fragile sites or activation of viral oncogenes.

Loss of fidelity of the splicing process occurs during tumor progression. This may affect the process of alternative splicing of the tumor suppressor gene *tsg101* (*tumor susceptibility gene 101*) {11p15.2–p15.1}. Aberrant transcripts of *tsg101*, which arise in carcinomata of the cervix, prostate, liver, and gastrointestinal tract, are generally shorter than the wild-type mRNA and are generated by exon skipping or splicing events. In breast cancer, mutations of *p53* can lead to the presence of aberrant *tsg101* transcripts [Lee and Feinberg 1997; Sun et al. 1997; Moyret-Lalle et al. 2001].

The *mdm2* oncogene produces more than 40 alternatively spliced transcripts that encode distinct protein forms. Some are only found in a range of cancers, but not in normal tissues [Bartel et al. 2002]. Some are also produced by healthy cells. Many of the splice variants show loss of P53 binding, consistent with a partial deletion of sequences encoding the P53 binding domain. They retain COOH-terminal zinc finger domains [Sigalas et al. 1996]. Transcripts of the *mdm2* gene may be aberrantly spliced in breast cancer [Hori et al. 2000b; Lukas et al. 2001].

Micro-RNAs (miRNAs, MIRs) are a large family of highly conserved noncoding genes involved in temporal and tissue-specific gene regulation, which play important regulatory roles in development. They constitute an abundant class of nucleic acids transcribed as short hairpin precursors of approximately 60–110 nucleotides and are processed into about 22 nucleotides in length. This precursor processing reaction requires Dicer (Helicase-MOI), RNase III, and Argonaute family members. miRNAs pair with target RNAs to specify the posttranscriptional repression of these messages. This may result in proto-oncogenic effects if the target sequences are tumor suppressor RNAs or in tumor suppressive effects if the target sequences are proto-oncogenic RNAs. *micro-RNA* genes are frequently located at fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification (minimal amplicons), or common breakpoint regions. Therefore, their disruption may contribute to carcinogenesis [Calin et al. 2004]. Such disruption may be due to

– The location of *miR* genes in cancer associated genomic regions. More than 50% of the known *miR* genes reside in genomic regions that are prone to alteration in cancer cells, including minimal regions of loss of heterozygosity, minimal regions of amplification, common breakpoint regions, and fragile sites.
– Altered epigenetic regulation of *miR* expression. Histone Deacetylase activity has a substantial effect on the expression of micro-RNAs. Furthermore, DNA methylation on CpG islands can alter the expression of micro-RNAs.
– Abnormalities in *miR* processing gene products. A failure of the RNAse III-processing step may account for a down-regulation of micro-RNAs in tumor cells. The expression levels of Dicer are reduced in a fraction of lung cancers, and this is associated with a poor prognosis.

- The genes for *miR-142*, *miR-15*, and *miR-16* are sites of translocation breakpoints or deletions in leukemias. Hemizygous or homozygous loss at 13q14 occurs in more than 50% of cases in CLL. *miR-15a* and *miR-16–1* {13q14.3} act as tumor suppressors. They are located within a 30 kb region

of loss in CLL, and both genes are deleted or down-regulated in the majority of CLL cases [Calin et al. 2002]. Their absence may prevent hematopoietic lineage differentiation and predispose to transformation.

- The 3′ untranslated region of the *ras* genes contains multiple *let-7* complementary sites, allowing *let-7* to regulate *ras* expression. *let-7* expression is lower in lung tumors than in normal lung tissue, while RAS protein is significantly higher in lung tumors, reflecting a possible mechanism for *let-7* in cancer [Johnson et al. 2005].
- *miR-143* and *miR-145*, consistently display reduced steady state levels of the mature miRNA at the adenomatous and cancer stages of colorectal neoplasia [Michael et al. 2003].
- Compared with normal breast tissue, miRNAs are aberrantly expressed in breast cancer. The overall miRNA expression is distinct in normal versus cancer tissues, with the most significantly dysregulated miRNAs being *miR-125b*, *miR-145*, *miR-21*, and *miR-155*. The expression of certain miRNAs correlates with specific breast cancer pathologic features, including *estrogen receptor* and *progesterone receptor* expression, tumor stage, vascular invasion, or proliferation index [Iorio et al. 2005].
- *miR-21* is overexpressed in multiple cancers. It inactivates *pten* and protects from Caspase-dependent apoptosis.
- *miR-155* and the cluster *miR17–92* {13q31} exert proto-oncogenic effects through *myc*.

cis-acting adenine- and uridine-rich elements destabilize mRNAs and play an important role in the control of gene expression. The ELAVL (Embryonic Lethal Abnormal Vision-Like) family of RNA-binding proteins includes ELAVL-1 (HU-R) {19p13.2}, ELAVL-2 (HU-B, HEL-N1) {9p21}, ELAVL-3 (HU-C) {19p13.2}, and ELAVL-4 (HU-D) {1p34}. They bind to adenine- and uridine-rich stability elements (AU-rich elements, AREs) in the 3′ untranslated regions of target mRNA. Sustained expression of the ELAVL family proteins is accomplished by RNA stabilization and may promote tumor growth [Nabors et al. 2001].

A frequently occurring type of mutation causes a premature stop codon to appear within the coding sequence of messenger RNA (mRNA). Mutations of this nature can lead to transformation, as is the case for *brca1*, which causes familial breast cancer, or for *nf1*, which causes neurofibromatosis 1. A mechanism known as nonsense-mediated decay has evolved to detect these harmful RNAs. It is dependent on RENT1 (Regulator of Nonsense Transcripts 1) {19p13.2–p13.11}. The protein kinase SMG-1 is related to Phosphatidylinositol 3-Kinase. SMG-1 can associate with RENT1/SMG-2 and other components of the surveillance complex. It phosphorylates and activates RENT1/SMG-2 on specific serine residues in SQ motifs. Downstream exon–exon junctions of the transcripts may be critical determinants for discrimination between normal and premature stop codons. When a mutation occurs in the nonsense-mediated decay process itself, such as a loss-of-function mutation in RENT1, it can result in a population of truncated proteins. This may give rise to transformed cells.

- In a form of familial hereditary nonpolyposis colorectal cancer, a deletion of two nucleotides in codon 659 introduces a frameshift and a new stop codon in exon 17 of the DNA mismatch repair gene *mlh1*. This leads to skipping of the exon. This phenomenon occurs also when there are missense or nonsense mutations in this codon or in codon 461 within exon 12 [Nystrom-Lahti et al. 1999; Stella et al. 2001].
- Germline mutations in *brca1* cause a fraction of familial breast cancers. They are scattered over the 22 coding exons and most of them generate premature termination codons. All truncating mutations located in the 3.4 kb long central exon are subject to nonsense-mediated decay, irrespective of their distance to the downstream exon–exon junctions. Premature termination codons that do not lead to nonsense-mediated decay are either located in the last exon or very close to the translation initiation codon [Perrin-Vidoz et al. 2002].

RNA editing is an enzyme-catalyzed process, in which newly synthesized messenger RNA undergoes selective base modifications that can dramatically alter the function of the encoded protein.

- In glioblastoma multiforme, the mRNA encoding the Glutamate Receptor subunit B is substantially underedited at a nucleotide position that must be changed from adenosine to inosine for normal receptor function. Consistently, the tumors have reduced activity of the enzyme ADAR2 (Adenosine Deaminase Acting on RNA 2), which is responsible for this editing step [Maas et al. 2001].

- Aberrant C to U editing of *neurofibromin* mRNA occurs in 25% of peripheral nerve sheath tumors in neurofibromatosis type 1. The affected tumors overexpress the enzyme APOBEC-1 (Apolipoprotein B mRNA-Editing Enzyme Catalytic Polypeptide-1), which catalyzes this modification [Mukhopadhyay et al. 2002].

Fragile sites are recombinogenic and are induced by agents that retard DNA reduplication. Allelic asynchrony (differences in reduplication time of adjacent segments of DNA) is common in fragile sites. Therefore, gaps at these sites may reflect incompletely reduplicated DNA. A late-reduplicating genomic region may not be fully copied by the time the cell enters G_2 and mitosis. Common fragile sites do not share high sequence homology, but they do tend to contain high percentages of adenosine and thymidine. Clusters of sequence of high flexibility and low stability are a characteristic shared by common fragile sites [Huebner and Croce 2001]. Environmental factors, like cigarette smoking, also appear to play a role in the expression of instability at common fragile sites. Six of the fragile sites, including FRA10A, FRA11B, FRA16A, FRAXA, FRAXE, and FRAXF, are folate sensitive. Each is characterized by an expanded and methylated trinucleotide repeat, CGG or CCG.

– FRA3B is located on chromosome 3p14.2 and contains the gene *fhit*. Even though the *fhit* gene spans 1 Mb of DNA it gives rise to a small RNA transcript of 1.1 kb. FHIT is a histidine triad protein, containing the motif (P/K)HfHfHffPR, with f denoting any hydrophobic amino acid. FHIT can exert proapoptotic functions through a Caspase-mediated pathway. In renal cancer, a translocation t(3,8)(p14.2;q24) may occur, which affects the FRA3B site. The FHIT protein may be absent from, or reduced in cancers of the entire alimentary tract. FRA3B differs from other fragile sites insofar as instability occurs over a large region of DNA, encompassing at least 500 kb, and trinucleotide repeat motifs are absent.
– FRA6E is located on chromosome 6q26. 8 genes, *igf-2r*, *slc22A1*, *slc22A2*, *slc22A3*, *plg*, *lpa*, *map3k4*, and *park2* map within the large FRA6E region. *park2* expression is down-regulated in 60% of primary ovarian tumors.
– FRA7G is located on chromosome 7q32.1. It contains the genes *cav1* (*caveolin-1*), *cav2* (*caveolin-2*), and *tes* (*testin*). It is the site of presence of the endogenous retroviral sequence *herv-H* and of sequences with homology to small polydispersed circular DNAs. FRA7G displays loss of heterozygosity in ovarian, breast, and prostate cancer.
– FRA7H is located on chromosome 7q31. Its loss of heterozygosity is associated with breast and prostate cancer.
– FRA9E is located on chromosome 9q32–33. The distal end of this fragile site contains *pappa* (*pregnancy-associated plasma protein A*), whose expression is frequently lost in ovarian cancer.
– FRA11B is a folate-sensitive fragile site located on chromosome 11q23.3. It contains the 5′ end of the *cbl-2* proto-oncogene, which includes a CCG trinucleotide repeat.
– FRA12A is located on chromosome 12q13. *pcbp2* (*poly(rC)-binding protein*, *hnrpe2*, heterogenous nuclear riboprotein E2) maps to 12q13.12-q13.13, distal to FRA12A. The gene encodes an RNA-binding protein. The arrest of differentiation is a feature of chronic myelogenous leukemia cells harboring the fusion protein BCR–ABL. BCR–ABL induces PCBP2, which inhibits the translation of *c/ebpα* mRNA. C/EBPα acts as the principal regulator of granulocytic differentiation and its lack maintains the cells in a precursor state.
– FRA16D is located on chromosome 16q23.2. It contains the possible tumor suppressor gene *WWox* (*WW-containing oxidoreductase*, *for*, *wox1*) and has loss of heterozygosity in breast, prostate, ovarian, and liver cancer. It may be translocated in multiple myeloma.
– FRAXA is associated with the fragile X syndrome. The molecular basis of FRAXA is the extensive expansion of a CCG trinucleotide repeat in the 5′ untranslated portion of the first exon of *fmr1* and associated methylation of an adjacent CpG island. The number of trinucleotide repeats in families with fragile X syndrome has a propensity to expand. In fragile X syndrome, nasopharyngeal carcinoma may occur. The *fmr1* CGG repeat at the fragile X locus shows a marked level of instability in patients with hereditary nonpolyposis colorectal cancer.
– FRAXB is located on the X chromosome {Xp22.3}. It contains the genes *gs1* and *sts* (*microsomal steroid sulfatase locus*). FRAXB is deleted in some cancers [Arlt et al. 2002].

Viral genes capable of causing transformation typically belong to the latent group of genes, which allow the infected cells to stay alive. These

viral oncogenes are present in all of the resulting cancer cells.

DNA tumor viruses encode oncogenes that are essential for viral replication. The oncogenes of transforming viruses have sequences that frequently differ from human proto-oncogenes and are under the control of promoters that are 50- to 100-fold stronger than the promoters of human genes. The proteins E1A and E1B from human adenovirus, E6 and E7 from human papillomavirus, and large T and small T antigens from SV40, are viral oncoproteins. Both E1A and E7 interact physically with RAN GTPase. This interaction is key in uncoupling the centrosome cycle from the cell cycle. Like RB, P53 forms a complex with SV40 large T antigen, as well as with the E1B-transforming protein of adenovirus and with the E6 protein of human papilloma viruses. Complexing with these tumor antigens decreases the stability of the P53 protein. Furthermore, the complexes of viral tumor antigens and P53 render P53 incapable of binding to DNA and inducing the transcription of cell cycle inhibiting or apoptosis inducing genes.

Transforming retroviruses carry oncogenes derived from cellular genes that are involved in mitogenic signaling and growth control. Retroviruses that do not contain oncogenes may cause transformation through insertional mutagenesis,

– either by insertion of proviral DNA into a tumor suppressor gene, resulting in its inactivation,
– or by integration of proviral DNA into regulatory sequences of cellular proto-oncogenes (*myc*, *erbB*, *ras*, *myb*, *fms*, *mos*, *int-1*, *int-2*, *pim-1*, *lck*, *evi-1*, *il-2*, *il-3*, *csf-1*), resulting in excessive proto-oncogene expression through promoter action by the long terminal repeats (LTRs).

REFERENCES

Adams PD, Kaelin WG jr. 1998. Negative control elements of the cell cycle in human tumors. Current Opinion in Cell Biology 10:791–797.

Adelmant G, Gilbert JD, Freytag SO. 1998. Human translocation liposarcoma-CCAAT/enhancer binding protein (C/EBP) homologous protein (TLS-CHOP) oncoprotein prevents adipocyte differentiation by directly interfering with C/EBPβ function. The Journal of Biological Chemistry 273:15574–15581.

Adida C, Berrebi D, Peuchmaur M, Reyes-Mugica M, Altieri DC. 1998. Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. The Lancet 351:882–883.

Ahn MS, Hayashi GM, Hilsinger RL jr, Lalwani AK. 1999. Familial mixed tumors of the parotid gland. Head and Neck 21:772–775.

Aigner A, Brachmann P, Beyer J, Jager R, Raulais D, Vigny M, Neubauer A, Heidenreich A, Weinknecht S, Czubayko F, Zugmaier G. 2003. Marked increase of the growth factors pleiotrophin and fibroblast growth factor-2 in serum of testicular cancer patients. Annales of Oncology 14:1525–1529.

Ali S, Coombes RC. 2002. Endocrine-responsive breast cancer and strategies for combating resistance. Nature Reviews Cancer 2:101–112

Altucci L, Gronemeyer H. 2001. The promise of retinoids to fight against cancer. Nature Reviews Cancer 1:181–193.

Alvaro V, Levy L, Dubray C, Roche A, Peillon F, Querat B, Joubert D. 1993. Invasive human pituitary tumors express a point-mutated alpha-protein kinase-C. Journal of Clinical Endocrinology and Metabolism 77:1125–1129.

Alvaro V, Prevostel C, Joubert D, Slosberg E, Weinstein BI. 1997. Ectopic expression of a mutant form of PKCα originally found in human tumors: aberrant subcellular translocation and effects on growth control. Oncogene 14:677–685.

Alvaro V, Touraine P, Raisman Vozari R, Bai-Grenier F, Birman P, Joubert D. 1992. Protein kinase C activity and expression in normal and adenomatous human pituitaries. International Journal of Cancer 50:724–730.

Amarante-Mendes GP, Naekyung Kim C, Liu L, Huang Y, Perkins CL, Green DR, Bhalla K. 1998. Bcr-Abl exerts its anti-apoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome C and activation of caspase-3. Blood 91:1700–1705.

Ambrosini G, Adida C, Altieri DC. 1997. A novel anti-apoptosis gene, *survivin*, expressed in cancer and lymphoma. Nature Medicine 3:917–921.

Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, Reuter VE, Garvin AJ, Perez-Atayde AR, Fletcher JA, Beckwith JB, Bridge JA, Ladanyi M. 2001. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. American Journal of Pathology 159:179–192.

Argani P, Lui MY, Couturier J, Bouvier R, Fournet JC, Ladanyi M. 2003. A novel CLTC-TFE3 gene fusion in pediatric renal adenocarcinoma with t(X;17)(p11.2;q23). Oncogene 22:5374–5378.

Arlt MF, Miller DE, Beer DG, Glover TW. 2002. Molecular characterization of FRAXB and comparative common fragile site instability in cancer cells. Genes, Chromosomes and Cancer 33:82–92.

Arnaoutov A, Dasso M. 2003. The Ran GTPase regulates kinetochore function. Developmental Cell 5:99–111.

Arvanitakis L, Geras-Raaka E, Varma A, Gershengorn MC, Cesarman E. 1997. Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. Nature 385:347–350.

Ashar HR, Fejzo MS, Tkachenko A, Zhou X, Fletcher JA, Weremowicz S, Morton CC, Chada K. 1995. Disruption of the architectural factor HMGI-C: DNA-binding AT hook motifs fused in lipomas to distinct transcriptional regulatory domains. Cell 82:57–65.

Ashkenazi. A. 2002. Targeting death and decoy receptors of the tumor-necrosis factor super family. Nature Reviews Cancer 2:420–430.

Aurelio ON, Kong XT, Gupta S, Stanbridge EJ. 2000. p53 mutants have selective dominant-negative effects on apoptosis

but not growth arrest in human cancer cell lines. Molecular and Cellular Biology 20:770–778.

Bais C, Santomasso B, Coso O, Arvanitakis L, Raaka EG, Gutkind JS, Asch AS, Cesarman E, Gershengorn MC, Mesri EA. 1998. G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis factor. Nature 391:86–89.

Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, vanTuinen P, Ledbetter DH, Barker DF, Nakamura Y, White R, Vogelstein B. 1989. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science 244:217–221.

Ball DW, Azzoli CG, Baylin SB, Chi D, Dou S, Donis-Keller H, Cumaraswamy A, Borges M, Nelkin BD. 1993. Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. Proceedings of the National Academy of Sciences USA 90:5648–5652.

Barbaux S, Niaudet P, Gubler MC, Grunfeld JP, Jaubert F, Kuttenn F, Fekete CN, Souleyreau-Therville N, Thibaud E, Fellous M, McElreavey K. 1997. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. Nature Genetics 17:467–470.

Bartel F, Taubert H, Harris LC. 2002. Alternative and aberrant splicing of MDM2 mRNA in human cancer. Cancer Cell 2:9–15.

Beckwith JB. 1969. Macroglossia, omphalocele, adrenal cytomegaly, gigantism, and hyperplastic visceromegaly. Birth Defects Original Article Series V:188–196.

Behrens J, Jerchow B-A, Wuertele M, Grimm J, Alsbrand C, Wirtz R, Kuehl M, Wedlich D, Birchmeier W. 1998. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. Science 280:596–599.

Belinsky SA, Belinsky SA, Nikula KJ, Baylin SB, Issa JP. 1996. Increased cytosine DNA methyltransferase activity is target cell-specific and an early event in lung cancer. Proceedings of the National Academy of Sciences USA 93:4045–4050.

Bergsten E, Uutela M, Li X, Pietras K, Oestman A, Heldin CH, Alitalo K, Eriksson U. 2001. PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor. Nature Cell Biology 3:512–516.

Biegel JA, Kalpana G, Knudsen ES, Packer RJ, Roberts CW, Thiele CJ, Weissman B, Smith M. 2002. The role of INI1 and the SWI/SNF complex in the development of rhabdoid tumors: meeting summary from the workshop on childhood atypical teratoid/rhabdoid tumors. Cancer Research 62:323–328.

Bird A. 1999. DNA methylation de novo. Science 286:2287–2288.

Bjorge JD, Pang A, Fujita DJ. 2000. Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. The Journal of Biological Chemistry 275:41439–41446.

Boldin MP, Goncharov TM, Goltsev YV, Wallach D. 1996. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85:803–815.

Bolger GB, Stamberg J, Kirsch IR, Hollis GF, Schwarz DF, Thomas GH. 1985. Chromosomal translocation t(14;22) and oncogene (c-sis) variant in a pedigree with familial meningioma. New England Journal of Medicine 312:564–567.

Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. 2004. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119:591–602.

Bonetta L, Kuehn SE, Huang A, Law DJ, Kalikin LM, Koi M, Reeve AE, Brownstein BH, Yeger H, Williams BRG, Feinberg AP. 1990. Wilms' tumor locus on 11p13 defined by multiple CpG island-associated transcripts. Science 250:994–997.

Bongarzone I, Butti MG, Coronelli S, Borrello MG, Santoro M, Mondellini P, Pilotti S, Fusco A, Della Porta G, Pierotti MA. 1994. Frequent activation of ret protooncogene by fusion with a new activating gene in papillary thyroid carcinomas. Cancer Research 54:2979–2985.

Bonneau D, Longy M. 2000. Mutations of the human PTEN gene. Human Mutations 16:109–122.

Borner C, Wyss R, Regazzi R, Eppenberger U, Fabbro D. 1987. Immunological quantitation of phospholipid/Ca^{2+}-dependent protein kinase of human mammary carcinoma cells: inverse relationship to estrogen receptors. International Journal of Cancer 40:344–348.

Borrow J, Shearman AM, Stanton VP jr, Becher R, Collins T, Williams AJ, Dube I, Katz F, Kwong YL, Morris C, Ohyashiki K, Toyama K, Rowley J, Housman DE. 1996. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. Nature Genetics 12:159–167.

Bowman T, Broome MA, Sinibaldi D, Wharton W, Pledger WJ, Sedivy JM, Irby R, Yeatman T, Courtneidge SA, Jove R. 2001. Stat3-mediated Myc expression is required for Src transformation and PDGF-induced mitogenesis. Proceedings of the National Academy of Sciences USA 98:7319–7324.

Brou C, Logeat F, Gupta N, Bessia C, LeBail O, Doedens JR, Cumano A, Roux P, Black RA, Israel A. 2000. A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Molecular Cell 5:207–216.

Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, Kley N. 1995. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 377:646–649.

Buckley CD, Pilling D, Henriquez NV, Parsonage G, Threlfall K, Scheel-Toellner D, Simmons DL, Akbar AN, Lord JM, Salmon M. 1999. RGD peptides induce apoptosis by direct caspase-3 activation. Nature 397:534–539.

Bütschli O. 1876. Studien ueber die ersten Entwicklungsvorgaenge der Eizelle, der Zellteilung und die Conjugation der Infusorien. Abhandlungen der Senkenbergischen Naturforschungsgesellschaft 10:213–452.

Bulavin DV, Higashimoto Y, Popoff IJ, Gaarde WA, Basrur V, Potapova O, Appella E, Fornace AJ jr. 2001. Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. Nature 411:102–107.

Bullerdiek J, Haubrich J, Meyer K, Bartnitzke S. 1988. Translocation t(11;19)(q21;p13.1) as the sole chromosome abnormality in a cystadenolymphoma (Warthin's tumor) of the parotid gland. Cancer Genetics and Cytogenetics 35:129–132.

Bullerdiek J, Raabe G, Bartnitzke S, Boschen C, Schloot W. 1987. Structural rearrangements of chromosome Nr 8 involving 8q12–a primary event in pleomorphic ademona of the parotid gland. Genetica 72:85–92.

Bullock AN, Fersht AR, 2001. Rescuing the function of Mutant p53. Nature Review Cancer 1:68–76.

Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Secivy JM, Kinzler KW, Vogelstein B. 1998. Requirement for

p53 and p21 to sustain G_2 arrest after DNA damage. Science 282:1497–1501.

Butti MG, Bongarzone I, Ferraresi G, Mondellini P, Borrello MG, Pierotti MA. 1995. A sequence analysis of the genomic regions involved in the rearrangements between TPM3 and NTRK1 genes producing TRK oncogenes in papillary thyroid carcinomas. Genomics 28:15–24.

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences USA 99:15524–15529.

Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences USA 101:2999–3004.

Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE. 1990. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 60:509–520.

Carling T, Rastad J, Akerstrom G, Westin G. 1998. Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. The Journal of Clinical Endocrinology and Metabolism 83:2255–2259.

Cascino I, Papoff G, De Maria R, Testi R, Ruberti G. 1996. Fas/Apo-1 (CD95) receptor lacking the intracytoplasmic signaling domain protects tumor cells from Fas-mediated apoptosis. The Journal of Immunology 156:13–17.

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, Strong LC, White RL. 1983. Expression of recessive alleles by chromosomal mechanisms in retinoblasoma. Nature 305:779–784.

Chan, Y-M, Jan YN. 1998. Roles for proteolysis and trafficking in Notch maturation and signal transduction. Cell 94:423–426.

Chang HY, Nishitoh H,Yang X, Ichijo H, Baltimore D. 1998. Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adaptor protein Daxx. Science 281:1860–1863.

Chang S-H, Ai Y, Breyer RM, Lane TF, Hla T. 2005. The prostaglandin E2 receptor EP2 is required for cyclooxygenase 2-mediated mammary hyperplasia. Cancer Research 65:4496–4499.

Charest A, Kheifets V, Park J, Lane K, McMahon K, Nutt CL, Housman D. 2003a. Oncogenic targeting of an activated tyrosine kinase to the Golgi apparatus in a glioblastoma. Proceedings of the National Academy of Sciences USA 100:916–921.

Charest A, Lane K, McMahon K, Park J, Preisinger E, Conroy H, Housman D. 2003b. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Genes, Chromosomes and Cancer 37:58–71.

Chen PL, Riley DJ, Chen-Kiang S, Lee WH. 1996. Retinoblastoma protein directly interacts with and activates the transcription factor NF-IL6. Proceedings of the National Academy of Sciences USA 93:465–469.

Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. 1998. DNA hypomethylation leads to elevated mutation rates. Nature 395:89–93.

Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. 2003. VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 301:513–517.

Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR. 2004. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 303:1010–1014.

Choe G, Horvath S, Cloughesy TF, Crosby K, Seligson D, Palotie A, Inge L, Smith BL, Sawyers CL, Mischel PS. 2003. Analysis of the phosphatidylinositol 3′-kinase pathway in glioblastoma patients *in vivo*. Cancer Research 63:2742–2746.

Chuang L S-H, Ian H-I, Koh T-W, Ng H-H, Xu G, Li BFL. 1997. Human DNA-(Cytosine-5) methyltransferase-PCNA complex as a target for $p21^{WAF1}$. Science 277:1996–2000.

Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS. 1979. Hereditary renal-cell carcinoma associated with chromosomal translocation. New England Journal of Medicine 301:592–595.

Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, Vande Woude GF. 1984. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature 311:29–33.

Cooper G. 1997. The cell: a molecular approach. Herndon: ASM Press, p. 534.

Cooper GM. 1999. Oncogenes. Boston: Jones and Barlett.

Crew AJ, Clark J, Fisher C, Gill S, Grimer R, Chand A, Shipley J, Gusterson BA, Cooper CS. 1995. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. EMBO Journal 14:2333–22340.

Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR, Feinberg AP. 2003. Loss of *IGF2* imprinting: a potential marker of colorectal cancer risk. Science 299:1753–1755.

Cui H, Niemitz EL, Ravenel JD, Onyango P, Brandenburg SA, Lobanenkov VV, Feinberg AP. 2001. Loss of imprinting of insulin-like growth factor-II in Wilms' tumor commonly involves altered methylation but not mutations of CTCF or its binding site. Cancer Research 61:4947–4950.

Danilkovitch-Miagkova A, Zbar B. 2002. Dysregulation of Met receptor tyrosine kinase activity in invasive tumors. The Journal of Clinical Investigation 109:863–867.

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. 2002. Mutations in the BRAF gene in human cancer. Nature 417:949–954.

Denys P, Malvaux P, van den Berghe H, Tanghe W, Proesmans W. 1967. Association d'un syndrome anatomo-pathologique de pseudohermaphrodisme masculin, d'une tumeur de Wilms, d'une nephropathie parenchymateuse et d'un mosaicisme XX/XY. Archives Francaises de Pediatrie 24:729–739.

Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC. 1998. IAPs block apoptotic events induced by caspase-8 and

cytochrome c by direct inhibition of distinct caspases. EMBO Journal 17:2215–2223.

Deverman BE, Cook BL, Manson SR, Niederhoff RA, Langer EM, Rosova I, Kulans LA, Fu X, Weinberg JS, Heinecke JW, Roth KA, Weintraub SJ. 2002. Bcl-x_L deamidation is a critical switch in the regulation of the response to DNA damage. Cell 111:51–62.

De Vivo I, Huggins GS, Hankinson SE, Lescault PJ, Boezen M, Colditz GA, Hunter DJ. 2002. A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. Proceedings of the National Academy of Sciences USA 99:12263–12268.

Dhanasekaran N, Heasley LE, Johnson GL. 1995. G protein-coupled receptor systems involved in cell growth and oncogenesis. Endocrinology Reviews 16:259–270.

Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG. 2002. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295:1079–1082.

Dierlamm J, Baens M, Wlodarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, De Wolf-Peeters C, Hagemeijer A, Van den Berghe H, Marynen P. 1999. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood 93:3601–3609.

D'Orazi G, Cecchinelli B, Bruno T, Manni I, Higashimoto Y, Saito S, Gostissa M, Coen S, Marchetti A, Del Sal G, Piaggio G, Fanciulli M, Appella E, Soddu S. 2002. Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. Nature Cell Biology 4:11–19.

Di Renzo MF, Olivero M, Ferro S, Prat M, Bongarzone I, Pilotti S, Belfiore A, Costantino A, Vigneri R, Pierotti MA, Comiglio P. 1992. Overexpression of the c-MET/HGF receptor gene in human thyroid carcinomas. Oncogene 7:2549–2553.

Di Renzo MF, Olivero M, Giacomini A, Porte H, Chastre E, Mirossay L, Nordlinger B, Bretti S, Bottardi S, Giordano S, Plebani M, Gespach C, Comoglio PM. 1995. Overexpression and amplification of the MET/HGF receptor gene during the progression of colorectal cancer. Clinical Cancer Research 1:147–154.

Di Renzo MF, Olivero M, Katsaros D, Crepaldi T, Gaglia P, Zola P, Sismondi P, Comoglio PM. 1994. Overexpression of the MET/HGF receptor in ovarian cancer. International Journal of Cancer 58:658–662.

Di Renzo MF, Olivero M, Martone T, Maffe A, Maggiora P, Stefani AD, Valente G, Giordano S, Cortesina G, Comoglio PM. 2000. Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. Oncogene 19:1547–1555.

Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades HN. 1983. Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221:275–277.

Drash A, Sherman F, Hartmann WH, Blizzard RM. 1970. A syndrome of pseudohermaphroditism, Wilms' tumor, hypertension, and degenerative renal disease. The Journal of Pediatrics 76:585–593.

Dutton A, O'Neil JD, Milner AE, Reynolds GM, Starczynski J, Crocker J, Young LS, Murray PG. 2004. Expression of the cellular FLICE-inhibitory protein (c-FLIP) protects Hodgkin's lymphoma cells from autonomous Fas-mediated death. Proceedings of the National Academy of Sciences USA 101:6611–6616.

Duval A, Gayet J, Zhou XP, Iacopetta B, Thomas G, Hamelin R. 1999. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. Cancer Research 59:4213–4215.

Eads CA, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, Peters JH, DeMeester SR, DeMeester TR, Skinner KA, Laird PW. 2001. Epigenetic pattern in the progression of esophageal adenocarcinoma. Cancer Research 61:3410–3418.

el-Deiry WS, Nelkin BD, Celano P, Chiu Yen R-W, Falco JP, Hamilton SR, Baylin SB. 1991. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. Proceedings of the National Academy of Sciences USA 88:3470–3474.

el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. 1993. WAF1, a potential mediator of p53 tumor suppression. Cell 75:817–825.

Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, Sklar J. 1991. TAN-1, the human homolog of the Drosophila Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 66:649–661.

Enric Domingo, Simo Schwartz Jr. 2004. BRAF. Atlas Genet Cytogenet Oncol Haematol 8(4):622–631.

Essafi A, Fernandez de Mattos S, Hassen YA, Soeiro I, Mufti GJ, Thomas NS, Medema RH, Lam EW. 2005. Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells. Oncogene 24:2317–2329.

Esteller M, Corn PG, Baylin SB, Herman JG. 2001. A gene hypermethylation profile of human cancer. Cancer Research 61:3225–3229.

Ewen ME, Xing YG, Lawrence JB, Livingston DM. 1991. Molecular cloning, chromosomal mapping, and expression of the cDNA for p107, a retinoblastoma gene product-related protein. Cell 66:1155–1164.

Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J. 2001. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 410:842–847.

Feldman BJ, Feldman D. 2001. The development of androgen-independent prostate cancer. Nature Reviews Cancer 1:34–45.

Feng X-H, Liang Y-Y, Liang M, Zhai W, Lin X. 2002. Direct interaction of c-Myc with Smad2 and Smad3 to inhibit TGF-β-mediated induction of the CDK inhibitor $p15^{Ink4B}$. Molecular Cell 9:133–143.

Ferguson-Smith AC, Surani MA, 2001. Imprinting and the epigenetic asymmetry between parental genomes. Science 293:1086–1089.

Fernandez de Mattos S, Essafi A, Soeiro I, Pietersen AM, Birkenkamp KU, Edwards CS, Martino A, Nelson BH, Francis JM, Jones MC, Brosens JJ, Coffer PJ, Lam EW. 2004. FoxO3a and BCR-ABL regulate cyclin D2 transcription through a STAT5/BCL6-dependent mechanism. Molecular and Cellular Biology 24:10058–10071.

Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. 1998. The murine gene $p27^{Kip1}$ is haplo-insufficient for tumour suppression. Nature 396:177–180.

Ferracini R, Di Renzo MF, Scotlandi K, Baldini N, Olivero M, Lollini P, Cremona O, Campanacci M, Comoglio PM. 1995. The Met/HGF receptor is over-expressed in human osteosarcomas

and is activated by either a paracrine or an autocrine circuit. Oncogene 10:739–749.

Ferracini R, Olivero M, Di Renzo MF, Martano M, De Giovanni C, Nanni P, Basso G, Scotlandi K, Lollini PL, Comoglio PM. 1996. Retrogenic expression of the MET proto-oncogene correlates with the invasive phenotype of human rhabdomyosarcomas. Oncogene 12:1697–1705.

Filippova GN, Lindblom A, Meincke LJ, Klenova EM, Neiman PE, Collins SJ, Doggett NA, Lobanenkov VV. 1998. A widely expressed transcription factor with multiple DNA sequence specificity, CTCF, is localized at chromosome segment 16q22.1 within one of the smallest regions of overlap for common deletions in breast and prostate cancers. Genes, Chromosomes and Cancer 22:26–36.

Fitzgerald K, Harrington A, Leder P. 2000. Ras pathway signals are required for notch-mediated oncogenesis. Oncogene 19:4191–4198.

Flanagan JG, Chan DC, Leder P. 1991. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell 64:1025–1035.

Flynn A, Proud CG. 1996. The role of eIF4 in cell proliferation. Cancer Survey 27:293–310.

Fodde R, Smith R, Cleuers H. 2001. APC, Signal transduction and genetic instability in colorectal cancer. Nature Reviews Cancer 1:55–67.

Frame MC. 2002. Src in cancer: deregulation and consequences for cell behaviour. Biochimica et Biophysica Acta – Reviews on Cancer 1602:114–130.

Franceschini P, Licata D, Di Cara G, Guala A, Bianchi M, Ingrosso G, Franceschini D. 1999. Bladder carcinoma in Costello syndrome: report on a patient born to consanguineous parents and review. American Journal of Medical Genetics 86:174–179.

Frasier SD, Bashore RA, Mosier HD. 1964. Gonadoblastoma associated with pure gonadal dysgenesis in monozygotic twins. Journal of Pediatrics 64:740–745.

Friedrich CA. 2001. Genotype–phenotype correlation in von Hippel-Lindau syndrome. Human Molecular Genetics 10:763–767.

Fu X-Y, Schindler C, Improta T, Aebersold R, Darnell JE Jr. 1992. The proteins of ISGF-3: the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. Proceedings of the National Academy of Sciences USA 89:7840–7843.

Fujiwara Y, Ohata H, Emi M, Okui K, Koyama K, Tsuchiya E, Nakajima T, Monden M, Mori T, Kurimasa A, Oshimura M, Nakamura Y. 1994. A 3-Mb physical map of the chromosome region 8p21.3-p22, including a 600-kb region commonly deleted in human hepatocellular carcinoma, colorectal cancer, and non-small cell lung cancer. Genes, Chromosomes and Cancer 10:7–14.

Fujiwara Y, Ohata H, Kuroki T, Koyama K, Tsuchiya E, Monden M, Nakamura Y. 1995. Isolation of a candidate tumor suppressor gene on chromosome 8p21.3-p22 that is homologous to an extracellular domain of the PDGF receptor β gene. Oncogene 10:891–895.

Furukawa M, He YJ, Borchers C, Xiong Y. 2003. Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nature Cell Biology 5:1001–1007.

Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, Della Porta G, Vecchio G. 1987. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature 328:170–172.

Gartel AL, Schors K. 2003. Mechanisms of c-myc-mediated transcriptional repression of growth arrest genes. Experimental Cell Research 283:17–21.

Gauwerky CE, Croce CM. 1995. Molecular genetics and cytogenetics of hematopoetic malignancies. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 18–37.

Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GA. 1990. Homozygous deletion in Wilms' tumours of a zinc-finger gene identified by chromosome jumping. Nature 343:774–778.

Gigoux V, L'Hoste S, Raynaud F, Camonis J, Garbay C. 2002. Identification of Aurora kinases as RasGAP Src homology 3 domain-binding proteins. The Journal of Biological Chemistry 277:23742–23746.

Glotzer M, Murray AW, Kirschner MW. 1991. Cyclin is degraded by the ubiquitin pathway. Nature 349:132–138.

Golstein P. 2000. FasL binds preassembled Fas. Science 288:2328–2329.

Goodman JE, Bowman ED, Chanock SJ, Alberg AJ, Harris CC. 2004. Arachidonate lipoxygenase (ALOX) and cyclooxygenase (COX) polymorphisms and colon cancer risk. Carcinogenesis 25:2467–2472.

Greco A, Pierotti MA, Bongarzone I, Pagliardini S, Lanzi C, Della Porta G. 1992. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene 7:237–242.

Greer EL, Brunet A. 2005. Foxo transcription factors at the interface between longevity and tumor suppression. Oncogene 24:7410–7425.

Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A, Vecchio G. 1990. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60:557–563.

Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D Abderrahim H, Cohen D, Leppert M, White R. 1991. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66:589–600.

Gronbaek K, Straten PT, Ralkiaer E, Ahrenkiel V, Klarskov Andersen M, Hansen NE, Zeuthen J, Hou-Jensen K, Guldberg P. 1998. Somatic Fas mutations in non-Hodgkin's lymphoma: association with extranodal disease and autoimmunity. Blood 92:3018–3024.

Gu W, Roeder RG. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90:595–606.

Guacci V, Koshland D, Strunnikov A. 1997. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cervisiae*. Cell 91:47–57.

Guan XY, Xu J, Anzick SL, Zhang H, Trent JM, Meltzer PS. 1996. Hybrid selection of transcribed sequences from microdissected DNA: isolation of genes within amplified region at 20q11-q13.2 in breast cancer. Cancer Research 56:3446–3450.

Guasparri I, Keller SA, Cesarman E. 2004. KSHV vFLIP is essential for the survival of infected lymphoma cells. The Journal of Experimental Medicine 199:993–1003.

Guedez L, Mansoor A, Birkedal-Hansen B, Lim MS, Fukushima P, Venzon D, Stetler-Stevenson WG, Stetler-Stevenson M. 2001. Tissue inhibitor of metalloproteinases 1 regulation of interleukin-10 in B-cell differentiation and lymphomagenesis. Blood 97:1796–1802.

Guikema JE, Hovenga S, Vellenga E, Conradie JJ, Abdulahad WH, Bekkema R, Smit JW, Zhan F, Shaughnessy J jr., Bos NA. 2003. CD27 is heterogeneously expressed in multiple myeloma: low CD27 expression in patients with high-risk disease. British Journal of Haematology 121:36–43.

Guikema JE, Vellenga E, Abdulahad WH, Hovenga S, Bos NA. 2004. CD27-triggering on primary plasma cell leukaemia cells has anti-apoptotic effects involving mitogen activated protein kinases. British Journal of Haematology 124:299–308.

Guo HG, Browning P, Nicholas J, Hayward GS, Tschachler E, Jiang YW, Sadowska M, Raffeld M, Colombini S, Gallo RC, Reitz MS jr. 1997. Characterization of a chemokine receptor-related gene in human herpesvirus 8 and its expression in Kaposi's sarcoma.Virology 228:371–378.

Guo X, Knudsen BS, Peehl DM, Ruiz A, Bok D, Rando RR, Rhim JS, Nanus DM, Gudas LJ. 2002. Retinol metabolism and lecithin:retinol acyltransferase levels are reduced in cultured human prostate cancer cells and tissue specimens. Cancer Research 62:1654–1661.

Gura T. 1997. How TRAIL kills cancer cells, but not normal cells. Science 277:768.

Haber DA, Timmers HT, Pelletier J, Sharp PA, Housman DE. 1992. A dominant mutation in the Wilms tumor gene WT1 cooperates with the viral oncogene E1A in transformation of primary kidney cells. Proceedings of the National Academy of Sciences USA 89:6010–6014.

Haberstroh K, Heigold S, Bauer G. 2002. Transformed cell-derived reactive oxygen species support and inhibit nitric oxide-mediated apoptosis induction. International Journal of Oncology 21:145–151.

Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. 1996a. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85:841–851.

Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE. 1996b. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271:350–353.

Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Schneider P, Bornand T, Fontana A, Lienard D, Cerottini J, Tschopp J. 1996c. Melanoma cell expression of Fas (Apo-1/CD95) ligand: implications for tumor immune escape. Science 274:1363–1366.

Hainaut P, Hollstein M. 2000. p53 and human cancer: the first ten thousand mutations. Advances in Cancer Research 77:81–137.

Halevy O, Michalovitz D, Oren M. 1990. Different tumor-derived p53 mutants exhibit distinct biological activities. Science 250:113–116.

Half E, Tang XM, Gwyn K, Sahin A, Wathen K, Sinicrope FA. 2002. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma *in situ*. Cancer Research 62:1676–1681.

Hanai J, Chen LF, Kanno T, Ohtani-Fujita N, Kim WY, Guo WH, Imamura T, Ishidou Y, Fukuchi M, Shi MJ, Stavnezer J, Kawabata M, Miyazono K, Ito Y. 1999. Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline Cα promoter. The Journal of Biological Chemistry 274:31577–31582.

Hansson A, Manetopoulos C, Jonsson JI, Axelson H. 2003. The basic helix-loop-helix transcription factor TAL1/SCL inhibits the expression of the $p16^{INK4A}$ and pTα genes. Biochemical and Biophysical Research Communication 312:1073–1081.

Harper JW. 1997. Cyclin dependent kinase inhibitors. Cancer Surveys 29:91–107.

Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805–816.

Harris AL. 2002. Hypoxia-A key regulatory factor in tumor growth. Nature Reviews Cancer 2:38–47.

Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, Alessi DR, Hardie DG. 2003. Complexes between the LKB1 tumor suppressor, STRADα/β and MO25α/β are upstream kinases in the AMP-activated protein kinase cascade. Journal of Biology 2:28.

Hecht JT, Hogue D, Wang Y, Blanton SH, Wagner M, Strong LC, Raskind W, Hansen MF, Wells D. 1997. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. American Journal for Human Genetics 60:80–86.

Heimann P, Housni HE, Ogur G, Weterman MAJ, Petty EM, Vassart G. 2001. Fusion of a novel gene, *RCC17*, to the *TFE3* gene in t(X;17)(p11.2;q25.3)-bearing papillary renal carcinomas. Cancer Research 61:4130–4135.

Heinen CD, Goss KH, Cornelius JR, Babcock GF, Knudsen ES, Kowalik T, Groden J. 2002. The APC tumor suppressor controls entry into S-phase through its ability to regulate the cyclin D/RB pathway. Gastroenterology 123:751–763.

Henry GL, Melton DA. 1998. Mixer, a homeobox gene required for endoderm development. Science 281:91–96.

Hernandez L, Kozlov S, Piras G, Stewart CL. 2003. Paternal and maternal genomes confer opposite effects on proliferation, cell-cycle length, senescence, and tumor formation. Proceedings of the National Academy of Sciences USA 100:13344–13349.

Hershko A, Ganoth D, Pehrson J, Palazzo RE, Cohen LH. 1991. Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts. The Journal of Biological Chemistry 266:16376–16379.

Hersey P, Zhang XD. 2001. How melanoma cells evade TRAIL-included Apoptosis. Nature Reviews Cancer 1:142–150.

Hibbard MK, Kozakewich HP, Dal Cin P, Sciot R, Tan X, Xiao S, Fletcher JA. 2000. *PLAG1* fusion oncogenes in lipoblastoma. Cancer Research 60:4869–4872.

Hirao A, Kong Y-Y, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW. 2000. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science 287:1824–1827.

Hocevar BA, Burns DJ, Fields AP. 1993. Identification of protein kinase C (PKC) phosphorylation sites on human lamin B. Potential role of PKC in nuclear lamina structural dynamics. The Journal of Biological Chemistry 268:7545–7552.

Hoffman AD, Engelstein D, Bogenrieder T, Papandreou CN, Steckelman E, Dave A, Motzer RJ, Dimitrovsky E, Albino AP,

Nanus DM. 1996. Expression of retinoic acid receptor β in human renal cell carcinomas correlates with sensitivity to the antiproliferative effects of 13-cis-retinoic acid. Cancer Research 2:1077–1082.

Hofmann TG, Moller A, Sirma H, Zentgraf H, Taya Y, Droge W, Will H, Schmitz ML. 2002. Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. Nature Cell Biology 4:1–10.

Hopkins-Donaldson S, Bodmer J-L, Bourloud KB, Brognara CB, Tschopp J, Gross N. 2000. Loss of caspase-8 expression in highly malignant human neuroblastoma cells correlates with resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. Cancer Research 60:4315–4319.

Hori M, Shimazaki J, Inagawa S, Itabashi M, Hori M. 2000a. Alternatively spliced MDM2 transcripts in human breast cancer in relation to tumor necrosis and lymph node involvement. Pathology International 50:786–792.

Hori T, Takaori-Kondo A, Kamikubo Y, Uchiyama T. 2000b. Molecular cloning of a novel human protein kinase, kpm, that is homologous to warts/lats, a Drosophila tumor suppressor. Oncogene 19:3101–3109.

Howe JR, Bair JL, Sayed MG, Anderson ME, Mitros FA, Petersen GM, Velculescu VE, Traverso G, Vogelstein B. 2001. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nature Genetics 28:184–187.

Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IP, Houlston RS, Bevan S, Mitros FA, Stone EM, Aaltonen LA. 1998. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science 280:1086–1088.

Hoyt MA, Totis L, Roberts BT. 1991. *S. cervisiae* genes required for cell cycle arrest in response to loss of microtubulte function. Cell 66:507–517.

Hu Y, Benedict MA, Wu D, Inohara N, Nunez G. 1998. Bcl-X_L interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proceedings of the National Academy of Sciences USA 95:4386–4391.

Huang C, Ma WY, Dawson MI, Rincon M, Flavell RA, Dong Z. 1997. Blocking activator protein-1 activity, but not activating retinoic acid response element, is required for the antitumor promotion effect of retinoic acid. Proceedings of the National Academy of Sciences USA 94:5826–5830.

Huang DC, Adams JM, Cory S. 1998. The conserved N-terminal BH4 domain of Bcl-2 homologues is essential for inhibition of apoptosis and interaction with CED-4. EMBO Journal 17:1029–1039.

Huebner K, Croce CM. 2001. *FRA3B* and other common fragile sites: the weakest links. Nature Reviews Cancer 1:214–221.

Igney FH, Krammer FH. 2002. Death and anti-death: Tumor resistance Apoptosis. Nature Reviews Cancer 2:277–278.

Imoto I, Yang ZQ, Pimkhaokham A, Tsuda H, Shimada Y, Imamura M, Ohki M, Inazawa J. 2001. Identification of *cIAP1* as a candidate target gene within an amplicon at 11q22 in esophageal squamous cell carcinoma. Cancer Research 61:6629–6634.

Inohara N, Koseki T, Chen S, Wu X, Nunez G. 1998. CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. EMBO Journal 17:2526–2533.

Inoue K, Zindy F, Randle DH, Rehg JE, Sherr CJ. 2001. *Dmp1* is haplo-insufficient for tumor suppression and modifies the frequencies of Arf and p53 mutations in Eμ-myc-induced lymphomas. Genes and Development 15:2934–2939.

Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. 2005. MicroRNA gene expression deregulation in human breast cancer. Cancer Research 65:7065–7070.

Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ. 1999. Activating SRC mutation in a subset of advanced human colon cancers. Nature Genetics 21:187–190.

Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J. 1997. Inhibition of death receptor signals by cellular FLIP. Nature 388:190–195.

Ivanov VN, Ronai Z. 2000. p38 protects human melanoma cells from UV-induced apoptosis through down-regulation of NF-κB activity and Fas expression. Oncogene 19:3003–3012.

Jallepalli PV, Lengauer C. 2001. Chromosome segregation and cancer cutting through the mystery. Nature Reviews Cancer 1:109–117.

James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S. 2003. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. Journal of Nutrition 133:3740S–3747S.

Javelaud D, Wietzerbin J, Delattre O, Besancon F. 2000. Induction of $p21^{Waf1/Cip1}$ by TNFα requires NF-κB activity and antagonizes apoptosis in Ewing tumor cells. Oncogene 19:61–68.

Jensen MR, Factor VM, Fantozzi A, Helin K, Huh CG, Thorgeirsson SS. 2003. Reduced hepatic tumor incidence in cyclin G1-deficient mice. Hepatology 37:862–870.

Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH jr., Scott MP. 1996. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272:1668–1671.

Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. 2005. RAS is regulated by the let-7 microRNA family. Cell 120:635–647.

Joslyn G, Carlson M, Thliveris A, Albertsen H, Gelbert L, Samowitz W, Groden J, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R. 1991. Identification of deletion mutations and three new genes at the familial polyposis locus. Cell 66:601–613.

Jost CA, Marin MC, Kaelin WG Jr. 1997. p73 is a human p53-related protein that can induce apoptosis. Nature 389:191–194.

Kacinski BM, Scata KA, Carter D, Yee LD, Sapi E, King BL, Chambers SK, Jones MA, Pirro MH, Stanley ER, Rohrschnieder LR. 1991. FMS (CSF-1 receptor) and CSF-1 transcripts and protein are expressed by human breast carcinomas in vivo and in vitro. Oncogene 6:941–952.

Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, Minty A, Chalon P, Lelias J-M, Dumont X, Ferrara P, McKeon F, Caput D. 1997. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90:809–819.

Kalab P, Pu RT, Dasso M. 1999. The ran GTPase regulates mitotic spindle assembly. Current Biology 9:481–484.

Kalinichenko VV, Major ML, Wang X, Petrovic V, Kuechle J, Yoder HM, Dennewitz MB, Shin B, Datta A, Raychaudhuri P, Costa RH. 2004. Foxm1b transcription factor is essential for development of hepatocellular carcinomas and is negatively regulated by the $p19^{ARF}$ tumor suppressor. Genes and Development 18:830–850.

Kamemura K, Hayes BK, Comer FI, Hart GW. 2002. Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmic proteins: alternative glycosylation/phosphorylation of THR-58, a known mutational hot spot of c-Myc in lymphomas, is regulated by mitogens. Journal of Biological Chemistry 277:19229–19235.

Kamps MP, Murre C, Sun XH, Baltimore D. 1990. A new homeobox gene contributes the DNA binding domain of the t(1;19) translocation protein in pre-B ALL. Cell 60:547–555.

Kastan MB, Zhan Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ jr. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71:587–597.

Katayama Y, Sakai A, Oue N, Asaoku H, Otsuki T, Shiomomura T, Masuda R, Hino N, Takimoto Y, Imanaka F, Yasui W, Kimura A. 2003. A possible role for the loss of CD27-CD70 interaction in myelomagenesis. British Journal of Haematology 120:223–234.

Kazmierczak B, Rosigkeit J, Wanschura S, Meyer-Bolte K, Van de Ven WJ, Kayser K, Krieghoff B, Kastendiek H, Bartnitzke S, Bullerdiek J. 1996. HMGI-C rearrangements as the molecular basis for the majority of pulmonary chondroid hamartomas: a survey of 30 tumors. Oncogene 12:515–521.

Kerr B, Eden OB, Dandamudi R, Shannon N, Quarrell O, Emmerson A, Ladusans E, Gerrard M, Donnai D. 1998. Costello syndrome: two cases with embryonal rhabdomyosarcoma. Journal of Medical Genetics 35:1036–1039.

Kerr JFR, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biologic phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer 26:239–257.

Kidd S, Kelley MR, Young MW. 1986. Sequence of the notch locus of Drosophila melanogaster: relationship of the encoded protein to mammalian clotting and growth factors. Molecular and Cellular Biology 6:3094–3108.

Kim J-S, Crooks H, Dracheva T, Nishanian TG, Singh B, Jen J, Waldman T. 2002. Oncogenic β-catenin is required for bone morphogenic protein 4 expression in human cancer cells. Cancer Research 62:2744–2748.

Kim RH, Peters M, Jang Y, Shi W, Pintilie M, Fletcher GC, DeLuca C, Liepa J, Zhou L, Snow B, Binari RC, Manoukian AS, Bray MR, Liu FF, Tsao MS, Mak TW. 2005. DJ-1, a novel regulator of the tumor suppressor PTEN. Cancer Cell 7:263–273.

Kinzler KW, Bigner SH, Bigner DD, Trent JM, Law ML, O'Brien SJ, Wong AJ, Vogelstein B. 1987. Identification of an amplified, highly expressed gene in a human glioma. Science 236:70–73.

Kinzler KW, Nilbert MC, Su L-K, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Miyoshi Y, Miki Y, Nishisho I, Nakamura Y. 1991. Identification of FAP locus genes from chromosome 5q21. Science 253:661–665.

Knauf JA, Elisei R, Mochly-Rosen D, Liron T, Chen XN, Gonsky R, Korenberg JR, Fagin JA. 1999. Involvement of protein kinase Cε (PKCε) in thyroid cell death. A truncated chimeric PKCε cloned from a thyroid cancer cell line protects thyroid cells from apoptosis. Journal of Biological Chemistry 274:23414–23425.

Kondo S, Shinomura Y, Miyazaki Y, Kiyohara T, Tsutsui S, Kitamura S, Nagasawa Y, Nakahara M, Kanayama S, Matsuzawa Y. 2000. Mutations of the *bak* gene in human gastric and colorectal cancers. Cancer Research 60:4328–4330.

Kondoh N, Noda M, Fisher RJ, Schweinfest CW, Papas TS, Kondoh A, Samuel KP, Oikawa T. 1996. The S29 ribosomal protein increases tumor suppressor activity of Krev-1 gene on v-Kras-transformed NIH3T3 cells. Biochimica et Biophysica Acta 1313:41–46.

Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC. 1995. Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Research 55:4471–4478.

Kristensen VN, Harada N, Yoshimura N, Haraldsen E, Lonning PE, Erikstein B, Karesen R, Kristensen T, Borresen-Dale AL. 2000. Genetic variants of CYP19 (aromatase) and breast cancer risk. Oncogene 19:1329–1333.

Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA. 2000. PAX8-PPARγ1 fusion in oncogene human thyroid cascinoma. Science 289:1357–1360.

Krontiris T, Devlin B, Karp D, Robert NJ, Risch N. 1993. An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. The New England Journal of Medicine 329:517–523.

Krygier S, Djakiew D. 2001. Molecular characterization of the loss of $p75^{NTR}$ expression in human prostate tumor cells. Molecular Carcinogenesis 31:46–55.

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proceedings of the National Academy of Sciences USA 93:5925–5930.

Kuiper RP, Schepens M, Thijssen J, van Asseldonk M, van den Berg E, Bridge J, Schuuring E, Schoenmakers EF, van Kessel AG. 2003. Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. Human Molecular Genetics 12:1661–1669.

Küppers R. 2005. Mechanisms of B-cell lymphoma pathogenesis. Nature Reviews Cancer 5:251–262.

Kyriakis JM, Avruch J. 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiology Reviews 81:807–869.

Lacronique V, Boureux A, Della Valle V, Poirel H, Tran Quang C, Mauchauffe' M, Berthou C, Lessard M, Berger R, Ghysdael J, Bernard OA. 1997. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science 278:1309–1312.

Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S, Winawer S, Offit K, Luce MC, Kinzler KW, Vogelstein B. 1997. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. Nature Genetics 17:79–83.

Lamb J, Ramaswamy S, Ford HL, Contreras B, Martinez RV, Kittrell FS, Zahnow CA, Patterson N, Golub TR, Ewen ME. 2003. A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. Cell 114:323–334.

Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L. 1989. GTPase inhibiting mutations activate the α chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature 340:692–696.

Landowski TH, Qu N, Buyuksal I, Painter JS, Dalton WS. 1997. Mutations in the Fas antigen in patients with multiple myeloma. Blood 90:4266–4270.

Larue L, Dougherty N, Porter S, Mintz B. 1992. Spontaneous malignant transformation of melanocytes explanted from Wf/Wf mice with a Kit kinase-domain mutation. Proceedings of the National Academy of Sciences USA 89:7816–7820.

Lasorella A, Noseda M, Beyna M, Yokota Y, Iavarone A. 2000. Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins. Nature 407:592–598.

Lassus P, Opitz-Araya X, Lazebnik Y. 2002. Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. Science 297:1352–1354.

Lee JH, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF. 2000. A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene 19:4947–4953.

Lee MH, Reynisdottir I, Massague J. 1995. Cloning of $p57^{KIP2}$, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. Genes and Development 9:639–649.

Lee MP, Feinberg AP. 1997. Aberrant splicing but not mutations of TSG101 in human breast cancer. Cancer Research 57:3131–3134.

Lee SB, Huang K, Palmer R, Truong VB, Herzlinger D, Kolquist KA, Wong J, Paulding C, Yoon SK, Gerald W, Oliner JD, Haber DA. 1999a. The Wilms tumor suppressor WT1 encodes a transcriptional activator of amphiregulin. Cell 98:663–673.

Lee SB, Kim SH, Bell DW, Wahrer DC, Schiripo TA, Jorczak MM, Sgroi DC, Garber JE, Li FP, Nichols KE, Varley JM, Godwin AK, Shannon KM, Harlow E, Haber DA. 2001. Destabilization of CHK2 by a missense mutation associated with Li-Fraumeni syndrome. Cancer Research 61:8062–8067.

Lee SH, Shin MS, Park WS, Kim SY, Dong SM, Pi JH, Lee HK, Kim HS, Jang JJ, Kim CS, Kim SH, Lee JY, Yoo NJ. 1999b. Alterations of Fas (APO-1/CD95) gene in transitional cell carcinomas of urinary bladder. Cancer Research 59:3068–3072.

Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. 1994. Cytidine methylation of regulatory sequences near the π-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proceedings of the National Academy of Sciences USA 91:11733–11737.

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947.

Li LY, Luo X, Wang X. 2001. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412:95–99.

Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W. 2003a. Mono-versus polyubiquitination: differential control of p53 fate by Mdm2. Science 302:1972–1975.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2003b. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109:113–124.

Li R, Murray AW. 1991. Feedback control of mitosis in budding yeast. Cell 66:519–531.

Li S, Perlman DM, Peterson MS, Burrichter D, Avdulov S, Polunovsky VA, Bitterman PB. 2004. Translation initiation factor 4E blocks endoplasmic reticulum-mediated apoptosis. Journal of Biological Chemistry 279:21312–21317.

Li Y, Pei J, Xia H, Ke H, Wang H, Tao W. 2003. Lats2, a putative tumor suppressor, inhibits G1/S transition. Oncogene 22:4398–4405.

Li YS, Milner PG, Chauhan AK, Watson MA, Hoffman RM, Kodner CM, Milbrandt J, Deuel TF. 1990. Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. Science 250:1690–1694.

Liang L, Zhao M, Xu Z, Yokoyama KK, Li T. 2003. Molecular cloning and characterization of CIDE-3, a novel member of the cell-death-inducing DNA-fragmentation-factor (DFF45)-like effector family. Biochemical Journal 370:195–203.

Lin B, Chen GQ, Xiao D, Kolluri SK, Cao X, Su H, Zhang XK. 2000a. Orphan receptor COUP-TF is required for induction of retinoic acid receptor β, growth inhibition, and apoptosis by retinoic acid in cancer cells. Molecular and Cellular Biology 20:957–970.

Lin Y, Ma W, Benchimol S. 2000b. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. Nature Genetics 26:124–127.

Liu Y, Lee M-O, Wang H-G, Li Y, Hashimoto Y, Klaus M, Reed JC, Zhang X-K. 1996. Retinoic acid receptor β mediates the grtowth-inhibitory effect of retinoic acid by promoting apoptosis in human breast cacner cells. Molecular and Cellular Biology 16:1138–1149.

Lobanenkov VV, Nicolas RH, Adler VV, Paterson H, Klenova EM, Polotskaja AV, Goodwin GH. 1990. A novel sequence-specific DNA binding protein which interacts with three regularly spaced direct repeats of the CCCTC-motif in the 5′-flanking sequence of the chicken c-myc gene. Oncogene 5:1743–1753.

Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell JE. 1999[4]. Molecular cell biology. New York: W. H. Freeman.

Lohka MJ, Hayes MK, Maller JM. 1988. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. Proceedings of the National Academy of Sciences USA 85:3009–3013.

Look AT. 1997. Oncogenic transcription factors in the human acute leukemias. Science 278:1059–1064.

Ludwig L, Kessler H, Wagner M, Hoang-Vu C, Dralle H, Adler G, Bohm BO, Schmid RM. 2001. Nuclear factor-κB is constitutively active in C-cell carcinoma and required for RET-induced transformation. Cancer Research 61:4526–4535.

Lukas J, Gao DQ, Keshmeshian M, Wen WH, Tsao-Wei D, Rosenberg S, Press MF. 2001. Alternative and aberrant messenger RNA splicing of the mdm2 oncogene in invasive breast cancer. Cancer Research 61:3212–3219.

Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. 2001. Negative control of p53 by Sir2α promotes cell survival under stress. Cell 107:137–148.

Luttrell LM, Lefkowitz RJ. 2002. The role of β-arrestins in the termination and transduction of G-protein-coupled receptor signals. Journal of Cell Science 115:455–465.

Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR, McCormick F. 1990. Two G protein oncogenes in human endocrine tumors. Science 249:655–659.

Ma ZQ, Liu Z, Ngan ES, Tsai SY. 2001. Cdc25B functions as a novel coactivator for the steroid receptors. Molecular and Cellular Biology 21:8056–8067.

Maas S, Patt S, Schrey M, Rich A. 2001. Underediting of glutamate receptor GluR-B mRNA in malignant gliomas. Proceedings of the National Academy of Sciences USA 98:14687–147692.

MacLeod AR, Szyf M. 1995. Expression of antisense to DNA methyltransferase mRNA induces DNA demethylation and inhibits tumorigenesis. The Journal of Biological Chemistry 270:8037–8043.

Maeda T, Hobbs RM, Merghoub T, Guernah I, Zelent A, Cordon-Cardo C, Teruya-Feldstein J, Pandolfi PP. 2005. Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. Nature 433:278–285.

Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J, Lukas J. 2000. Rapid destruction of human Cdc25A in response to DNA damage. Science 288:1425–1429.

Maira SM, Galetic I, Brazil DP, Kaech S, Ingley E, Thelen M, Hemmings BA. 2001. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. Science 294:374–380.

Majewski M, Nieborowska-Skorska M, Salomoni P, Slupianek A, Reiss K, Trotta R, Calabretta B, Skorski T. 1999. Activation of mitochondrial Raf-1 is involved in the antiapoptotic effects of Akt. Cancer Research 59:2815–2819.

Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, Mangelsdorf DJ. 2002. Vitamin D receptor as an intestinal bile acid sensor. Science 296:1313–1316.

Mancini A, Koch A, Wilms R, Tamura T. 2002. c-Cbl associates directly with the C-terminal tail of the receptor for the macrophage colony-stimulating factor, c-Fms, and down-modulates this receptor but not the viral oncogene v-Fms. The Journal of Biological Chemistry 277:14635–14640.

Mangelsdorf DJ, Thummel C, Beato M, Herlich P, Schutz G, Umesono K, Blumbery B, Kastner P, Mark M, Chanson P, Evan RM. 1995. The nuclear receptor super family: the decade. Cell 83:835–839.

Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, Kane LS, Gow AJ, Stamler JS. 1999. Fas-induced caspase denitrosylation. Science 284:651–654.

Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, Bodurtha J, Crowe C, Curtis MA, Dasouki M, Dunn T, Feit H, Geraghty MT, Graham JM jr., Hodgson SV, Hunter A, Korf BR, Manchester D, Miesfeldt S, Murday VA, Nathanson KL, Parisi M, Pober B, Romano C, Eng C, Cancer Research Campaign Human Cancer Genetics Research Group University of Cambridge. 1999. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Human Molecular Genetics 8:1461–1472.

Martin-Zanca D, Hughes SH, Barbacid M. 1986. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. Nature 319:743–748.

Masaki T, Okada M, Tokuda M, Shiratori Y, Hatase O, Shirai M, Nishioka M, Omata M. 1999. Reduced C-terminal Src kinase (Csk) activities in hepatocellular carcinoma. Hepatology 29:379–384.

Masui Y, Markert CL. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. Journal of Experimental Zoology 177:129–145.

Mathas S, Lietz A, Anagnostopoulos I, Hummel F, Wiesner B, Janz M, Jundt F, Hirsch B, Johrens-Leder K, Vornlocher HP, Bommert K, Stein H, Dorken B. 2004. c-FLIP mediates resistance of Hodgkin/Reed-Sternberg cells to death receptor-induced apoptosis. The Journal of Experimental Medicine 199:1041–1052.

Mathur M, Das S, Samuels HH. 2003. PSF-TFE3 oncoprotein in papillary renal cell carcinoma inactivates TFE3 and p53 through cytoplasmic sequestration. Oncogene 22:5031–5044.

Matsumoto T, Jiang J, Kiguchi K, Ruffino L, Carbajal S, Beltran L, Bol DK, Rosenberg MP, DiGiovanni J. 2003. Targeted expression of c-Src in epidermal basal cells leads to enhanced skin tumor promotion, malignant progression, and metastasis. Cancer Research 63:4819–4828.

Matsuoka S, Edwards MC, Bai C, Parker S, Zhang P, Baldini A, Harper JW, Elledge SJ. 1995. $p57^{KIP2}$, a structurally distinct member of the $p21^{CIP1}$ Cdk inhibitor family, is a candidate tumor suppressor gene. Genes and Development 9:650–662.

Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, Friedman SL, Kojima S. 2001. Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma. Cancer Research 61:7675–7682.

Maxwell M, Naber SP, Wolfe HJ, Galanopoulos T, Hedley-Whyte ET, Black PM, Antoniades HN. 1990. Coexpression of platelet-derived growth factor (PDGF) and PDGF-receptor genes by primary human astrocytomas may contribute to their development and maintenance. Journal of Clinical Investigation 86:131–140.

Mayo LD, Donner DB. 2001. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proceedings of the National Academy of Sciences USA 98:11598–11603.

McKee T, McKee JR. 2003. Biochemistry: the molecular basis of life. New York: McGraw-Hill, p. 645.

McPherson JP, Lemmers B, Chahwan R, Pamidi A, Migon E, Matysiak-Zablocki E, Moynahan ME, Essers J, Hanada K, Poonepalli A, Sanchez-Sweatman O, Khokha R, Kanaar R, Jasin M, Hande MP, Hakem R. 2004. Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 304:1822–1826.

Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Sloetjes AW, de Witte T, Waksman G, Korsmeyer SJ. 1998. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. Blood 91:2991–2997.

Melino G, De Laurenzi V, Vousden KH. 2002. p73: friend or foe in tumorigenesis. Nature Reviews Cancer 2:605–615.

Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. 2003. Reduced accumulation of specific microRNAs in colorectal neoplasia. Molecular Cancer Research 1:882–891.

Michaelis C, Ciosk R, Nasmyth K. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91:35–45.

Michalovitz D, Halevy O, Oren M. 1991. p53 mutations – gains or losses. Journal of Cellular Biochemistry 45:22–29.

Midis GP, Shen Y, Owen-Schaub LB. 1996. Elevated soluble Fas (sFas) levels in nonhematopoietic human malignancy. Cancer Research 56:3870–3874.

Milner J, Medcalf EA. 1991. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. Cell 65:765–774.

Mitchison T, Kirschner M. 1984a. Microtubule assembly regulated by isolated centrosomes. Nature 312:232–237.

Mitchison T, Kirschner M. 1984b. Dynamic instability of microtubule growth. Nature 312:237–242.

Miyamoto K, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S, Inoue Y, Morita K, Takeda E, Pike JW. 1997. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. Molecular Endocrinology 11:1165–1179.

Modur V, Nagarajan R, Evers BM, Milbrandt J. 2002. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. The Journal of Biological Chemistry 277:47928–47937.

Morgan R, Hecht BK, Sandberg AA, Hecht F, Smith SD. 1986. Chromosome 5q35 breakpoint in malignant histiocytosis. The New England Journal of Medicine 314:1322.

Morley GM, Uden M, Gullick WJ, Dibb NJ. 1999. Cell specific transformation by c-fms activating loop mutations is attributable to constitutive receptor degradation. Oncogene 18:3076–3084.

Mosselman S, Polman J, Dijkema R. 1996. ERβ: identification and characterization of a novel human estrogen receptor. FEBS Letters 392:49–53.

Moyret-Lalle C, Duriez C, Van Kerchove J, Gilbert C, Wang Q, Puisieux A. 2001. p53 induction prevents accumulation of aberrant transcripts in cancer cells. Cancer Research 61:486–488.

Mukhopadhyay D, Anant S, Lee RM, Kennedy S, Viskochil D, Davidson NO. 2002. C– > U editing of neurofibromatosis 1 mRNA occurs in tumors that express both the type II transcript and apobec-1, the catalytic subunit of the apolipoprotein B mRNA-editing enzyme. American Journal of Human Genetics 70:38–50.

Mumm JS, Schroeter EH, Saxena MT, Griesemer A, Tian X, Pan DJ, Ray WJ, Kopan R. 2000. A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. Molecular Cell 5:197–206.

Murakami MS, Strobel MC, vande Woude GF. 1995. Cell cylce regulation, oncogenes, and antineoplastic drugs. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 3–17.

Muramatsu H, Zou K, Sakaguchi N, Ikematsu S, Sakuma S, Muramatsu T. 2000. LDL receptor-related protein as a component of the midkine receptor. Biochemical and Biophysical Research Communications 270:936–941.

Murray NR, Fields AP. 1997. Atypical protein kinase C ι protects human leukemia cells against drug-induced apoptosis. The Journal of Biological Chemistry 272:27521–27524.

Myers S, Bottolo L, Freeman C, McVean G, Donnelly P. 2005. A fine-scale map of recombination rates and hotspots across the human genome. Science 310:321–324.

Nabors LB, Gillespie GY, Harkins L, King PH. 2001. HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3′ untranslated regions of cytokine and angiogenic factor mRNAs. Cancer Research 61:2154–2161.

Nagakubo D, Taira T, Kitaura H, Ikeda M, Tamai K, Iguchi-Ariga SM, Ariga H. 1997. DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. Biochemical and Biophysical Research Communications 231:509–513.

Nakagawa H, Chadwick RB, Peltomaki P, Plass C, Nakamura Y, de La Chapelle A. 2001. Loss of imprinting of the insulin-like growth factor II gene occurs by biallelic methylation in a core region of H19-associated CTCF-binding sites in colorectal cancer. Proceedings of the National Academy of Sciences USA 98:591–596.

Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J. 2000. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β. Nature 403:98–103.

Nakamura T, Largaespada DA, Lee MP, Johnson LA, Ohyashiki K, Toyama K, Chen SJ, Willman CL, Chen IM, Feinberg AP, Jenkins NA, Copeland NG, Shaughnessy JD jr. 1996. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nature Genetics 12:154–158.

Nasmyth K. 1996. Viewpoint: putting the cell cycle in order. Science 274:1643–1645.

Nigro JM. 1989. Mutations in the p53 gene occur in diverse human tumour types. Nature 342:705–708.

Nikiforova MN, Stringer JR, Bpough R, Medvedovic M, Fagin JA, Nikiforov YE. 2000. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science 290:138–141.

Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, Utsunomiya J, Baba S, Hedge P, Markham A, Krush AJ, Petersen G, Hamilton SR, Nilbert MC, Levy DB, Bryan TM, Preisinger AC, Smith KJ, Su L-K, Kinzler KW, Vogelstein B. 1991. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253: 665–669.

Nister M, Libermann TA, Betsholtz C, Pettersson M, Claesson-Welsh L, Heldin CH, Schlessinger J, Westermark B. 1988. Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor-alpha and their receptors in human malignant glioma cell lines. Cancer Research 48:3910–3918.

Nonet GH, Stampfer MR, Chin K, Gray JW, Collins CC, Yaswen P. 2001. The *ZNF217* gene amplified in breast cancers promotes immortalization of human mammary epithelial cells. Cancer Research 61:1250–1254.

Nourse J, Mellentin JD, Galili N, Wilkinson J, Stanbridge E, Smith SD, Cleary ML. 1990. Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. Cell 60:535–545.

Nurcombe V, Fraser N, Herlaar E, Heath JK. 1992. MK: a pluripotential embryonic stem-cell-derived neuroregulatory factor. Development 116:1175–1183.

Nystrom-Lahti M, Holmberg M, Fidalgo P, Salovaara R, de la Chapelle A, Jiricny J, Peltomaki P. 1999. Missense and nonsense mutations in codon 659 of MLH1 cause aberrant splicing of messenger RNA in HNPCC kindreds. Genes, Chromosomes and Cancer 26:372–375.

Obeid LM, Linardic CM, Karolak LA, Hannun YA. 1993. Programmed cell death induced by ceramide. Science 259:1769–1771.

Okamoto K, Li H, Jensen MR, Zhang T, Taya Y, Thorgeirsson SS, Prives C. 2002. Cyclin G recruits PP2A to dephosphorylate Mdm2. Molecular Cell 9:761–771.

Olefsky JM. 2001. Nuclear receptor minireview series. Minireview prologue. The Journal of Biological Chemistry 276:36863–36864.

Onate SA, Tsai SY, Tsai MJ, O'Malley BW. 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357.

Oro AE, Higgins KM, Hu Z, Bonifas JM, Epstein EH jr., Scott MP. 1997. Basal cell carcinomas in mice overexpressing sonic hedgehog. Science 276:817–821.

Osborne CK, Monaco ME, Kahn CR, Huff K, Bronzert D, Lippman ME. 1979. Direct inhibition of growth and antagonism of insulin action by glucocorticoids in human breast cancer cells in culture. Cancer Research 39:2422–2428.

Paggi MG, Giordano A. 2001. Who is the boss in the retinoblastoma family? The point of view of *Rb2/p130*, the little brother. Cancer Research 61:4651–4654.

Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, Pena C, Peiro S, Puig I, Rodriguez R, de la Fuente R, Bernad A, Pollan M, Bonilla F, Gamallo C, de Herreros AG, Munoz A. 2004. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. Nature Medicine 10:917–919.

Pan G, O'Rourke K, Dixit VM. 1998. Caspase-9, Bcl-X_L, and Apaf-1 form a ternary complex. The Journal of Biological Chemistry 273:5841–5845.

Park WS, Dong SM, Kim SY, Na EY, Shin MS, Pi JH, Kim BJ, Bae JH, Hong YK, Lee KS, Lee SH, Yoo NJ, Jang JJ, Pack S, Zhuang Z, Schmidt L, Zbar B, Lee JY. 1999. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. Cancer Research 59:307–310.

Pasche B, Kolachana P, Nafa K, Satagopan J, Chen YG, Lo RS, Brener D, Yang D, Kirstein L, Oddoux C, Ostrer H, Vineis P, Varesco L, Jhanwar S, Luzzatto L, Massague J, Offit K. 1999. *TβR-I(6A)* is a candidate tumor susceptibility allele. Cancer Research 59:5678–5682.

Pedone PV, Tirabosco R, Cavazzana AO, Ungaro P, Basso G, Luksch R, Carli M, Bruni CB, Frunzio R, Riccio A. 1994. Mono- and bi-allelic expression of insulin-like growth factor II gene in human muscle tumors. Human Molecular Genetics 3:1117–1121.

Pekarsky Y, Hallas C, Croce CM. 2001. Molecular basis of mature T-cell leukemia. The Journal of the American Medical Association 286:2308–2314.

Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM, Mazoyer S. 2002. The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. Human Molecular Genetics 11:2805–2814.

Petit MM, Schoenmakers EF, Huysmans C, Geurts JM, Mandahl N, Van de Ven WJ. 1999. LHFP, a novel translocation partner gene of HMGIC in a lipoma, is a member of a new family of LHFP-like genes. Genomics 57:438–441.

Piao X, Bernstein A. 1996. A point mutation in the catalytic domain of c-kit induces growth factor independence, tumorigenicity, and differentiation of mast cells. Blood 87:3117–3123.

Pitti RM, Marsters SA, Lawrence DA, Roy M, Kischkel FC, Dowd P, Huang A, Donahue CJ, Sherwood SW, Baldwin DT, Godowski PJ, Wood WI, Gurney AL, Hillan KJ, Cohen RL, Goddard AD, Botstein D, Ashkenazi A. 1998. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature 396:699–703.

Planas-Silva MD, Shang Y, Donaher JL, Brown M, Weinberg RA. 2001. AIB1 enhances estrogen-dependent induction of cyclin D1 expression. Cancer Research 61:3858–3862.

Powers CJ, McLeskey SW, Wellstein A. 2000. Fibroblast growth factors, their receptors and signaling. Endocrine-Related Cancer 7:165–197.

Powzaniuk M, McElwee-Witmer S, Vogel RL, Hayami T, Rutledge SJ, Chen F, Harada S, Schmidt A, Rodan GA, Freedman LP, Bai C. 2004. The LATS2/KPM tumor suppressor is a negative regulator of the androgen receptor. Molecular Endocrinology 18:2011–2023.

Prasad KV, Ao Z, Yoon Y, Wu MX, Rizk M, Jacquot S, Schlossman SF. 1997. CD27, a member of the tumor necrosis factor receptor family, induces apoptosis and binds to Siva, a proapoptotic protein. Proceedings of the National Academy of Sciences USA 94:6346–6351.

Prives C, Manley JL. 2001. Why is p53 acetylated? Cell 107:815–818.

Qureshi SA, Alexandropoulos K, Rim M, Joseph CK, Bruder JT, Rapp UR, Foster DA. 1992. Evidence that Ha-Ras mediates two distinguishable intracellular signals activated by v-Src. The Journal of Biological Chemistry 267:17635–17639.

Radtke F, Raj K. 2003. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nature Reviews Cancer 3:756–767.

Ram PT, Horvath CM, Iyengar R. 2000. Stat3-Mediated Transformation of NIH-3T3 Cells by the Constitutively Active Q205L G_o Protein. Science 287:142–144.

Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. 1997. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 275:967–969.

Ravi R, Bedi A. 2002. Sensitization of tumor cells to Apo2 ligand/TRAIL-induced apoptosis by inhibition of casein kinase II. Cancer Research 62:4180–4185.

Reeve AE, Eccles MR, Wilkins RJ, Bell GI, Millow LJ. 1985. Expression of insulin-like growth factor-II transcripts in Wilms' tumour. Nature 317:258–260.

Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. 1990. FMS mutations in myelodysplastic, leukemic, and normal subjects. Proceedings of the National Academy of Sciences USA 87:1377–1380.

Riegel AT, Wellstein A. 1994. The potential role of the heparin-binding growth factor pleiotrophin in breast cancer. Breast Cancer Research and Treatment 31:309–314.

Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzalez FA, Jones PA. 1999. The human DNA methyltransferases (DNMTs) 1, 3a, and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Research 27:2291–2298.

Romashkova JA, Makarov SS. 1999. NF-κB is a target of AKT in anti-apoptotic PDGF signalling. Nature 401:86–90.

Roose J, Huls G, van Beest M, Moerer P, van der Horn K, Goldschmeding R, Logtenberg T, Clevers H. 1999. Synergy between tumor suppressor APC and the β-catenin-Tcf4 target Tcf1. Science 285:1923–1926.

Roth W, Isenmann S, Nakamura M, Platten M, Wick W, Kleihues P, Bahr M, Ohgaki H, Ashkenazi A, Weller M. 2001. Soluble decoy receptor 3 is expressed by malignant gliomas and suppresses CD95 ligand-induced apoptosis and chemotaxis. Cancer Research 61:2759–2765.

Rouleau J, MacLeod AR, Szyf M. 1995. Regulation of the DNA methyltransferase by the Ras-AP-1 signaling pathway. The Journal of Biological Chemistry 270:1595–1601.

Roussel MF, Downing JR, Rettenmier CW, Sherr CJ. 1988. A point mutation in the extracellular domain of the human CSF-1 receptor (c-fms proto-oncogene product) activates its transforming potential. Cell 55:979–988.

Ruiz-Carrillo A, Renaud J. 1987. Endonuclease G: a (dG)n X (dC)n-specific DNase from higher eukaryotes. EMBO Journal 6:401–407.

Rutter JL, Goldstein AM, Davila MR, Tucker MA, Struewing JP. 2003. CDKN2A point mutations D153spl(c.457G > T) and IVS2 + 1G > T result in aberrant splice products affecting both $p16^{INK4a}$ and $p14^{ARF}$. Oncogene 22:4444–4448.

Sadot E, Geiger B, Oren M, Ben-Ze'ev A. 2001. Down-regulation of β-catenin by activated p53. Molecular and Cellular Biology 21:6768–6781.

Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E. 1998. DNA damage activates p53 through a phosphorylation-acetylation cascade. Genes and Development 12:2831–2841.

Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. 2004. High frequency of mutations of the PIK3CA gene in human cancers. Science 304:554.

Samuels-Lev Y, O'Connor DJ, Bergamaschi D, Trigiante G, Hsieh JK, Zhong S, Campargue I, Naumovski L, Crook T, Lu X. 2001. ASPP proteins specifically stimulate the apoptotic function of p53. Molecular Cell 8:781–794.

Satyamoorthy K, Li G, Vaidya B, Patel D, Herlyn M. 2001. Insulin-like growth factor-1 induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and beta-catenin pathways. Cancer Research 61:7318–7324.

Schechtman D, Mochly-Rosen D. 2001. Adaptor proteins in protein kinase C-mediated signal transduction. Oncogene 20:6339–6347.

Schindler C, Fu X-Y, Improta T, Aebersold R, Darnell JE Jr. 1992. Proteins of transcription factor ISGF-3: one gene encodes the 91 and 84 kDa ISGF-3 proteins that are activated by inteferon-α. Proceedings of the National Academy of Sciences USA 89:7836–7839.

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Zbar B. 1997. Germline and somatic mutations in the tyrosine kinase domain of the *MET* proto-oncogene in papillary renal carcinomas. Nature Genetics 16:68–73.

Schmitt JM, Stork PJS. 2001. Cyclic AMP-mediated inhibition of cell growth requires the small G protein Rap1. Molecular and Cellular Biology 21:3671–3683.

Scholl SM, Crocker P, Tang R, Pouillart P, Pollard JW. 1993. Is colony-stimulating factor-1 a key mediator of breast cancer invasion and metastasis? Molecular Carcinogenesis 7:207–211.

Schonhoff CM, Gaston B, Mannick JB. 2003. Nitrosylation of cytochrome c during apoptosis. The Journal of Biological Chemistry 278:18265–18270.

Schutze S, Potthoff K, Machleidt T, Berkovic D, Wiegmann K, Kronke M. 1992. TNF activates NF-κB by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell 71:765–776.

Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. 2003. BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. Science 300:135–139.

Scott J, Cowell J, Robertson ME, Priestley LM, Wadey R, Hopkins B, Pritchard J, Bell GI, Rall LB, Graham CF, Knott TJ. 1985. Insulin-like growth factor-II gene expression in Wilms' tumour and embryonic tissues. Nature 317:260–262.

Sears R, Leone G, DeGregori J, Nevins JR. 1999. Ras enhances Myc protein stability. Molecular Cell 3:169–179.

Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM. 1999. Regulation of β-catenin signaling by the B56 subunit of protein phosphatase 2A. Science 283:2089–2091.

Sehouli J, Mustea A, Konsgen D, Katsares I, Lichtenegger W. 2002. Polymorphism of IL-1 receptor antagonist gene: role in cancer. Anticancer Research 22:3421–3424.

Shankar DB, Sakamoto KM. 2004. The role of cyclic-AMP binding protein (CREB) in leukemia cell proliferation and acute leukemias. Leukemia and Lymphoma 45:265–270.

Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA, Cantley LC. 2004. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell 6:91–99.

Sherbet GV, Lakshmi MS. 1997. The genetics of cancer: genes associated with cancer invasion, metastasis and cell proliferation. San Diego/London/Boston/New York/Sydney/Tokyo/Toronto: Academic Press.

Shikama N, Lee CW, France S, Delavaine L, Lyon J, Krstic-Demonacos M, La Thangue NB. 1999. A novel cofactor for p300 that regulates the p53 response. Molecular Cell 4:365–376.

Shin MS, Kim HS, Lee SH, Park WS, Kim SY, Park JY, Lee JH, Lee SK, Lee SN, Jung SS, Han JY, Kim H, Lee JY, Yoo NJ. 2001. Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. Cancer Research 61:4942–4946.

Shridhar V, Rivard S, Shridhar R, Mullins C, Bostick L, Sakr W, Grignon D, Miller OJ, Smith DI. 1996. A gene from human chromosomal band 3p21.1 encodes a highly conserved arginine-rich protein and is mutated in renal cell carcinomas. Oncogene 12:1931–1939

Shridhar R, Shridhar V, Rivard S, Siegfried JM, Pietraszkiewicz H, Ensley J, Pauley R, Grignon D, Sakr W, Miller OJ, Smith DI. 1997a. Mutations in the arginine-rich protein gene, in lung, breast, and prostate cancers, and in squamous cell carcinoma of the head and neck. Cancer Research 56:5576–5578

Shridhar V, Rivard S, Wang X, Shridhar R, Paisley C, Mullins C, Beirnat L, Dugan M, Sarkar F, Miller OJ, Vaitkevicius VK, Smith DI. 1997b. Mutations in the arginine-rich protein gene (ARP) in pancreatic cancer. Oncogene 14:2213–2216

Sidhar SK, Clark J, Gill S, Hamoudi R, Crew AJ, Gwilliam R, Ross M, Linehan WM, Birdsall S, Shipley J, Cooper CS. 1996. The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene PRCC to the TFE3 transcription factor gene. Human Molecular Genetics 5:1333–1338.

Siegelmann-Danieli N, Buetow KH. 1999. Constitutional genetic variation at the human aromatase gene (Cyp19) and breast cancer risk. British Journal of Cancer 79:456–463.

Sigalas I, Calvert AH, Anderson JJ, Neal DE, Lunec J. 1996. Alternatively spliced mdm2 transcripts with loss of p53

binding domain sequences: transforming ability and frequent detection in human cancer. Nature Medicine 2:912–917.

Simon M-P, Pedeutour F, Sirvent N, Grosgeorge J, Minoletti F, Coindre J.-M, Terrier-Lacombe M-J, Mandahl N, Craver RD, Blin N, Sozzi G, Turc-Carel C, O'Brien KP, Kedra D, Fransson I, Guilbaud C, Dumanski JP. 1997. Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. Nature Genetics 15:95–98.

Skinnider BF, Kapp U, Mak TW. 2002. The role of interleukin 13 in classical Hodgkin lymphoma. Leukemia and Lymphoma 43:1203–1210.

Smidt M, Kirsch I, Ratner L. 1990. Deletion of Alu sequences in the fifth c-sis intron in individuals with meningiomas. The Journal of Clinical Investigations 86:1151–1157.

Smith LT, Lin M, Brena RM, Lang JC, Schuller DE, Otterson GA, Morrison CD, Smiraglia DJ, Plass C. 2006. Epigenetic regulation of the tumor suppressor gene *TCF21* on 6q23-q24 in lung and head and neck cancer. Proceedings of the National Academy of Sciences USA 103:982–987.

Soengas MS, Alarcon RM, Yoshida H, Giaccia AJ, Hakem R, Mak TW, Lowe SW. 1999. Apaf-1 and Caspase-9 in p53-dependent apoptosis and tumor inhibition. Science 284:156–159.

Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW. 2001. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature 409:207–211.

Sozzi G, Bongarzone I, Miozzo M, Borrello MG, Blutti MG, Pilotti S, Della Porta G, Pierotti MA. 1994. A t(10;17) translocation creates the RET/PTC2 chimeric transforming sequence in papillary thyroid carcinoma. Genes, Chromosomes and Cancer 9:244–250.

Steiner DF, Oyer PE. 1967. The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proceedings of the National Academy of Sciences USA 57:473–480.

Stella A, Wagner A, Shito K, Lipkin SM, Watson P, Guanti G, Lynch HT, Fodde R, Liu B. 2001. A nonsense mutation in MLH1 causes exon skipping in three unrelated HNPCC families. Cancer Research 61:7020–7024.

Strand S, Hofmann WJ, Hug H, Müller M, Otto G, Strand D, Mariani SM, Stremmel W, Krammer PH, Galle PR. 1996. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumore cells – a mechanism of immune evasion? Nature Medicine 2:1361–1366.

Strobeck MW, Knudsen KE, Fribourg AF, DeCristofaro MF, Weissman BE, Imbalzano AN, Knudsen ES. 2000. BRG-1 is required for RB-mediated cell cycle arrest. Proceedings of the National Academy of Sciences USA 97:7748–7753.

Sun Z, Pan J, Bubbley G, Balk SP. 1997. Frequent abnormalities of TSG101 transcripts in human prostate cancer. Oncogene 15:3121–3125.

Suzuki H, Itoh F, Toyota M, Kikuchi T, Kakiuchi H, Imai K. 2000. Inactivation of the 14-3-3σ gene is associated with 5′ CpG island hypermethylation in human cancers. Cancer Research 60:4353–4357.

Takahashi M, Fujita M, Furukawa Y, Hamamoto R, Shimokawa T, Miwa N, Ogawa M, Nakamura Y. 2002. Isolation of a novel human gene, APCDD1, as a direct target of the β-Catenin/T-cell factor 4 complex with probable involvement in colorectal carcinogenesis. Cancer Research 62:5651–5656.

Takahashi R, Deveraux Q, Tamm I, Welsh K, Assa-Munt N, Salvesen GS, Reed JC. 1998. A single BIR domain of XIAP sufficient for inhibiting caspases. The Journal of Biological Chemistry 273:7787–7790.

Takakuwa T, Dong Z, Takayama H, Matsuzaka F, Nagata S, Aozasa K. 2001. Frequent mutations of *Fas* gene in thyroid lymphoma. Cancer Research 61:1382–1385.

Takeo S, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, Oga A, Hirano T, Yoshida T, Okita K, Sasaki K. 2001. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridisation analysis. Cancer Genetics and Cytogenetics 130:127–132.

Talamonti MS, Roh MS, Curley SA, Gallick GE. 1993. Increase in activity and level of $pp60^{c\text{-}src}$ in progressive stages of human colorectal cancer. Journal of Clinical Investigation 91:53–60.

Tambe Y, Isono T, Haraguchi S, Yoshioka-Yamashita A, Yutsudo M, Inoue H. 2004. A novel apoptotic pathway induced by the drs tumor suppressor gene. Oncogene 23:2977–2987.

Tang Q, Gonzales M, Inoue H, Bowden GT. 2001. Roles of Akt and glycogen synthase kinase 3β in the ultraviolet B induction of cyclooxygenase-2 transcription in human keratinocytes. Cancer Research 61:4329–4332.

Tao W, Zhang S, Turenchalk GS, Stewart RA, St John MA, Chen W, Xu T. 1999. Human homologue of the Drosophila melanogaster lats tumour suppressor modulates CDC2 activity. Nature Genetics 21:177–181.

Tassan JP, Jaquenoud M, Fry AM, Frutiger S, Hughes GJ, Nigg EA. 1995. In vitro assembly of a functional human CDK7-cyclin H complex requires MAT1, a novel 36 kDa RING finger protein. EMBO Journal 14:5608–5617.

Tevosian SG, Shih HH, Mendelson KG, Sheppard KA, Paulson KE, Yee AS. 1997. HBP1: a HMG box transcriptional repressor that is targeted by the retinoblastoma family. Genes and Development 11:383–396.

Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, Dunson DB, Rogan EG, Morham SG, Smart RC, Langenbach R. 2002. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Research 62:3395–3401.

Tinel A, Tschopp J. 2004. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 304:843–846.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM. 2005. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310:644–648.

Trasler J, Deng L, Melnyk S, Pogribny I, Hiou-Tim F, Sibani S, Oakes C, Li E, James SJ, Rozen R. 2003. Impact of Dnmt1 deficiency, with and without low folate diets, on tumor numbers and DNA methylation in Min mice. Carcinogenesis 24:39–45.

Trusolino L, Comoglio PM. 2002. Scatter-Factor and senaphosin receptors: cell signalling for invasive growth. Nature Reviews Cancer 2:289–300.

Tuck AB, Park M, Sterns EE, Boag A, Elliott BE. 1996. Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. American Journal of Pathology 148:225–232.

Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. 1997. Expression of hepatocyte growth factor and its receptor, the

c-Met proto-oncogene, in hepatocellular carcinoma. Hepatology 25:619–623.

Ugurel S, Rappl G, Tilgen W, Reinhold U. 2001. Increased soluble CD95 (sFas/CD95) serum level correlates with poor prognosis in melanoma patients. Clinical Cancer Research 7:1282–1286.

Ulbricht U, Brockmann MA, Aigner A, Eckerich C, Muller S, Fillbrandt R, Westphal M, Lamszus K. 2003. Expression and function of the receptor protein tyrosine phosphatase ζ and its ligand pleiotrophin in human astrocytomas. Journal of Neuropathology and Experimental Neurology 62:1265–1275.

Ullrich A, Schlessinger J. 1990. Signal transduction by receptors with tyrosine kinase activity. Cell 61:203–212.

Valette A, Gas N, Jozan S, Roubinet F, Dupont M, Bayard F. 1987. Influence of 12-O-tetradecanoylphorbol-13-acetate on proliferation and maturation of human breast carcinoma cells (MCF-7): relationship to cell cycle events. Cancer Research 47:1615–1620.

van der Plas DC, Soekarman D, van Gent AM, Grosveld G, Hagemeijer A. 1991. bcr-abl mRNA lacking abl exon a2 detected by polymerase chain reaction in a chronic myelogeneous leukemia patient. Leukemia 5:457–461.

van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, Burley M, Jeremiah S, Woodward K, Nahmias J, Fox M, Ekong R, Osborne J, Wolfe J, Povey S, Snell RG, Cheadle JP, Jones AC, Tachataki M, Ravine D, Kwiatkowski DJ. 1997. Identification of the tuberous sclerosis gene *TSC1* on chromosome 9q34. Science 277:805–808.

Varticovski L, Daley GQ, Jackson P, Baltimore D, Cantley LC. 1991. Activation of phosphatidylinositol 3-kinase in cells expressing abl oncogene variants. Molecular and Cellular Biology 11:1107–1113.

Vaux DL, Cory S, Adams JM. 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335:440–442.

Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA. 2001. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 107:149–159.

Villunger A, Michalak EW, Coultas L, Muellauer F, Boeck G, Ausserlechner MJ, Adams JM, Strasser A. 2003. p53- and drug-induced apoptotic responses mediated by BH3-only proteins Puma and Noxa. Science 302:1036–1038.

Vivanco I, Sawyers CL. 2002. The Phosphatidylinositol 3-kinase-Akt pathway in human cancer. Nature Reviews Cancer 2:489–501.

Voegel JJ, Heine MJ, Zechel C, Chambon P, Gronemeyer H. 1996. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO Journal 15:3667–3675.

Vogt PK, Bos TJ, Doolittle RF. 1987. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene jun. Proceedings of the National Academy of Sciences USA 84:3316–3319.

von Recklinghausen F. 1882. Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen. Berlin (August Hirschwald).

Vucic D, Stennicke HR, Pisabarro MT, Salvesen GS, Dixit VM. 2000. ML-IAP, a novel inhibitor of apoptosis that is preferentially expressed in human melanomas. Current Biology 10:1359–1366.

Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, van der Heijden MS, Parmigiani G, Yan H, Wang TL, Riggins G, Powell SM, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. 2004. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science 304:1164–1166.

Wei G, Li AG, Liu X. 2005. Insights into selective activation of p53 DNA binding by c-Abl. Journal of Biological Chemistry 280:12271–12278.

Weinberg RA. 1996. How cancer arises. Scientific American 275:62–70.

Weinert T. 1997. A DNA damage checkpoint meets the cell cycle engine. Science 277:1450–1451.

Weinstein IB, Carothers AM, Santella RM, Perera FP. 1995. Molecular mechansims of mutagenesis and multistage carcinogenesis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 59–85.

Weisenberg RC, Borisy GG, Taylor EW. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. Biochemistry 7:4466–4479.

Wellstein A, Fang WJ, Khatri A, Lu Y, Swain SS, Dickson RB, Sasse J, Riegel AT, Lippman ME. 1992. A heparin-binding growth factor secreted from breast cancer cells homologous to a developmentally regulated cytokine. The Journal of Biological Chemistry 267:2582–2587.

Weng L-P, Yuan J, Yu Q. 1998. Overexpression of the transmembrane tyrosine phosphatase LAR activates the caspase pathway and induces apoptosis. Current Biology 8:247–256.

Wertz IE, O'Rourke KM, Zhang Z, Dornan D, Arnott D, Deshaies RJ, Dixit VM. 2004. Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. Science 303:1371–1374.

Weston CR, Davis RJ. 2001. Signaling specificity – a complex affair. Science 292:2439–2440.

Weterman MAJ, van Groningen JJM, den Hartog A, van Kessel AG. 2001. Transformation capacities of the papillary renal cell carcinoma-associated PRCCTFE3 and TFE3PRCC fusion genes. Oncogene 20:1414–1424.

Weterman MJ, van Groningen JJM, Jansen A, van Kessel AG. 2000. Nuclear localization and transactivating capacities of the papillary renal cell carcinoma-associated TFE3 and PRCC (fusion) proteins. Oncogene 19:69–74.

Wharton KA, Johansen KM, Xu T, Artavanis-Tsakonas S. 1985. Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats. Cell 43:567–581.

White MF. 2003. Insulin signaling in health and disease. Science 302:1710–1711.

Whitmarsh AJ, Davis RJ. 2000. A central control for cell growth. Nature 403:255–256.

Wiedemann H-R. 1964. Complexe malformatif familial avec hernie ombilicale et macroglossie – un 'syndrome nouveau'? Journal de Genetique Humaine 13:223–232.

Wischhusen J, Jung G, Radovanovic I, Beier C, Steinbach JP, Rimner A, Huang H, Schulz JB, Ohgaki H, Aguzzi A, Rammensee HG, Weller M. 2002. Identification of CD70-mediated apoptosis of immune effector cells as a novel immune escape pathway of human glioblastoma. Cancer Research 62:2592–2599.

Wong JC, Lee SB, Bell MD, Reynolds PA, Fiore E, Stamenkovic I, Truong V, Oliner JD, Gerald WL, Haber DA. 2002. Induction of the interleukin-2/15 receptor β-chain by the EWS-WT1 translocation product. Oncogene 21:2009–2019.

Wu K-J, Polack A, Dalla-Favers R. 1999. Coordinated regulation of iron-controlling genes, H-ferritin and *IRP2*, by c-MYC. Science 283:676–679.

Wyss R, Fabbro D, Regazzi R, Borner C, Takahashi A, Eppenberger U. 1987. Phorbol ester and epidermal growth factor receptors in human breast cancer. Anticancer Research 7:721–727.

Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, Rosenthal A, Epstein EH jr., de Sauvage FJ. 1998. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature 391:90–92.

Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. 1993. p21 is a universal inhibitor of cyclin kinases. Nature 366:701–704.

Xu B, Kim S-T, Lim D-S, Kastan MB. 2002. Two molecularly distinct G_2/M checkpoints are induced by ionizing radiation. Molecular and Cellular Biology 22:1049–1059.

Xu FJ, Ramakrishnan S, Daly L, Soper JT, Berchuck A, Clarke-Pearson D, Bast RC jr. 1991. Increased serum levels of macrophage colony-stimulating factor in ovarian cancer. American Journal for Obstetrics and Gynecology 165:1356–1362.

Xu N, Voyno-Yasenetskaya T, Gutkind JS. 1994. Potent transforming activity of the G_{13} α subunit defines a novel family of oncogenes. Biochemical and Biophysical Research Communications 201:603–609.

Xu N, Bradley L, Ambdukar I, Gutkind JS. 1993. A mutant α subunit of G_{12} potentiates the eicosanoid pathway and is highly oncogenic in NIH 3T3 cells. Proceedings of the National Academy of Sciences USA 90:6741–6745.

Xu XC, Wong WY, Goldberg L, Baer SC, Wolf JE, Ramsdell WM, Alberts DS, Lippman SM, Lotan R. 2001. Progressive decreases in nuclear retinoid receptors during skin squamous carcinogenesis. Cancer Research 61:4306–4310.

Yang G, Niendorf KB, Tsao H. 2004. A novel methionine-53-valine mutation of p16 in a hereditary melanoma kindred. Journal of Investigative Dermatology 123:574–575.

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. 1997. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 275:1129–1132.

Yang X, Li DM, Chen W, Xu T. 2001. Human homologue of Drosophila lats, LATS1, negatively regulate growth by inducing G_2/M arrest or apoptosis. Oncogene 20:6516–6523.

Yang X, Yu K, Hao Y, Li DM, Stewart R, Insogna KL, Xu T. 2004. LATS1 tumour suppressor affects cytokinesis by inhibiting LIMK1. Nature Cell Biology 6:609–617.

Yeung RS, Bell DW, Testa JR, Mayol X, Baldi A, Grana X, Klinga-Levan K, Knudson AG, Giordano A. 1993. The retinoblastoma-related gene, RB2, maps to human chromosome 16q12 and rat chromosome 19. Oncogene 8:3465–3468.

Yin Y, Liu Y-X, Jin YJ, Hall EJ, Barrett JC. 2003. PAC1 phosphatase is a transcription target of p53 in signaling apoptosis and growth suppression. Nature 422:527–531.

York JD, Odom AR, Murphy R, Ives EB, Wente SR. 1999. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. Science 285:96–100.

Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. 2001. PUMA induces the rapid apoptosis of colorectal cancer cells. Molecular Cell 7:673–682.

Yu H, Jove R. 2004. The STATs of cancer–new moleculars targets come of age. Nature Reviews Cancer 4:97–105.

Yuan J, Shaham S, Ledoux S, Ellis H, Horvitz H. 1993. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 β-converting enzyme. Cell 75:641–652.

Zahnow CA. 2002. CCAAT/enhancer binding proteins in normal mammary development and breast cancer. Breast Cancer Research 4:113–121.

Zaidi SK, Sullivan AJ, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2002. Integration of Runx and Smad regulatory signals at transcriptionally active subnuclear sites. Proceedings of the National Academy of Sciences USA 99:8048–8053.

Zhang QX, Borg A, Wolf DM, Oesterreich S, Fuqua SA. 1997. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. Cancer Research 57:1244–1249.

Zhang T, Otevrel T, Gao Z, Gao Z, Ehrlich SM, Fields JZ, Boman BM. 2001. Evidence that APC regulates survivin expression: a possible mechanism contributing to the stem cell origin of colon cancer. Cancer Research 62:8664–8667.

Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. 1999. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature 400:687–693.

Zhu Y, Bond J, Thomas P. 2003a. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proceedings of the National Academy of Sciences USA 100:2237–2242.

Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. 2003b. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proceedings of the National Academy of Sciences USA 100:2231–2236.

Ziv E, Cauley J, Morin PA, Saiz R, Browner WS. 2001. Association between the T29 C polymorphism in the transforming growth factor β1 gene and breast cancer among elderly white women. The study of osteoporotic fractures. The Journal of the American Medical Association 285:2859–2863.

Zucman J, Delattre O, Desmaze C, Epstein AL, Stenman G, Speleman F, Fletchers CD, Aurias A, Thomas G. 1993. EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nature Genetics 4:341–345.

Zwijsen RM, Wientjens E, Klompmaker R, van der Sman J, Bernards R, Michalides RJ. 1997. CDK-independent activation of estrogen receptor by cyclin D1. Cell 88:405–415.

CHAPTER 4

CELLULAR SENESCENCE

Tumors contain cells that continue to divide beyond normal limits. This is in contrast to most somatic cells, which arrest their cell cycle or die after a finite number of cell divisions, a phenomenon described as senescence. Among the exceptions are certain stem cells that do not senesce throughout an individual's life span. Germline cells, however, have not aged during the existence of mankind.

Cellular senescence is genetically dominantly controlled by senescence genes [Bringold and Serrano 2000; Campisi 1997, 2000], which act in part by inducing cell cycle arrest. Hence, many tumor suppressor genes also act as senescence genes. This has led to the hypothesis that cellular senescence evolved as a defense against cancer. Cells can respond to carcinogenic stimuli by entering a senescent state. Cellular senescence thereby consigns cells to mortality in order to protect them against transformation.

The phenotype of replicative senescence is characterized by an altered metabolism, irreversible arrest of cell proliferation, and frequently increased resistance to apoptotic stimuli. Senescent cells undergo a number of morphological changes, such as increase in cell size, increase in nuclear and nucleolar size, loss of the ability to utilize all the available growth surface, and appearance of multinucleated cells. Senescent cells exhibit changes in the expression of proteins that regulate the cell cycle. Typically, they fail to express CDC2, Cyclin A, and c-FOS in response to mitogenic stimulation. The expression of PAI-1 and β-Galactosidase activity are markers of senescence in various cell types. The expression of *β-galactosidase* is not differentially regulated during senescence. An increase in lysosomal mass may account for the increase in β-Galactosidase activity in senescence.

– Senescent prostate epithelial cells display increased expression of *mmp-14*, *cathepsin B*, *fibronectin-1*, and *integrin* β_4, whereas the pro-apoptotic gene *bad* is downregulated. In contrast, *e2f4* is expressed at elevated levels in immortalized prostate epithelial cells [Untergasser et al. 2002].
– In fibroblasts, close to 50% of genes specifically upregulated in senescence encode membrane associated proteins, 10% relate to apoptosis, and 16% to transport. Furthermore, Stromelysin-1 and Stromelysin-2, PAI-1 and PAI-2, Urokinase Plasminogen Activator (UPA), as well as the inflammatory regulators MCP-1 (Monocyte Chemotactic Protein-1), GRO-α, and Interleukin-15 (IL-15) are increased. The proteins encoded by 60% of the downregulated genes represent nuclear proteins, 18% are involved in cell cycle regulation, and 21% are involved in transcription. Senescent fibroblasts significantly underexpress Prostaglandin-1 Synthase, Elastin, and Stromelysin-3 [Shelton et al. 1999; Zhang et al. 2003].
– Keratinocytes undergoing replicative senescence predominantly express P16. Their resistance to apoptosis is mediated, in part, by NF-κB. Immortalized keratinocytes have undetectable P16 due to hypermethylation of the gene promoter, dysfunctional NF-κB, and a diminished capacity to respond to antiproliferative agents. They remain sensitive to apoptosis through pathways that involve the cleavage of Caspases-3 and -8 [Chaturvedi et al. 1999].

Mitotic time. There is a finite number of possible population doublings by nontransformed differentiated cells, the so-called Hayflick limit [Hayflick and Moorehead 1961; Hayflick 1965]. Fibroblasts can undergo 60–80 population doublings before ceasing division and developing a senescent phenotype. In activated $CD8^+$ T-lymphocytes, senescence is reached after about 23 population doublings [Perillo et al. 1989]. Per cell division, 30–200 nucleotides of telomeric sequence are lost from the 5′ end of the chromosomes. The progressive shortening of the chromosomal telomeres with every cell division may function as a mitotic clock, which leads to cell senescence. Replicative senescence can be linked to telomere shortening [Harley et al. 1990] if the latter is perceived by the cells as a form of DNA damage, or if critically short telomeres signal cell cycle arrest. The loss of about 100 base pairs per population doubling results in senescence after around 50 population doublings [Karlseder et al. 2002]. The average telomere length in lymphocytes reduces about 33 bp per year [Hastie et al. 1990]. In contrast, immortal cell lines and germline cells have stable chromosome ends and express Telomerase, thus overcoming senescence.

Metabolic time. On the level of the whole organism, the metabolic rate is inversely related to the average life span [Adelman et al. 1988]. In general, senescence associated loss of functional capacity is due to the accumulation of molecular oxidative damage caused by reactive oxygen intermediates, which escape elimination from the cell by protective enzyme systems, such as Superoxide Dismutase and Catalase [Harman 1956, 1987]. Oxidative stress and damage in every tissue increase with aging. Hyperoxia shortens the replicative life span, while low oxygen tension gives rise to extended replicative life span [von Zglinicki et al. 1995; Chen et al. 1995]. These reactions cause, among other changes, the peroxidation of membrane polyunsaturated fatty acid chains, various modifications of DNA, carbonylation, and loss of sulfhydryl groups in proteins. The rate of telomere shortening may be affected, in addition to the history of population doublings, by single-strand breaks derived from oxidative stress [Sitte et al. 1998]. Therefore, the activity of antioxidant enzymes may extend the life span.

The overexpression of *thioredoxin* {9q31} leads to life span extension. Thioredoxin (TRX, TXN) is a 12 kD protein with a redox active disulfide group within the conserved active site sequence CGPC (cysteine–glycine–proline–cysteine). Reduced Thioredoxin catalyzes the reduction of disulfide bonds in many proteins, while oxidized Thioredoxin is reversibly reduced by the action of Thioredoxin Reductase and NADPH. Thioredoxin-2 {22} is uniquely expressed in mitochondria, where it regulates the mitochondrial redox state and plays an important role in cell proliferation. Thioredoxin-2 binds to Cytochrome *c* and may prevent its release from the mitochondria in response to apoptotic stimuli. Methionine Sulfoxide Reductase A (MSRA) catalyzes the reduction of oxidized methionine in proteins by converting methionine sulfoxide to methionine. This enzyme reaction is completely dependent on the Thioredoxin redox system. Thioredoxin can serve as an electron donor in order to reduce the oxidized form of MSRA, therefore, the effective reaction of MSRA is closely associated with Thioredoxin, Thioredoxin Reductase, and NADPH. A lack of MSRA causes a reduced life span and high sensitivity towards oxidative stress. The mitochondria are a constant source of reactive oxygen intermediates, which can limit the cellular life span. Superoxide Dismutases constitute antioxidant enzymes that convert superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen. The mitochondrial form, Manganese Superoxide Dismutase (Mn-SOD) {6q25} is a homotetramer, which contains one manganese ion per subunit. It is synthesized in the cytosol as a precursor molecule and is transported to the mitochondria. The cellular content of Mn-SOD generally parallels the metabolic activity of the tissue, with the highest levels occurring in heart, brain, liver, and kidneys. The mitochondrial level of Catalase is a predictor of life span [Schriner et al. 2005].

Besides telomere shortening with every cell division and DNA strand breaks through oxidative damage, other degenerative processes may accumulate over time to induce senescence.

– With age, proteins, DNA, and other structural molecules develop cross-links to one another. These bonds decrease their mobility and elasticity, and may thus compromise function. Although damaged proteins are normally broken down by proteases, the presence of cross-linkages inhibits proteolysis and disposition. These damaged proteins, therefore, persist and can mediate senescent phenotypes [Abrams et al. 1995].

– Somatic mutations occur spontaneously and continuously. While most of them are corrected and eliminated, others accumulate and lead to malfunction. Cells have the capacity to repair DNA damage, but not all of those repairs are accurate or complete. Thus the damage progressively accumulates. This process may be a crucial component in senescence. It encompasses a role for mitochondria, which have little capacity for repair of DNA damage, so that free radicals can cause much irreversible damage [Wei 1998]. Somatic mutations in the DNA of the mitochondria accumulate with age, and are associated with an age-related decline in mitochondrial function.
– The rate of aging may be influenced by extracellular cues, such as the cumulative exposure to stress hormones, including cortisol, a steroid whose levels rise in the circulation under physically and emotionally stressful conditions. Circulating cortisol levels rise with age. While cortisol levels fall at night in younger adults, in older adults, the levels do not fall as far, increasing the exposure to high levels of cortisol. The decline in variability of cortisol levels is a reflection of deterioration after life-long exposure to stresses [Van Cauter et al. 1996].

In cancer, suppressors of senescence may be overactive, while mediators of senescence may be inhibited.

- Elevated levels of certain antioxidants can delay cell senescence. The antioxidant Thioredoxin is overexpressed in cancers, including pancreatic cancers [Nakamura et al. 2000].
- Thioredoxin is produced by the transformed cells in adult T-cell leukemia, for which the pathogenetic agent is Human T-Cell Leukemia Virus 1 (HTLV-1). Thioredoxin is also an autocrine growth factor derived from Epstein-Barr virus transformed cells.
- The Peroxiredoxins (PRX, Thioredoxin Peroxidases) are a family of six peroxidases (PRX I-VI) of about 25 kD that can reduce H_2O_2 using an electron from Thioredoxin or other substrates. They are located in the cytosol and play roles in the cell signaling system. Peroxiredoxin is greatly overexpressed in breast cancer tissues [Noh et al. 2001] and in malignant mesothelioma [Kinnula et al. 2002].
- In gastric carcinomata, Mn-SOD expression is significantly elevated compared to the surrounding tissue. It may be an adverse prognostic marker [Izutani et al. 1998; Janssen et al. 2000].
- In glioblastoma, Mn-SOD is associated with loss of differentiation, increased malignancy, and poor prognosis [Ria et al. 2001].

4.1 PATHWAYS TO SENESCENCE

Senescent cells are blocked in G_1 of the cell cycle and cannot enter S phase, even after mitogen stimulation [Smith and Pereira-Smith 1996]. Consistently, the pathways leading to cellular senescence overlap with the pathways associated with tumor suppressor gene products. According to immortal cell complementation mapping studies, where various immortal cell lines were fused with each other, all fall into four complementation groups, dubbed A, B, C, or D [Noda et al 1994; Hensler et al 1994; Ogata et al 1993; Leung and Pereira-Smith 2001]. The four genetically determined signaling pathways that contribute to senescence involve P53, RB1, downmodulators of c-FOS (Stress Induced Kinases), and PTEN. Inactivation of at least one of them is a necessary step toward immortality [Pereira-Smith and Smith 1988], which is reflected in indefinite proliferation. There is cross-talk among these pathways and typically during cellular senescence several of them are activated in unison. However, their involvement in the process of senescence varies in a cell-type specific manner.

- Induced senescence may be a specific response of tumor cells, but not normal cells, to external cues. Morphological changes indicative of senescence can be triggered in tumor cells by differentiating agents, including TGF-β and retinoids. Whether these agents induce senescence or differentiation is determined by characteristics intrinsic in the target cell. Senescence can also occur in response to chemotherapy or radiation. The induced senescent phenotype is independent of telomere erosion and is not prevented by the overexpression of Telomerase. The accelerated senescence of tumor cells in response to these agents is not absolutely dependent on *p53*, *p21*, or *p16*. Other tumor suppressor pathways may contribute as evidenced by the upregulation of various growth inhibitory gene products [Roninson 2003].

4.1.1 P53 pathway

The telomere signal that activates the program of senescence operates in part through P53. The replicative life span of normal fibroblasts is limited

by senescence mechanisms that respond to partial telomere shortening by triggering a P53 and $P21^{CIP1/WAF1}$ dependent growth arrest. The exposure of fibroblasts to γ-irradiation leads to a P53 dependent, prolonged G_1 arrest and induction of *p21CIP1/WAF1* expression that has the morphologic features of senescence. Telomerase activity is sufficient to evade this senescence and immortalize fibroblasts, retinal pigmented epithelial cells, vascular endothelial cells, and mesothelial cells. In addition, there is a P53-dependent and Telomerase-independent mechanism that contributes to the limited replicative potential of normal keratinocytes [Rheinswald et al. 2002]. (Figure 4.1.1.A).

Senescence is associated with increased phosphorylation of P53 at serine 15, threonine 18, and serine 376, and decreased phosphorylation at serine 392 [Webley et al. 2000]. This is distinct from the profile of changes in P53 in response to ionizing or UV radiation and is frequently associated with an inability by senescent cells to undergo P53 dependent apoptosis. The DNA binding and transcriptional activities of P53 increase with cell age. In most cases, this occurs in the absence of any marked increase in the level of P53. Functional inactivation of P53 rescues cells from senescence related growth arrest. Instead they enter crisis at a delayed time point.

In response to damage, P53 halts the cell cycle late in G_1. This occurs through stabilization of P53 and results in transcriptional activation of *p21CIP1/WAF1*. $P21^{CIP1/WAF1}$ may be the major effector of P53 mediated senescence. The *p21CIP1/WAF1* (*cdkn1A*) gene, which encodes an inhibitor of Cyclin-Dependent Kinases, is overexpressed in senescent cells. Conversely, inactivation of *p21CIP1/WAF1* is often sufficient to bypass senescence [Brown et al. 1997]. $P21^{CIP1/WAF1}$ is capable of binding to the Proliferating Cell Nuclear Antigen (PCNA) component of DNA Polymerases, thereby affecting the process of DNA reduplication. After the establishment of senescent growth arrest, the protein levels of $P21^{CIP1/WAF1}$ and P53 decrease and the CDK Inhibitor $P16^{INK4a}$ becomes constitutively upregulated, suggesting that $P16^{INK4a}$ may be responsible for the maintenance of growth arrest in senescent cells [Roninson 2003].

P53 is a positive regulator of *caveolin-1* (*cav1*) gene expression [Volonte et al. 2002]. The overexpression of *caveolin-1* promotes cellular senescence as reflected in a reduced proliferative life span, morphologic characteristics of senescence, and a senescence associated increase in β-Galactosidase activity. Sub-cytotoxic oxidative stress induces premature senescence in fibroblasts and increases the endogenous Caveolin-1 expression. While the upregulation of Caveolin-1 is induced by hydrogen peroxide, the antioxidant agent vitamin E prevents it and protects from the prematurely senescent phenotype. The *caveolin* gene family consists of *caveolin-1*,

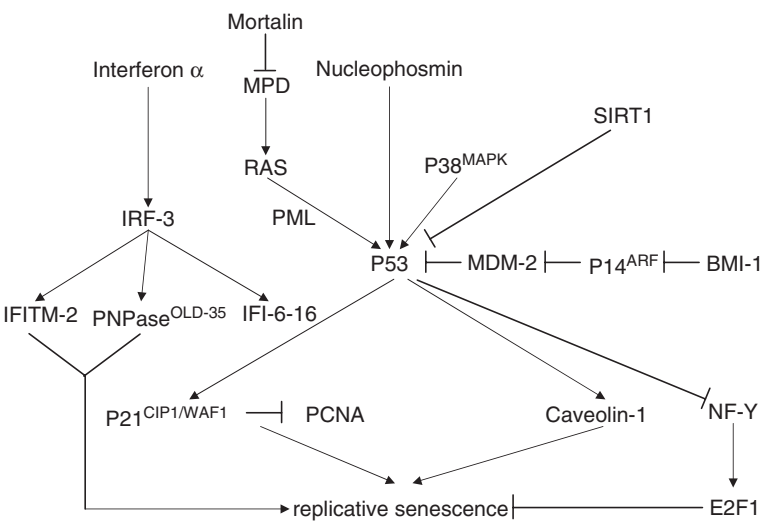


Figure 4.1.1.A. P53-dependent senescence. P53 induces senescence by activating $P21^{CIP1/WAF1}$ and Caveolin-1, and by inhibiting NF-Y. This pathway synergizes with Interferon-α induced senescence through IFI-6–16. The P53 pathway to replicative senescence can be stimulated by various upstream events. They may involve RAS signaling, Nucleophosmin, $P38^{MAPK}$, or SIRT1.

–2, and *–3*. Caveolin-1 {7q31.1} and Caveolin-2 {7q31.1} are co-expressed and form a hetero-oligomeric complex in many cell types, whereas the expression of Caveolin-3 {3p25} is muscle specific. The Caveolin homo- and hetero-oligomers directly interact with cholesterol and represent the functional assembly units of caveolae. Caveolin-1 is a 21 kD or 24 kD integral membrane protein, with the two forms being generated by transcription from alternative start sites. Structurally, Caveolin can be divided into three distinct regions, a hydrophilic cytosolic NH_2-terminal domain, a membrane spanning region, and a hydrophilic COOH-terminal domain. The COOH-terminal domain undergoes palmitoylation (S-acylation) on three cysteine residues, suggesting that both the membrane spanning region and the COOH-terminal domain of Caveolin are associated with the membrane.

P53 can suppress NF-Y (Nuclear Transcription Factor Y, Core Binding Factor, CBF), a CCAAT box-binding general transcription regulator that contains Histone fold motifs and specifically recognizes the consensus sequences 5′-CTGATTGG (C/T)(C/T)(A/G)(A/G)-3′ or 5′-(C/T)(C/T)(A/G)(A/G)CCAATCAG-3′. NF-Y is composed of three subunits, NF-Y α {6p21.3}, NF-Y β {12q22-q23}, and NF-Y γ {1p32}, the interaction of which is necessary for DNA binding. Target promoters for NF-Y include *cdc2*, *cyclin A*, and *cdc25C*. The phosphorylation of NF-Y by CDK2 is essential for the expression of these cell cycle-regulatory genes and therefore for cell cycle progression at both G_1/S and G_2/M [Chae et al. 2004]. The mRNA levels of the *nf-y* subunits do not change age-dependently, however, the protein level of NF-Y α exhibits a significant and progressive decrease during cell senescence [Matuoka and Chen 2000]. Activated P53 family proteins suppress the function of NF-Y, thereby downregulating a set of cell cycle related genes, including *e2f1*. This may play a role in senescence [Matuoka and Chen 2002].

Interferons are capable of inducing cell cycle arrest. Senescent prostate epithelial cells have elevated expression levels of IRF-3 (Interferon Regulatory Factor 3) {19q13.3–q13.4}, which transactivates Interferon responsive genes through sequence specific binding of Interferon response elements. The Interferon-α inducible protein IFI-6–16 (Interferon Induced Protein 6–16, G1P3) {1p35} is also dramatically increased. It is important for the P53-dependent transcription of $p21^{CIP1/WAF1}$ in fibroblasts [Xin et al. 2004]. Furthermore, the Interferon target gene *ifitm2* (*Interferon-induced transmembrane protein 2*) is induced during senescence [Untergasser et al. 2002]. The poly-nucleotide phosphorylase (3′-5′ RNA exonuclease) $PNPase^{OLD\text{-}35}$ is induced by type I Interferons. The protein is localized in the cytoplasm and regulates RNA degradation. $PNPase^{OLD\text{-}35}$ reduces colony formation, suggesting a role for RNA processing in growth control associated with terminal differentiation and cellular senescence [Leszczyniecka et al. 2002].

Nucleophosmin (NPM) is a ubiquitously expressed nucleolar phosphoprotein that continuously shuttles between the nucleus and cytoplasm. Nucleophosmin interacts directly with P53, regulates the increase in stability and transcriptional activation of P53 after various types of stress, and when overexpressed induces P53-dependent premature senescence [Colombo et al. 2002]. There are three forms of Nucleophosmin: NPM-1 {5q35}, NPM-2 {8p21.3}, and NPM-3 {10q24-q26}, encoded by distinct genes. *npm-1* may be expressed in multiple splice variants.

In nonimmortal cells, oncogenic RAS activates P53 to promote cellular senescence. In response to oncogenic RAS activation, $P38^{MAP\ Kinase}$ can phosphorylate and activate P53. RAS^{V12} also induces PML expression, which in turn facilitates P53 acetylation and transcriptional activation, resulting in the upregulation of $p21^{CIP1/WAF1}$ and subsequent senescence induction [Pearson et al. 2000]. RAS induced senescence is not associated with telomere shortening [Wei and Sedivy 1999], but depends on the generation of reactive oxygen species [Lee et al. 1999]. Mortalin (Mitochondrial Heat Shock Protein 70, MHSP 70, MOT2, GRP75, PBP74) {5q31.1} is involved in the regulation of cellular senescence and immortalization pathways. It binds to and inactivates P53 and influences the RAS→RAF→MAPK pathway [Dundas et al. 2005]. MPD (Mevalonate Pyrophosphate Decarboxylase) is a Mortalin binding partner. MPD furnishes prenyl groups required for the prenylation and activation of $P21^{RAS}$. Overexpression of Mortalin prevents the prenylation of RAS and results in reduced levels of RAS and phosphorylated ERK-2 [Wadhwa et al. 2003].

Elevated expression levels of MDM2 may immortalize cells through the destabilization of P53. $P14^{ARF}$ is an inhibitor of the P53 degrading activity

of MDM2, thus counteracting immortalization. $P14^{ARF}$ is transcriptionally activated by the accumulation of cell divisions and therefore is a marker of senescence. BMI-1 is a RING finger protein of the polycomb group and a senescence suppressor. It represses the transcription of $p14^{ARF}$. By virtue of this, BMI-1 supports the immortalization of cells by MDM2.

Life span may increase with elevated levels of the Histone Deacetylase and ADP-Ribosyl Transferase SIRT1 (Sirtuin-1, Mammalian Homolog of SIR2p), consistent with its role as a senescence suppressor. It targets the lysines 9 and 14 of Histone H3 and lysine 16 of Histone H4. The SIRT1 de-acetylation reaction generates two products, *O*-acetyl-ADP-ribose and nicotinamide. The Histone Deacetylase activity of SIR2 requires NAD^+ as a cofactor. This requirement may link SIR activity to the metabolic status, involving reduction and oxidation reactions. Mutations in the *sir* gene or a reduction of NAD^+ give way to toxicity of reactive oxygen intermediates and thus compromise the achievable life span. In addition,

– SIRT1 de-acetylates the tumor suppressor P53. Because acetylation is important for the transcriptional activity of P53, this may render cells resistant to damage-induced apopotosis [Hekimi and Guarente 2003].
– SIRT1 de-acetylates the DNA repair factor and end-joining protein KU70, causing it to sequester the pro-apoptotic factor BAX away from mitochondria, thereby inhibiting stress-induced apoptosis [Cohen et al. 2004]. Acetylation of KU70 disrupts its interaction with BAX.
– SIRT1 modulates the cellular response to stress by regulating the FOXO family of forkhead transcription factors, a family of proteins that function as sensors of Insulin signaling. SIRT1 de-acetylates FOXO3 in response to oxidative stress, which inhibits the ability of FOXO3 to induce cell death, but increases its ability to mediate cell cycle arrest and resistance to oxidative damage.

Because FOXO3 and P53 interact with each other under conditions of oxidative stress, they may synergize downstream of SIRT1 [Brunet et al. 2004]. SIRT1 activates fat mobilization in adipocytes, which is a critical component of calorie restriction [Cohen et al. 2004]. The senescence suppressing effect of SIRT1 may be accomplished, in part, by the negative regulation of pathways involving Insulin and related hormones. There are seven members of the Sirtuin family.

Various mechanisms can inactivate the senescence inducer gene *p53*:

– Most commonly, mutations of *p53* in carcinomata are missense mutations, while in sarcomata and some lymphoid tumors *p53* is often lost or rearranged.
– Amplification of the *mdm2* gene often arises in sarcomata. Its product acts as an inhibitor of P53-mediated transcription. MDM2 also targets P53 for proteasome-mediated degradation. Elevated MDM2 levels suppress P53 dependent senescence.
– Virus encoded oncogene products can inactivate P53 by diverse modes of interaction.
– Cytoplasmic retention of P53 may occur via an unknown mechanism [Levine 1995].

In cancer,

- P53 is phosphorylated at serine 33 and serine 46 by $P38^{MAPK}$. This sensitizes affected cells to oncogenic RAS. PPM1D (Magnesium-Dependent Protein Phosphatase 1δ; WIP1, Wild-Type P53 Induced Phosphatase 1) {17q22–23} blocks the $P38^{MAPK}$-mediated phosphorylation and prevents the senescence response that protects cells from the oncogenic effects of RAS. The phosphatase PPM1D is amplified in over 10% of breast tumors [Bulavin et al. 2002; Li et al. 2002].
- The constitutive activation of the RAS effector MEK (MAPKK) induces P53 and $P16^{INK4a}$ [Lin et al. 1998]. Inactivation of either P53 or $P16^{INK4a}$ in this pathway prevents the induction of senescence and leads to tumorigenesis [Serrano et al. 1997].
- PML facilitates P53 acetylation and transcriptional activation, resulting in the upregulation of $p21^{CIP1/WAF1}$ and subsequent senescence induction. The PML–RARα fusion protein promotes leukemogenesis by interfering with differentiation and the progression to senescence [Ferbeyre 2002].
- *caveolin-1* mRNA and protein expression are lost or reduced during cell transformation by activated oncogenes, such as *v-abl* and *H-ras* (G12V). Caveolin-1 expression is also compromised in cervical carcinogenesis during cell transformation by the activated HPV oncogene product E6 [Razani et al. 2000]. The induction of *caveolin-1* suppresses anchorage independent growth [Engelman et al. 1997] and supports replicative senescence.
- Certain mutations in *npm1* cause an aberrant cytoplasmic localization of the expressed protein. Leukemias with this dislocation of NPM constitute about one third of cases of primary AML in

adults. Most of them retain normal karyotypes and harbor mutations in the *flt3* gene. These leukemic cells do not express the multilineage stem cell marker CD34, suggesting that the cell of origin might be a relatively differentiated hematopoietic progenitor cell [Falini et al. 2005].

- Chromosomal translocations involving the *npm1* gene occur in myeloid and lymphoid cancers. The *npm1* gene rearranges with the *rar* (*retinoic acid receptor*) gene in acute promyelocytic leukemia. Rearrangements with *mlf1* (*myeloid leukemia factor 1*) occur in acute myelogenous leukemia, chronic myelogenous leukemia, and myelodysplasia. As a consequence of these translocations, the rearranged *npm1* allele encodes a fusion protein. The fusion proteins may form dimers with the wild type NPM protein and reduce its activity. The chromosomal translocation t(2;5)(p23;q35) occurs in most anaplastic large cell non-Hodgkin lymphomata arising from activated T-lymphocytes. It generates a hybrid protein, consisting of the NH_2-terminus of Nucleophosmin {5q35} and the catalytic domain of ALK (Anaplastic Lymphoma Kinase) {2p23}. Expressed in the small intestine, testis, and brain but not in normal lymphoid cells, ALK displays sequence similarity to the Insulin Receptor subfamily of kinases [Morris et al. 1994].
- IFN-β has tumor suppressor function. This is independent of its immunologic effects and is not due to cytotoxicity. IFN-β induces predominantly S phase accumulation and the promotion into a senescence-like state [Kaynor et al. 2002]. Defects in Interferon signaling that result in loss of expression of IFN inducible proteins are associated with cellular immortalization, an important early event in the development of cancer.
- Mortalin is overexpressed in colorectal adenocarcinomata and correlates with poor patient survival [Dundas et al. 2005].

4.1.2 RB-1 pathway

The telomere signal that activates the program of senescence operates in part through RB. During the mid-G_1 phase of the cell cycle, phosphorylation of RB by Cyclin D/CDK4 and Cyclin D/CDK6 complexes leads to the transcription of genes required for entry into S phase (Figure 4.1.2.A).

– In senescent cells, RB is under-phosphorylated. In this under-phosphorylated state, RB causes a posttranscriptional accumulation of the Cyclin-Dependent Kinase Inhibitor $P27^{KIP1}$ that is accompanied by an increase in specific binding of $P27^{KIP1}$ to Cyclin E and concomitant decrease in Cyclin E-associated kinase activity. The upregulation of $P27^{KIP1}$ does not require the interaction of RB with E2F [Alexander and Hinds 2001].
– RB proteins also repress the *cyclin E* promoter [Geng et al. 1996; Bandyopadhyay et al. 2002], in part through the recruitment of Histone Deacetylase [Brehm et al. 1998], thus further enhancing the senescence inducing effect.
– In the RB-dependent senescence pathway, CDK5 inhibits RAL and phosphorylates Ezrin. This alters the subcellular localization of Ezrin toward the membrane. Together with increased Ezrin expression in aged cells, this leads to cytoskeletal changes in senescence [Yang and Hinds 2003].

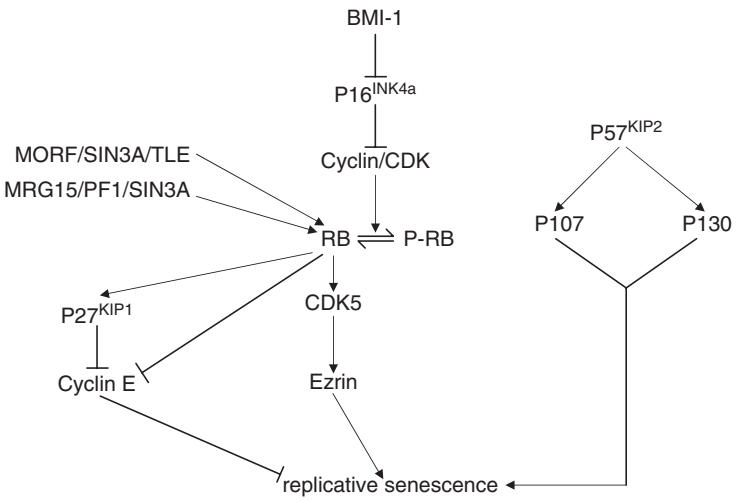


Figure 4.1.2.A. RB-dependent senescence. The RB family of proteins induces replicative senescence through the activation of CDK5 and Ezrin and through the inhibition of Cyclin E. The RB family proteins P107 and P130 may be activated by $P57^{KIP2}$ and also mediate replicative senescence.

For keratinocytes, melanocytes, bladder urothelial cells, and prostate epithelial cells, senescence is associated with the expression and accumulation of $P16^{INK4a}$. In mammary epithelial cells, senescence occurs after approximately 20 population doublings and is associated with the induction of *p16^{INK4a}*. Bypass of senescence is accomplished through the loss of the *cdkn2A* gene or through *p16^{INK4a}* promoter methylation [Mathon and Lloyd 2001]. In keratinocytes and mammary epithelial cells, Telomerase activity is not sufficient to cause immortalization, loss of P16 is also required [Rheinswald et al. 2002]. The replicative senescence of melanocytes is induced by $P16^{INK4a}$, by downregulation of Cyclin E, by hypophosphorylation of RB, or by downregulation of CDK2 and CDK4.

In senescent cells, the overexpression of *p16* inhibits G_1 Cyclin/CDK complexes, thereby preventing the phosphorylation of RB-1. This is followed by the sequestration of E2F, which prevents the transcriptional activation of *cdc2* or *cyclin A* [Smith and Pereira-Smith 1996]. The transcription factors ETS1 and ETS2, but not other members of the ETS family, induce *p16* expression. The helix-loop-helix protein ID-1 antagonizes ETS action and thus downregulates the expression of *p16*. Consistently, loss of ID function may be linked to replicative senescence. The oncogenic and immortalizing potential of BMI-1 is mediated, in part, by its ability to downregulate *p16^{INK4a}* gene expression [Jacobs et al. 1999]. PML acts as a regulator of the premature senescence that occurs in response to oncogenic RAS through increasing the levels of $P16^{INK4a}$ and hypophosphorylated RB [Ferbeyre 2002]. The Histone Acetyl Transferases P300 and CBP (cAMP-Responsive Element-Binding Protein) are phosphorylated in a cell cycle dependent manner, are targeted by certain viral oncoproteins, and may be required for G_1 to S transition. Their total cellular levels decrease dramatically with increasing population doublings of melanocytes. One consequence of the P300 depletion is the transcriptional downregulation of the *cyclin E* gene [Bandyopadhyay et al. 2002].

Fully differentiated cells exit the cell cycle and undergo senescence. A key step in skeletal muscle differentiation involves the downregulation of cell cycle activators such as Cyclins and CDKs, and upregulation of cell cycle inhibitors such as RB, $P21^{CIP1/WAF1}$, $P27^{KIP1}$, and $P57^{KIP2}$. Intestinal cell differentiation requires $P21^{CIP1/WAF1}$ and $P57^{KIP2}$ [Yan et al. 1997; Deschenes et al. 2001]. The growth arrest that precedes intestinal cell differentiation involves the activation of RB proteins and the inhibition of CDK2. $P57^{KIP2}$ is a paternally imprinted gene product that encodes a potent inhibitor of several Cyclin/CDK complexes. $P57^{KIP2}$ is primarily expressed in terminally differentiated cells, associates with G_1 CDKs, and can cause cell cycle arrest in G_1. It may also act as an upstream regulator of the RB-related proteins P107 and P130 [Yan et al. 1997].

The *morf* gene family comprises the senescence genes *morf4* {4q33-q34.1}, *mrgX* {Xq22}, and *mrg15* {15q24}, which encode transcriptional regulators. *morf4* (*mortality factor on chromosome 4*, *sen1*, *cell senescene related*, *csr*) [Bertram et al. 1999] suppresses the immortal phenotype in complementation group B cell types. The 235 amino acid MORF4 protein contains a bipartite nuclear localization signal, a helix-loop-helix domain, and a COOH-terminal leucine zipper motif. It also has a putative PKA phosphorylation site, a PKC phosphorylation site, and a tyrosine phosphorylation site. Its localization is predominantly nuclear. Repression of gene expression by MORFs requires their association with the Histone Deacetylase containing corepressors SIN3A and TLE (Transducin-Like Enhancer) of Split. Therefore, common functions of the MORFs are likely elicited through the action of a MORF/SIN3A/TLE complex. MRG15 contains a nuclear localization signal, a helix-loop-helix region, a leucine zipper, and a chromo-domain. MRG15, but not MRGX or MORF4, interacts with the homeodomain zinc finger protein PF1. Unique functions of MRG15 may be elicited through the action of an MRG15/PF1/SIN3A complex [Yochum and Ayer 2002]. Both the tumor suppressor RB and the 14 kD nuclear protein PAM14 (Protein Associated with MRG) specifically associate with MRG15 in a multiprotein complex. These interactions require the helix-loop-helix and leucine zipper domains of MRG15 [Leung et al. 2001].

- The pathway involving $P16^{INK4a}$, CDK4, Cyclin D_1, and RB is deregulated in the majority of human tumors either by loss of the senescence genes *p16^{INK4a}* or *rb*, or by activation of the oncogenes *cdk4* or *cyclin D_1* [Bringold and Serrano 2000]. In cancer, $P16^{INK4a}$ and RB exhibit a reciprocal pattern of alterations. Tumors that have lost $P16^{INK4a}$ expression retain RB, whereas tumors

that have lost RB retain $P16^{INK4a}$. In tumors that retain both $P16^{INK4a}$ and RB, CDK4 or Cyclin D_1 are frequently overexpressed [Ruas and Peters 1998]. The *cdk4* gene is often amplified in sarcomata and gliomata, and mutations that confer resistance to $P16^{INK4}$-mediated inhibition occur in sporadic melanomata and in the germline of patients with inherited susceptibility to melanoma [Greene 1999]. Cyclin D_1 is the only D-type Cyclin that is frequently activated in human cancers. In particular, amplification and overexpression of Cyclin D_1 occurs in breast tumors, and overexpression of Cyclin D_1 by translocation is a characteristic signature of mantle cell lymphomata [Donnellan and Chetty 1998].

- Loss-of-function alterations of $P16^{INK4a}$ commonly occur in lymphoid malignancies, but are consistently absent in pre-B-cell leukemias induced by the chimeric oncoprotein E2A-PBX1, which is created by t(1;19) chromosomal translocations. E2A-PBX1 enhances the expression of *bmi1*, a lymphoid proto-oncogene whose product functions as a transcriptional repressor of the *cdkn2A* tumor suppressor locus that encodes $P16^{INK4a}$ and $P14^{ARF}$. This oncogenic pathway is likely to play a role in the pathogenesis of lymphoid leukemias through the downregulation of the *cdkn2A* gene [Smith et al. 2003].
- The EWS-FLI1 fusion protein is generated by chromosomal translocation and may lead to Ewing sarcoma. EWS-FLI1 downregulates, possibly through an indirect mechanism, the transcription of $p57^{KIP2}$. This facilitates cell cycle progression through G_1. The modulation of $p57^{KIP2}$ expression by EWS-FLI1 may be a fundamental step in Ewing tumorigenesis [Dauphinot et al. 2001].
- Normal astrocytes express $P57^{KIP2}$. In contrast, this protein is absent from astrocytomata, although the gene does not contain mutations. $p57^{KIP2}$ gene expression is silenced in these cancers.

4.1.3 Stress-Induced Kinase pathway

Replicative senescence is characterized by the loss of proliferative capacity and by impaired inducibility of the immediate early gene *c-fos* {14q24.3} [Seshadri and Campisi 1990]. The transcription of *c-fos* in response to mitogens depends on the activation of a multiprotein complex formed on the serum response element (SRE) in the *c-fos* promoter, which includes the transcription factor SRF (Serum Response Factor) and ternary complex factors (TCFs) of the ETS-domain family. The transcription factor ELK-1 {Xp11.2} binds to a conserved ETS motif adjacent to the SRF recognition site and forms a ternary complex with SRF at the *c-fos* serum response element. TCFs, including ELK-1, are activated after phosphorylation by ERK-1 or ERK-2 (Extracellular Signal-Regulated Kinase-1 or -2), two kinases of the RAF→MEK→ERK signaling pathway. Although the total activity of ERK is not compromised in senescent cells, the kinase is unable to efficiently phosphorylate its nuclear targets. The AP-1 (Activator Protein-1) complex is formed by c-FOS together with c-JUN. AP-1 proteins are differentially expressed in normal and immortalized fibroblasts. While normal fibroblasts only express a 44 kD JUN-B species, immortalized fibroblasts express this 44 kD and a 34 kD JUN-B species. All fibroblasts express 2 JUN-D proteins, but the smaller 39 kD species is more prominent in normal cells, whereas the larger 44 kD protein is more prominent in immortalized cells. The expression of the AP-1 transcription factor *fra2* (*Fos-related antigen-2*, *fosl2*) {2p23-p22} is very low in normal cells, but very evident in immortalized cells [Sheerin et al. 2002].

The inappropriate activation of mitogenic signaling pathways by expression of oncogenic RAS or oncogenic RAF triggers entry into senescence in fibroblasts [Zhu et al. 1998; Mathon and Lloyd 2001]. Among the causative molecular changes for the induction of cellular aging is the repression of the proto-oncogene product c-FOS, which is downstream of RAS, This leads to an inhibition of proliferation [Smith and Pereira-Smith 1996].

Cellular senescence can be induced by chronic exposure to stress, such as UV light, oxidizing agents, or DNA damaging agents. Excessive expression of an activated form of MKK6 (MEK6), a direct activator of the stress induced $P38^{HOG}$ mitogen-activated protein kinase pathway, is sufficient for inducing senescence. Consistent with the senescent phenotype, $P38^{HOG}$ activation induces cell cycle arrest in G_1, which is permanent and irreversible after about 4 days. MKK6 also induces biochemical features of senescence in a $P38^{HOG}$ dependent manner, including enhanced expression of $p21^{CIP1/WAF1}$. $P38^{HOG}$ is part of an intracellular pathway that activates a senescence checkpoint in tumor cells and may play a role in RAS dependent or stress induced senescence. Constitutive $P38^{HOG}$ Kinase activation

mediates permanent cell cycle arrest and senescence [Haq et al. 2002].

- C-FOS expression is frequently elevated in osteosarcomata and may play a causal role in its tumorigenesis [Wu et al. 1990].
- The expression of *c-fos* is associated with ACTH secretion and the corticotroph phenotype in bronchial carcinoid tumors [Pascual-Le Tallec et al. 2002].
- In hepatocellular carcinoma, a reduction in signaling through the $P38^{HOG}$ cascade may account, in part, for the resistance to apoptosis, leading to unrestricted cell growth [Iyoda et al. 2003].

4.1.4 PTEN pathway

The balance between PTEN and Phosphatidylinositol 3-Kinase determines the levels of phosphatidylinositol trisphosphate in the cells. Decreased levels of phosphatidylinositol trisphosphate lead to upregulation of $P27^{KIP1}$, which contributes to senescence. $P27^{KIP1}$ is downregulated by phosphorylation and proteasome degradation in cells lacking PTEN. The inhibition of Phosphatidylinositol 3-Kinase decreases the life span of fibroblasts [Tresini et al. 1998]. Replicative senescence is characterized by increased expression of *collagenase* (*mmp-1*, *matrix metalloproteinase-1*). *collagenase* expression is activated by the transcription factor FKHRL1, which is under negative regulation by the Phosphatidylinositol 3-Kinase→PKB pathway. Although the total activity of PKB is not diminished in senescent cells, PKB cannot efficiently phosphorylate its nuclear targets [Mawal-Dewan et al. 2002; Lorenzini et al. 2002].

- PTEN mutations are implicated in the development of a variety of neoplasias, including high-grade glioblastoma, prostate, breast, endometrial, and thyroid carcinoma.
- The catalytic subunit of Phosphatidylinositol 3-Kinase, p110α, is frequently amplified in ovarian cancers. In these tumors, the $P27^{KIP1}$ protein levels are often substantially diminished, particularly at advanced stages [Bringold and Serrano 2000; Masciullo et al. 2000].
- Germline mutations of *pten* cause multiple hamartoma conditions, including Cowden syndrome, Lhermitte-Duclos disease (LD, cerebelloparenchymal disorder VI), Bannayan-Riley-Ruvalcaba syndrome (Bannayan-Zonana syndrome, BZS), and Proteus syndrome, all resulting in increased susceptibility to the development of cancer.

4.2 CRISIS

Limitations to continued cell division are imposed in two phases. Mortality Stage 1 (M1), occurs when there are still at least several thousand base pairs of telomeric sequences left at the ends of most of the chromosomes. It is possible that M1 is induced by the activation of genes located in the immediately sub-telomeric region of the chromosomes or by a DNA damage signal produced by the shortest of the 92 chromosomal telomeres. The M1 mechanism causes growth arrest mediated by the tumor suppressor proteins RB and P53. If their actions are blocked, either by mutation or by binding to proto-oncoproteins, the cells can continue to divide and the telomeres continue to shorten until the Mortality Stage 2 (M2) is reached. M2 probably represents the physiological result of critically short telomeres when cells are no longer able to protect the ends of the chromosomes, so that end degradation and end-to-end fusion occur, resulting in multicentric chromosomes (chromosomes with multiple centromeres) with breaks occurring in mitosis, finally causing genomic instability and crisis. The state of crisis is an aspect of senescence, which occurs in cells with extremely shortened telomeres and is characterized by marked genetic instability and massive cell death. The shortened telomeres initiate checkpoint signals that trigger apoptosis. This may occur through two variant processes:

– Cells that retain functional P53 may be able to avoid crisis. As these dividing cells approach a growth plateau, termed agonescence [Yaswen and Stampfer 2002], their populations accumulate chromosome abnormalities. In contrast to crisis, agonescent cells can maintain a high level of viability. They form clusters of the telomere-associated protein TIN2.
– Coincident with severe telomere shortening and associated genomic instability, P53 is activated, leading to growth arrest or apoptosis. A lack of functional P53 initially significantly attenuates the adverse cellular and organismal effects of telomere dysfunction, but at later stages supports the process of transformation. This stage of advanced crisis has been termed genetic catastrophe [Chin et al. 1999].

Due to the genetic instability during crisis, a small fraction of cells in the population is

frequently altered in a fashion that allows them to continue to divide. These cells often have overcome senescence permanently and are immortalized [Wright et al. 1989]. Immortalization bears a higher risk of full transformation because a protective barrier has been overcome.

4.3 SENESCENCE SUPPRESSOR GENES

4.3.1 Telomerase

DNA damage activates a signaling pathway that leads to cell cycle arrest (checkpoint). Due to the linear structure of the chromosomes, telomeres (repeats of TTAAGGG) are needed to prevent the chromosome ends from being recognized as strand breaks, which would activate such checkpoints and block the continuation of cell division [Szostak and Blackburn 1982; Kipling 1995]. Even in immortalized cells, protection of the chromosome ends, at least by short telomeres, is still necessary. The progression to senescence is regulated by telomere length, telomere structure, and telomere interacting proteins.

– The length of the shortest telomere determines the replicative capacity of a cell. In germline cells, the terminal restriction fragment is about 15 kb, in primary somatic cells it is about 10 kb, in senescent cells 5–7 kb, and in tumor cells 2–4 kb [Effros and Pawelec 1997]. Stretches of RNA are required to prime DNA Polymerases. These primers leave gaps on the lagging strand, which cannot be filled in and result in chromosome shortening with every cell division. This has been referred to as the end replication problem. While the end replication problem contributes to telomere shortening it accounts only for the shortening of about five nucleotides per cell cycle according to primer length. In contrast, chromosome ends have overhangs of 150–200 bp. The estimated loss of 50–200 bp of DNA per reduplication cycle is consistent with the relative telomere length of young and senescent fibroblasts, which diminishes from 20–25 kb to 8–10 kb [Urquidi et al. 2000; Counter et al. 1992]. The difference may be accounted for by oxidative strand breaks. Although telomere shortening is an important component of cell aging, it is by itself not sufficient to account for senescence. The rate of telomere erosion varies among cell types and there is no consistent telomere length, at which senescence predictably takes place [Ouellette et al. 2000].
– In addition to telomere length, telomere structure may be important in senescence [Kim et al. 2003]. Telomeres have a 3′ overhang, known as the G strand overhang (G-rich strand, contains the hexanucleotide repeat TTAGGG; the complementary strand is C-rich and contains the repeat CCCTAA). The single-strand telomeric overhang, a key component of telomere structure, is eroded at senescence. The expression of Telomerase protects from overhang loss, suggesting that this enzyme prevents senescence by maintaining proper telomere structure. In contrast, progressive overhang loss occurs in cells that avoid senescence through the inactivation of P53 and RB, indicating that overhang erosion is the result of continuous cell division and not a consequence of senescence [Stewart et al. 2003]. A telomere is considered to be capped when it is sufficiently stable to signal continued proliferation to the cell. Telomeric DNA is protected from recognition by the DNA repair system by the formation of T-loops (the bare ends of telomeres bend back and tuck into double stranded DNA), in which the 3′ G strand extension invades the duplex part of the telomere, thereby forming a displacement loop (D-loop) (Figure 4.3.1.A,B). This loop formation is facilitated by the telomere binding proteins TRF1 and TRF2 (Terminal Restriction Fragment 1 and 2). Functional telomeres require at least three factors, a minimum length of TTAAGGG repeats, the integrity of the 3′ overhang, and functional telomere binding proteins [Moyzis et al. 1988].
– Replicative senescence is induced by a change in the protected status of shortened telomeres rather than by a complete loss of telomeric DNA. Telomere-binding proteins play an important role in determining this status. TRF1 (Telomeric Repeat Binding Factor 1, Peptidyl-Prolyl cis/trans Isomerase NIMA-Interacting 2, PIN2) [Chong et al. 1995] {8q13} and TRF2 [Broccoli et al. 1997] {6q22.1} are key components of telomeres and bind to double-stranded telomeric DNA as homodimers (Table 4.3.1.A). Dimerization involves the TRF homology (TRFH) domain, which also mediates interactions with other telomeric proteins. The amount of TRF1 and TRF2 bound to the chromosome ends inversely correlates with the length of the telomere. TRF1 and TRF2 act as negative regulators of telomere length but affect distinct aspects of telomere dynamics. Neither TRF1 nor TRF2 affect the

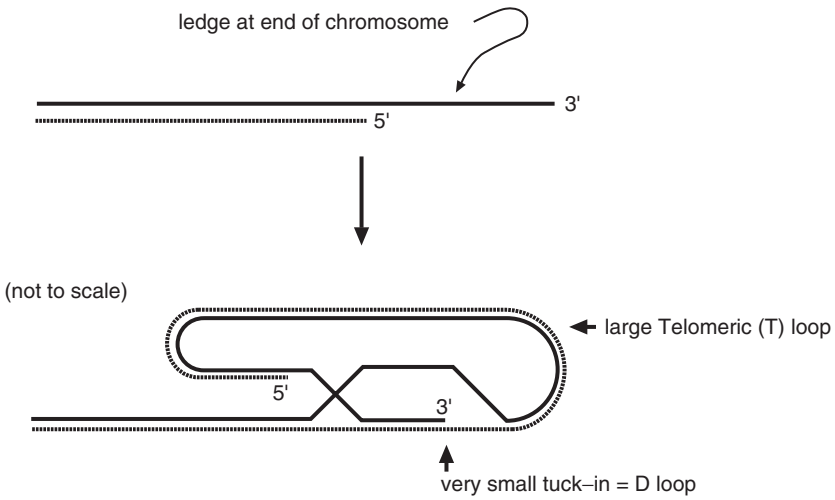


Figure 4.3.1.A. Telomere looping. The telomere DNA loops back on itself, forming a lariat structure. The 3′ G strand extension invades the duplex telomeric repeats and forms a D loop (displacement loop). Duplex DNA telomere binding proteins bind along the length of the telomere repeats. A specialized telomere binding protein binds the D loop at the junction of the lariat. TRF2 may play this role in stabilizing or allowing formation of the D loops. [Reproduced from: http://www.cns.pdx.edu/~newmanl/TelomereLoop4.gif. There are instances where we have been unable to trace or contact the copyright holder. If notified the publisher will be pleased to rectify any errors or omissions at the earliest opportunity.]

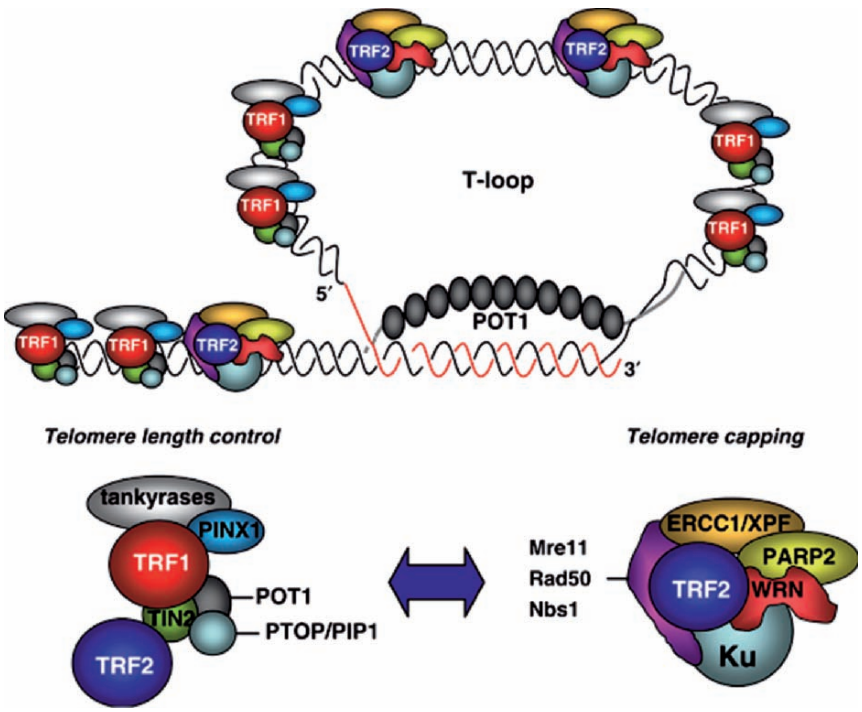


Figure 4.3.1.B. Telomere looping. Telomere-binding proteins. Scheme showing the telomere in a T-loop conformation bound to various protein complexes. The TRF1 complex influences telomere length, while the TRF2 complex influences both telomere length and telomere capping. [Reproduced from Macmillan Blasco 2005 The EMBO Journal 24:1096 With permission.]

Table 4.3.1.A. Telomere-binding proteins. Telomere maintenance requires the coordinated functions of multiple gene products. The telomere binding proteins exert catalytic and structural effects.

Telomerase (TERT, TERC)	Elongates telomeres
POT1	Binds single-stranded TTAAGGG repeats Telomere length maintenance and protection
TRF1	Binds double-stranded TTAAGGG repeats Present in T-loops Negative regulator of telomere length
TRF2	Binds double-stranded TTAAGGG repeats Present in T-loops Negative regulator of telomere length
TANK1	Telomere-associated Poly(ADP-Ribose) Polymerase 1, ribosylates TRF1 Regulator of telomere length
TANK2	Telomere-associated Poly(ADP-Ribose) Polymerase 2, ribosylates TRF1 Positive regulator of telomere length
TIN2	TRF1-binding protein Negative regulator of telomere length
RAP1	TRF2-binding protein regulates telomere length
RAD50/NBS1/ MRE11	DNA repair complex that binds TRF2 Possible role in T-loop formation
PINX1	TRF1/TIN2 interacting protein Potent Telomerase inhibitor
KU86	Negative regulator of telomere length Role in telomere capping
DNA-PK	Role in telomere capping

expression level or enzymatic activity of Telomerase. TRF2 is localized at the junction where the 3′ G strand invades the duplex tract, implying a role for TRF2 in strand invasion. Elevated expression of the telomere binding protein TRF2 reduces the senescence set-point (telomere length at senescence) from 7 to 4 kb. The ability of TRF2 to delay senescence may be due to its protection of critically short telomeres from fusion and its repression of chromosome end-fusions in pre-senescent cells [Karlseder et al. 2002]. A loss of TRF2 function disrupts the normal telomere structure. It leads to chromosome abnormalities and the activation of the ATM→P53 DNA damage response pathway, finally resulting in apoptosis or rapid onset of senescence. Telomere length is influenced by TRF1, its interacting proteins Tankyrase (a PARP homolog) [Smith et al. 1998] and TIN2, and heterogenous nuclear ribonucleoproteins (hnRNPs), including A1. TIN2 (TRF1-Interacting Nuclear Factor 2) [Kim et al. 1999, 2003] is a negative regulator of telomere length. Its NH_2-terminal domains are essential mediators of TRF1 function, suggesting that TRF1 is by itself insufficient for the control of telomere length. The telomere end binding protein POT1 (Protection of Telomeres 1) is also essential for telomere maintenance [Baumann and Cech 2001]. The interaction between the TRF1 complex and POT1 may affect the loading of POT1 on the single-stranded telomeric DNA, thus transmitting information about telomere length to the telomere terminus, where Telomerase is regulated. KU is a heterodimer of 70 kD and 80 kD subunits, which plays an important role in the regulation and maintenance of telomeres and in DNA double strand break repair. KU70 and KU80 also associate with the catalytic subunit DNA-PKcs to form DNA-PK (DNA-Dependent Protein Kinase). KU70 interacts with the telomere binding protein TRF2 and with HP-1α (Hetero-Chromatin Protein 1α) [Song et al. 2001]. HP-1, which is one of the three HP-1 family proteins, is a non-Histone chromosomal protein suppressor of position effect variegation. It is associated with the heterochromatin region and with telomeres, where it prevents telomere fusion.

Telomeric DNA sequences can influence the expression of genes located close to them. A telomere position effect results in the reversible silencing of genes near telomeres, the extent of which correlates with telomere length [Baur et al. 2001]. This raises the possibility that pre-senescent changes could be programmed by the progressive shortening of telomeres with ongoing cell division, leading to altered patterns of gene expression. This position effect on gene expression arises through transcriptional repression. Silencing is accomplished, in part, by Histone deacetylation. Telomeres may also influence the timing of replication of adjacent genomic regions. Sub-telomeric DNA is late reduplicating. The position effect variegation (a form of epigenetic gene silencing caused by placement of a gene near a heterochromatic region of the chromosome, where it is variably silenced, resulting in variable degrees of expression) decreases exponentially with the distance of a gene from the telomere [Kipling 1995]. The 47 kD, 399 amino acid telomere binding protein RAP1 (TRF2IP, TRF-2 Interacting Protein) {16} has a NH_2-terminal BRCT domain, a central

helix-turn-helix motif, and an acidic COOH-terminus featuring a 33 amino acid coiled-coil region and a bipartite nuclear localization signal. RAP1 exerts transcriptional repression of sub-telomeric genes through the recruitment of SIR proteins. Mutations of RAP1 have a dramatic influence on telomeric position effects.

The synthesis of DNA at chromosome ends by Telomerase may be necessary for indefinite cell proliferation. Telomerase (Telomere Terminal Transferase) is a ribonucleoprotein that synthesizes telomeric DNA onto chromosome ends using a segment of its RNA component as a template [Nakamura et al. 1997]. The enzyme can therefore be viewed as a specialized reverse transcriptase. It is able to recognize the 3′ end of telomeric sequences as primer DNA and add the next nucleotide, a process that can still be accomplished if up to 30 bp of nontelomeric sequence are interposed between primer and DNA terminus [Barnett et al. 1993; Kipling 1995]. Telomerase consists of the catalytic subunit TERT (TCS1, EST2) {5p15.33} and the RNA component TERC (TRC3, TR) {3q21-q28}. *tert* consists of 16 exons and 15 introns, spanning approximately 35 kb. *terc* consists of 451 nucleotides. The template sequence of TERC comprises 11 bases that are complementary to the telomere. Differentiation inducing agents inhibit *telomerase* expression in immortal cells and mediate senescence [Sherbet and Lakshmi 1997]. Conversely, the overexpression of *telomerase* is frequently sufficient to reverse the senescent phenotype [Bodnar et al. 1998; Vaziri and Benchimol 1998] (Table 4.3.1.B). The expression of hTERT is, however, not always sufficient to bypass senescence [Kiyono et al. 1998].

- Telomerase activity is important in cancer to allow the continued proliferation of the transformed cells. However, extreme telomere shortening in the absence of Telomerase activity abolishes the protection of the chromosome ends and causes genomic instability, which also predisposes to cancer [Rudolph et al. 1999]. When the intrinsic mutation rates of the affected cells are low, the genomic instability induced by telomere shortening promotes cancer. In backgrounds with high mutation rates, the protective role of shortened telomeres predominates.
- Telomerase activity is repressed in normal somatic tissues, but is reactivated in cancer [Counter et al. 1992; Shay and Wright 1996; Kim et al. 1994]. Despite increased Telomerase activity in malignant cells, their telomere length may be reduced [Hastie et al. 1990]. This implies that Telomerase is synthesized only after the initiation of uncontrolled proliferation, allowing affected cells to become immortal [Greider and Blackburn 1996].
- When telomeres decline to a threshold level, a signal is emitted that prevents the cells from dividing further. This may be because critically shortened telomeres are recognized by checkpoint proteins as DNA strand breaks, resulting in cell cycle arrest. In cancer, such safety signals may be blocked due to loss of function in checkpoint singaling and the cells continue to divide. Telomere stabilization is requisite for tumor development. When telomeres are completely lost, most cells die, only those cells that generate Telomerase will be rescued and maintained. Telomerase activation under these circumstances follows a crisis period of increase in chromosomal aberrations, including aneuploidy and dicentrics (chromatids with two centromeres) [Kipling 1995]. Telomere maintenance is active in virtually all types of malignant tumors [Shay and Bacchetti 1997].

Table 4.3.1.B. Modifications of Telomerase activity. Telomerase activity is typically restricted to cells in early development and stem cells. While Telomerase activity is absent from most healthy cells in the adult organism, it is high in almost all cancer cells.

Protein	Effect
Dyskerin	Stabilizes the TERC component Facilitates Telomerase activity
HSP90	Chaperone that binds to TERT Increases Telomerase activity
P23	Chaperone that binds to TERT Increases Telomerase activity
BCL-2	Increases Telomerase activity
AKT	Phosphorylates TERT Increases Telomerase activity
PTEN	Decreases Telomerase activity
PINX1	Inhibition of Telomerase
c-ABL	Phosphorylates TERT Inhibits Telomerase activity
retinoic acid	Inhibits Telomerase activity
14-3-3	Binds TERT and affects its subcellular localization
c-MYC	Increases *tert* transcription
ER	Increases *tert* transcription
SP1	Increases *tert* transcription
NF-κB	Increases *tert* transcription
SP3	Decreases *tert* transcription
MAD1	Decreases *tert* transcription
P53	Decreases *tert* transcription Possible localization at T-loops

Telomerase activity is upregulated in 85–90% of tumors. In the remaining 10–15% alternative lengthening of telomeres occurs [Bryan et al. 1995].

4.3.2 Regulation of Telomerase activity

The promoter of the catalytic subunit of *telomerase* (*tert*) is inactive in Telomerase negative cells and is active in Telomerase positive cells. A core promoter, 283 bp upstream of the transcription initiation site, is sufficient for maximum promoter activity. The *tert* promoter is GC rich and lacks TATA or CAAT boxes. It contains transcription factor binding elements for SP1, c-MYC, AP-2, AP-4, and NF-1. The promoter contains two E-boxes (CACGTG) and a MT-box (CGTGGGAAG), which function cooperatively [Braunstein et al. 2001]. c-MYC plays an essential role in the regulation of Telomerase activity by inducing the gene expression of *tert* [Fujimoto and Takahashi 1997; Wang et al. 1998].

Inhibitory elements are present within the larger promoter sequence. Retinoids and the tumor suppressor gene product WT1 downregulate *tert* RNA levels through repression of transcription [Oh et al. 1999; Pendino et al. 2001]. The repression of the *tert* promoter by P53 is mediated by $P21^{CIP1/WAF1}$ and E2F. This repression is enhanced by RB [Shats et al. 2004].

Beside the transcriptional regulation, processing of *tert* RNA occurs on the level of splicing. The transcript has at least six alternate splicing sites, comprising four insertion sites and two deletion sites. Variants containing both or either of the deletion sites are present during development and in a large number of cancer cells. Several splice variants of *tert* RNA that encode enzymatically inactive Telomerases are expressed during embryonic development and in some immortalized cells. Intron containing, immature RNA is then spliced in the nucleus to generate the mature product. Deletion of the β site and all four insertions cause premature translation terminations, whereas the deletion of the α site eliminates part of the reverse transcriptase motif. The -α deletion variant lacks 36 bp from exon 6. The -β splice variant deletes 182 bp from exons 7 and 8. A -γ variant is characterized by an in-frame deletion of 189 bp spanning nucleotides 2710–2898, corresponding to the complete loss of exon 11. The TERT splice variant +α, +β (full length transcript) is catalytically active, wile the -α variant acts as a dominant negative, and -β hTERT variant is inactive.

Small RNPs are RNA/protein complexes that function in RNA processing in the nucleus, including pre-mRNA splicing and pre-mRNA 3′ end processing in the nucleoplasm, and pre-rRNA processing in the nucleolus. They are also active in *telomerase* RNA processing.

– The H/ACA (hairpin-hinge-hairpin-tail) RNPs consist of four core proteins and a function-specifying RNA. They reside in nucleoli and Cajal bodies. The H/ACA RNPs guide the site directed pseudouridylation of target RNAs, such as ribosomal and spliceosomal small nuclear RNAs, process ribosomal RNA, and stabilize *telomerase* RNA. The function of H/ACA RNPs is essential for telomere maintenance.
– The 36 kD hnRNP A2 has alternatively spliced variants, the 37 kD hnRNP B1 and the 38 kD B2. The heterogenous ribonucleoprotein hnRNP A2/B1 binds with some sequence specificity to telomeric DNA sequences. The protein is involved with primary RNA transcripts in spliceosomes and may function as a telomere binding protein [Kipling 1995; McKay and Cooke 1992]. A2 and B1 form a major part of the protein component of hnRNP particles and are abundant nuclear proteins.

The small nucleolar ribonucleoprotein Dyskerin (DKC1, NAP57, CBF5p) binds to the H/ACA motif containing 3′ end of the Telomerase RNA component and may be important for the biogenesis, processing and turnover of the Telomerase ribonucleoprotein [Mitchell et al. 1999]. A lack of Dyskerin function leads to reduced levels of Telomerase RNA, lower levels of Telomerase activity, and shorter telomeres than healthy cells. Catalysis of the reaction is mediated by the putative pseudouridylase Dyskerin. Dyskerin promotes the interaction of Telomerase with the nucleolus, possibly facilitating Telomerase RNA processing or RNP.

The chaperones P23 (Cytosolic Prostaglandin E Synthase, CPGES, Unactive Progesterone Receptor) and HSP90 (HSPC) are often associated with each other. They physically and functionally interact with the catalytic subunit of Telomerase. P23 and HSP90 are essential for the assembly of active Telomerase [Holt et al. 1999]. HSP90 and PKB bind to TERT, resulting in TERT phosphorylation. HSP90 prevents the de-phosphorylation of PKB by PP2A. This is mediated through a COOH-terminal domain of HSP90 and is necessary for Telomerase activity.

The disruption of this process may result in apoptosis [Haendeler et al. 2003].

The Telomerase-associated protein TEP1 (Telomerase Protein Component 1, TP1) {14q11.2} binds to the RNA component and the catalytic subunit of Telomerase [Harrington et al. 1997]. However, it may not contribute to Telomerase activity or the levels of Telomerase RNA.

The proto-oncogene *znf217* (*zinc finger protein 217*) {20q13.2} encodes alternatively spliced Krüppel-like transcription factors, which contain 8 C_2H_2 zinc fingers and a proline-rich transcription activation domain. *znf217* overexpressing mammary epithelial cells give rise to immortalized cells. This is associated with initial telomere erosion, followed by an increase in Telomerase activity and telomere length stabilization [Nonet et al. 2001].

- Various oncogene products activate the expression of TERT. Overexpression of c-MYC activates *tert* transcription. The human papilloma virus oncogene E6 can directly upregulate *telomerase* gene expression [Klingelhutz et al. 1996]. In various tumors, including melanoma [Villa et al. 2001], multiple splice variants of *telomerase* are expressed.
- 1,25-dihydroxy-vitamin D_3 is a differentiating agent for many cell types. In ovarian carcinoma, 1,25-dihydroxy-vitamin D_3 decreases the stability of *telomerase* mRNA. This results in diminished Telomerase activity and apoptosis [Jiang et al. 2004].
- Dyskeratosis congenita (DKC, Zinsser–Cole–Engeman syndrome, Hoyeraal–Hreidarsson syndrome) is a rare inherited disorder that involves a multisystem failure. The symptoms of dyskeratosis congenita appear with variable onset in those tissues that proliferate rapidly and have the greatest need for telomere maintenance, such as gut epithelia and bone marrow. The mucosal leucoplakia in patients with this disease can transform into spinocellular carcinoma. Furthermore, there is an age dependent increase in the risk for developing certain cancers, such as epithelial tumors of the skin and gastrointestinal tract. This is consistent with a telomeric maintenance disorder that leads to chromosomal instability, telomeric rearrangements, and cancer progression.
- The nuclear protein PIN-X1 binds to TRF1 and the Telomerase catalytic subunit and inhibits Telomerase activity. The *pinX1* gene is located on chromosome 8p23, which is frequently affected by loss of heterozygosity in liver, breast, prostate, colorectal, lung, head, and neck cancer [Zhou and Lu 2001].
- The chromosomal region 20q13.2, which contains the *znf217* gene, is frequently amplified in breast and ovarian carcinomata [Collins et al. 1998; Tanner et al. 2000].
- Mutations in either of the two KU subunits leads to enhanced instability of telomeres by increasing their sensitivity to either degradation or recombination reactions.

4.3.3 Other senescence suppressor genes

Exemptions from the correlation between telomere length/Telomerase activity and replicative senescence exist. The activation of Telomerase is not sufficient to cause tumors. Conversely, Telomerase activity is not an absolute prerequisite for the generation of tumors because fibroblasts that lack the RNA component of Telomerase can form tumors after transformation with oncogenes, even if the telomeres are profoundly shortened [Blasco et al. 1997]. An alternative, recombination based pathway (alternative lengthening of telomeres, ALT) allows telomere length maintenance in the absence of Telomerase activity. Other potential mechanisms may also contribute, including changes in DNA methylation and genes affected in progeric conditions, including the *Werner syndrome gene* (*wrn*) [Smith and Pereira-Smith 1996] {8p12-p11.2} or the gene causing Hutchinson–Gilford disease (*lamin A*) {1q21.2}.

The TYRO family receptor Tyrosine Kinases TYRO-3 (SKY, ETK-2), TYRO-7 (AXL), and TYRO-12 (MER) can immortalize cells, in part by preventing apoptosis [Darby et al. 2000; Healy et al. 2001; Gutteridge et al. 2002]. They have an extracellular region composed of two Immunoglobulin-like domains and two Fibronectin-like domains, and an intracellular kinase domain that contains a KWIAIES motif. These receptors activate SRC family kinases and signal transduction pathways downstream of GRB-2. The ligands for the TYRO receptors, the anticoagulant Protein S and GAS6 (Growth Arrest Specific Gene 6), are expressed by many cell types [Stitt et al. 1995]. GAS6 is able to induce cell cycle reentry and protect cells from apoptotic cell death.

- The TYRO family receptors are overexpressed by many tumors and can immortalize cells. TYRO-7 expression occurs in 60% of non-small cell lung cancer cells. It may be a consequence of cellular adhesion, and possibly influences differentiation in lung cancers [Wimmel et al. 2001]. *tyro-7* gene expression is associated with cutaneous malignant melanoma [Quong et al. 1994]. TYRO-7 is localized in the membrane of breast cancer cells and the number of cells expressing TYRO-7 is higher in cancerous tissue than in normal breast [Berclaz et al. 2001].
- Uterine leiomyoma is a common benign smooth muscle cell tumor of the myometrium, occurring in as many as 30% of women over 35 years. Leiomyoma growth is dependent on ovarian steroids and local growth factors. In addition, GAS6 and TYRO-7 signal transduction is aberrantly stimulated in uterine leiomyoma, possibly related to its growth [Sun et al. 2003].

REFERENCES

Abrams WB, Beers MH, Berkow R (eds). 1995. The Merck manual of geriatrics. New Jersey: Merck Research Laboratories.

Adelman R, Saul RL, Ames BN. 1988. Oxidative damage to DNA: relation to species metabolic rate and life span. Proceedings of the National Academy of Sciences USA 85:2706–2708.

Alexander K, Hinds PW. 2001. Requirement for $p27^{KIP1}$ in retinoblastoma protein-mediated senescence. Molecular and Cellular Biology 21:3616–3631.

Bandyopadhyay D, Okan NA, Bales E, Nascimento L, Cole PA, Medrano EE. 2002. Down-regulation of p300/CBP histone acetyltransferase activates a senescence checkpoint in human melanocytes. Cancer Research 62:6231–6239.

Barnett MA, Buckle VJ, Evans EP, Porter ACG, Rout D, Smith AG, Brown WRA. 1993. Telomere directed fragmentation of mammalian chromosomes. Nucleic Acids Research 21:27–36.

Baumann P, Cech TR. 2001. Pot1, the putative telomere end-binding protein in fission yeast and humans. Science 292:1171–1175.

Baur JA, Zou Y, Shay JW, Wright WE. 2001. Telomere position effect in human cells. Science 292:2075–2077.

Berclaz G, Altermatt HJ, Rohrbach V, Kieffer I, Dreffer E, Andres AC. 2001. Estrogen dependent expression of the receptor tyrosine kinases axl in normal and malignant human breast. Annals of Oncology 12:819–824.

Bertram MJ, Berube NG, Hang-Swanson X, Ran Q, Leung JK, Bryce S, Spurgers K, Bick RJ, Baldini A, Ning Y, Clark LJ, Parkinson EK, Barrett JC, Smith JR, Pereira-Smith OM. 1999. Identification of a gene that reverses the immortal phenotype of a subset of cells. Molecular and Cell Biology 19:1479–1485.

Blasco MA. 2005. Mice with bad ends: mouse models for the study of telomerase in cancer and aging. EMBO Journal 24:1095–1103.

Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. 1997. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25–34.

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. 1998. Extension of life-span by introduction of telomerase into normal human cells. Science 279:349–352.

Braunstein I, Cohen-Barak O, Shachaf C, Ravel Y, Yalon-Hacohen M, Mills GB, Tzukerman M, Skorecki KL. 2001. Human telomerase reverse transcriptase promoter regulation in normal and malignant human ovarian epithelial cells. Cancer Research 61:5529–5536.

Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T. 1998. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature 391:597–601.

Bringold F, Serrano M. 2000. Tumor suppressors and oncogenes in cellular senescence. Experimental Gerontology 35:317–329.

Broccoli D, Smogorzewska A, Chong L, de Lange T. 1997. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. Nature Genetics 17:231–235.

Brown JP, Wei W, Sedivy JM. 1997. Bypass of senescence after disruption of $p21^{CIP1/WAF1}$ gene in normal diploid human fibroblasts. Science 277:831–834.

Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. 2004. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303:2011–2015.

Bryan TM, Engelzou A, Gupta J, Bacchetti S, Reddel RR. 1995. Telomere elongation in immortal human cells without detectable telomerase activity. EMBO Journal 14:4240–4248.

Bulavin DV, Demidov ON, Saito S, Kauraniemi P, Phillips C, Amundson SA, Ambrosino C, Sauter G, Nebreda AR, Anderson CW, Kallioniemi A, Fornace AJ Jr, Appella E. 2002. Amplification of PPM1D in human tumours abrogates p53 tumour suppressor activity. Nature Genetics 31:210–215.

Campisi J. 1997. Aging and cancer: the double-edged sword of replicative senescence. Journal of the American Geriatric Society 45:482–488.

Campisi J. 2000. Cancer, aging and cellular senescence. In Vivo 14:183–188.

Chae HD, Yun J, Bang YJ, Shin DY. 2004. Cdk2-dependent phosphorylation of the NF-Y transcription factor is essential for the expression of the cell cycle-regulatory genes and cell cycle G1/S and G2/M transitions. Oncogene 23:4084–4088.

Chaturvedi V, Qin JZ, Denning MF, Choubey D, Diaz MO, Nickoloff BJ. 1999. Apoptosis in proliferating, senescent, and immortalized keratinocytes. The Journal of Biological Chemistry 274:23358–23367.

Chen Q, Fisher A, Reagan JD, Yan LJ, Ames BN. 1995. Oxidative DNA damage and senescence of human diploid fibroblast cells. Proceedings of the National Academy of Sciences USA 92:4337–4341.

Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider CW, DePinho RA. 1999. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 97:527–538.

Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, de Lange T. 1995. A human telomeric protein. Science 270:1663–1667.

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA. 2004.

Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305:390–392.

Collins C, Rommens JM, Kowbel D, Godfrey T, Tanner M, Hwang SI, Polikoff D, Nonet G, Cochran J, Myambo K, Jay KE, Froula J, Cloutier T, Kuo WL, Yaswen P, Dairkee S, Giovanola J, Hutchinson GB, Isola J, Kallioniemi OP, Palazzolo M, Martin C, Ericsson C, Pinkel D, Gray JW. 1998. Positional cloning of ZNF217 and NABC1: genes amplified at 20q13.2 and overexpressed in breast carcinoma. Proceedings of the National Academy of Sciences USA 95:8703–8708.

Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. 2002. Nucleophosmin regulates the stability and transcriptional activity of p53. Nature Cell Biology 4:529–533.

Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. 1992. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO Journal 11:1921–1929.

Darby C, Giannola DM, Couzens MS, Emerson SG. 2000. ETK2 receptor tyrosine kinase promotes survival of factor-dependent FDC-P1 progenitor cells. Experimental Hematology 28:716–725.

Dauphinot L, De Oliveira C, Melot T, Sevenet N, Thomas V, Weissman BE, Delattre O. 2001. Analysis of the expression of cell cycle regulators in Ewing cell lines: EWS-FLI-1 modulates $p57^{KIP2}$ and c-Myc expression. Oncogene 20:3258–3265.

Deschenes C, Vezina A, Beaulieu JF, Rivard N. 2001. Role of $p27^{Kip1}$ in human intestinal cell differentiation. Gastroenterology 120:423–438.

Donnellan R, Chetty R. 1998. Cyclin D1 and human neoplasia. Molecular Pathology 51:1–7.

Dundas SR, Lawrie LC, Rooney PH, Murray GI. 2005. Mortalin is over-expressed by colorectal adenocarcinomas and correlates with poor survival. The Journal of Pathology 205:74–81.

Effros RB, Pawelec G. 1997. Replicative senescence of T cells: does the Hayflick limit lead to immune exhaustion. Immunology Today 18:450–454.

Engelman JA, Wycoff CC, Yasuhara S, Song KS, Okamoto T, Lisanti MP. 1997. Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. The Journal of Biological Chemistry 272:16374–16381.

Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF, GIMEMA Acute Leukemia Working Party. 2005. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. The New England Journal of Medicine 352:254–266.

Ferbeyre G. 2002. PML a target of translocations in APL is a regulator of cellular senescence. Leukemia 16:1918–1926.

Fujimoto K, Takahashi M. 1997. Telomerase activity in human leukemic cell lines is inhibited by antisense pentadecadeoxynucleotides targeted against c-myc mRNA. Biochemical and Biophysical Research Communications 241:775–781.

Geng Y, Eaton EN, Picon M, Roberts JM, Lundberg AS, Gifford A, Sardet C, Weinberg RA. 1996. Regulation of cyclin E transcription by E2Fs and retinoblastoma protein. Oncogene 12:1173–1180.

Greene MH. 1999. The genetics of hereditary melanoma and nevi: 1998 update. Cancer 86(S11):2464–2477.

Greider CW, Blackburn EH. 1996. Telomeres, Telomerase and cancer. Scientific American 274:92–97.

Gutteridge KL, Luft JC, Dawson TL, Kozlowska E, Mahajan NP, Varnum B, Earp HS. 2002. Mer receptor tyrosine kinase signaling: prevention of apoptosis and alteration of cytoskeletal architecture without stimulation or proliferation. The Journal of Biological Chemistry 277:24057–24066.

Haendeler J, Hoffmann J, Rahman S, Zeiher AM, Dimmeler S. 2003. Regulation of telomerase activity and anti-apoptotic function by protein-protein interaction and phosphorylation. FEBS Letters 536:180–186.

Haq R, Brenton JD, Takahashi M, Finan D, Rottapel R, Zanke B. 2002. Constitutive p38HOG mitogen-activated protein kinase activation induces permanent cell cycle arrest and senescence. Cancer Research 62:5076–5082.

Harley CB, Futcher AB, Greider CW. 1990. Telomeres shorten during aging of human fibroblasts. Nature 345:458–460.

Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology 11:298–300.

Harman D. 1987. The free radical theory of aging. In: Warner HR, Butler RN, Sprott RL, Schneider EL (eds). Modern biological theories of aging. New York: Raven Press, pp. 81–87.

Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I, Robinson MO. 1997. A mammalian telomerase-associated protein. Science 275:973–976.

Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. 1990. Telomere reduction in human colorectal carcinoma and with ageing. Nature 346:866–868.

Hayflick L. 1965. The limited *in vitro* lifetime of human diploid cell strains. Experimental Cell Research 37:614–636.

Hayflick L, Moorehead PS. 1961. The limited in vitro lifetime of human diploid cell strains. Experimantal Aging Research 25:585–621.

Healy AM, Schwartz JJ, Zhu X, Herrick BE, Varnum B, Farber HW. 2001. Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells. American Journal of Physiology: Lung Cellular and Molecular Physiology 280:L1273–L1281.

Hekimi S, Guarente L. 2003. Genetics and the specificity of the aging process. Science 299:1351–1354.

Hensler PJ, Annab LA, Barrett JC, Pereira-Smith OM. 1994. A gene involved in control of human cellular senescence on human chromosome 1Q. Molecular and Cellular Biology 14:2291–2297.

Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE, White MA. 1999. Functional requirement of p23 and Hsp90 in telomerase complexes. Genes and Development 13:817–826.

Iyoda K, Sasaki Y, Horimoto M, Toyama T, Yakushijin T, Sakakibara M, Takehara T, Fujimoto J, Hori M, Wands JR, Hayashi N. 2003. Involvement of the p38 mitogen-activated protein kinase cascade in hepatocellular carcinoma. Cancer 97:3017–3026.

Izutani R, Asano S, Imano M, Kuroda D, Kato M, Ohyanagi H. 1998. Expression of manganese superoxide dismutase in esophageal and gastric cancers. Journal of Gastroenterology 33:816–822.

Janssen AM, Bosman CB, van Duijn W, Oostendorp-van de Ruit MM, Kubben FJ, Griffioen G, Lamers CB, van Krieken JH,

van de Velde CJ, Verspaget HW. 2000. Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. Clinical Cancer Research 6:3183–3192.

Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. 1999. The oncogene and Polycomb group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature 397:164–168.

Jiang F, Bao J, Li P, Nicosia SV, Bai W. 2004. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D_3 through the down-regulation of telomerase. The Journal of Biological Chemistry 279:53213–53221.

Karlseder J, Smogorzewska A, de Lange T. 2002. Senescence induced by altered telomere state, not telomere loss. Science 295:2446–2449.

Kaynor C, Xin M, Wakefield J, Barsoum J, Qin XQ. 2002. Direct evidence that IFN-β functions as a tumor-suppressor protein. Journal of Interferon and Cytokine Research 22:1089–1098.

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. 1994. Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015.

Kim SH, Han S, You YH, Chen DJ, Campisi J. 2003. The human telomere-associated protein TIN2 stimulates interactions between telomeric DNA tracts in vitro. EMBO Report 4:685–691.

Kim SH, Kaminker P, Campisi J. 1999. TIN2, a new regulator of telomere length in human cells. Nature Genetics 23:405–412.

Kinnula VL, Lehtonen S, Sormunen R, Kaarteenaho-Wiik R, Kang SW, Rhee SG, Soini Y. 2002. Overexpression of peroxiredoxins I, II, III, V, and VI in malignant mesothelioma. Journal of Pathology 196:316–323.

Kipling D. 1995. The telomere. Oxford/New York/Tokyo: Oxford University Press.

Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ. 1998. Both Rb/$p16^{INK4a}$ inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 396:84–88.

Klingelhutz AJ, Foster SA, McDougall JK. 1996. Telomerase activation of the E6 gene product of human papilloma virus type 16. Nature 380:79–82.

Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, Finkel T. 1999. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. The Journal of Biological Chemistry 274:7936–7940.

Leszczyniecka M, Kang DC, Sarkar D, Su ZZ, Holmes M, Valerie K, Fisher PB. 2002. Identification and cloning of human polynucleotide phosphorylase, $hPNPase^{old-35}$, in the context of terminal differentiation and cellular senescence. Proceedings of the National Academy of Sciences USA 99:16636–16641.

Leung JK, Berube N, Venable S, Ahmed S, Timchenko N, Pereira-Smith OM. 2001. MRG15 activates the B-myb promoter through formation of a nuclear complex with the retinoblastoma protein and the novel protein PAM14. The Journal of Biological Chemistry 276:39171–39178.

Leung JK, Pereira-Smith OM. 2001. Identification of genes involved in cell senescence and immortalization: potential implications for tissue ageing. Novartis Foundation Symposium 235:105–110.

Levine A. 1995. Tumor suppressor genes. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 86–104.

Li J, Yang Y, Peng Y, Austin RJ, van Eyndhoven WG, Nguyen KC, Gabriele T, McCurrach ME, Marks JR, Hoey T, Lowe SW, Powers S. 2002. Oncogenic properties of PPM1D located within a breast cancer amplification epicenter at 17q 23. Nature Genetics 31:133–134.

Lin AW, Barradas M, Stone JC, vanAelst L, Serrano M, Lowe SW. 1998. Premature senescence involving p53 and $p16^{INK4a}$ is activated in response to constitutive MEK7/MAPK mitogenic signaling. Genes and Development 12:3008–3019.

Lorenzini A, Tresini M, Mawal-Dewan M, Frisoni L, Zhang H, Allen RG, Sell C, Cristofalo VJ. 2002. Role of the Raf/MEK/ERK and the PI3K/Akt(PKB) pathways in fibroblast senescence. Experimental Gerontology 37:1149–1156.

Masciullo V, Ferrandina G, Pucci B, Fanfani F, Lovergine S, Palazzo J, Zannoni G, Mancuso S, Scambia G, Giordano A. 2000. $p27^{Kip1}$ expression is associated with clinical outcome in advanced epithelial ovarian cancer: multivariate analysis. Clinical Cancer Research 6:4816–4822.

Mathon NF, Lloyd AC. 2001. Cell senescence and cancer. Nature Reviews Cancer 1:203–213.

Matuoka K, Chen KY. 2000. Possible role of subunit A of nuclear factor Y (NF-YA) in normal human diploid fibroblasts during senescence. Biogerontology 1:261–271.

Matuoka K, Chen KY. 2002. Transcriptional regulation of cellular ageing by the CCAAT box-binding factor CBF/NF-Y. Ageing Research Reviews 1:639–652.

Mawal-Dewan M, Lorenzini A, Frisoni L, Zhang H, Cristofalo VJ, Sell C. 2002. Regulation of collagenase expression during replicative senescence in human fibroblasts by Akt-forkhead signaling. The Journal of Biological Chemistry 277:7857–7864.

McKay SJ, Cooke H. 1992. A protein which specifically binds to single stranded $TTAGGG_n$ repeats. Nucleic Acids Research 20:1387–1391.

Mitchell JR, Wood E, Collins K. 1999. A telomerase component is defective in the human disease dyskeratosis congenita. Nature 402:551–555.

Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT. 1994. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263:1281–1284.

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. 1988. A highly conserved repetitive DNA sequence, $(TTAGGG)_n$, present at the telomeres of human chromosomes. Proceedings of the National Academy of Sciences USA 85:6622–6626.

Nakamura H, Bai J, Nishinaka Y, Ueda S, Sasada T, Ohshio G, Imamura M, Takabayashi A, Yamaoka Y, Yodoi J. 2000. Expression of thioredoxin and glutaredoxin, redox-regulating proteins, in pancreatic cancer. Cancer Detection and Prevention 24:53–60.

Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR. 1997. Telomerase catalytic subunit homologs from fission yeast and human. Science 277:955–959.

Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR. 1994. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Experimental Cell Research 211:90–98.

Noh DY, Ahn SJ, Lee RA, Kim SW, Park IA, Chae HZ. 2001. Overexpression of peroxiredoxin in human breast cancer. Anticancer Research 21:2085–2090.

Nonet GH, Stampfer MR, Chin K, Gray JW, Collins CC, Yaswen P. 2001. The *ZNF217* gene amplified in breast cancers promotes immortalization of human mammary epithelial cells. Cancer Research 61:1250–1254.

Ogata T, Ayusawa D, Namba M, Takahashi E, Oshimura M, Oishi M. 1993. Chromosome 7 suppresses indefinite division of non-tumorigenic immortalized human fibroblast cell lines kmst-6 and susm-1. Molecular and Cellular Biology 13:6036–6043.

Oh S, Song Y, Yim J, Kim TK. 1999. The Wilms' tumor 1 tumor suppressor gene represses transcription of the human telomerase reverse transcriptase gene. The Journal of Biological Chemistry 274:37473–37478.

Ouellette MM, Liao M, Herbert BS, Johnson M, Holt SE, Liss HS, Shay JW, Wright WE. 2000. Subsenescent telomere lengths in fibroblasts immortalized by limiting amounts of telomerase. The Journal of Biological Chemistry 275:10072–10076.

Pascual-Le Tallec L, Dulmet E, Bertagna X, de Keyzer Y. 2002. Identification of genes associated with the corticotroph phenotype in bronchial carcinoid tumors. Journal of Clinical Endocrinology and Metabolism 87:5015–5022.

Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. 2000. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 406:207–210.

Pendino F, Flexor M, Delhommeau F, Buet D, Lanotte M, Segal-Bendirdjian E. 2001. Retinoids down-regulate telomerase and telomere length in a pathway distinct from leukemia cell differentiation. Proceedings of the National Academy of Sciences USA 98:6662–6667.

Perillo NL, Walford RL, Newman MA, Effros RB. 1989. Human T lymphocytes possess a limited in vitro life span. Experimental Gerontology 24:177–187.

Pereira-Smith OM, Smith JR. 1988. Genetic Analysis of Indefinite Division in Human Cells: Identification of Four Complementation Groups. Proceedings of the National Academy of Sciences USA 85:6042–6046.

Quong RY, Bickford ST, Ing YL, Terman B, Herlyn M, Lassam NJ. 1994. Protein kinases in normal and transformed melanocytes. Melanoma Research 4:313–319.

Razani B, Altschuler Y, Zhu L, Pestell RG, Mostov KE, Lisanti MP. 2000. Caveolin-1 expression is down-regulated in cells transformed by the human papilloma virus in a p53-dependent manner. Replacement of caveolin-1 expression suppresses HPV-mediated cell transformation. Biochemistry 39:13916–13924.

Rheinswald JG, Hahn WC, Ramsey MR, Wu JY, Guo Z, Tsao H, De Luca M, Catricala C, O'Toole KM. 2002. A two-stage, $p16^{INK4A}$- and p53-dependent keratinocyte senescence mechanism that limits replicative potential independent of telomere status. Molecular and Cellular Biology 22:5157–5172.

Ria F, Landriscina M, Remiddi F, Rosselli R, Iacoangeli M, Scerrati M, Pani G, Borrello S, Galeotti T. 2001. The level of manganese superoxide dismutase content is an independent prognostic factor for glioblastoma. Biological mechanisms and clinical implications. British Journal of Cancer 84:529–534.

Roninson IB. 2003. Tumor cell senescence in cancer treatment. Cancer Research 63:2705–2715.

Ruas M, Peters G. 1998. The $p16^{INK4a}$/CDKN2A tumor suppressor and its relatives. Biochimica et Biophysica Acta 1378:115–177.

Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA. 1999. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96:701–712.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS. 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308:1909–1911.

Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SE. 1997. Oncogenic Ras provokes premature senescence associated with accumulation of p53 and $p16^{INK4a}$. Cell 88:593–602.

Seshadri T, Campisi J. 1990. Repression of c-fos transcription and an altered genetic program in senescent human fibroblasts. Science 247:205–209.

Shats I, Milyavsky M, Tang X, Stambolsky P, Erez N, Brosh R, Kogan I, Braunstein I, Tzukerman M, Ginsberg D, Rotter V. 2004. p53-dependent down-regulation of telomerase is mediated by $p21^{waf1}$. The Journal of Biological Chemistry 279:50976–50985.

Shay JW, Bacchetti S. 1997. A survey of telomerase activity in human cancer. European Journal of Cancer 33:787–791.

Shay JW, Wright WE. 1996. Telomerase activity in human cancer. Current Opinion in Oncology 8:66–71.

Sheerin A, Thompson KS, Goyns MH. 2002. Altered composition of the AP-1 transcription factor in immortalized compared to normal proliferating cells. Cancer Letters 177:83–87.

Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. 1999. Microarray analysis of replicative senescence. Current Biology 9:939–945.

Sherbet GV, Lakshmi MS. 1997. The genetics of cancer: genes associated with cancer invasion, metastasis and cell proliferation. San Diego/London/Boston/New York/Sydney/Tokyo/Toronto: Academic Press.

Sitte N, Saretzki G, von Zglinicki T. 1998. Accelerated telomere shortening in fibroblasts after extended periods of confluency. Free Radical Biology and Medicine 24:885–893.

Smith JR, Pereira-Smith OM. 1996. Replicative senescence: implications for in vivo aging and tumor suppression. Science 273:63–67.

Smith KS, Chanda SK, Lingbeek M, Ross DT, Botstein D, van Lohuizen M, Cleary ML. 2003. Bmi-1 regulation of INK4A-ARF is a downstream requirement for transformation of hematopoietic progenitors by E2a-Pbx1. Molecular Cell 12:393–400.

Smith S, Giriat I, Schmitt A, de Lange T. 1998. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 282:1484–1487.

Song K, Jung Y, Jung D, Lee I. 2001. Human Ku70 interacts with heterochromatin protein 1α. The Journal of Biological Chemistry 276:8321–8327.

Stewart SA, Ben-Porath I, Carey VJ, O'Connor BF, Hahn WC, Weinberg RA. 2003. Erosion of the telomeric single-strand overhang at replicative senescence. Nature Genetics 33:492–496.

Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Mattsson K, Fisher J, Gies DR, Jones PF, Masiakowski P, Ryan TE, Tobkes NJ, Chen DH, DiStefano PS, Long GL, Basilico C, Goldfarb MP, Lemke G, Glass DJ, Yancopoulos GD. 1995. The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell 80:661–670.

Sun WS, Fujimoto J, Tamaya T. 2003. Clinical implications of coexpression of growth arrest-specific gene 6 and receptor tyrosine kinases Axl and Sky in human uterine leiomyoma. Molecular Human Reproduction 9:701–707.

Szostak JW, Blackburn EH. 1982. Cloning yeast telomeres on linear plasmid vectors. Cell 29:245–255.

Tanner MM, Grenman S, Koul A, Johannsson O, Meltzer P, Pejovic T, Borg A, Isola JJ. 2000. Frequent amplification of chromosomal region 20q12-q13 in ovarian cancer. Clinical Cancer Research 6:1833–1839.

Tresini M, Mawal-Dewan M, Cristofalo VJ, Sell C. 1998. A phosphatidylinositol 3-kinase inhibitor induces a senescent-like growth arrest in human diploid fibroblasts. Cancer Research 58:1–4.

Untergasser G, Koch HB, Menssen A, Hermeking H. 2002. Characterization of epithelial senescence by serial analysis of gene expression: identification of genes potentially involved in prostate cancer. Cancer Research 62:6255–6262.

Urquidi V, Tarin D, Goodison S. 2000. Role of telomerase in cell senescence and oncogenesis. Annual Reviews in Medicine 52:65–79.

Van Cauter E, Leproult R, Kupfer DJ. 1996. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. Journal of Clinical Endocrinology and Metabolism 81:2468–2473.

Vaziri H, Benchimol S. 1998. Reconstitution of telomerase activity in normal cells leads to elongation of telomeres and extended replicative life span. Current Biology 8:279–282.

Villa R, Porta CD, Folini M, Daidone MG, Zaffaroni N. 2001. Possible regulation of telomerase activity by transcription and alternative splicing of telomerase reverse transcriptase in human melanoma. The Journal of Investigative Dermatology 116:867–873.

Volonte D, Zhang K, Lisanti MP, Galbiati F. 2002. Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. Molecular Biology of the Cell 13:2502–2517.

von Zglinicki T, Saretzki G, Docke W, Lotze C. 1995. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? Experimental Cell Research 220:186–193.

Wadhwa R, Yaguchi T, Hasan MK, Taira K, Kaul SC. 2003. Mortalin-MPD (mevalonate pyrophosphate decarboxylase) interactions and their role in control of cellular proliferation. Biochemical and Biophysical Research Communications 302:735–742.

Wang J, Xie LY, Allan S, Beach D, Hannon GJ. 1998. Myc activates telomerase. Genes and Development 12:1769–1774.

Webley K, Bond JA, Jones CJ, Blaydes JP, Craig A, Hupp T, Wynford-Thomas D. 2000. Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. Molecular and Cellular Biology 20:2803–2808.

Wei S, Sedivy JM. 1999. Expression of catalytically active telomerase does not prevent premature senescence caused by overexpression of oncogenic Ha-Ras in normal human fibroblasts. Cancer Research 59:1539–1543.

Wei YH. 1998. Oxidative stress and mitochondrial DNA mutations in human aging. Proceedings of the Society for Experimental Biology and Medicine 217:53–63.

Wimmel A, Glitz D, Kraus A, Roeder J, Schuermann M. 2001. Axl receptor tyrosine expression in human lung cancer cell lines correlates with cellular adhesion. European Journal of Cancer 37:2264–2274.

Wright WE, Pereira-Smith OM, Shay JW. 1989. Reversible cellular senescence: implications for immortalization of normal human diploid fibroblasts. Molecular and Cellular Biology 9:3088–3092.

Wu JX, Carpenter PM, Gresens C, Keh R, Niman H, Morris JW, Mercola D. 1990. The proto-oncogene c-fos is over-expressed in the majority of human osteosarcomas. Oncogene 5:989–1000.

Xin H, Pereira-Smith OM, Choubey D. 2004. Role of IFI 16 in cellular senescence of human fibroblasts. Oncogene 23:6209–6217.

Yan Y, Frisen J, Lee MH, Massague J, Barbacid M. 1997. Ablation of the CDK inhibitor $p57^{Kip2}$ results in increased apoptosis and delayed differentiation during mouse development. Genes and Development 11:973–983.

Yang HS, Hinds PW. 2003. Increased Ezrin expression and activation by CDK5 coincident with acquisition of the senescent phenotype. Molecular Cell 11:1163–1176.

Yaswen P, Stampfer MR. 2002. Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. International Journal of Biochemistry and Cell Biology 34:1382–1394.

Yochum GS, Ayer DE. 2002. Role for the mortality factors MORF4, MRGX, and MRG15 in transcriptional repression via associations with Pf1, mSin3A, and Transducin-like enhancer of split. Molecular and Cellular Biology 22: 7868–7876.

Zhang H, Pan KH, Cohen SN. 2003. Senescence-specific gene expression fingerprints reveal cell-type-dependent physical clustering of up-regulated chromosomal loci. Proceedings of the National Academy of Sciences USA 100:3251–3256.

Zhou XZ, Lu KP. 2001. The Pin2/TRF-1interacting protein PinX1 is a potent telomerase inhibitor. Cell 107:347–359.

Zhu J, Woods D, McMahon M, Bishop JM. 1998. Senescence of human fibroblasts induced by oncogenic Raf. Genes and Development 12:2997–3007.

CHAPTER 5

INVASIVENESS

The extracellular matrix supports the adhesion of cells and transmits signals through cell surface adhesion receptors. The basement membrane separates the epithelial cells from the underlying stroma, thereby providing the first barrier against invasion of malignant growths. The destruction of the basement membrane is a prerequisite for the dissemination of cancer cells and loss of its continuity is distinguishing the earliest sign of invasiveness. This is followed by intravasation into local blood or lymph vessels. The basement membrane is 20–200 nm thick and consists of Collagens, noncollagenous glycoproteins, and proteoglycans. Networks are formed through the self-organization of Laminins and Collagen IV. Entactin forms connections between these two networks. Heparan sulfate proteoglycans are linked to the membrane through their interactions with Laminin. Alternative extracellular matrix constituents, such as Tenascin, Fibronectin, and variant forms of Laminin, are existent in tumors and can affect cancer progression.

– Collagen molecules have three α chains that form a triple helix. Through self-assembly, the Collagen types I, II, III, V, and XI shape fibrils, and interact with Integrins on cells. Basement membrane Collagens (type IV) form networks. Cells interact with Collagen type IV molecules through Integrins, Laminin, and heparin sulfate proteoglycans. Proteolytic fragments of these Collagens, such as Tumstatin, can inhibit tumor angiogenesis. The Multiple-Triple-Helix-Domains-With-Interruptions Collagens, including the Multiplexins (type XV and XVIII), are mainly located in the basement membranes of internal organs. Endostatin, a proteolytic fragment of the αl chain of Collagen type XVIII, is an inhibitor of angiogenesis. There are several other groups of Collagens, including the transmembrane Collagens that are a part of focal adhesion sites. Gelatin is denatured Collagen, the product of Collagenase digestion.
– The extracellular glycoproteins include Laminins, which are heterotrimeric glycoproteins composed of α, β, and γ chains. They are primarily located in basement membranes, where they form networks with Collagen IV and Nidogen. Laminins bind to Integrins and non-Integrin receptors. Fibronectins are dimeric glycoproteins that are present in the extracellular matrix and in the blood. They form fibrils and affect cell morphology, adhesion, migration, and differentiation by binding to Integrins.
– Proteoglycans have posttranslational modifications of glycosaminoglycan chains such as heparan, keratan, and chondroitin sulfate. Perlecan is the most common heparin sulfate proteoglycan of the basement membrane and is associated with tumor stroma. Other important proteoglycans are Decorin, which associates with Collagen fibrils, and Aggrecan, which exists as large aggregates in cartilagenous tissues. Versican is the main chondroitin sulfate proteoglycan of noncartilagenous tissues. Syndecans, Glypicans, and CD44 are cell surface proteoglycans. Hyaluronan is a glycosaminoglycan, but not a proteoglycan, as it is not covalently attached to any protein. The ability of heparan sulfate to interact with extracellular matrix macromolecules and with various attachment sites on cellular plasma membranes makes it

essential in the assembly and insolubility of the extracellular matrix.

5.1 EXTRACELLULAR MATRIX DEGRADING ENZYMES

The loss of epithelial basement membranes is a hallmark of invasion. The extracellular matrix needs to be sufficiently degraded to facilitate cell passage, but not to the extent that cellular traction is lost. The maximum rate of migration occurs at intermediate levels of adhesiveness. At low levels of adhesiveness, weakly attached cells cannot generate sufficient traction to move efficiently. At high levels of adhesiveness, cells cannot break contact and are therefore immobile.

Malignant tumors secrete various proteolytic enzymes that degrade the basement membrane, thus allowing for invasive behavior. A positive correlation exists between tumor aggressiveness and protease levels for all known classes of proteases, including seryl-, aspartyl-, cysteinyl-, and metal atom-dependent proteases (Table 5.1.A). The activity of these enzymes is tightly regulated by proteolytic processing of precursors, cascades of proteases, and binding to inhibitors. Many of the

Table 5.1.A. Proteases associated with cancer invasion. The secretion of proteases is necessary during invasion, predominantly to degrade the extracellular matrix. Diverse groups of proteases contribute to this process. They are under tight regulation, in part by secreted protease inhibitors

Enzyme	Target	Structural class	Tumor
Matrix Metalloproteinases			
MMP-1 (Interstitial Collagenase)	Collagen type I, fibrillar Collagens II,III,VII,X	Simple Hemopexin domain	Head and neck cancer
MMP-2 (72 kD Collagenase type IV, Gelatinase A)	Collagens IV,V,VII,IX,X, Gelatin, Fibronectin, Elastin	Gelatin binding	Skin cancer, colon cancer, stomach cancer, ovarian cancer, thyroid cancer
MMP-3 (Stromelysin-1, Transin-1)	Proteoglycan Core Protein, Laminin, Fibronectin, Gelatin, basement membrane Collagens	Simple Hemopexin domain	
MMP-4	α-chain of type I Collagen		
MMP-5	Native 3/4 Collagen fragments		
MMP-6 (Acid Metalloproteinase)	Cartilage proteoglycan		
MMP-7 (Matrilysin, PUMP-1)	Gelatin types I, III, IV, V, Collagen, Fibronectin	Minimal domain	Gastric cancer, colon cancer
MMP-8 (Collagenase 2, Neutrophil Collagenase)	Collagen I	Simple Hemopexin domain	
MMP-9 92 kD Collagenase type IV, Gelatinase B)	Collagens IV,V,VII,IX,X, Gelatin, Fibronectin, Elastin	Gelatin binding	Skin cancer
MMP-10 (Stromelysin-2, Transin)	Proteoglycan Core Protein, Laminin, Fibronectin, Gelatin, basement membrane Collagens	Simple Hemopexin domain	Head and neck cancer
MMP-11 (Stromelysin-3)	Proteoglycan Core Protein, Laminin, Fibronectin, Gelatin, basement membrane Collagens	Furin activated and secreted	Breast cancer
MMP-12 (Metalloelastase, Macrophage Elastase)	Elastin	Simple Hemopexin domain	Breast cancer, desmoid tumors
MMP-13 (Collagenase 3)	Collagen	Simple Hemopexin domain	Malignant peripheral nerve sheath tumors, colorectal cancer

Table 5.1.A. (continued)

MMP-14 (MT1-MMP)	pro-MMP-2	Transmembrane	Gastric cancer, squamous cell lung cancer
MMP-15 (MT2-MMP)		Transmembrane	Lung adenocarcinoma, prostate cancer
MMP-16 (MT3-MMP)		Transmembrane	
MMP-17 (MT4-MMP)	pro-Gelatinase A Gelatin	GPI anchored Zn^{2+} and Ca^{2+} dependent	Breast cancer
MMP-19 (RASI-1)		Simple Hemopexin domain	
MMP-20 (Enamelysin)	Amelogenin	Simple Hemopexin domain	
MMP-23 (Femalysin)		Type II transmembrane	
MMP-24 (MT5-MMP)	pro-MMP-2	Transmembrane Hemopexin domain	Brain tumors
MMP-25 (MT6-MMP)		GPI anchored	
MMP-26 (Matrilysin-2, Endometase)		Minimal domain	Endometrial cancer
MMP-27		Simple Hemopexin domain	
MMP-28 (Epilysin)		Furin activated and secreted	Fibrosarcoma
Fibrinolytic Proteinases			
Urokinase Type Plasminogen Activator	Plasminogen		Breast cancer, melanoma
Tissue Type Plasminogen Activator	Plasminogen		Breast cancer
Plasmin	Laminin, type IV Collagen		
Thrombin	PAR-1		Pancreas cancer, oral squamous cell carcinoma
Cathepsins			
Cathepsin D			Glioblastoma, hepatoma, melanoma, thyroid cancer
Cathepsin B	Amyloid β, pro-Urokinase		Glioblastoma, meningioma, lung cancer
Cathepsin G			
Cathepsin H			Prostate cancer
Cathepsin K			Breast cancer, prostate cancer
Cathepsin L	α-1 Protease Inhibitor, Collagen, Elastin		
Cathepsin O			Breast carcinoma
Cathepsin S	Elastin		Astrocytoma
Other Proteinases			
Elastase	Elastin		Colorectal carcinoma, breast cancer, lung cancer
Protease inhibitors			
TIMP-1	92 kD Collagenase type IV Interstitial Collagenase Stromelysin		
TIMP-2	72 kD Collagenase type IV		
TIMP-3			
TIMP-4	Pro-MMP-2		
PAI-1	Plasminogen Activator		
PAI-2	Plasminogen Activator		

proteases secreted by invasive tumor cells have high enzymatic activity for the cleavage of Collagen IV to allow the penetration of the stroma. The proteolytic cleavage of Laminin-5 or Collagen IV by MMPs exposes cryptic sites that promote cell migration.

5.1.1 Matrix Metalloproteinases

MMPs (Matrix Metalloproteinases) can regulate the tumor microenvironment. Their expression and activation is increased in almost all cancers.

According to their structure, there are eight classes of MMPs. They include:
– Minimal domain MMPs
– Simple Hemopexin domain containing MMPs
– Gelatin binding MMPs
– Furin-activated secreted MMPs
– Vitronectin-like insert MMPs
– Transmembrane MMPs
– GPI anchored MMPs
– Type II transmembrane MMPs

The members of the Metalloproteinase family have four major domains, the translocation signal domain that targets the molecule to the endoplasmic reticulum, the pro-form domain that maintains a latent state, the catalytic domain, and the Hemopexin domain that binds to inhibitors (Figure 5.1.1.A). The auto-inhibitory pro-form domain contains the conserved sequence PRCGXPDV, in which the cysteine residue interacts with the Zn^{2+} atom in the catalytic site. Disruption of the Zn^{2+}–cysteine interaction exposes the Zn^{2+} in the active site, allowing H_2O to bind and activating the Metalloproteinase by a mechanism known as the cysteine switch. Activation of the cysteine switch may involve S-nitrosylation of the reactive cysteine and further oxidation, which forms a stable sulfinic or sulfonic acid, leading to MMP activation.

Extracellular matrix fragments or cryptic sites, when unmasked by proteinases, can affect tissue remodeling and cancer progression. MMP-2 cleaves the Laminin-5 γ2 subunit at residue 587. This cleavage of Laminin-5 by MMP-2 reveals a cryptic site that triggers cell motility. The cleaved form of Laminin-5 arises in tumors and in tissues undergoing remodeling, but not in quiescent tissues [Giannelli et al. 1997]. The cleavage of Laminin-5 by MMP-14 also reveals a cryptic site that triggers cell motility, and MMP-14 co-localizes with Laminin-5 in cancer [Koshikawa et al. 2000].

The function of transmembrane molecules can frequently be altered by the cleavage and subsequent release of their ectodomains. MMP-3 and MMP-7 cleave E-Cadherin. The released 80 kD fragment of E-Cadherin promotes tumor cell invasion in a paracrine manner [Noe et al. 2001]. Physiologically, the shedding of the E-Cadherin ectodomain is important in epithelial repair after damage. In prostate cancer, HGF (Hepatocyte Growth Factor, Scatter Factor) induces the cleavage of the extracellular domain of E-Cadherin by MMP-7. This results in the dissociation of the Cadherin/Catenin complex and increased invasiveness.

MMP-14 (MT1-MMP, Membrane-Type 1 Matrix Metalloproteinase) is a potent modulator of the pericellular environment through its proteolytic activity. Migratory cells, including invasive tumor cells, frequently express CD44 and MMP-14. During cell migration, MMP-14 binds to the standard form of CD44 (CD44s, CD44H) through the Hemopexin domain and localizes at the migration front. CD44s acts as a linker that connects MT1-MMP to the actin cytoskeleton, thus playing a role in directing MT1-MMP to the migration front. Conversely, MMP-14 acts as a processing enzyme for CD44s, releasing it into the medium as a soluble 70 kD fragment. This processing event stimulates cell motility. The Hemopexin (PEX, HPX) domain of MT1-MMP is indispensable in promoting cell migration and CD44s shedding. Due to the ability of the Hemopexin domains to bind CD44s, other members of the MT-MMP family induce varied extents of CD44s shedding, depending on the catalytic domains. CD44s may act as a core molecule assembling multiple MT-MMPs on the cell surface [Kajita et al. 2001; Suenaga et al. 2005].

Unlike oncogenes, *mmp* genes are not upregulated in cancer by gene amplification or activating mutations. Their elevated expression is due to transcriptional activation.
– *mmp* gene expression is frequently induced as a result of oncogenic signaling. Consistently, the expression of many *mmp*s is inhibitable by

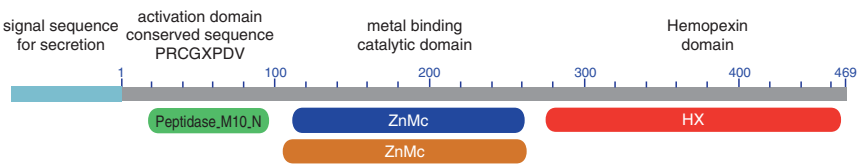


Figure 5.1.1.A. Functional domains of MMPs. Model for the functional domains of Metalloproteinases. Modified from a domain model for MMP-1 according to NCBI structure. [From http://www.ncbi.nlm.nih.gov/Structure/]

differentiating or tumor suppressive agents, including retinoids, corticosteroids, and TGF-β.
– Activity of the transcription factors Snail and SIP-1 (SMAD Interacting Protein-1) is associated with epithelial–mesenchymal transition. The expression of MMP-1, MMP-2, MMP-7, and MMP-14 is strongly upregulated by Snail and SIP-1.
– Various polymorphisms in *mmp* promoters exist, which affect gene transcription and influence cancer susceptibility.

The expression of *mmp-1* (*fibroblast collagenase*, *interstitial collagenase*) is induced by Interferons and growth factors (EGF, bFGF, PDGF, TNF). In osteoblasts, a signal transduction pathway involving cyclic AMP and PKA can activate *mmp-1* transcription. The *mmp-1* gene promoter contains a composite PPARγ/AP-1 cis-acting element at –83 to –77. The PPARγ and c-FOS/c-JUN (AP-1) proteins bind this site in a mutually exclusive way, with PPARγ binding resulting in repression and AP-1 binding resulting in transcriptional activation. A single nucleotide polymorphism at position 1607 in the *mmp-1* promoter partially regulates gene expression. It contains either G or GG. The presence of GG creates a functional ETS binding site that is adjacent to an AP-1 site and enhances transcription, leading to elevated levels of MMP-1 [Rutter et al. 1998].

mmp-2, which encodes the 72 kD type IV Collagenase (Gelatinase A, Neutrophil Gelatinase), is induced by TNF, EGF, and 1,25-$(OH)_2$ vitamin D_3. *mmp-2* is uniquely regulated on the transcriptional level. In contrast to *stromelysins* and *interstitial collagenase*, it is not upregulated by phorbol esters due to the absence of an AP-1 site in its promoter region and it is not decreased by TGF-β_1 due to the absence of a TGF-β_1 inhibitory element (TIE). A common C→T transition at position –1306 of the *mmp-2* promoter disrupts an SP-1 site (CCACC box). This polymorphism is associated with a substantially lower promoter activity in the T allele due to the abolition of SP-1 binding [Price et al. 2001].

mmp-3 (*stromelysin*, *transin*) is induced by Interferons, Interleukin-1, TNF, bFGF, and heat shock. Signaling through PKC, cyclic AMP, ETS-1, or ETS-2 leads to *mmp-3* expression. The gene is repressed by glucocorticosteroids. A polymorphism in the *mmp-3* gene contains either 5A or 6A. The 6A allele has 50% the transcriptional activity of the 5A allele [Egeblad/Werb 2002].

mmp-7 (*matrilysin*, *pump-1*) is inducible by VEGF and Interleukin-8. It is upregulated through the combined activation of the transcription factors PEA3, c-JUN, β-Catenin, and LEF-1, all of which are downstream of proto-oncogene pathways.

The degrading nature of MMP functions necessitates the tight regulation of their activation. This may occur in cascades, where pro-forms are sequentially activated by proteolytic processing. The pro-forms of MMPs are kept inactive by the interaction between a cysteine sulfhydryl group in the pro-peptide domain and the zinc ion bound in the catalytic domain. Activation requires the proteolytic removal of the propeptide pro-domain. MT-MMPs can be activated by intracellular Furin-like serine proteinases before they reach the cell surface. pro-MMP-1 (Interstitial pro-Collagenase) and pro-MMP-3 (pro-Stromelysin) can be activated by Plasmin. MMP-3, in turn, may activate MMP-1 (Interstitial Collagenase) and MMP-9 (92 kD Collagenase type IV). The 72 kD type IV pro-Collagenase (pro-MMP-2) is processed in response to mitogenic stimuli. Type IV Collagenases are secreted in latent forms complexed with their Tissue Inhibitors of Metalloproteinases (TIMPs) [Stracke and Liotta 1995].

The 450 kD glycoprotein Thrombospondin-1 (TSP-1) interacts in multiple ways with MMP-2 and MMP-9, depending in part on the nature of proteolytic Thrombospondin fragments released by the action of proteases [Donnini et al. 2004]. The 25 kD TSP-1 fragment may enhance the expression of *mmp-2* and *mmp-9*. In endothelial cells, Thrombospondin-1 upregulates MMP-9 expression and promotes cell invasion. Thrombospondin-1 also induces MMP-2 activation through posttranslational mechanisms. This mode of MMP-2 activation may be relevant to the induced migration of vascular smooth muscle cells [Lee et al. 2003]. In contrast, the 140 kD proteolytic TSP-1 fragment blocks the stimulation of *mmp-2* and *mmp-9* expression and inhibits MMP activity by preventing the activation of pro-MMP-2 [Bein/Simons 2000]. Thrombospondins-1 and -2 interact through their Properdin-like type 1 repeats (TSR) with the NH_2-terminal region of MMP-2, which contains the catalytic domain. Neither TSP-1 nor TSP-2 is degraded by MMP-2.

Secreted Metalloproteinases can localize to the cell surface by interactions with cell surface associated heparin sulfate proteoglycans, Collagen IV, or EMMPRIN. The Integrin $\alpha_V\beta_3$ can bind the MMP-2

on the surface of invasive tumor cells [Brooks et al. 1996], which leads to the degradation of surrounding Collagen. Negative feedback regulation of this Protease binding to Integrin is mediated by the MMP-2 dependent generation of Hemopexin fragments (PEX), encoded in the COOH-terminal domain of MMP-2, which block protease activation by competing with MMP-2 for the binding to Integrin $\alpha_V\beta_3$ [Brooks et al. 1998]. Similarly, CD44 may bind and present the MMP-9 to the surrounding stroma [Yu/Stamenkovic 1999].

Invasive cells secrete MMP. Mesenchymal cells, particularly fibroblasts, are also a major source of MMP. Gelatinolytic activity in the liver is high, in the kidneys low, and it is absent from the lungs. In most healthy adult tissues, the levels of MMP-1 are usually low. By contrast, its expression is elevated when the system faces a disturbance, such as wound healing, repair, or remodeling processes. MMP-11 (Stromelysin-3) is transiently expressed by cells of mesenchymal origin in association with remodeling processes that occur during embryogenesis and tissue involution. MMP-11 acts at epithelial–connective interfaces and is involved in epithelium homeostasis.

- The expression of *mmp-1* is induced by Interferons and growth factors (EGF, bFGF, PDGF, TNF). It is also associated with enhanced susceptibility to lung cancer and ovarian cancer.
- A single nucleotide polymorphism in the *mmp-1* promoter contains either G or GG. The presence of GG in tumors from patients leads to elevated levels of MMP-1 as compared to G homozygotes. The frequency of the 2G/2G genotype is higher in cancer patients than in the healthy population, and it is associated with increased likelihood of tumor invasiveness [Rutter et al. 1998] in ovarian cancer [Kanamori et al. 1999], colorectal cancer [Ghilardi et al. 2001], and melanoma [Ye et al. 2001].
- HSP90α is expressed on the surface of invasive cells, including fibrosarcoma and breast cancer, where it interacts with and activates MMP-2. A decrease in the activity of HSP90α leads to reduced invasiveness [Eustace et al. 2004].
- In ovarian cancer, lysophosphatidic acid induces the secretion of pro-MMP-2. This may depend on the upregulation of Integrin β_1 expression by lysophosphatidic acid. β_1 Integrin clustering on the cell surface promotes the activation of pro-MMP-2 and the processing of Membrane Type 1-MMP [Fishman et al. 2001].
- *mmp-2* mRNA is expressed by stromal cells in breast tumors, whereas the MMP-2 protein is present on stromal and cancer cell membranes. MMP-2 localizes to the carcinomatous breast, where the degree of activation of MMP-2 correlates well with tumor grade and patient prognosis.
- In invasive cells, predominantly melanomata and gliomata, the pro-form of MMP-2 is cleaved and activated on the cell surface by a multimeric complex, which contains Integrin $\alpha_V\beta_3$, MMP-14 (MT1-MMP), and TIMP-2.
- MMP-2 and MMP-9 may be important for the metastasis of lung cancer cells. A single nucleotide polymorphism at position -1306 in the *mmp-2* promoter sequence generates (C allele) or destroys (T allele) a SP-1 site and thus affects gene expression. Subjects with the CC genotype have a twofold increased risk for developing lung cancer [Yu C et al. 2002]. Bearers of the CC genotype of the *mmp-2* promoter also have a threefold increased risk for gastric adenocarcinoma [Miao et al. 2003]. In addition, MMP-9 is related to gastric cancer metastasis. Stromal cells release this MMP, which affects tumor aggressiveness. Furthermore, MMP-9 is upregulated by Thrombospondin-1 in gastric cancer [Albo et al. 2002].
- Squamous cell carcinoma may depend on the secretion of MMP-9 by stromal cells.
- VEGF signaling in squamous cell carcinoma induces the expression of MMP-9 and MMP-13 in stromal cells, increased turnover of extracellular matrix components, and neovascularization.
- A polymorphism in the *mmp3* gene contains either 5A or 6A. The frequency of homozygotes for the 6A allele, which has 50% the transcriptional activity of the 5A allele, is lower in cancer patients than in the healthy population [Egeblad/Werb 2002].
- Activation of SRC or the WNT pathway are frequent occurrences in colon carcinogenesis. They synergize in transactivating the *mmp-7* promoter. SRC signaling induces c-JUN binding to the proximal AP-1 binding site (–61 to –67). C-JUN interacts with the HMG box transcription factor LEF-1, which binds to its cognate site (–109 to –194) [Rivat et al. 2003].

5.1.2 Proteinases of the Fibrinolytic System

Components of the fibrinolytic system (Plasminogen activation system) are important for the degradation of the extracellular matrix. The zymogen Plasminogen is synthesized in the liver and deposited

in tumors in response to hyper-permeability. The conversion of Plasminogen to Plasmin is regulated by the two Plasminogen activators TPA and UPA. UPA can be localized at the tumor cell surface by binding to a specific receptor, UPAR. Plasmin facilitates tumor cell migration, invasion, and metastasis by degrading Fibrin and other matrix proteins directly and by activating several Metalloproteinases that additionally degrade the extracellular matrix.

Plasminogen {6q26} is the zymogen in the circulating blood, from which Plasmin is formed. Plasminogen is a single chain glycoprotein with 790 amino acid residues. Its activation to the active form, Plasmin, involves cleavage at the arginine–valine bond between residues 560 and 561, resulting in the formation of the two-chain Plasmin molecule, held together by two disulfide linkages. Plasmin belongs to the family of serine proteinases, in which the active site catalytic triad histidine, asparagine, and serine is situated in the light chain. Plasmin digests Fibrin in blood clots. It is also is capable of degrading the extracellular matrix components Laminin and type IV Collagen. Cleavage by Plasmin can activate other proteases. Collagenase type IV becomes active after cleavage by Stromelysin, while pro-Stromelysin and Interstitial pro-Collagenase are activated by Plasmin.

UPA {10q24} is secreted as a single chain inactive pro-enzyme, which is cleaved by other proteinases, including Cathepsin B, into two chains connected by a disulfide bridge. The active UPA converts Plasminogen to Plasmin. Plasminogen Activator may also activate HGF (Hepatocyte Growth Factor, Scatter Factor) by cleaving the single chain form to generate the active two-chain form. UPA binds to Plasminogen Activator Receptors on cell surfaces and is consecutively internalized.

Tissue-Type Plasminogen Activator (TPA, PLAT) {8p12} is a serine protease that activates the pro-enzyme Plasminogen to Plasmin. TPA is synthesized in vascular endothelial cells as a single polypeptide chain, the proteolytic cleavage of which at a centrally located arginine-isoleucine bond gives rise to a disulfide linked heterodimer, composed of a NH_2-terminally derived heavy chain and a COOH-terminal light chain. The activities of TPA and UPA are negatively regulated by PAI-1 (Plasminogen Activator Inhibitor-1, PLANH-1, Serpin E1) {7q21.3-q22} [Ginsburg et al. 1986].

Thrombin (Coagulation Factor II) {11p11-q12} is correlated with the tumor stage in various carcinomata and is associated with cell invasion and extracellular matrix degradation [Koivunen et al. 1991; Walz/Fenton 1995]. A cell surface receptor for Thrombin is PAR-1. It belongs to the family of protease-activated receptors. These receptors are coupled to G-Proteins and undergo proteolytic cleavage of their NH_2-terminus and subsequent auto-activation by a tethered peptide ligand. PAR-1 exhibits an extended NH_2-terminus containing both a putative Thrombin cleavage site ($LDPR^{41}S$) and a Hirudin-like domain ($K^{52}YEPF$) complementary to the anion binding exo-site in Thrombin. Thrombin binds to PAR-1 with increased affinity through this Hirudin-like site and subsequently cleaves the extended NH_2-terminus with the motif SFLLRN. The second extracellular loop of PAR-1 contains several residues important for ligand/receptor interactions. The intracellular loops are sites for G-Protein coupling and the cytoplasmic tail contains numerous sites for receptor phosphorylation, which leads to receptor inactivation and internalization. In many cells, PAR signaling results in the activation of RHO-A and other members of the RHO family of small GTPases that are involved in cytoskeletal re-organization. PAR-1 is widely distributed among cells and tissues, consistent with the widespread effects of Thrombin.

Activation of PAR-1 increases the phosphorylation of FAK (Focal Adhesion Kinase) and Paxillin, and the induced formation of focal contact complexes. PAR-1 activation affects the Integrin distribution on the cell surface without altering their expression levels. The Integrin $\alpha_V\beta_5$ is recruited to focal contact sites [Even-Ram et al. 2001]. The ligation of Integrin $\alpha_V\beta_3$ on invasive cells induces the transcription of *upar* and *pai-1* and results in a significant increase in cell surface associated Plasmin levels.

- Exposure of breast carcinoma cells to estrogens induces the expression of *upa* and *tpa*. Osteopontin induces *upa* expression in breast tumor cells [Tuck et al. 1999]. This activates Plasminogen, which is required for the degradation of Collagen I during bone metastasis by breast cancer. Plasminogen Activator Receptors are expressed in breast carcinoma, but not in normal breast tissue. Breast cancer cell invasion is promoted by the activation of PAR-1 in cooperation with the Integrin $\alpha_V\beta_5$.
- Glioblastomata have high Plasminogen Activator and PAR content [Gladson et al. 1995].

- The UPA system is associated with the development of melanoma, with PAR-1 being localized in melanoma cells.
- High levels of Plasminogen Activator arise at the zone between colon adenocarcinomata and normal epithelium. Similarly, gastric carcinomata express high Plasminogen Activator.
- In ovarian cancer, lysophosphatidic acid may induce the secretion of UPA through EDG (Endothelial Cell Differentiation Gene) Receptors (EDG-2 or EDG-4). EDG Receptors are G-Protein coupled receptors that activate RHO-GTPases.

5.1.3 Cathepsins

Cathepsins represent major components of the lysosomal proteolytic system. There are 11 Cathepsins (B, C, F, H, K, L, O, S, V, W, X). They are synthesized as inactive precursors consisting of a signal sequence, a pro-peptide, and a catalytically active mature region. Activation of the pro-enzyme usually occurs following cleavage and dissociation of the NH_2-terminal pro-region. Peptides corresponding to the pro-regions of cysteine proteases are potent, selective inhibitors of the parent enzymes. Cathepsins contain phospho-mannosyl residues, which bind to Mannose-6-Phosphate Receptors. They are routed to the endosomal/lysosomal compartment via the Mannose 6-Phosphate Receptor pathway. The Cathepsins B, H, and L are lysosomal thiol proteases. The Cathepsins H, L, B, and S are Papain family cysteine proteases involved in pro-enzyme activation, enzyme inactivation, antigen presentation, hormone maturation, tissue remodeling, and bone matrix resorption. All of them are glycoproteins and contain an essential cysteine residue in their active site, but they differ in their substrate specificities and pH stability.

Cathepsins D (CTSD) {11p15.5} and E (CTSE) {1q31} are members of the Pepsin protease family expressed in the gastrointestinal tract. Cathepsin D is an aspartyl proteinase that is secreted as a pro-form. It is regulated by intracellular pH, growth factors, hormones, and endogenous inhibitors. Cathepsin E is an intracellular proteinase with the highest concentration in the surface of epithelial, mucus producing cells of the stomach. Multiple transcripts result from alternative poly-adenylation of the primary transcripts of the *ctse* (*cathepsin E*) gene.

- Cathepsin D is induced in hepatomata, thyroid cancers, melanomata, bladder cancers, as well as in gastric and colon carcinomata. Because Cathepsin D is an aspartic proteinase expressed very early in the fetal stomach and is also expressed in more than 50% of gastric cancers, it has the characteristics of an oncofetal gene product.
- In breast cancer, Cathepsin D is induced by estrogen, IGF-1 (Insulin-Like Growth Factor 1), basic FGF, or EGF.
- Cathepsin D levels are elevated in high-grade astrocytoma. Glioblastoma invasion depends on Cathepsin D, suggesting that the enzyme activity is involved in the invasion process [Levicar et al. 2002].

Cathepsin B (Amyloid Precursor Protein Secretase, APPS) is a 29 kD cysteinyl proteinase that is associated with the plasma membrane and with endosomes. It is secreted as an inactive 42 kD pro-form. Cathepsin B cleaves the Amyloid β peptide, and it converts pro-Urokinase into an active form. The *cathepsin B* gene {8p22} spans nearly 27 kb and contains 12 exons. Two alternatively spliced exons, designated 2a and 2b, exist between exons 2 and 3 in the 5′ untranslated region. In addition, there are at least three upstream translation initiation codons. The 339 amino acid pre-pro-Cathepsin B contains a 17 residue NH_2-terminal pre-peptide, followed by a 62 residue pro-peptide, 254 residues in the mature Cathepsin B, and a six-residue COOH-terminal extension.

Cathepsin K (CTSK, CTSO2) {1q21} is a cysteine protease that exhibits strong degradative activity against the extracellular matrix and is involved in osteoclast mediated bone destruction. It is expressed as a single transcript of 1.7 kb and yields a protein of 329 amino acids.

Cathepsin L {9q21-q22} is a lysosomal cysteinyl proteinase with a major role in intracellular protein catabolism. It also shows the most potent collagenolytic and elastinolytic activity of the Cathepsins. Cathepsin L proteolytically inactivates α-1 Protease Inhibitor, a major controlling element of Neutrophil Elastase activity. Two distinct *cathepsin L* (*ctsL*) mRNAs, encoded by a single gene, are concurrently expressed in adenocarcinoma, hepatoma, and renal cancer cells. The 3′ end

of the first intron contains the 5′ portion of the alternative mRNA and is contiguous to the second exon of the gene, suggesting either the possibility of splicing or the presence of a second promoter within the first intron of the *ctsL* gene [Cauhan et al. 1993].

- Elevated levels of Cathepsin B occur in gliomata, lung, pancreas, prostate, breast, and stomach carcinomata.
- In high-grade astrocytoma, transcript abundance, protein level, and activity of Cathepsin B are increased compared with low-grade astrocytoma and normal brain. The Cathepsin B level in glioblastomata correlates with invasion and serves as a marker for prognosis.
- A high level of Cathepsin B protein is a marker for invasive types of meningioma, distinguishing between invasive meningiomata and noninvasive clear-benign meningiomata [Levicar et al. 2002].
- In addition to its high expression in osteoclasts, where it plays an essential role in the degradation of protein components of bone matrix, Cathepsin K is expressed in a significant fraction of breast and prostate cancers, where it can contribute to tumor invasiveness and bone destruction.
- Giant cell tumors are neoplasms of bones characterized by a localized osteolytic lesion (Figure 5.1.3.A). Cathepsin K, Cathepsin L, and MMP-9 are the preferentially expressed. While there is high Cathepsin K activity, MMP-9 is primarily present as an inactive pro-enzyme. Cathepsin K, its associated proton pump V-H^+-ATPase, and MMP-9 are exclusively expressed in the osteoclast-like giant cells, whereas Cathepsin L expression is confined to mononuclear cells. The osteoclast-like giant cells are responsible for osteolysis in this condition [Lindeman et al. 2004].

The 28 kD Cathepsin H is a glycoprotein that possesses amidase and esterase activity, but does not have carboxypeptidase activity. Two forms of Cathepsin H {15q24-q25}, the full length form and a truncated form with a 12 amino acid deletion in its signal peptide region are expressed. This deletion occurs likely at the level of RNA processing. The deletion within the signal peptide region affects the trafficking of Cathepsin H, with the truncated form being secreted and having a reduced lysosomal association as compared with the full length Cathepsin H. The truncated form remains enzymatically active and may affect tumor progression [Waghray et al. 2002].

- There is a significant increase in *cathepsin H* expression in high-grade prostatic intraepithelial neoplasia and in carcinoma of the prostate.

Cathepsin S {1q21} is a lysosomal protease that has a restricted tissue distribution, with highest levels in spleen, heart, and lung macrophages. The high expression in the spleen and in phagocytes implies specific functions in the immune system. The *cathepsin S* promoter contains two SP-1 binding sites, at least 18 AP-1 binding sites, and CA microsatellites.

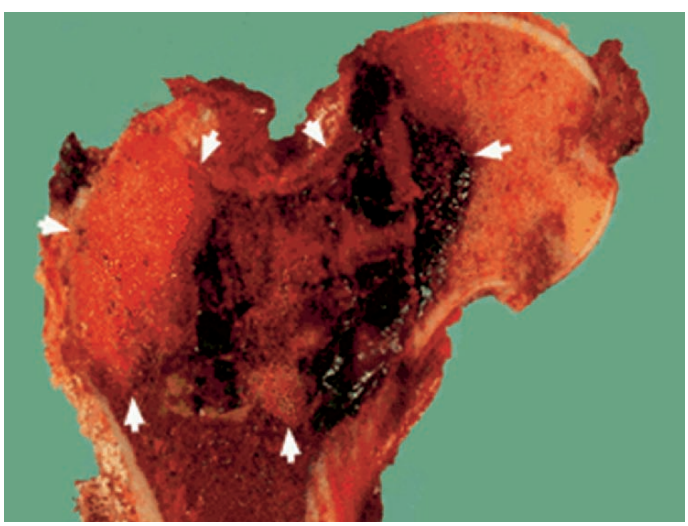


Figure 5.1.3.A. Giant cell tumor. Giant cell tumors of the bone (osteoclastomata) are usually large and distinctly red. Areas of hemorrhage and cystic degeneration are frequent. The large central lesion surrounded by white arrows is tumor. Note the areas of hemorrhage, which is a common feature in giant cell tumors. Histologically, the tumor is composed of mononuclear cells and many multinucleate cells, often with 40–100 nuclei. Giant cell tumors usually arise in the third to the fifth decade of life. They are slightly more common in females. These are locally aggressive tumors that usually do not metastasize distantly. The most common location is around the knee joint, but any other bone may be involved. Symptoms are usually related to the involvement of the joint, including pain and restriction of movement. Occasionally, the patient may present with a fracture. The tumor has a tendency to local recurrence after conservative treatment. [Reproduced from: http://pathweb.uchc.edu. With permission.]

- Cathepsin S expression is upregulated in astrocytoma cells and there is evidence for a potential role by Cathepsin S in invasion [Flannery et al. 2003].

Cathepsin O (CTSO1) is a polypeptide of 321 amino acids, which is widely expressed.

- Cathepsin O is expressed in breast carcinoma [Velasco et al. 1994].

5.1.4 Kallikreins

Secreted proteases may contribute to invasiveness through the modulation of the tumor microenvironment. Kallikreins are a family of several single chain, secreted proteases, which contain the conserved catalytic triad of serine proteases. Their molecular mass is 25–30 kD. In addition to the catalytic triad of histidine, asparagine, and serine, Kallikreins contain 5–6 conserved disulfide bonds.

Kallikreins are synthesized as pre-proenzymes containing a NH_2-terminal signal sequence that directs them to the endoplasmic reticulum for secretion, a pro-peptide that maintains them as inactive precursors, and a serine protease domain responsible for catalytic activity. The proteolytic activity of Kallikreins is regulated by zymogen activation, complex formation with endogenous inhibitors, inhibition by inorganic ions, and proteolysis. Kallikrein-1 activates MMP-2 and MMP-9, resulting in the degradation of the extracellular matrix. Kallikreins-2 and -4 activate the pro-form of UPA and Kallikrein-2 inactivates PAI-1, the combination of which supports invasiveness. Kallikrein-6 is able to auto-activate as well as proteolyse itself, leading to inactivation. It cleaves with much higher efficiency after arginine than lysine and with a preference for serine or proline in the P2 position. It efficiently degrades Fibrinogen and Collagen types I and IV [Magklara et al. 2003]. Once activated, Kallikreins are tightly controlled by endogenous inhibitors, mainly α_2-Macroglobulin and Serpins in fluids and tissues.

The *kallikrein* locus spans about 300 kb on the long arm of chromosome 19 {19q13.4}. It includes 15 tandemly located genes belonging to the *kallikrein* family. All *kallikrein* genes consist of five coding exons and four intervening introns with a conserved intron phase pattern (I, II, I, 0). All *kallikrein* genes give rise to multiple transcripts due to alternative splicing, use of alternative start sites, or variant poly-adenylation. The expression of many *kallikrein* genes is regulated by steroid hormones. *klk2* and *klk3* are androgen regulated genes expressed almost exclusively in the prostate. Complexes of androgen and Androgen Receptor bind to androgen response elements (AREs) within the proximal promoter and enhancer regions of *klk2* and *klk3* to stimulate their transcription. In comparison, *klk5* and *klk6* are more responsive to estrogens.

Kallikrein expression and proteolytic activity are dysregulated in tumors, mainly in adenocarcinomata, and are often associated with patient prognosis [Borgoño/Diamandis 2004].

- Kallikreins-2 (Prostase) and -3 (PSA, Prostate Specific Antigen), as well as Kallikrein-11 are markers for prostate cancer progression. Kallikrein-3 is also implicated in the development of osteoblastic bone metastasis in prostate cancer through its support of interactions between prostate cancer cells and bone endothelial cells.
- Kallikrein-5 (Human Stratum Corneum Tryptic Enzyme, HSCTE) is physiologically predominantly expressed in skin, testis, breast, and brain. It is differentially expressed in testicular, breast, and ovarian cancers and may be a marker for these malignancies [Yousef et al. 2003].
- In ovarian carcinoma, 12 *kallikrein* genes are concurrently upregulated. Kallikrein-8 (Neuropsin) in serum and ascites is a marker of ovarian carcinoma [Kishi et al. 2003]. Kallikrein-11 may also be a marker for ovarian carcinomata.

5.1.5 Other Enzymes

Elastase. Elastase is a seryl protease. The Elastin fragments liberated by the Elastin-mediated enzymatic digestion of insoluble Elastin stimulate tumor cell chemotaxis [Yusa et al. 1989], further supporting dissemination. Elastase-1 (ELA-1, Pancreatic Elastase) {12q13} is a member of the pancreatic family of serine proteases. The 31 kD Elastase-2 (ELA-2, HLE, Medullasin, Neutrophil Elastase) {19p13.3} contains 267 amino acids, including a possible leader sequence of 29 amino acids. The protein contains two *N*-linked carbohydrate side chains, and is joined together by two disulfide bonds.

- Elastinolytic activity exists in extracts of human breast carcinomata and is exponentially related to the age of the patient. There is a parallel

neo-synthesis of Elastin, which also increases with the age of the patient [Hornebeck/Robert 1977].

- Polymorphonuclear Elastase activity is present in colorectal carcinomata with particular enrichment at the tumor–host interface. This Elastase is mainly produced by infiltrating neutrophils, whereas the colon carcinoma cells do not express it. This suggests an interaction between tumor cells and Elastase producing leukocytes [Bjornland et al. 1998].
- Elastin surrounds micro-vessels in the pulmonary circulation and may pose a barrier to the extravasation of metastatic tumor cells. Lung colonizing melanoma cells produce Elastase activity.

Heparanase. Heparan sulfate proteoglycans are proteins substituted with glycosaminoglycan side chains, predominantly of the heparan sulfate type. They are among the main components of basement membranes and extracellular matrix. In addition to proteinases, the endoglycosidase Heparanase [Hulett et al. 1999; Vlodavsky et al. 1999], which cleaves haparan sulfate chains, is needed for efficient degradation of the extracellular matrix. It is implicated in the degradation of the subendothelial basement membrane by leukocytes and cancer cells. In healthy tissues, the expression of Heparanase is restricted primarily to lymphoid organs and the placenta [Parish et al. 2001].

Heparanase is a polypeptide of 543 amino acids and a molecular weight of 61 kD. It is processed by the cleavage of an internal 6 kD peptide to yield a heterodimer of the NH_2-terminal 8 kD peptide (amino acids 36–109) with the COOH-terminal 50 kD peptide (amino acids 158–543). The processed form is at least 100-fold more active than its precursor [Vlodavsky et al. 1999]. Like other extracellular matrix degrading enzymes, Heparanase may be displayed on the cell surface, possibly by the 300 kD Mannose Phosphate Receptor [Parish et al. 2001].

Angiogenic factors, such as Fibroblast Growth Factors, often have high affinity to heparin, which anchors them in the basement membrane or extracellular matrix. Heparanase activity can release angiogenic FGFs from the extracellular matrix. Heparanase may therefore participate in both tumor cell invasion and angiogenesis through degradation of the extracellular matrix heparin sulfate and mobilization of angiogenic factors.

- Heparanase is expressed in carcinomata of the breast, colon, and liver [Vlodavsky et al. 1999]. High expression levels of Heparanase by tumor cells enhance their invasiveness [Koliopanos et al. 2001]. Its overexpression in primary and metastatic pancreatic cancer is associated with reduced life expectancy.

5.2 HOMING RECEPTORS AND THEIR LIGANDS

The migration of leukocytes such as neutrophils, monocytes, and lymphocytes into lesions is among the critical events of inflammation. During the acute phase of inflammatory and allergic diseases, the predominantly migrating cells are neutrophils and mast cells, respectively. In the subsequent chronic phase, mainly monocytes and lymphocytes migrate. Proteases and cytokines mediate leukocyte migration and may have a role in the transition from acute inflammation to chronic inflammation and delayed type hypersensitivity [Tani et al. 2001]. The interaction of circulating white cells and vascular endothelium involves five families of adhesion molecules: Selectins, members of the Immunoglobulin superfamily, Integrins, Carbohydrate-Rich Proteins, and Chemokine Receptors. The expression of chemokine receptors and Carbohydrate-Rich Proteins, such as CD44, enable the white blood cells to home to a site of damage. Inflammatory cytokines cause the immediate expression of P-Selectins on endothelial cells for binding to carbohydrate residues on circulating neutrophils, leading to rolling. Within minutes, P-Selectin is lost and replaced by E-Selectin on endothelial cells and L-Selectin on leukocytes, which bind to GlyCAMs or Mucins during the ensuing 1–2 h. Subsequently, Immunoglobulin superfamily molecules are expressed on endothelium and Integrins are expressed on leukocytes forming stable adhesions. After retraction of the endothelial cells, the expression of other Integrins on neutrophils leads to invasion into the surrounding tissue [Buck 1995].

5.2.1 Selectins

Selectins are homologous proteins comprising the three groups of L-Selectins, E-Selectins, and P-Selectins [Bevilacqua et al. 1991]. They are most prominently involved in heterotypic adhesion between blood cells and endothelial cells. The

interactions of Selectins with their ligands are important for lymphocyte homing into the skin, but not for lymphocyte extravasation into visceral organs. The extracellular portion of each Selectin is composed of three distinct domains, a C-type lectin domain of 120 amino acids in the NH_2-terminus, an Epidermal Growth Factor type domain of 30–40 amino acids, and 2–9 short consensus repeats (around 60 amino acids) that are characteristic of Complement Regulatory Proteins. Selectins are anchored in the membrane through a single helicoidal transmembrane domain that is connected to a short cytoplasmic tail (Figure 5.2.1.A).

Selectins recognize sialylated, fucosylated carbohydrates, specifically glycans containing a terminal sialyl Lewis^X (SLe^X). This oligosaccharide molecule is predominantly expressed on the surface of granulocytes, natural killer cells, and monocytes. The sialyl Lewis sugars alone have only a weak affinity for Selectins, whereas the presenter proteins have no binding capacity by themselves. Therefore, the loss of sialic acids (derivatives of neuraminic acid, where the amino-group is substituted by either an acetyl- or glycolyl-group) from the surface of cells abolishes their ability to bind to Selectins. Likewise the free tetrasaccharide sialyl Lewis^X (Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc) competitively blocks cell adhesion to Selectins.

Glycosylation-Dependent Cell Adhesion Molecule-1 (GlyCAM-1) and CD34 are Mucin-like ligands for L-Selectin that are both presented by endothelial cells. GlyCAM-1 on high endothelial venules (HEVs) facilitates the homing of lymphocytes to lymph nodes. Mucosal Addressin Cell Adhesion Molecule-1 (MadCAM-1) is a L-Selectin ligand on the surface of endothelial cells in mesenteric venules. α-(1,3)-Fucosyltransferases synthesize sialyl Lewis^X and sialyl Lewis^A, the ligands for E- and P-Selectins. There are 7 *α-(1,3)-fucosyltransferase* genes *fuc-T I*, *II*, *III*, *IV*, *V*, *VI*, and *VII*. The message of *fuc-T III* is expressed in most normal epithelial cells, correlating with the surface expression of these carbohydrate determinants. Leukocytes also contain *fuc-T IV* mRNA. Two receptors for P-Selectin on myeloid cells and lymphocytes are CD24 and P-Selectin Glycoprotein Ligand-1 (PSGL-1). CLA is a variant of PSGL-1 that is generated through glycosylation by Fucosyltransferase VII (Fuc-T VII). Deficiency of α-1,3-Fucosyltransferase VII (Fuc-T VII) is characterized by a deficiency of E-, P-, and L-Selectin ligand activity, as reflected in reduced CD4$^+$ T-lymphocyte-mediated contact hypersensitivity reactions as well as reduced CD8$^+$ T-lymphocyte-mediated delayed-type hypersensitivity reactions. These reduced inflammatory reactions of the skin are due to inefficient lymphocyte extravasation. Because Langerhans cell migration to local lymph nodes as well as CD4$^+$ and CD8$^+$ T-lymphocyte induction are normal, the afferent arm of these reactions is not impaired. Extravasation of CD4$^+$ and CD8$^+$ T-lymphocytes into visceral organs is not compromised in the absence of α-1,3-Fucosyltransferase [Erdmann et al. 2002].

- About 25–35% of patients with breast, colon, thyroid, and gastric cancers display significant levels of sialyl Lewis^X and have a poorer prognosis for survival than patients with low levels of this sugar.
- While the sialyl Lewis^X and sialyl Lewis^A antigens are expressed on many epithelial cancer cells, leukemia cells are positive for sialyl Lewis^X, but they are not normally positive for sialyl Lewis^A.

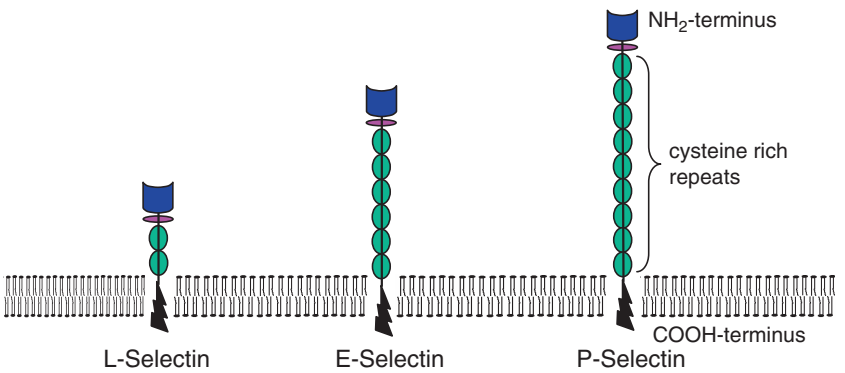


Figure 5.2.1.A. Structure of Selectins. The three types of Selectins comprise L-Selectin, E-Selectin, and P-Selectin. Their COOH-terminal ends constitute the cytoplasmic tail and their NH_2-termini are extracellular. The Selectins differ from each other in the number of cysteine-rich repeats.

- Exposure to Neuraminidase may abrogate the adhesion of tumors to endothelial cells by interfering with the binding to Selectins [Buck 1995].
- The RNA message of *fuc-T III* is expressed in most normal epithelial cells and epithelial cancer cells, correlating with the surface expression of these carbohydrate determinants. The messages of *fuc-T IV* and *fuc-T VI* are present in most epithelial cancer cells, while the message of *fuc-T V* is absent from most of them. The messages of *fuc-T IV*, *V*, and *VI* are expressed in most leukemia cells. The frequent occurrence of *fuc-T IV* mRNA in epithelial cancer cells may be related to their dedifferentiation associated with tumorigenesis [Yago et al. 1993].

L-Selectin. L-Selectin (Lymphocyte Adhesion Molecule 1, LAM-1, CD62L) {1q23-q25} mediates leukocyte adhesion to the endothelium and extravasation in lymph nodes and in sites of inflammation. L-Selectin expression can be down-modulated by shedding from surface membranes. This involves Tyrosine Kinase dependent, PKC-independent pathways of signal transduction.

- Lymphoma cells expressing the lymph node homing receptor L-Selectin metastasize extensively and exclusively to peripheral lymph nodes, a homing phenotype displayed by mature T-cells leaving the thymus [Bargatze et al. 1995; Baumhueter et al. 1994].

E-Selectin. E-Selectin (Endothelial Leukocyte Adhesion Molecule 1, ELAM-1) {1q23-q25} is expressed on endothelial cells after induction by the cytokines Interleukin-1, TNF, or Interferon-γ, but not at rest and not on lymphocytes. The Cutaneous Lymphocyte-Associated Antigen (CLA) and its counterreceptor E-Selectin are involved in the selective targeting of memory T-lymphocytes, reactive with skin associated antigens, to cutaneous inflammatory sites [Santamaria Babi et al. 1995]. E-Selectin can be proteolytically cleaved and released into the circulation. There, it may act as a decoy receptor. Following the stimulation of endothelial cells with TNF-α, three pathways converge on the activation of the *E-selectin* gene promoter. They comprise the NF-κB pathway, the SAPK1 (JNK1, MAPK8) pathway, and the SAPK2 ($P38^{MAPK}$, MAPK14, CSBP1, MXI2) pathway. While the SAPK1 and SAPK2 pathways mediate increases in *E-selectin* promoter activity through activation of the transcription factors c-JUN and ATF2, the SAPK2-mediated activation is ancillary [Laferriere et al. 2002]. The interaction of E-Selectin with its counterreceptors or ligands induces bidirectional signaling in the endothelial cells and in the adhering cells. This regulates the secondary interactions of both types of cells through other adhesion receptors and ultimately leads to shape modifications that allow the transmigration of the adhering cells.

- CLA is expressed by the malignant cells in chronic-phase cutaneous T-cell lymphoma (mycosis fungoides, Sézary syndrome). Mycosis fungoides may progress to the advanced form called Sézary syndrome. In this state, the entire skin is affected. There may also be patches, plaques, or tumors on the skin. Cancerous T-cells are present in the blood.
- Colorectal carcinomata with increased metastatic potential and with poor prognosis are characterized by a high content of sialyl $Lewis^X$ carbohydrate antigens. E-Selectin on activated endothelial cells can bind to colon carcinoma cells [Lauri et al. 1991].
- In breast cancer patients at advanced stages, high concentrations of circulating soluble E-Selectin may be associated with liver metastasis. In contrast, low levels of circulating E-Selectin are associated with a strong prognostic value for overall and disease-free survival in patients with lymph node negative breast cancer [Laferriere et al. 2002].

P-Selectin. P-Selectin (CD62, GMP-140, SELP) {1q23-q25} exists in platelets and endothelial cells, where it is stored in α-granules and Weibel-Palade bodies, respectively. P-Selectin is expressed on the cell surface of platelets following activation by Thrombin and on endothelial cells following activation by histamine, Interleukin-8, Substance P, or peroxides. It mediates the interaction of activated platelets or endothelial cells with leukocytes. The high affinity counterreceptor for P-Selectin on myeloid cells and stimulated T-lymphocytes is P-Selectin Glycoprotein Ligand (PSGL-1) {12q24}. Another receptor is the cell surface antigen CD24 {6q21}, a sialoglycoprotein that is anchored to the cell surface by a glycosylphosphatidylinositol (GPI) linkage [Van der Schoot et al 1989; Fischer et al 1990]. It is expressed on many B-lineage cells and on mature granulocytes, but not on most other hematopoietic cells. The enzymatic activity of Fucosyltransferase VII can place sialyl $Lewis^X$ residues on CD24.

- P-Selectin can bind to a number of carcinomata, including colon, lung, and breast, but it does not

bind to melanoma [Arrufo et al. 1992; Buck 1995].
- CD24 is a ligand for P-Selectin on various carcinoma cells [Aigner et al. 1997]. It serves as a ligand for P-Selectin under flow conditions. The interaction of tumor cells with P-Selectin via CD24 may be an important adhesion pathway in breast cancer metastasis [Aigner et al. 1998].

5.2.2 Immunoglobulin Superfamily Receptors

The Immunoglobulin superfamily is one of the most diverse families of receptors known. They have in common extracellular repeats of Immunoglobulin domains. This superfamily includes Cell–Cell Recognition Molecules (CAMs), as well as AGER, TAX-1, and CEA.

NCAM. NCAM1 (CD56) {1q23.1} is a membrane-bound glycoprotein that contributes to cell–cell and cell–matrix adhesion through both its homophilic and heterophilic binding activity. Structural diversity in NCAM is due to transcriptional and posttranslational variations, which are under tight developmental control. NCAM is expressed on myoblasts and myotubes until innervation. During nerve regeneration, NCAM is upregulated in the dorsal root ganglion and downregulated in the ventral horn [Cunningham 1991]. In the developing brain, there are no radial glia, on which progenitors could migrate from the subventricular zone to the olfactory bulb. They move by homotypic interactions of poly-sialylated NCAM (PSA NCAM) between the migrating progenitor cells and tube-like structures formed by specialized astrocytes. PSA NCAM is also required for migration to the dentate gyrus [Crossin et al. 1990].

- Epithelial–mesenchymal transition describes the transformation of polar epithelial cells into spindle-shaped motile cells that can pass through the basement membrane. Epithelial–mesenchymal transitions are frequently associated with the loss of NCAM, followed by its reexpression [Crossin et al. 1990].
- The expression of highly sialylated, embryonic, less avidly adhesive forms of NCAM (PSA NCAM) on neuroblastomata, rhabdomyosarcomata, Ewing sarcomata, Wilms tumors, pituitary adenomata, pheochromocytomata, and small cell lung carcinomata is associated with increased metastatic potential [Cunningham 1991; Buck 1995; Stracke/Liotta 1995].
- In some tumors, the loss of adhesion through NCAM may facilitate dissemination. The absence of NCAM function in pancreatic cancer causes lymph node metastasis via VEGF-C and VEGF-D mediated lymphangiogenesis [Crnic et al. 2004].

PECAM. PECAM-1 (Platelet Endothelial Cell Adhesion Molecule, CD31) {17q23} is a 130 kD protein with six extracellular Immunoglobulin-like domains, one transmembrane domain, and one cytoplasmic domain. PECAM is expressed on leukocytes and endothelial cells. It is implicated in transendothelial migration and angiogenesis. PECAM-1 may engage in homophilic and heterophilic interactions. Ligands for heterophilic interactions include CD38 and the Integrin $\alpha_v\beta_3$, which may bind directly to the Immunoglobulin domain two of PECAM-1 [Imhof et al. 1996]. The cytoplasmic tail of PECAM-1 is subject to alternative splicing.

- Breast tumors show significantly higher vascularization than normal breast tissue and the density of blood vessels is significantly associated with node metastasis. PECAM is a marker for angiogenesis in this setting [Horak et al. 1992; Charpin et al. 1995].
- Ligands for heterophilic interactions with PECAM include CD38, a transmembrane glycoprotein that is expressed on B-cell chronic lymphocytic leukemia [Ibrahim et al. 2003].

ICAM. The ICAMs (Inter-Cellular Adhesion Molecules) comprise ICAM-1, -2, and -3. ICAMs are ligands for LFA-1. ICAM-1 (CD54) {19p13.3-p13.2} is a 90 kD glycoprotein, encoded by a 3.3 kb RNA message. Its extracellular portion of 453 residues contains five Immunoglobulin domains. ICAM-1 is expressed on hematopoietic cells, vascular endothelial cells, mucosal epithelial cells, and T-lymphocytes. It can be induced by NF-κB. ICAM-1 is strongly upregulated by cytokine stimulation and plays a key role in the arrest of leukocytes in blood vessels at sites of inflammation and injury. Marginal zone B-lymphocytes express elevated levels of the Integrins LFA-1 and $\alpha_4\beta_1$ and bind to the ligands ICAM-1 and VCAM-1. In the absence of this interaction, they are rapidly released from the marginal zone [Lu and Cyster 2002]. ICAM-2 {17q23-q25} is an integral membrane protein that has two Immunoglobulin domains [Staunton et al. 1989]. It can bind to leukocyte Integrins and activate leukocyte adhesion. ICAM-3 {19p13.3-p13.2} is closely

related to ICAM-1, consists of five Immunoglobulin domains, and binds LFA-1 through its two NH_2-terminal domains.

- ICAM-1 on melanoma cells is an indicator of poor prognosis [Stracke and Liotta 1995]. TNF may upregulate the expression of ICAM-1 during the progression of malignant melanoma.
- While ICAM-1 is not expressed on normal hepatocytes it is invariably present on hepatocellular carcinoma cells.
- ICAM-1 is expressed on the majority of renal carcinomata, however it is absent from renal tubular cells. The expression of ICAM-1 on renal cell cancer can be modified by cytokines, produced by tumor infiltrating mononuclear cells. It is possible that this expression of ICAM-1 augments the host immune reaction to the tumor [Tomita et al. 1990].

VCAM. VCAM-1 (Vascular Cell Adhesion Molecule-1, CD106) is a glycoprotein expressed by cytokine-activated endothelial cells. It mediates the adhesion of monocytes and lymphocytes. The *vcam-1* gene {1p32-p31} contains nine exons, spanning about 25 kb of DNA. At least two distinct VCAM-1 precursors can be generated from the gene as a result of alternative mRNA splicing events, which include or exclude exon 5.

- Melanoma adhesion to endothelial cells frequently occurs through the interaction between VCAM-1 and Integrin $\alpha_4\beta_1$.
- In gastric carcinoma, the expression of VCAM-1 is closely related to tumor progression.
- The overexpression of VCAM-1 is likely a major source of elevated serum VCAM-1 [Ding et al. 2003]. In women with early breast cancer, serum levels of VCAM-1 (but not of E-Selectin or von Willebrandt Factor) correlate closely with microvessel density in the tumors. Furthermore, the serum VCAM-1 levels rise in women with advanced breast cancer whose disease progresses [Byrne et al. 2000].

MCAM. MCAM (Melanoma Cell Adhesion Molecule, MelCAM, MUC-18, Gicerin, CD146) {11q23.3} is a 113 kD glycoprotein adhesion receptor. It is expressed on smooth muscle cells, endothelial cells, and Schwann cells, but not on epithelial or hematopoietic cells. This is regulated by AP-2. The presence of binding sites for the transcriptional factors AP-1, AP-2, and CREB in the *mcam* gene is consistent with modulation of its expression by stress induced signaling. T-lymphocytes, upon activation, express MCAM. This occurs in $CD3^+$ cells infiltrating delayed type hypersensitivity lesions of the skin, in synovial fluid T-lymphocytes of rheumatoid arthritis patients, and on distinct T-leukemia cells. MCAM expression on activated T-lymphocytes suggests that this adhesion molecule is involved in extravasation and homing [Pickl et al. 1997].

- Extravasation and tissue infiltration of leukocytes and metastatic tumor cells require the regulated expression and function of adhesive surface molecules. MCAM occurs consistently on neoplasms of mesenchymal origin. Osteosarcoma metastasis to the lungs depends on MCAM [McGary et al. 2003].
- Contact between keratinocytes and melanocytic cells inhibits MCAM expression. This raises the possibility that escape from keratinocyte control during melanoma development contributes to the malignant phenotype [Shih et al. 1994]. MCAM is not present on normal melanocytes or on the cells of benign naevi, but is highly expressed on malignant melanoma cells [Lehmann et al. 1989] and is associated with melanoma metastasis.

ALCAM. ALCAM (Activated Leukocyte Cell Adhesion Molecule, MEMD, CD166) {3q13.1-q13.2} is a 500 amino acid polypeptide, which has a secretory signal sequence and five Immunoglobulin domains. The *alcam* gene encodes mRNA of 5.2 kb in length and is expressed by mitogen-activated, but not by resting leukocytes [Bowen et al. 1995]. ALCAM is a counterreceptor for CD6, a receptor expressed on T-lymphocytes, which is involved in cell adhesion interactions between thymocytes and thymic epithelial cells. ALCAM may also mediate homophilic clustering.

- ALCAM is prevalent in tumors, including malignant melanoma, where it is preferentially expressed in the highly metastasizing forms [Degen et al. 1998; van Kempen et al. 2000].
- ALCAM is upregulated in low-grade prostate cancer and is progressively lost in high-grade lesions. This may reflect a complex role of ALCAM in prostate cancer progression [Kristiansen et al. 2003].

Not all CAMs are important in tumor dissemination. LCAM (Liver Cell Adhesion Molecule) is homologous to E-Cadherin and mediates cell–cell

adhesion of liver epithelium in a calcium-dependent manner. LCAM is essential for compaction in blastocysts and is expressed on all cells at early embryonic stages [Cunningham 1991]. Consistent with this cell cohesive function, no role for LCAM has been defined in metastasis formation.

AGER. AGER (Receptor for Advanced Glycation End products, RAGE) {6p21.3} is a receptor of the Immunoglobulin superfamily that has multiple ligands. The heparin binding protein Amphoterin (High Mobility Group Protein 1, HMG1) {13q12} binds to AGER through a COOH-terminal motif (amino acids 150–183). This induces signaling through the GTPases CDC42 and RAC. Amphoterin ia a key protein in normal neurite outgrowth, reflecting its contributions to cellular migration [Taguchi et al. 2000]. Amphoterin at the cell surface can act as a nucleating site for the generation of the protein degrading complex Plasmin, which activates Matrix Metalloproteinases, including MMP-2 and MMP-9.

- AGER and Amphoterin are functionally important in glioma dissemination [Taguchi et al. 2000].
- AGER expression is closely associated with invasion and metastasis in gastric cancer. AGER-positive cancer cells tend to be distributed at the invasive front of primary tumors and are present in all metastatic foci in lymph nodes [Kuniyasu et al. 2002].
- The coexpression of RAGE and its ligand Amphoterin associates closely with metastasis of colorectal cancer [Kuniyasu et al. 2003b]. In colon cancer cells, the phosphorylation of ERK-1 and -2, RAC1, and PKB, and the expression of MMP-9 are increased to a greater degree by Amphoterin than by advanced glycation end products [Kuniyasu et al. 2003a]. Amphoterin produced by colon cancer cells may also inhibit the function of tumor infiltrating macrophages.
- In prostate cancer, androgen deprivation induces the expression of Amphoterin in prostatic stromal cells. This provides a paracrine interaction between cancer and stromal cells through AGER and is associated with metastasis [Kuniyasu et al. 2003c].

TAX. The axonal cell adhesion molecule TAX-1 (Contactin 2, CNTN2, Transiently-Expressed Axonal Glycoprotein, TAG1, Axonin 1) {1q32.1} is a glycosylphosphatidylinositol (GPI) anchored member of the Immunoglobulin superfamily that is expressed as a 135 kD glycoprotein. It also occurs as a secreted form. TAX-1 contains four Fibronectin III-like domains and six C2 Immunoglobulin-like domains. The *tax-1* gene contains 23 exons spanning about 40 kb. TAX-1 binds to NGCAM and it can form homophilic interactions with other TAX-1 molecules expressed on adjacent cells. It is exclusively expressed in neurons, where it contributes to neurite outgrowth.

- Pathologically, TAX-1 occurs on high-grade gliomata and plays a role in glioma migration [Rickman et al. 2001]. In this setting, *tax-1* is coamplified with *mdm4* and other genes on chromosome 1q32 [Riemenschneider et al. 2003].

CEA. CEA proteins are cell surface glycoproteins involved in cell adhesion. The *cea* gene family encodes products belonging to the Immunoglobulin superfamily of molecules. Members of the CEA family consist of a single N domain with structural homology to the Immunoglobulin variable domains, followed by a variable number of Immunoglobulin constant like A or B domains. The *cea* family consists of 29 genes, 18 of which are normally expressed, while the others are pseudogenes. Based on sequence similarity and functional characteristics, the CEA family is subdivided into the CEACAM subgroup (CEACAM 1-8) and the PSG (Pregnancy-Specific Glycoprotein) subgroup. Members of the CEACAM subgroup are anchored in the cell membrane, whereas members of the PSG subgroup are secreted. Genes in the *ceacam* and *psg* subgroups have a similar gene structure and organization. Exon 1, denoted L, encodes the 5′ untranslated region and part of the signal peptide. Exon 2, denoted L/N, encodes the rest of the signal peptide and the N domain. Depending on the gene, exon 2 is followed by up to six exons, each encoding an A or B domain.

- CEA is commonly expressed on many carcinoma cell types and enhances metastases. CEA is associated with tumors of the gut and lung. High serum levels of CEA are a marker for breast cancer progression [Cheung et al. 2000] and for liver metastasis in colorectal cancers [Duffy 2001].
- CEAs exist intercellularly in the intestine of embryos and adults and are anchored to the cell surface via a phosphatidylinositol linkage [Buck 1995]. Polarization is an early event in colon epithelial differentiation. The abundance of membrane associated CEA is increased when colon carcinoma cells are prevented from polarizing. Rounded,

unpolarized cells express increased levels of CEA molecules throughout their cell surface, where they act as intercellular adhesion molecules, allowing unpolarized cells to form random cell–cell contacts. The pattern of random, multilayered associations of tumor cells may be due to intercellular adhesion mediated by CEA, which is upregulated and expressed throughout the cell surface of unpolarized tumor cells [Yan et al. 1993].

5.2.3 Integrins

Integrins are a large family of cell surface glycoproteins that mediate cell–cell and cell–matrix communication. They are constituted of noncovalently bound heterodimers of α and β chains. There are eight β subunits and 18 α subunits, which are organized into at least 24 different Integrins (Figure 5.2.3.A). Most cells express multiple Integrins on their surface. Integrin α chains comprise 1000–2000 amino acids. They contain a large NH_2-terminal extracellular domain with seven conserved repeats of potential metal binding domains, a transmembrane segment, and a short COOH-terminal cytoplasmic tail. Some also contain an insertion domain (I) of approximately 200 amino acids in the extracellular head region, and some undergo cleavage near the transmembrane domain. The β subunits are shorter, entailing 700–800 amino acids. Each subunit comprises a large extracellular domain, together forming the globular head region, which represents the ligand binding site. The cytoplasmic domains of both, the α and the β subunit, are relatively short, with 30–40 and 40–50 residues respectively. Both segments are linked by a single transmembrane domain. Integrins form a connection between their ligands in the extracellular matrix and the microfilaments inside the cell via intracellular scaffolding proteins. These interactions are essential to determine cell migration, adhesion, or spreading.

The binding of ligands to Integrin receptors leads to conformational changes in the interactions between the α and β chains and to Integrin clustering in focal contacts (hemidesmosomes). The combination of occupancy and lateral recruitment of receptors initiates intracellular signals. The cytoplasmic tail of Integrin β chains binds Talin, Paxillin, and Vinculin. Signal transduction through Integrin receptors depends in part on cell morphology and may proceed in two phases, haptotaxis ("cell crawling") up a gradient of immobilized ligand, followed by adhesion and spreading. Integrins support the formation of contacts during migration. The force transmitted to the sites of adhesion derives from the interaction of Myosin II with Actin filaments that attach to these sites. Myosin II activity is positively regulated by MYLK (Myosin Light Chain Kinase, MLCK) and ROCK. It is negatively regulated by the trimeric MLC Phosphatase, which consists of the catalytic subunit PP1Cδ and the two regulatory subunits MYPT (Myosin Phosphatase Target) and M20.

Integrins are subject to inside-out signaling, which is the activation to a high affinity state for their ligands by cytoplasmic signals. In an inactive state, the extracellular domain of Integrin heterodimers may be bent over, without binding to extracellular ligands. Upon activation, the Integrins

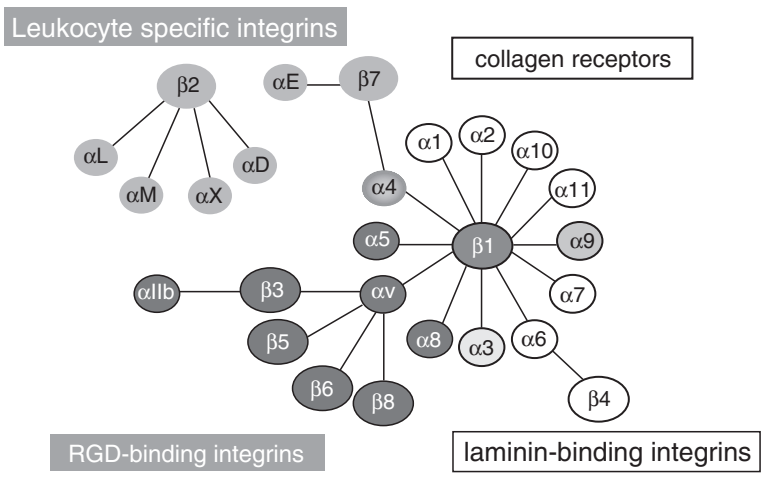


Figure 5.2.3.A. The Integrin family of adhesion receptors. The 18 α and 8 β subunits associate to 24 distinct Integrins, which can be classified into several subfamilies, with regard to their ligand specificity and expression patterns: Integrins that recognize the RGD motif, Laminin binding Integrins, Collagen Receptors, and the leukocyte specific receptors. [Reproduced from Arndt et al. 2005. With permission from Horizon Press.]

are stabilized by their ligands and by intracellular cytoskeletal proteins, such as Talin. Furthermore, lateral clustering of Integrins is stabilized by homo-oligomerization of the α and β transmembrane domains [Li et al. 2003; Hynes 2003]. The control of Integrin affinity for its ligands (Integrin activation) is essential for cell adhesion and migration. Specific binding of the cytoskeletal protein Talin to the cytoplasmic tail of the Integrin β subunit leads to the conformational rearrangements of Integrin extracellular domains that increase their affinity. The two forms of this protein are Talin-1 and Talin-2 [Tadokoro et al. 2003]. The affinity of integrins for their ligands can be increased in invasive tumor cells.

Integrin $\alpha_V\beta_3$. The Integrin $\alpha_V\beta_3$ (CD51/CD61) is typically expressed on macrophages and monocytes, activated leukocytes, and cytokine stimulated endothelial cells, but not on epithelial cells. Multiple ligands engage this Integrin, including Vitronectin, Osteopontin, Entactin, and CYR61.

Vitronectin (VTN, Somatomedin B) {7q11} is a 75 kD glycoprotein in plasma and tissue. It promotes the attachment and spreading of various cells. It contains the amino acid motif RGD, which is involved in Integrin-mediated cell attachment. Vitronectin circulates as a single chain moiety of 75 kD and a two-chain moiety of 65 kD plus 10 kD. It interacts with platelets and the vessel wall in the early stages of blood clotting. When immobilized on surfaces, low concentrations of Vitronectin promote endothelial cell attachment and induce the migration and spreading of cells.

Osteopontin (OPN, Secreted Phosphoprotein-1, SPP1, Early T-Cell Activation-1, ETA-1) {4q21–25} is a widely expressed extracellular protein containing a GRGDS cell surface receptor binding motif, which mediates cell attachment and spreading [Oldberg et al. 1986; Oldberg/Ruoslahti 1986]. Osteopontin is an acidic glycoprotein with a protein backbone of 32.5 kD. It is rich in aspartate, glutamate, and serine and contains about 30 monosaccharides, including 10 sialic acids. Carbohydrate is present as 1 *N*-glycosyl and 5–6 *O*-glycosyl side chains. Phosphorylation occurs to a variable extent, possibly on up to 28 sites, distributed throughout the molecule. Osteopontin function is extensively regulated on the posttranslational level. The molecule has a protease hypersensitive site, which separates the Integrin binding domain from the CD44 binding domain. Proteolytic cleavage and phosphorylation of Osteopontin are required for efficient Integrin ligation.

Entactin (Nidogen-1, NID) {1q43} is a 150 kD sulfated glycoprotein, which is a major component of basement membranes and forms a highly stable non-covalent complex with Laminin. Entactin is structurally and functionally organized into four distinct domains, G1, G2, E, and G3. Cleavage of Entactin with the MMP-7 (Matrilysin) liberates the functional peptides. The E domain contains the single RGD motif and is sufficient for the ligation of β_3 Integrin resulting in neutrophil chemotaxis [Gresham et al. 1996].

The immediate early response gene product CYR61 (Cysteine-Rich Angiogenic Inducer 61, IGF-BP10) {1p22.3} is a secreted cysteine-rich protein that is associated with the cell surface and the extracellular matrix. CYR61 can bind to heparin, and it is a ligand for Integrin $\alpha_V\beta_3$, through which it mediates cell adhesion, migration, and angiogenesis.

- CYR61 is differentially expressed on Estrogen Receptor negative, Heregulin positive breast cancer cells [Tsai et al. 2000].
- Overexpression of the Integrin β_3 upregulates molecules associated with adhesion, including Osteonectin (SPARC). Osteonectin is critical for melanoma progression, possibly because it inhibits the adhesion of Integrin $\alpha_V\beta_3$ expressing cells to Vitronectin or Fibronectin. In this setting, the ligation of Integrin $\alpha_V\beta_3$ may increase invasiveness through signal transduction leading to the secretion of Collagenase and UPA. These connections make Integrin $\alpha_V\beta_3$ a key contributor to melanoma dissemination and a marker of a poor prognosis.
- Malignant astrocytoma cells express the Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ and these receptors are markers for an invasive phenotype.
- While its occurrence on primary breast cancers is variable, Integrin $\alpha_V\beta_3$ is consistently abundant in all breast cancer bone metastases. Bone metastases by breast and prostate cancer depend on the ligation of Integrin $\alpha_V\beta_3$ by Osteopontin or Vitronectin. Integrin $\alpha_V\beta_3$ also facilitates lytic bone metastases through its functional role on osteoclasts. It contributes to bone resorption by mediating osteoclast-bone recognition.

The extracellular matrix protein and secreted glycoprotein SPARC (Secreted Protein Acidic and Rich in Cysteine, Osteonectin, BM-40) {5q31.3-q32} is a matrix-associated protein that is expressed during tissue remodeling, such as in cutaneous wound healing, bone formation, adipogenesis, and

angiogenesis. It is a 32 kD bone specific phosphoprotein that binds selectively to hydroxyapatite and to Collagen fibrils at distinct sites. SPARC can be selectively expressed by the endothelium in response to injury. It affects the barrier functions of endothelial cells. SPARC modulates cell adhesion and migration, in part by exerting de-adhesive effects. SPARC induces loss of focal adhesion and regulates the expression of MMPs and Plasminogen Activator. TRAIL is a receptor for SPARC.

- SPARC selectively supports the migration of highly metastatic prostate cancer cells to bone. Increased migration in response to SPARC is mediated by the Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ on the tumor cells. As a consequence, VEGF is secreted and initiates an autocrine loop between VEGF and VEGF Receptor-2 on the tumor cells [De et al. 2003].
- Breast cancer metastasis to the lungs is characterized by a unique gene expression signature. Among the lung metastasis signature genes is SPARC. Patients expressing the lung metastasis signature have poor lung metastasis free survival, but not bone metastasis free survival, compared to subjects without the signature [Minn et al. 2005].
- SPARC is expressed by invasive, but not by benign, meningiomata as well as by highly invasive astrocytic tumors [Rempel et al. 1999] and it promotes glioma invasion.
- The expression of SPARC mediates adhesion and invasion of melanoma cells [Ledda et al. 1997].

Integrin $\alpha_V\beta_6$. The Integrin $\alpha_V\beta_6$ engages Tenascin-C {9q33} or TGFβ1-LAP (Tumor Growth Factor β1–Latency-Associated Peptide) as ligands. Integrin $\alpha_V\beta_6$ activates latent TGF-β in the lungs and skin. This Integrin is activated during the healing of skin wounds, where it acts as a potential inducer of skin stem cell activation and migration.

The extracellular matrix glycoprotein Tenascin (TN, Cytotactin, Hexabrachion) is a hexameric, multidomain protein with disulfide-linked subunits of 190–240 kD. Tenascin exists as multiple forms generated through alternative splicing. In the central nervous system, Tenascin-C is expressed primarily by astrocytes. It is physiologically expressed in lung parenchyma. High levels of Tenascin may disrupt cell-substrate adhesion and allow cells to migrate through the extracellular matrix.

TGF-β family members are secreted in inactive complexes with an LAP, which is derived from the NH_2-terminal region of the TGF-β gene product. Extracellular activation of these complexes is a critical step in the regulation of TGF-β function. Cells that express Integrin $\alpha_V\beta_6$ induce spatially restricted activation of TGF-β1 [Munger et al. 1999].

- Tenascin is elevated in invasive breast tumors and astrocytomata. The forms containing exon 14 and exons 14 plus 16 are significantly associated with the invasive phenotype. Tenascin contributes to tumor progression in intrahepatic cholangiocarcinoma and, especially when expressed at the invasive front, is a marker for an unfavorable prognosis [Aishima et al. 2003].
- The overexpression of Integrin $\alpha_V\beta_6$ in serous epithelial ovarian cancer regulates extracellular matrix degradation via the Plasminogen activation cascade. Through this mechanism, it may play a role in metastasis [Ahmed et al. 2002].
- The Integrin $\alpha_V\beta_6$ is expressed in invasive oral squamous cell carcinoma and is correlated with tumor progression. Integrin $\alpha_V\beta_6$ may also be involved in the lymphatic metastasis by gastric carcinomata.

Integrin β_1. Integrin $\alpha_2\beta_1$ plays an essential role in platelet adhesion to blood vessel walls under flow conditions. Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ are major receptors for Collagen. Each recognizes a variety of Collagens, including Collagen I, the most abundant and widely distributed form. The triple-helical Collagen peptide GFOGERGVEGPOGPA (O stands for hydroxy-proline) is also recognized by other β_1 Integrins, with the binding site locating to the motif GFOGER [Knight et al. 2000].

In glial cell migration, detachment depends on Integrin α_3 signaling. The Integrin $\alpha_3\beta_1$ (CD49c/CD29) is a receptor for Laminin and Entactin. Laminins are extracellular glycoproteins that are present in all basement membranes. They are composed of one α chain, one β chain, and one γ chain. The α chains are generally large (around 400 kD) and contain a COOH-terminal G-domain that consists of five modules (LG1–LG5). There are at least 15 distinct forms. Laminin-10 (α5β1γ1) and Laminin-11 (α5β2γ1) are ligands for Integrin $\alpha_3\beta_1$. Entactin is structurally and functionally organized into four distinct domains, G1, G2, E, and G3. Cleavage of Entactin by MMP-7 (Matrilysin) liberates the functional peptides. In neutrophils, the G2 domain signals for Fc Receptor-mediated phagocytosis

through Integrin $\alpha_3\beta_1$, only after Integrin activation [Gresham et al. 1996].

- The interactions of Collagen with Integrin $\alpha_2\beta_1$ are implicated in tumor metastasis. The expression of Integrin $\alpha_2\beta_1$ increases the metastatic potential of rhabdomyosarcoma cells [Chan et al. 1991] and of melanoma cells [Stracke and Liotta 1995].
- Integrin β_1 is essential for invasiveness by fibrosarcomata and bladder carcinomata. The ligation of β_1 Integrins by Fibronectin or Collagen on lung cancer cells activates Focal Adhesion Kinase and down-regulates ICAM-1 expression.
- The interaction of Integrin $\alpha_3\beta_1$ and Laminin plays important roles in the process of brain metastasis by non-small cell lung cancer [Yoshimasu et al. 2004].
- The migration of colon carcinoma cells on Laminin is mediated by Integrin $\alpha_3\beta_1$ [Pouliot et al. 2001]. The migration inducing $\gamma 2$ chain of Laminin-5 is a marker of malignancy. It is overexpressed by infiltrating tumor cells at the invasive front of colorectal carcinoma.
- Arrest of circulating tumor cells in distant organs is required for hematogenous metastasis. the tumor cell Integrin $\alpha_3\beta_1$ makes an important contribution to arrest in the lung and to early colony formation. Its ligand Laminin-5 is available to the tumor cells in preexisting patches of exposed basement membrane within the pulmonary vasculature [Wang et al. 2004].

Integrin α_6. The Integrin α_6 is expressed in almost all blood vessels on the luminal and basal cell membrane. The $\alpha_6\beta_4$ heterodimer is predominantly expressed on epithelial cells. It is essential for the attachment of the epidermis and other epithelia. The Integrin $\alpha_6\beta_1$ is a receptor for Laminin.

- The expression of Integrin α_6 on tumor cells is frequently associated with increased metastatic potential and poor prognosis [Imhof et al. 1996].
- The Integrin $\alpha_6\beta_4$ may associate with MET independently of engagement by its ligand Laminin-5. Activation of MET increases the phosphorylation of the β_4 chain. This enhances the activation of the downstream SHC and Phosphatidylinositol 3-Kinase and facilitates invasion by colon carcinoma.
- The Integrin $\alpha_6\beta_4$ is not expressed on normal thyroid cells. Its expression on thyroid carcinoma correlates with progression to invasiveness.
- The migration of colon carcinoma cells on Laminin is mediated by Integrin $\alpha_6\beta_4$ [Pouliot et al. 2001].

Integrin α_9. The Integrin α_9 (a-RLC, a-Related-to-Lung-Cancer) protein contains 1,006 amino acids (after cleavage of the 29 amino acid NH_2-terminal signal sequence) and is structurally similar to Integrin α_4. Two transcripts may result from the use of alternative poly-adenylation signals.

- The expression of Integrin α_9 is elevated on cells of small cell lung cancer.

Various Integrins contribute to the dissemination of cancer cells.

- Integrin $\alpha_L\beta_2$ (LFA-1, CD11a/CD18) adheres to ICAM-1, -2, or -3. Whereas the invasion of lymph nodes by lymphoma cells is dependent on CD44 and hyaluronate, but not on Integrin $\alpha_L\beta_2$, the invasion of the spleen by the same tumor is dependent on CD18 but not on CD44 [Zahalka et al. 1995].
- T-lymphocyte tumors metastasize predominantly into the liver but spare the lungs. Chemical mutants of these T-lymphocyte tumors that lack Integrin $\alpha_L\beta_2$ display reduced metastatic acitvity [Roos 1993].
- Melanoma, colon carcinoma, and lung carcinoma may express Integrin $\alpha_{IIb}\beta_3$. It can be activated by exposure to Thrombin and leads to increased metastasis formation [Buck 1995].

IAP. Integrin-Associated Protein (IAP, CD47, MER6, OA3) associates with various Integrins in the cell membrane. The interactions of CD47 with Integrins and heterotrimeric G-Proteins depend on cholesterol, which also regulates CD47 conformation. CD47 is a receptor for Thrombospondin family members and a counterreceptor for the transmembrane signaling protein SIRPα (SHPS-1). CD47 modulates a range of cell activities, including leukocyte adhesion, migration, and phagocytosis.

IAP is a receptor for the COOH-terminal cell-binding domain of Thrombospondin-1. Peptides containing a VVM motif in the COOH-terminal domain of Thrombospondins are agonists for CD47, initiating heterotrimeric G_i protein signaling that augments the functions of Integrins of the β_1, β_2, and β_3 families. Thrombospondin (*thbs1*) {15q15} has a domain structure. The heparin binding NH_2-terminus of Thrombospondin stimulates chemotaxis in a manner that is inhibitable by heparin and fucoidan, while the COOH-terminus mediates haptotaxis in a RGD inhibitable fashion. TSP-1 has six domains of repeating homologous amino acid sequences: NH_2-terminal,

pro-Collagen homology (pro-CH), type 1 repeat, type 2 repeat, type 3 repeat/RGD (T3), and COOH-terminal. Through its binding to IAP, Thrombospondin modulates the functions of certain Integrins.

- IAP (OA3, Ovarian Cancer Antigen 3) is a marker for ovarian cancer [Campbell et al. 1992; Mawby et al. 1994].
- CD47 binds to and thereby downregulates SHPS-1 on adjacent cells, resulting in inhibition of cell motility. Resistance to this inhibitory mechanism may contribute to the metastatic potential tumor cells.

5.2.4 Carbohydrate-rich proteins

Malignant transformation is typically accompanied by increased β–1,6-GlcNAc branching of *N*-glycans in mature glycoproteins [Dennis et al. 1987]. The medial Golgi enzyme β–1,6-*N*-Acetylglucosaminyl Transferase V (GNT-V, Mannoside Acetyl Glucosaminyl Transferase 5, MGAT5) {2q21} catalyzes the addition and branching of this sugar. MGAT5 products may occur on various metastasis-associated receptors, including Integrin $\alpha_L\beta_2$ (LFA-1, CD11a/CD18), Integrin $\alpha_1\beta_5$, Integrin β_1 (CD29), and CD44. The expression of MGAT5 leads to loss of contact inhibition and increased cell motility. Expression of the *mgat5* gene is induced by oncogene signaling, specifically by the RAS→RAF→ETS pathway [Granovsky et al. 2000]. The increase in β–1,6 branching of *N*-glycans on tumor cells results from an increased expression of GlcNAc Transferase V (GlcNAc-T V, *O*-linked *N*-Acetylglucosamine Transferase, OGT). This occurs primarily at the transcriptional level, can be induced by carcinogenic events, and is exerted through specific features of the upstream promoter region of *GlcNAc transferase V* {Xq13}. Cells with increased GlcNAc-T V expression show an increased frequency of metastasis, and spontaneous revertants for loss of this enzyme activity also lose the metastatic phenotype.

CD44 (PGP-1, Hermes, MDU3) {11pter-p13} is highly glycosylated with sugar accounting for about 40–120 kD of its molecular weight. The NH_2-terminal domain is extracellular, the transmembrane domain spans 21 amino acids. The molecule has 10 variant exons that can be spliced into the proximal extracellular domain (downstream of exon 5), adding up to a total of 20 exons. An additional splice site in the intracellular domain (exons 19 and 20) can lead to the generation of a long (72 amino acids) or a short (3 amino acids) cytoplasmic tail.

CD44 functions as a homing receptor [Jalkanen et al. 1986]. The standard form, without alternatively spliced exons, is expressed on resting lymphoid cells. A splice variant of CD44 is transiently expressed on B-lymphocytes, on T-lymphocytes, and on macrophages after antigenic stimulation, as well as on memory cells, and in the postnatal period [Budd et al. 1987; Arch et al. 1992]. CD44 plays important roles in the regulation of cellular and humoral immune responses [Ashkar et al. 2000]. CD44 is also expressed in multiple tissues outside the immune system, most prominently in epithelial cells and fibroblasts. The form expressed on epithelial cells contains variant exons v8–10 and is 130–160 kD in size. A variant containing exons v3–10 is expressed on keratinocytes. The variant exon 3 of CD44 contains a heparan sulfate attachment site, which has the capacity to bind certain soluble factors through a heparin bridge.

Like all receptors associated with migration, CD44 interacts with cytoskeletal proteins. The ligation of CD44 on cancer cells initiates interconnected signal transduction cascades (Figure 5.2.4.A). Ankyrins mediate the linkage of integral membrane proteins with the Spectrin-based skeleton in regulating attachment and migration. All three Ankyrins (ANK1, ANK2, and ANK3) are monomers comprised of an Ankyrin repeat domain, a Spectrin binding domain, and a variable domain.

– The cytoplasmic domain of CD44 can be acetylated with palmitic acid, which may play a role in its interactions with Ankyrin.
– The interaction between CD44 and Ankyrin may be mediated by RHO-A and a 160 kD RHO Kinase. RHO Kinase phosphorylates CD44, which enhances its interaction with Ankyrin.

The cytoplasmic domain of CD44 has two PKC phosphorylation sites, it may also bind guanosine trisphosphate and exert GTPase activity [Lokeshwar and Bourguignon 1992]. The NH_2-terminus of all ERM proteins can interact with CD44. They may act as molecular linkers between the cytoplasmic domain of CD44 and Actin cytoskeleton.

The SMARCA4 (BRG-1, Brahma-Related Gene-1) subunit of the SWI/SNF complex regulates gene transcription through ATPase-dependent remodeling of chromatin. SMARCA4 is an important regulator of *cd44* expression, and this depends on the

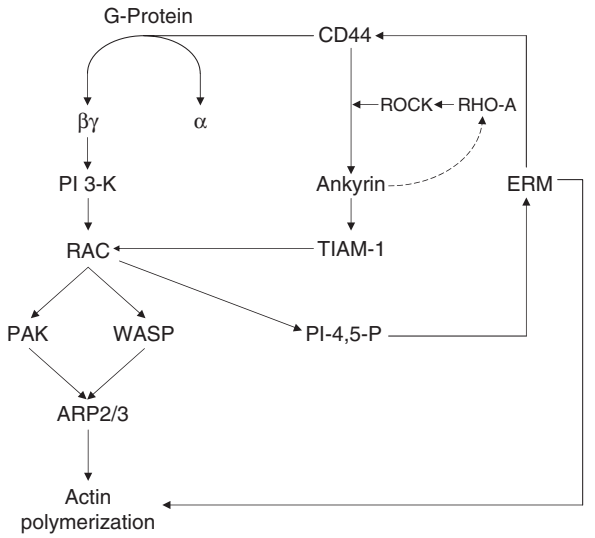


Figure 5.2.4.A. CD44 signaling in migration. Upon engagement by a cognate ligand, CD44 transduces signals through multiple pathways, involving G-Proteins, Ankyrin, and ERM proteins. These signaling pathways cross-communicate, engaging multiple positive feedback loops. Together, they lead to rearrangement of the Actin cytoskeleton.

ATPase activity of SMARCA4. Loss of SMARCA4 is frequently sufficient to cause a lack of CD44 expression [Strobeck et al. 2001].

Hyaluronate and Osteopontin are the main physiologic ligands for CD44 [Arrufo et al. 1990; Weber et al. 1996]. They support cell migration and dissemination in distinct ways. Osteopontin induces chemotaxis but not homotypic aggregation, whereas the interaction between CD44 and hyaluronate exerts an inverse effect.

Osteopontin functions include the induction of migration. The COOH-terminal domain of Osteopontin binds to the variant exon 6 of CD44 via a peptide interaction [Ashkar et al. 2000], independently of posttranslational modifications. Osteopontin may also bind CD44v3 via a heparin bridge. Through alternative splicing, three distinct forms of Osteopontin are generated, which differ in their far NH_2-terminal domains (Figure 5.2.4.B). The shortest form, Osteopontin-c, is most effective in inducing anchorage independence [He et al. 2006], which is a prerequisite for the dissemination of solid tumors. Organs that physiologically produce Osteopontin such as bone (osteocytes), lymph nodes (T-lymphocytes), lung (alveolar macrophages), and liver (Kupffer cells) may also provide micro-environments that are conducive for the chemoattraction of CD44 bearing cells. These organs are commonly targets for cancer metastasis.

The carbohydrate hyaluronate may bind to the NH_2-terminal domain of CD44, which is present in all splice forms. In some forms of the receptor, however, this domain is sterically blocked. Hyaluronate plays roles in the processes of tumor invasion and metastasis. The synthesis of hyaluronate is catalyzed by Hyaluronan Synthase (HAS). Three forms of this enzyme, HAS-1 {19q13.3-q13.4}, HAS-2 {8q24.12}, and HAS-3 {16q22.1}, exist.

- Carcinomata often express several forms of CD44 [Sy et al. 1996]. The presence of variant CD44 on the cell surface is a prerequisite for metastasis formation by various tumors [Matsumura and Tarin 1992; Günthert et al. 1991; Seiter et al. 1993; Tanabe et al. 1993]. CD44 variants may account for the malignant phenotype of colon cancer, breast cancer, non-Hodgkin lymphomata, and pancreas carcinoma. CD44 gene products are essential for the dissemination, but not for early stages of transformation, of certain solid tumors [Weber et al. 2002]. The variant exon 3 of CD44 contains a heparan sulfate attachment site, which has the capacity to bind certain soluble factors. The expression of this variant can cause metastasis, but has no effect on primary tumor development [Barbour et al. 2003].
- The repeat domain of Ankyrin interacts with CD44 and may promote ovarian tumor cell migration [Zhu and Bourguignon 2000].
- Metastasizing tumors express a variant of the CD44 molecule. In addition, metastasizing tumors secrete Osteopontin (Figure 5.2.4.C). This may reflect autocrine activation of migration. Osteopontin expression correlates with the metastatic potential of various tumor cells [Craig et al. 1990]. Osteopontin is the major

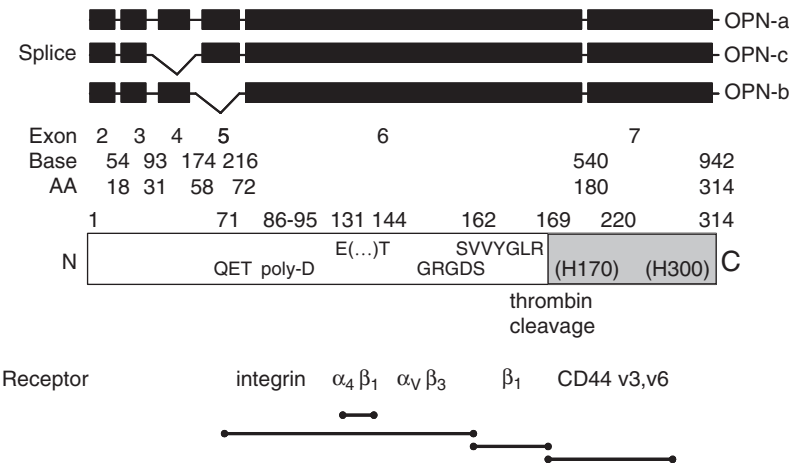


Figure 5.2.4.B. The molecular structure of Osteopontin. Top: The gene has six translated exons. The splice variant lacking exon 4 is referred to as Osteopontin-c, and the splice variant lacking exon 5 is Osteopontin-b. Middle: Two main domains on the protein are separated by a protease sensitive site, a NH_2-terminal fragment encompasses the Integrin binding domains, while the CD44v binding domain lies on the COOH-terminal part of the molecule. Bottom: The Integrin binding site covers the sequence GRGDS. The smallest integrin $\alpha_V\beta_3$ binding peptide identified starts at AA71. Binding to β_1 containing Integrins occurs through the noncanonical sequence SVVYGLR, unless the β_1 chain is paired with α_4, in which case the binding site ranges from AA131 to AA144. The CD44v6 binding site covers the region from AA169 to AA220. Heparin bridges between Osteopontin and CD44v3 may be formed via the heparin binding sites on AA170 and 300. The bases of the coding sequence and the corresponding amino acids are numbered such that the start site, or initiating methionine is 1. The scheme is not drawn to scale.

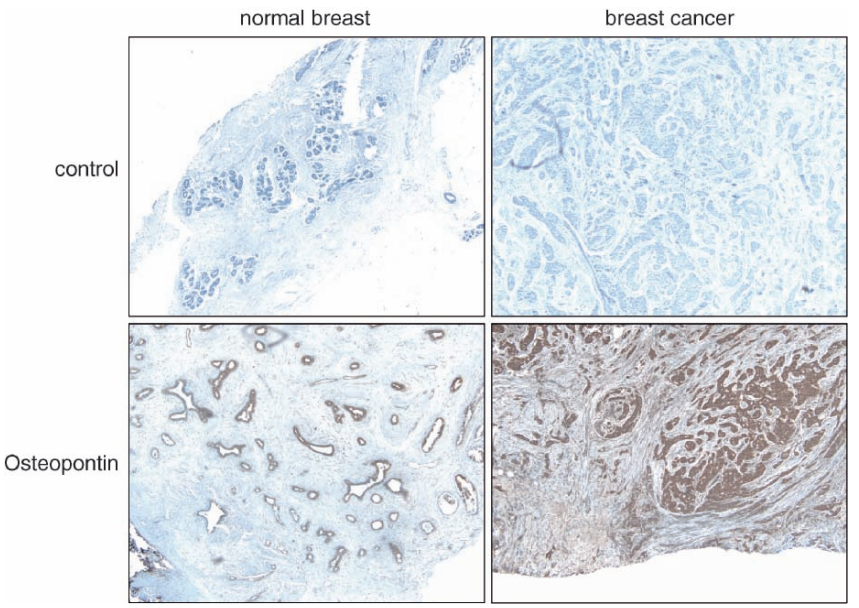


Figure 5.2.4.C. Osteopontin expression in breast cancer. Histologic staining of breast tissue (*left*: normal, *right*: cancer) with an antibody to osteopontin (*bottom*) or a nonspecific control antibody (*top*). While, the presence of Osteopontin is limited to the ducts in normal breasts, it is abundant in breast cancers.

phosphoprotein secreted by transformed cells, which is elevated 4- to 10-fold in sera from patients with advanced metastatic cancer [Senger et al, 1989]. It may be a marker for ovarian cancer [Kim et al. 2002] or colon cancer [Agrawal et al. 2002].

- The expression of *osteopontin* by tumor cells increases their malignant phenotype [Denhardt and Guo 1993], whereas the suppression of *osteopontin* expression yields populations with reduced malignant potential [Behrend et al. 1994; Gardner et al. 1994].

- Osteopontin expression is induced in cells transformed by *v-myc* or *ras* and correlates with the resulting metastatic ability [Chambers et al. 1993].
- In colon cancer, there is a significant correlation between poor survival and the *has-1* transcript level. At Dukes stage C, the transcript levels of *has-1* and *cd44v6* correlate, implying that HAS1 plays a role in the malignant progression of colon cancer cells [Yamada et al. 2004].

5.2.5 Chemokine Receptors

Chemokines are a superfamily of small cytokines that induce directional cell migration. Chemokines are 8–10 kD proteins with 20–70% homology in amino acid sequences (Figure 5.2.5.A). According to the number and spacing of conserved cysteine residues in their sequence, there are four major groups, consisting of:

– CC containing Chemokines and their corresponding receptors (CCR1 through CCR10)
– CXC containing Chemokines (with receptors CXCR1 through CXCR5)
– A CXXXC Chemokine (Fractalkine, with receptor CXXXCR1/V28)
– A XC Chemokine (with receptor XCR1).

Several chemokines undergo NH_2-terminal proteolytic processing after secretion, which alters their activity.

Chemokines induce cell migration and activation by binding to specific seven transmembrane spanning, G-Protein coupled cell surface receptors on target cells (Figure 5.2.5.B). The heterotrimeric G-Proteins then dissociate into α and $\beta\gamma$ subunits, which engage target enzymes. Mostly, they are functionally linked to Phospholipases and to members of the RHO family. There are four CXC Chemokine Receptors (CXCR1 through CXCR4), eight CC Chemokine Receptors (CCR1 through CCR8), and one CXXXC Chemokine Receptor (CXXXCR1) (Figure 5.2.5.C). Chemokine receptors are expressed differentially on various leukocyte subsets (Table 5.2.5.A). Chemokines and their receptors are critically important in tissue specific leukocyte homing. Some Chemokine Receptors are also expressed on non-hematopoietic cells, including neurons, astrocytes, epithelial cells, and endothelial cells. Chemokine Receptor expression may be constitutive or inducible. The recruitment of leukocytes to inflamed tissues involves several related chemotactic cytokines that attract and activate leukocytes. The CXC Chemokines activate primarily neutrophil leukocytes, while CC Chemokines act on monocytes, basophils, and eosinophils. The Chemokine Receptor CCR7 is expressed on all naïve T-lymphocytes, some memory T-lymphocytes, B-lymphocytes, and mature dendritic cells. It plays a central role in lymphocyte trafficking and homing to lymph nodes.

Chemokines interact with DARC (Duffy Antigen Receptor for Chemokines, FY) {1q21-q22}, a nonsignaling erythrocyte Chemokine Receptor and the determinant of the Duffy blood group. DARC is expressed on erythrocytes and endothelial cells. Although DARC is structurally related to Chemokine Receptors, it is distinctive in that both CXC and CC Chemokines bind to it and this interaction does not induce calcium flux. DARC may function as a decoy receptor and a sink for Chemokines, clearing them from the circulation.

- Chemokine receptors are expressed on various lymphomata, including Burkitt lymphoma [Dobner et al. 1992]. Acute lymphoblastic leukemia (ALL) is a malignancy with the potential to infiltrate liver, spleen, lymph nodes, and brain. The Chemokine Receptor CXCR4 is expressed on ALL cells and its ligand is abundant at sites associated with ALL-induced organ infiltration. This results in the chemotaxis of leukemic cells from the bone marrow via the circulation to preferential sites of extramedullary organ infiltration, and it is associated with shorter disease-free survival [Crazzolara et al. 2001].
- The Chemokine Receptor CXCR4 is typically expressed on a sub-population of ovarian cancer cells. The peritoneum is a frequent target organ for ovarian carcinoma metastasis, and the CXCR4 ligand CXCL12 is present in patients' ascites. Receptor ligation by CXCL12 elicits calcium flux, changes in Integrin expression, and directed migration of ovarian cancer cells [Scotton et al. 2001].
- The chemokine receptors CXCR4 and CCR7 are highly expressed on breast cancer cells and their metastases, but not, or at low levels, on normal breast cells. Their ligands, CXCL12 (SDF-1α) and CCL21 (6Ckine), exhibit peak levels of expression in organs that represent target tissues for metastasis formation, including lymph nodes, bone marrow, lungs, and liver. In particular, CCL21 may be important for homing

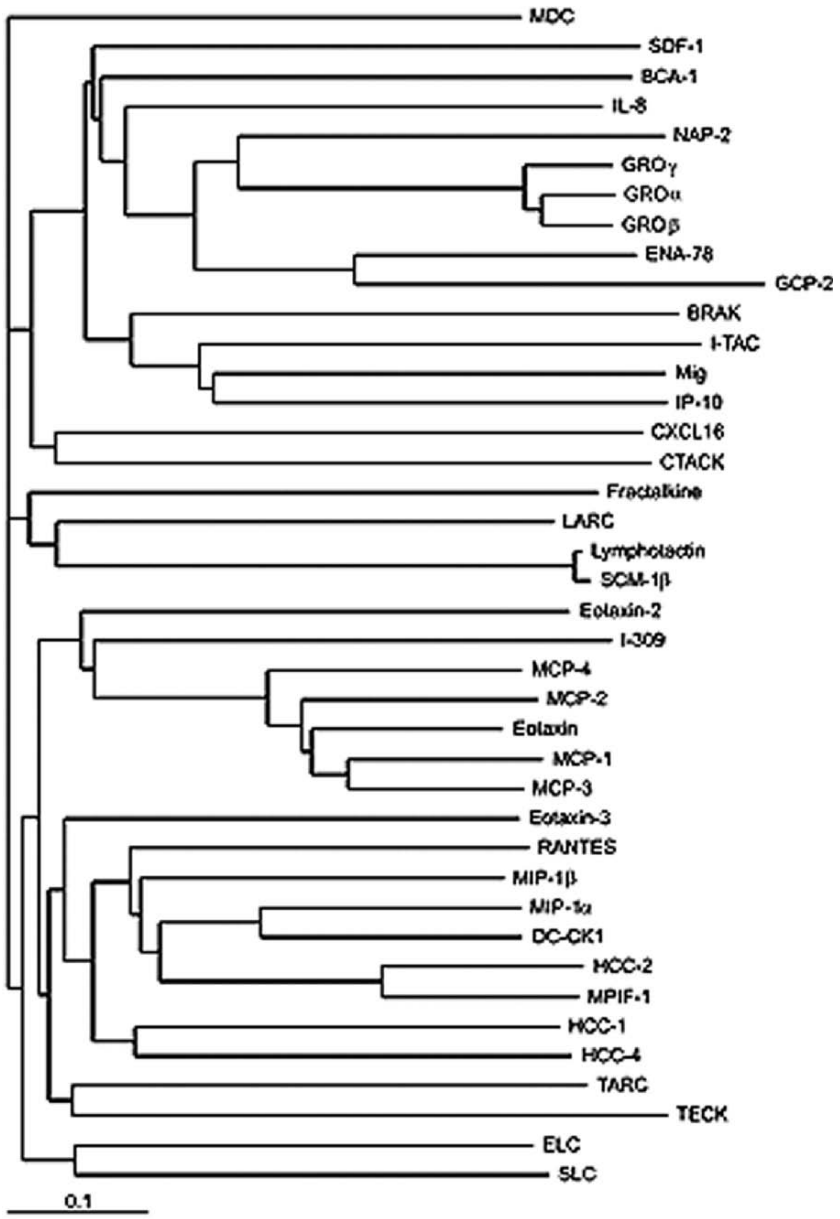


Figure 5.2.5.A. Chemokines. Dendrogram showing the amount of protein sequence similarity among all known human Chemokines. Protein sequences were obtained from the National Center for Biotechnology Information protein database. The phylogenetic tree was constructed using the Clustalw program provided by the European Bioinformatics Institute and analyzed using TreeView. The scale bar reflects the horizontal distance at which sequences diverge by 10% (90% identity). Amino acid identity between a pair of Chemokines is given by 1-x, where x is the sum of the two horizontal distances to the right of the pair's vertical branch point. MDC = Macrophage-Derived Chemokine, SDF = Stromal Cell-Derived Factor, BCA = B-Cell Activating Chemokine, IL = Interleukin, NAP = Neutrophil-Activating Protein, GRO = Growth-Related Oncogene, ENA = Epithelial Cell-Derived Neutrophil-Activating Factor, I-TAC = Interferon Inducible T-Cell Chemoattractant, MIG = Monokine-Induced by γ-Interferon, IP = Inducible Protein, CTACK = Cutaneous T-Cell Attracting Chemokine, LARC = Liver and Activation-Induced Chemokine, RANTES = Regulated on Activation Normal T-cell Expressed and Secreted, MIP = Macrophage Inflammatory Protein, DC = Dendritic Cell, HCC = Hemofiltrate CC Chemokine, MPIF = Myeloid Progenitor Inhibitory Factor, TARC = Thymus- and Activation-Related Chemokine, TECK = Thymus Expressed Chemokine, ELC = Epstein–Barr Virus-Induced Receptor Ligand Chemokine, SLC = Secondary Lymphoid Tissue Chemokine. [Reproduced from Olson and Ley 2002. With permission.]

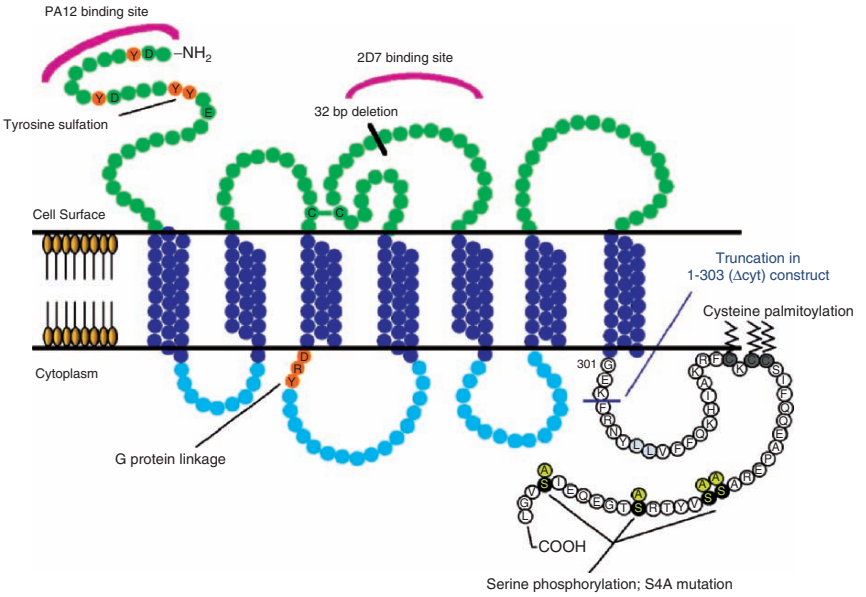


Figure 5.2.5.B. Structure and sequence of CCR5. Graphic of the CCR5 molecule showing the general orientation in the membrane. Sites recognized by two monoclonal antibodies (PA12 and 2D7) are shown, as is the truncation point caused by the Δ32 mutation. Mutations that block activity as a Chemokine Receptor but preserve function as an HIV Receptor include COOH-terminal truncations and mutations that impact phosphorylation. Sulfation of Tyrosine on the exposed NH_2-terminal domain is required for HIV-1 infection, but is dispensable for Chemokine signaling. [Reproduced from http://www.scripps.edu/newsandviews/e_20041018/mosier.html. With permission.]

to secondary lymphoid organs. The engagement of the Chemokine Receptors on breast cancer cells by their cognate ligands triggers Actin polymerization, pseudopodia formation, and directed migration. Neutralization of the interaction between CXCL12 and CXCR4 leads to a significant reduction of lymph node and lung metastases [Mueller et al. 2001]. Interleukin-8 is a member of the CXC Chemokine family. IL-8 is physiologically secreted by osteoclasts. Elevated expression of IL-8 by breast carcinoma may correlate with bone metastasis.

- Malignant melanoma preferentially develops lymph node, lung, liver, and bone marrow metastases. It also disseminates within the skin. Melanoma cells express the Chemokine Receptors CXCR4 and CCR7. In addition, they display high levels of CCR10. Its ligand, CCL27 (CTACK), is abundant in the skin, which represents a typical target tissue for metastasis [Mueller et al. 2001].
- CXCR4 is commonly expressed on small cell lung cancer cells. In addition, CXCL8 (IL-8), CXCL1 (GROα), and CXCL5 (ENA-78) are important in the progression of non-small cell lung carcinoma (NSCLC).
- The majority of gastric carcinomata express the receptor CCR7 for the Chemokine CCL21 (6Ckine). Its expression correlates with lymphatic invasion and lymph node metastases [Mashino et al. 2002].

5.2.6 Other Receptors

Based on their structures and sequence relationships, Ephrins are divided into the Ephrin-A (EFNA) class, which are anchored to the membrane by a glycosylphosphatidylinositol linkage (GPI linkage), and the Ephrin-B (EFNB) class, which are transmembrane proteins. The EPH family of receptors are divided into two groups based on the similarity of their extracellular domain sequences and their affinities for binding Ephrin-A or Ephrin-B ligands. Ephrins induce cell migration by signaling through the STE20 Kinase HGK (Hematopoietic Progenitor Kinase/Germinal Center Kinase-Like Kinase, MAP4K4). Ephrin Receptor A2 (EPH-A2) is a transmembrane receptor Tyrosine Kinase that is upregulated on many aggressive carcinoma cells, including those of the breast, prostate, colon, lung, and skin. It fails to bind its ligand, Ephrin-A1, which is anchored to the membrane on adjacent cells. EPH-A2 has kinase activity that is independent of ligand binding, however, ligand binding causes EPH-A2 to negatively regulate tumor cell growth and migration. EPH-A2 does not increase the growth rate, but facilitates anchorage independence [Carles-Kinch et al. 2002]. Ephrin B1 (ELK) is an oncogene. Ephrin B is regulated by the oncogenic transcription factor TCF. The Ephrin-B Receptor inhibits chemotaxis to SDF-1.

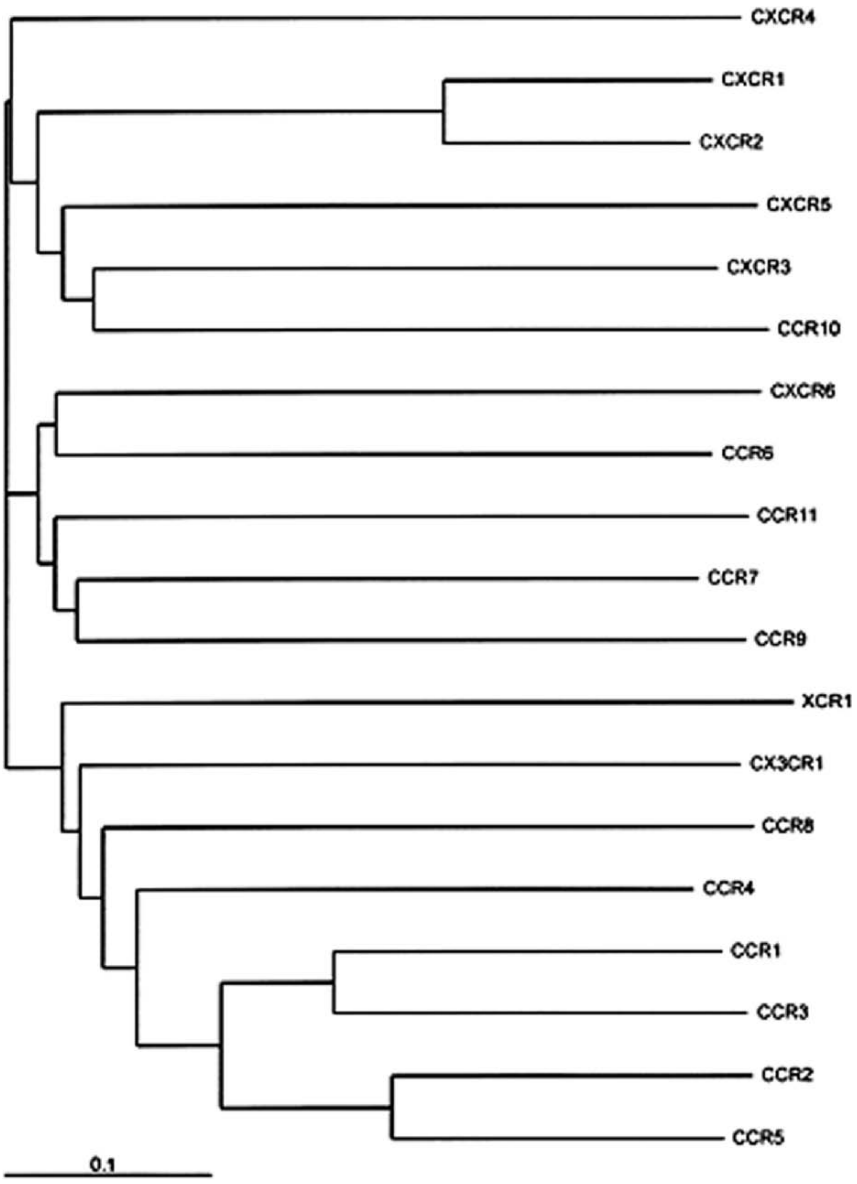


Figure 5.2.5.C. Chemokine Receptors. Dendrogram showing the amount of protein sequence similarity among all known human Chemokine Receptors. Protein sequences were obtained from the National Center for Biotechnology Information protein database. The phylogenetic tree was constructed with Clustalw program provided by the European Bioinformatics Institute and analyzed with TreeView. The scale bar reflects the horizontal distance at which sequences diverge by 10% (90% identity). Amino acid identity between a pair of Chemokine Receptors is given by 1-x, where x is the sum of the two horizontal distances to the right of the pair's vertical branch point. [Reproduced from Olson and Ley 2002. With permission.]

Plexins are cell surface receptors for Semaphorin molecules, and their interaction governs cell adhesion and migration in a variety of tissues. The receptor for Semaphorin 4D (SEMA4D), Plexin-B1, directly stimulates the intrinsic guanosine triphosphatase (GTPase) activity of R-RAS, a member of the RAS superfamily of small G-Proteins that is implicated in promoting cell adhesion and neurite outgrowth. This activity requires the interaction of Plexin-B1 with RND-1, a small G-Protein of the RHO family [Oinuma et al. 2004]. Over-expression of the Semaphorin SEMA3C is associated with metastatic lung carcinoma [Martin-Satue and Blanco 1999] and with squamous cell carcinomata [Yamada et al. 1997].

Table 5.2.5.A. Chemokine interactions with their receptors. Cells of the immune system variably and inducibly express Chemokine Receptors. This conveys responsiveness to specific subsets of Chemokines.

Chemokine	Receptors	Cell type
MCP-3, MCP-4, MIP-1α, RANTES	CCR1	Eosinophil
MCP-3, MCP-4, Eotaxin-1, Eotaxin-2, RANTES	CCR3	
MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR2	Basophil
MCP-3, MCP-4, Eotaxin-1, Eotaxin-2, RABTES	CCR3	
MCP-3, MCP-4, MIP-1α, RANTES	CCR1	Monocyte
MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR2	
MIP-1α, MIP-1β, RANTES	CCR5	
I309	CCR8	
SDF-1	CXCR4	
Fractalkine	CX_3CR1	
IL-8, GCP-2	CXCR1	Neutrophil
IL-8, GCP-2, GRO-α, GRO-β, GRO-γ, ENA-78	CXCR2	
SDF-1	CXCR4	Resting T-cell
MCP-3, MCP-4, MIP-1α, RANTES	CCR1	Activated T-cell
MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR2	
TARC	CCR4	
MIP-1α, MIP-1β, RANTES	CCR5	
MIP-3β	CCR7	
IP-10, MIG, I-TAC	CXCR3	
Fractalkine	CX_3CR1	
MCP-3, MCP-4, MIP-1α, RANTES	CCR1	Dendritic cell
MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR2	
MCP-3, MCP-4, Eotaxin-1, Eotaxin-2, RABTES	CCR3	
TARC	CCR4	
MIP-1α, MIP-1β, RANTES	CCR5	
MIP-3α	CCR6	
SDF-1	CXCR4	
MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR2	NK-cell
MIP-1α, MIP-1β, RANTES	CCR5	
IP-10, MIG-1, I-TAC	CXCR3	
Fractalkine	CX_3CR1	

5.3 INDUCTION OF METASTASIS GENES

Metastasis genes are stress response genes. In cancer cells, their induction occurs downstream of dysregulated growth signals. In highly differentiated cells, the signal transduction pathways leading to cell division do not activate stress responses. Growth factor-mediated signal transduction does, however, activate stress responses in precursor cells. Those cells are physiologically induced to divide in response to tissue damage so that the activation of genetic stress response programs in such cells concomitantly with cell division is physiologically important. Growth factor signal transduction pathways in precursor cells and in cancer cells induce transcription factors of the immediate early response, including AP, NF-κB, ETS, GLI, MTA, and EGR. This effect is enhanced at advanced tumor stages by a stress microenvironment, which includes hypoxia, acidosis, and the excess of reactive oxygen intermediates [Xie and Huang 2003].

AP. AP-1 constitutes a group of related dimeric, basic region, leucine zipper proteins that belong to the JUN, FOS, MAF, and ATF subfamilies. JUN [Maki et al. 1987] and FOS [Curran and Teich 1982] heterodimerize and form a leucine zipper. The composition of the leucine zipper is also responsible for the specificity and the stability of homo- and heterodimers formed by the various JUN and FOS proteins. JUN can form homodimers, but FOS cannot. JUN can also form heterodimers with some

members of the ATF family. The stress response transcription factor AP-1 (composed of homodimers of c-JUN or heterodimers of c-JUN and c-FOS) binds to specific sequences, TGACTCA, referred to as the TPA response element (TRE). This site is present in the promoters of the *metallothionein* and *collagenase* genes [Lee et al. 1987]. The JUN/ATF2 dimer binds preferentially to the related sequence TGACGTCA. AP-1 activity promotes the expression of various MMPs. The JUN transcription factors include c-JUN, JUN-B, and JUN-D, which are upregulated in response to growth signals [Ryder et al. 1988; Nakabeppu et al. 1988]. The FOS protein family comprises FOS, FOS-B, FRA-1, and FRA-2. Additionally, some members of the ATF subfamily (including ATF-a, ATF-2, and ATF-3) and JDP subfamily (JDP-1, JDP-2), which share structural similarities and form heterodimeric complexes with AP-1 proteins, can bind to TRE-like sequences. The regulation of AP-1 activity can be achieved through:

– Changes in transcription of genes encoding AP-1 subunits
– Control of the stability of their mRNAs
– Posttranslational processing and turnover of existing AP-1 subunits
– Specific interactions between AP-1 proteins and other transcription factors

Mutual repression of transcriptional activation is mediated between AP-1 and RARs [Chen et al. 1995].

- AP-1 may be induced by over-activation of the kinase PKB. This occurs in ERBB2 overexpressing breast cancer cells and may mediate the transcription of *osteopontin*, which contributes prominently to breast cancer dissemination [Zhang et al. 2003].
- AP-1 is a major target of asbestos induced signaling, which leads to mesothelioma. The FOS family gene product FRA-1 is required for this process.

NF-κB. NF-κB activation often occurs in situations in which rapid and decisive action is required for cell survival, such as during activation of the innate immune response, the first line of defense against bacterial, viral, and fungal infections. Frequently, NF-κB is important for the prevention of apoptosis (programmed cell death). It activates the transcription of *ciap*, *c-flip*, *a1* (*bfl1*), and *bcl-*X_L. NF-κB also induces the secretion of MMPs and Chemokines. Target genes of NF-κB include *interleukin-8*, which is important in angiogenesis. Consistent with its role in stress responses, NF-κB is preformed in the cytoplasm and shuttles into the nucleus upon activation.

The NF-κB family of proteins contains five transcription factors, NF-κB1 (P105, which is processed to yield P50), NF-κB2 (P100, which is processed to yield P52), c-REL, REL-A (P65), and REL-B. All NF-κB proteins contain a REL homology domain (RHD), which mediates their interactions with the inhibitory I-κB proteins, their dimerization, and their binding to DNA. The REL homology domain contains two Immunoglobulin-like folds connected by a flexible linker region. Both folds contact the DNA. Loops within the NH_2-terminal fold are primarily responsible for sequence specific recognition, whereas the COOH-terminal fold contains the dimer interface. Dimerization is mediated by extensive hydrophobic interactions along the interface surface, which is formed by a three-stranded β-sheet packing against a similar sheet in the opposing molecule [Karin and Ben-Neriah 2000]. At the COOH-terminal end of the REL homology region is the nuclear localization signal. One subgroup of NF-κB proteins requires proteolytic processing of precursor proteins (NF-κB1, NF-κB2) another does not (REL-A, REL-B, c-REL) (Figure 5.3.A).

NF-κB transcription factors act as dimers. Distinct NF-κB dimers exhibit different binding affinities for their cognate sites bearing the consensus sequence GGGRNNYYCC (R denotes purines, Y denotes pyrimidines, and N represents any base). The REL proteins differ in their abilities to activate transcription, such that only $P65^{REL\text{-}A}$ and c-REL contain potent transcriptional activation domains. Subunits that lack transactivation domains may induce transcriptional activation from noncanonical sites after heterodimerization with proteins that contain transactivation domains. BCL-3 {9q13} contains multiple Ankyrin repeats; it can act as a transcriptional coactivator for P50 or P52. Alternatively, dimers composed solely of NF-κB proteins that lack transcriptional activation domains, such as P50, may mediate transcriptional repression.

The ability of NF-κB to regulate gene expression is controlled by chemical modifications, such as phosphorylation, and by interactions with other proteins. Gene expression is typically regulated by multisubunit transcription factor complexes. A large number of gene promoters and enhancers contain recognition sites for the transcription factor NF-κB.

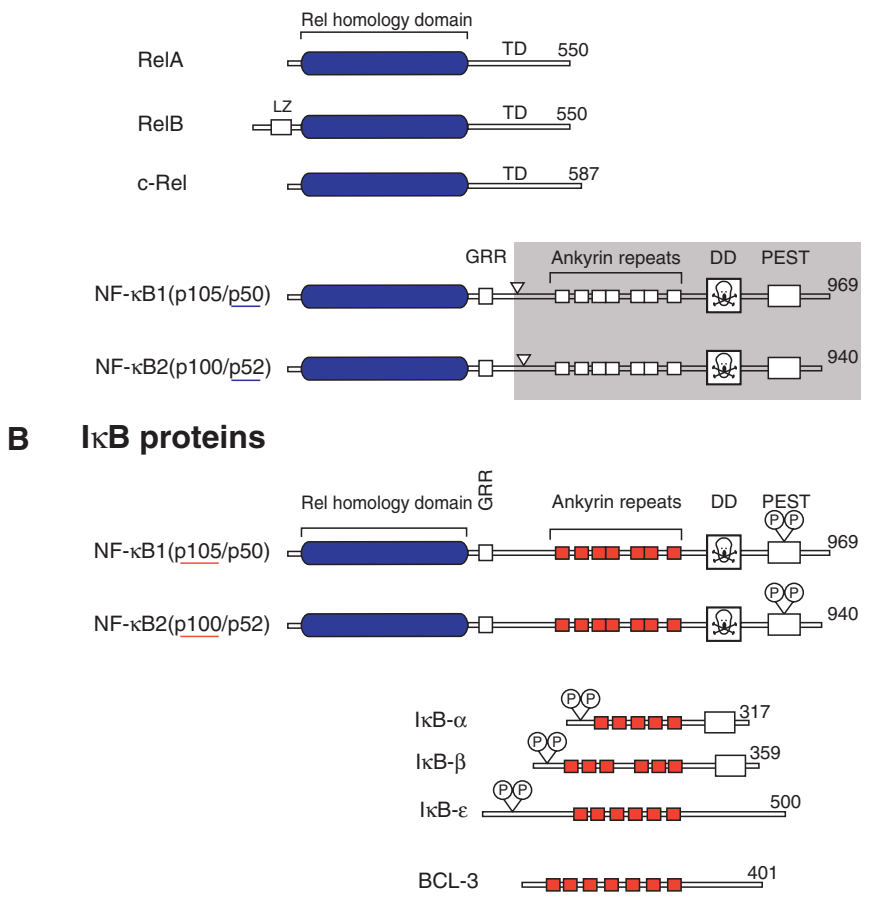


Figure 5.3.A. NF-κB structure. NF-κB (REL) proteins are characterized by their conserved NH_2-terminal REL homology domains (RHDs) (blue), which mediate DNA binding, nuclear localization, and subunit dimerization. I-κB proteins each contain Ankyrin repeat regions (red), which interact with RHDs of NF-κB subunits to prevent nuclear translocation. The numbers of amino acids in each protein are shown on the right. (a) arrowheads point to the COOH-terminal residues of P50 and P52, which are produced by proteolytic processing of the COOH-terminal domains of P105 and P100, respectively (grey shading). (b) The positions of the IKK phosphorylation sites, which regulate the inducible proteolysis of I-κB proteins, are shown. LZ = leucine zipper, TD = transactivation domain. [Reproduced from Beinke and Ley 2004. With permission.]

While the release of P65/P50 or P65/P52 into the nucleus is insufficient to differentially regulate transactivation from those sites, additional biochemical mechanisms control which genes driven by NF-κB sequences are activated at any particular time. One such mechanism is the formation of complexes of NF-κB subunits with transcription factors that recognize adjacent promoter sequences. Several DNA binding NF-κB subunits are substrates for cGMP-dependent kinase (PKG) and their transactivation from cognate sites is induced by phosphorylation. The phosphorylation of multiple transcription factors by an upstream kinase, such as PKG, can lead to the formation of transcription factor complexes and differential transactivation from a subset of NF-κB sites. Synergisitc amplification can be achieved by the phosphorylation and activation of several member proteins of a transcription factor complex, such as P50 and CREB, by one single kinase. Alternatively, PKA phosphorylates NF-κB P65, which induces transactivation. This is accomplished through the recruitment of P300/CBP by the phosphorylated P65. These constitute distinct modes of NF-κB activation, driven by kinases that promote the assemblies of different transcription factor complexes [Zhong et al. 1998; Zhong et al. 2002; He and Weber 2003, 2004].

A20 (TNFAIP3, TNF-α Induced Protein 3) is a cytoplasmic zinc finger protein that inhibits NF-κB activity and TNF-mediated programmed cell death. Cells deficient in A20 fail to terminate TNF induced NF-κB responses. These cells are also more susceptible than A20 expressing cells to undergo

TNF-mediated programmed cell death. A20 is critical for limiting inflammation [Lee et al. 2000].

In the cytoplasm, NF-κB is maintained in an inactive form by sequestration through its interaction with inhibitory proteins, the I-κBs. Proteolytic degradation of I-κB immediately precedes, and is required for NF-κB nuclear translocation. This step in the signaling pathway constitutes a commitment to transcriptional activation (Figure 5.3.B). The signal is eventually terminated through cytoplasmic re-sequestration of NF-κB, which depends on nuclear export, as well as on I-κBα synthesis, a process requiring NF-κB transcriptional activity. While the release of P50/REL-A or P50/c-REL dimers into the nucleus is negatively regulated through their interactions with the inhibitor I-κB(α, β, ε), the release of P100/REL-B dimers is positively regulated by proteolytic cleavage of P100. In contrast, the proteolytic processing of P105 is constitutive.

I-κB proteins are cytoplasmic inhibitors of NF-κB that engage the DNA binding subunits. All I-κB proteins contain 6–7 Ankyrin repeats, which mediate their binding to the REL homology domains. Inhibitors of the I-κB family (I-κBα, I-κBβ, and I-κBε) bind to NF-κB and retain it in the cytoplasm. Upon stimulation, I-κB Kinases (IKKα, IKKβ) phosphorylate I-κB on two conserved serine residues and target it for degradation in the Ubiquitin–proteasome pathway. This allows NF-κB P50/REL-A heterodimers to accumulate in the nucleus and transactivate the expression of target genes. Inducible I-κB phosphorylation, one of the earliest events in the common activation pathway, occurs at serines 32 and 36 in I-κBα. Phosphorylation leads to the immediate recognition of I-κBα by the F-Box/WD40 E3RS$^{I\text{-}\kappa B}$/β-TrCP, which consequently results in poly-ubiquitination of I-κBα, primarily at lysines 21 and 22 by a SKp1/Cullin/F-Box (SCF) type E3. This modification then targets I-κBα for rapid degradation by the proteasome. The degradation of its inhibitor exposes the nuclear localization sequence of NF-κB, resulting in binding to Karyopherins and translocation of NF-κB to the nucleus. The nonphosphorylated form of I-κBα can be sumolated. The such altered form of I-κBα is resistant to

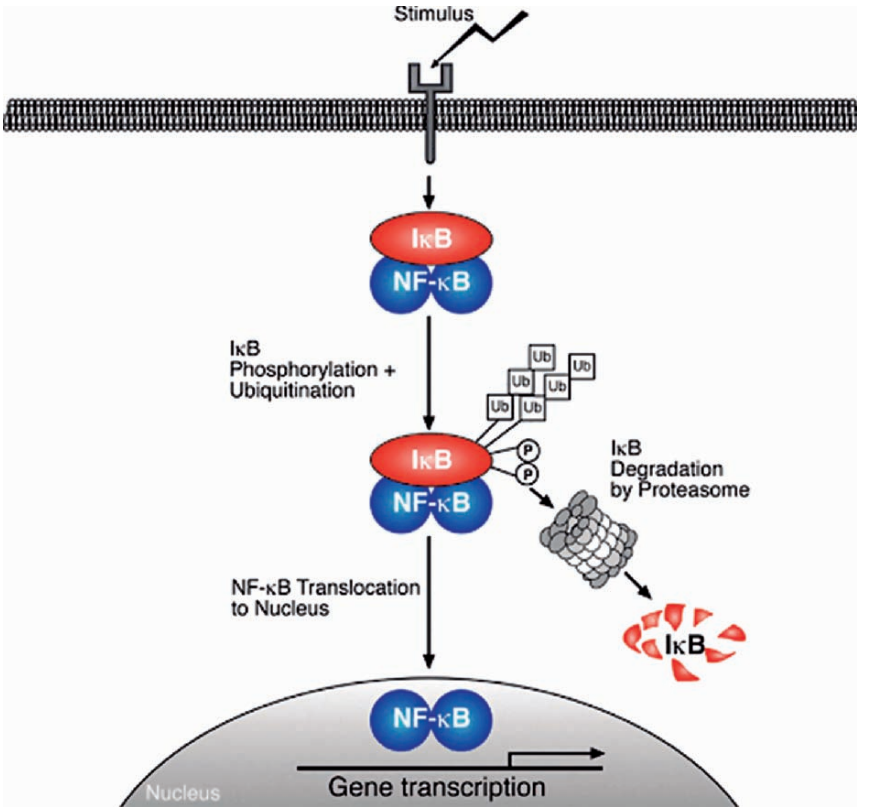


Figure 5.3.B. NF-κB function. Various stimuli induce the phosphorylation and subsequent poly-ubiquitination of I-κBs, which are then targeted for degradation by the 26 S proteasome. Associated NF-κB dimers are thereby released to translocate into the nucleus, where they bind to the promoter regions of NF-κB responsive genes to modulate their expression. The transactivating capacity of nuclear NF-κB dimers can also be regulated by phosphorylation. [Reproduced from: Beinke and Ley 2004. With permission.]

ubiquitnation and protesome degradation, because modification by Ubiquitin or SUMO1 occurs on the same lysine residue. Through a cognate mechanism, phosphorylation of P100 by IKKα triggers the Ubiquitin-dependent degradation of its COOH-terminus and releases the P52/REL-B heterodimer into the nucleus.

IKK (I-κB Kinase) is an upstream effector of the NF-κB pathway that has at least three components. IKKα (IKK1) and IKKβ (IKK2) are 85 kD and 87 kD proteins, respectively. The third component of IKK is the 48 kD regulatory subunit IKKγ (NF-κB Essential Modulator, NEMO, IKK-Associated Protein 1, IKKAP1, FIP-3, Type 2 Adenovirus E3 14.7 kD Interacting Protein). The ubiquitous 105 kD protein ELKS is an essential regulatory subunit of the IKK complex. In the absence of ELKS, the induced expression of NF-κB target genes is blocked [Ducut Sigala et al. 2004]. Two pathways lead to NF-κB activation:

– In the classical pathway, pro-inflammatory stimuli and genotoxic stress mediate the phosphorylation and proteasomal degradation of I-κB through IKK -α, -β, and -γ; this leads to the nuclear translocation of the P50/REL-A heterodimer.
– In the alternative pathway, TNF family cytokines activate IKKα, but not IKK -β or -γ, to induce the degradation of P100 to P52; this leads to the nuclear translocation of the P52/REL-B heterodimer.

The proto-oncogene product COT (TPL-2, Tumor Progression Locus 2, MAP3K8) {10p11.2} activates MAP and SAP Kinases. It activates NF-κB1 by stimulating the proteasome-mediated proteolysis of its precursor P105.

- The transcription factor NF-κB is activated in a range of cancers and promotes tumor progression, mainly due to its ability to protect transformed cells from apoptosis. In RAS transformed breast cancer, the IKK-2→I-κBα degradation→NF-κB pathway is required for the induction and maintenance of epithelial–mesenchymal transition. Inhibition of NF-κB activity leads to an abrogation of metastasis formation [Huber et al. 2004].
- Advanced breast cancers frequently metastasize to bone, resulting in osteolytic lesions. NF-κB plays a crucial role in this process by stimulating osteoclastogenesis. *gm-csf* (*granulocyte macrophage–colony stimulating factor*) as a key transcriptional target of NF-κB. The secretion of GM-CSF by the cancer cells stimulates osteoclast development and leads to osteolysis [Park et al. 2007].
- NF-κB contributes importantly to prostate cancer metastasis. It transcribes angiogenic genes, such as *il-8* and *vegf*.
- In colon carcinogenesis, the activation of NF-κB is linked to increased epithelial apoptosis during tumor promotion [Greten et al. 2004]. MIG-6 is an adapter molecule containing a CRIB domain, a SRC homology domain, and a 14-3-3 binding domain. The expression of *mig-6* is induced by K-RAS in colon cancer cells. MIG-6 induces the transcriptional activation of NF-κB by competing for the binding to I-κBα.
- In myelogenous leukemia, the fusion protein BCR-ABL is capable of activating NF-κB.
- A translocation t(4;16) in T-cell lymphomata activates BCMA (B-Cell Maturation Antigen), which mediates IKK activation and leads to constitutive NF-κB activity.
- Lymphomata of the intestinal mucosa frequently are characterized by translocations involving *malt1*. The translocations occurring in MALT lymphomata generate a chimeric protein containing the NH_2-terminal portion of cIAP-2 and the COOH-terminal domain of MALT1 that activates NF-κB. CARMA-1 and BCL-10 activate NF-κB through MALT1. The excessive levels of MALT1 (Para-Caspase) or BCL-10 that occur in some lymphomata mediate constitutive IKK activation.

ETS. The *ets* family comprises 27 genes, which encode sequence specific transcription factors.

– The PEA-3 subfamily of ETS transcription factors consists of PEA-3 (E1AF), ER-81 (ETV-1), and ERM. The expression of PEA-3 is most restricted, occurring principally in the brain and epididymis.
– Members of the TCF (Ternary Complex Factor) subfamily of ETS transcription factors include NET, ELK-1, and SAP-1.

The signature of the ETS family of transcription factors is the ETS domain (winged helix-turn-helix structure) of approximately 85 amino acids, which binds to sites that contain the central GG(A/T) motif (Figure 5.3.C). Several ETS transcription factors directly control the expression of the immediate early response genes. ETS and AP-1 jointly regulate the expression of *upa*, *mmp-1*, *-3*, *-7*, *-9*, and *osteopontin* (Figure 5.3.D).

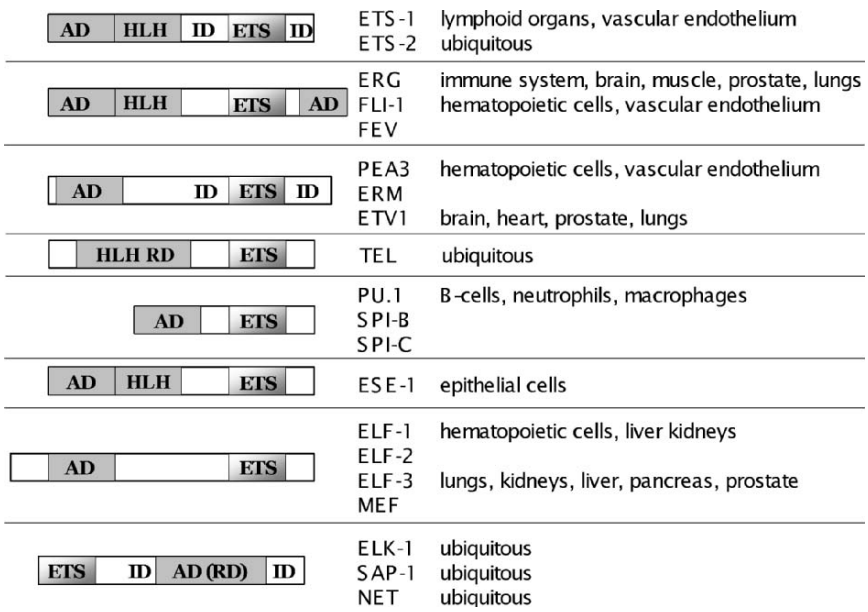


Figure 5.3.C. Structure of ETS transcription factors. Multiple ETS subfamilies share the ETS DNA binding domain (ETS). Most ETS family members have the signature ETS domain in their COOH-terminal portion. Some ETS family transcription factors contain a helix–loop–helix (HLH) domain through which they interact with other proteins. AD = activation domain, RD = repression domain, ID = autoinhibitory domain.

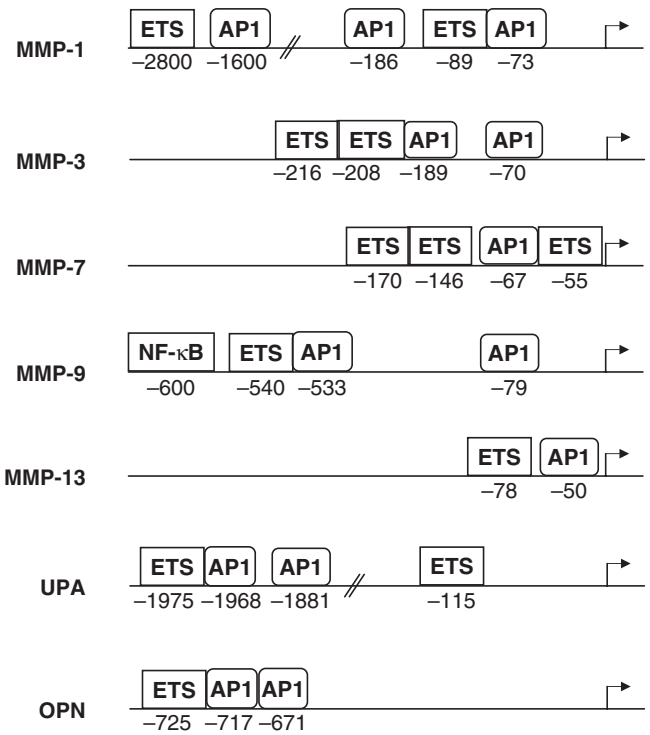


Figure 5.3.D. ETS recognition sites in promoters of metastasis genes. Depicted are the gene promoters, position 1 is the start site. The boxes indicate cis-acting elements. The indicated positions are ETS consensus sites. Also shown are recognition sites for the other metastasis-associated transcription factors AP-1 and NF-κB. An ETS binding site adjacent to an AP-1 binding site is referred to as RAS responsive element (RRE). UPA = Urokinase Plasmin Activator, OPN = Osteopontin

ETS family transcription factors may play roles in cancers (Table 5.3.A).

- *ets* genes may be activated by chromosome translocations, which generate *ets* fusion genes. In the Ewing family of tumors, *ews* {22q12} can be fused with three distinct ETS transcription factor genes: *fli-1* {11q24}, *erg* {21q22.3}, or *etv-1* {7p22}. *ets-1* can be involved in translocations in acute myelomonocytic leukemia and small cell lymphoma. TEL fusion proteins, generated by translocations, are associated with AML and CML.

Table 5.3.A. Aberrant expression of ETS family members in cancer. Transcription factors of the ETS family may be excessively active in cancers. Specific defects of individual ETS members underlie particular cancers.

ETS protein	Defect	Product	Cancer
ETS1	Amplification		Myelomonocytic leukemia
	t(9;11)(p22;q23)		Acute monocytic leukemia
ETS2	t(8;21)(q22;q22)		AML-M2
FLI1	t(11;22)	EWS-FLI1	Ewing sarcoma
ERG	t(21;22)	EWS-ERG	Ewing sarcoma
	t(16;21)	FUS-ERG	AML
		TMPRSS2-ERG	Prostate cancer
ETV1	t(7;22)(p22;q12)	EWS-ETV1	Ewing sarcoma
		TMPRSS2-ETV1	Prostate cancer
ETV4	t(17;22)	EWS-ETV4	Ewing sarcoma
	Overexpression		Breast cancer
ELF3	Overexpression		Breast cancer
ELK2	t(X;18)(p11.2;q11.2)		Synovial sarcoma
SPI1	Mutation		AML
TEL	t(12;15)(p13;q25)	TEL-NTRK3	Congenital fibrosarcoma
	t(12;21)	TEL-AML1	AML
	t(5;12)	TEL-PDGFRβ	Chronic myelomonocytic leukemia
	t(12;22)(p13;q11)	TEL-MN1	Myeloid malignancies
	t(5;12)(q31;p13)	TEL-ACS2	Acute eosinophilic leukemia

- Increased levels of *ets-1* transcripts in breast tumors predict a poor prognosis [Span et al. 2002], and PEA-3 group members are overexpressed in metastatic breast cancer [de Launoit et al. 2000]. Their expression activates the transcription of *matrix metalloproteinases* and *icam-1*, and may be sufficient to generate a metastatic phenotype. ETS transcription factors, particularly PEA-3, enhance the transcriptional activation of *osteopontin* by β-Catenin and LEF-1 and by c-JUN. This may play a role in breast cancer dissemination [El-Tanani et al. 2004].
- The levels of ETS-1 are associated with grade and prognosis in lung cancer and colorectal cancer. A correlation also exists between the expression level of PEA3 (E1AF) and tumor progression in ovarian cancer.
- In liver cancer, ETS-1 is involved in invasiveness and metastasis by upregulating the expression of *mmp-7* and *N-acetylglucosaminyl transferase*.
- The increases in GlcNAc Transferase V (GlcNAc-T V, *O*-linked *N*-Acetylglucosamine Transferase, OGT) expression after oncogenic transformation are most likely caused by direct effects on the *GlcNAc transferase V* promoter by the ETS family of transcriptional activators, which are upregulated by a cellular proliferation signaling pathway. This pathway is initiated by growth factor receptors that activate Tyrosine Kinases at the cell surface and proceeds through SRC, RAS, and RAF.

GLI. In the nucleus, the Krüppel family zinc finger protein GLI (Glioma-Associated Oncogene) acts as a repressor of *hedgehog* target genes. In normal tissues, *gli* gene products are mainly active in precursor cells. There are 4 GLI proteins, which reside in the nucleus and the cytoplasm. They comprise GLI1 {12q13.2-q13.3}, GLI2 {2q14}, GLI3 {7p13}, and GLI4 {8q24.3}. In the cytoplasm, they are components of multiprotein complexes that are tethered to the cytoskeleton. In the absence of SHH, and upstream signaling molecule, GLI is cleaved by the proteasome and its COOH-terminally truncated forms translocate to the nucleus.

- Overexpression of GLI in epithelial cells strongly enhances *osteopontin* gene expression. This may contribute to tumor progression in some epithelial cancers.
- Metastatic cancers adopt certain properties of normal cells in regenerating organs, such as the ability to alter tissue organization. Activity of the Hedgehog signaling pathway is required for regeneration of prostate epithelium. The continuous activation of this pathway distinguishes metastatic from localized prostate cancer and it is important for invasiveness [Karhadkar et al. 2004].
- Basal cell carcinoma is one of the most prevalent cancers in the Western world. Activation of the SHH signaling pathway because of PTCH-1

inactivation is a key event in sporadic and familial basal cell carcinoma development and is associated with the transcriptional activation of several target genes through GLI.

- Pallister-Hall syndrome [Hall et al. 1980] is caused by frameshift mutations in the *gli3* gene. Typically, they result in the synthesis of truncated GLI proteins. The condition is characterized by hypothalamic hamartoblastoma, hypopituitarism, imperforate anus, and postaxial polydactyly.

MTA. *mta1* (*metastasis-associated gene-1*) {14q32.3} encodes a protein of 703 amino acids and an approximate molecular mass of 80 kD [Toh et al. 1994]. The protein contains several potential phosphorylation sites and a proline-rich stretch at the COOH-terminal end, which constitutes a SH3 (SRC Homology 3) domain binding motif [Toh et al. 1995]. Because MTA1 is localized in the nucleus and contains nuclear localization signals, a GATA-like zinc finger, a leucine zipper, a SANT domain, and five copies of a SPXX motif it is likely to be a gene regulatory protein [Nawa et al. 2000; Toh et al. 2000]. MTA1 binds to the Histone Deacetylase HDAC1. MTA1 is physiologically expressed at low levels in most organs and at high levels in the testes.

– MTA1-ZG29p is an NH_2-terminally truncated form of MTA1 and is present in the zymogen granules of the pancreas.
– MTA1s is a COOH-terminally truncated form present in the cytoplasm. MTA1s binds to the Estrogen Receptor and inhibits its nuclear functions by sequestering it within the cytoplasm, stimulating the MAPK pathway.

In the MTA family, there are three distinct genes (*mta1*, *mta2*, and *mta3*) and six protein isoforms (MTA1, MTA1s, MTA1-ZG29p, MTA2, MTA3, MTA3L). MTA1, MTA2, and MTA3 are components of the NURD (Nucleosome Remodeling and Deacetylation) complex, which is associated with adenosine triphosphate (ATP) dependent chromatin remodeling and transcriptional regulation. MTA proteins, as a part of the NURD complex, may modulate transcription by influencing the status of chromatin remodeling.

- The *mta1* gene is induced severalfold in metastatic cancers. MTA1 overexpression is closely correlated with an aggressive course in highly metastatic melanomata, cervical carcinomata, and ovarian carcinomata.
- MTA1 enhances migration, invasion, and anchorage independent survival of keratinocytes. It is frequently overexpressed in aggressive epithelial neoplasms, including squamous carcinoma cells [Mahoney et al. 2002].
- Mammary adenocarcinomata with no or low Estrogen Receptor in the nucleus exhibit elevated levels of MTA1s and cytoplasmic subcellular localization of the Estrogen Receptor.

EGR. The immediate early gene product EGR-1 (Early Growth Response 1, NGFIA, Nerve Growth Factor-Induced Clone A, KROX24) [Forsdyke 1985] is a protein with three DNA binding zinc fingers that maps to 5q31.1. EGR1 regulates the expression of the *tgf-β1*, *fibronectin-1*, and *mt1-mmp* genes. It may contribute to the transcription of the *cd44* gene. The expression of *egr-2* (KROX20) {10q21.1-q22.1} is coregulated with *egr-1* by fibroblast and lymphocyte mitogens. Alternative splicing of *egr-2* may occur at the 5′ end of the coding region. The *egr-3* gene {8p23–21} encodes for a predicted 387 amino acid protein containing three C_2H_2 zinc fingers that are nearly identical to those of EGR-1 and EGR-2. The *egr-3* gene has one intron. The gene is induced in various brain regions in response to stress or following focal brain injury.

- EGR1 mRNA expression is increased in gastric cancer tissues compared to normal mucosa. Moreover, EGR1 expression is higher in cases with metastasis to lymph nodes or remote organs. EGR1 may have a significant role in canacer progression [Kobayashi et al. 2002].
- In various cancers, microvascular endothelial cell growth, neovascularization, tumor angiogenesis, and tumor growth are processes that are dependent on EGR1.
- In prostate cancer, EGR1 confers resistance to apoptotic signals through its ability to inhibit CD95 expression. This leads to insensitivity to CD95L.

Epithelial–mesenchymal transition. Epithelial cell plasticity can lead to remodeling and reversible conversion to a mesenchmal appearance. Epithelial–mesenchymal transition is a distinct process in carcinogenesis [Greenburg and Hay 1982]. Carcinomata lose most of their epithelial characteristics during tumor progression. This process may be triggered by loss of E-Cadherin function, TGF-β signaling, or stimulation by HGF.

Type 1 Cadherins (E-Cadherins) mediate homotypic interactions through the binding of their extracellular Immunoglobulin domains. They connect to Actin microfilaments through α-Catenin or β-Catenin in the cytoplasm. There is an inverse correlation between the levels of E-Cadherin expression and tumor grade or patient survival [Birchmeyer and Behrens 1994; Hirohashi 1998]. E-Cadherin is one of the caretakers of the epithelial phenotypes and its downregulation is an important step in epithelial–mesenchymal transition, a process that correlates with the presence of β-Catenin in the nucleus [Thiery 2002].

– A large portion of lobular breast carcinomata and gastric carcinomata contain *E-cadherin* nonsense mutations or frameshift mutations [Berx et al. 1995; Berx et al. 1998]. This leaves the E-Cadherin protein either absent or truncated and unable to mediate adhesion. The truncation mutants may exert a dominant negative effect on other Cadherins.
– In cancer, *E-cadherin* may be downregulated by transcriptional repression. A strong repressor of *E-cadherin* expression is the helix-loop-helix transcription factor E12/E47, both proteins are gene products of *e2A* (*tcf3*, *itf1*) {9p13.3}. The transcription factor FOS also induces epithelial–mesenchymal transition that is accompanied by the loss of E-Cadherin.
– Snail is a zinc finger containing transcriptional repressor that binds strongly to two E-boxes proximal to the transcriptional start site of *E-cadherin*. Snail is expressed in high-grade breast carcinomata and in lymph node positive tumors [Blanco et al. 2002]. Snail and its close family member Slug are expressed at sites of epithelial–mesenchymal transition. Slug is essential for this process. Moreover, the small G-Protein RHO-B, which is involved in cell motility, is encoded by a target gene of Slug.
– SIP-1 (SMAD Interacting Protein-1), an E-box binding protein and transcriptional target of the TGF-β pathway, acts as a strong repressor of *E-cadherin*. SIP-1 binding sites overlap Snail binding sites.

Besides E-Cadherin, other adhesion molecules regulate epithelial–mesenchymal transitions. N-Cadherin is expressed in some epithelial cells that have lost E-Cadherin and acts as a weak intercellular adhesion system. Epithelial–mesenchymal transformations are frequently associated with the loss of NCAM, followed by its reexpression [Crossin et al. 1990].

The outcome of TGF-β signaling is context dependent and may mediate epithelial–mesenchymal transition. This morphologic transition is characterized by a switch from a cytoskeleton composed of mainly Cytokeratin intermediate filaments to one comprising predominantly Vimentin.

– TGF-β induces Slug, possibly through $P38^{MAPK}$ and RHO-A. RHO-A normally functions to maintain apical–basal polarity and cell junctions.
– TGF-β signaling leads to SMAD-2 phosphorylation and nuclear import. Activated SMAD-2 cooperates with H-RAS in inducing the expression of α-Smooth Muscle Actin and Integrin $\alpha_V\beta_3$, leading to epithelial–mesenchymal transition.
– The dissolution of tight junctions is an early event in epithelial–mesenchymal transition. PAR-6 is a regulator of epithelial cell polarity and tight junction assembly. It is a substrate for phosphorylation by the TGF-β type 2 Receptor. PAR-6 phosphorylation activates its interaction with the E3 Ubiquitin Ligase SMURF-1 and targets RHO-A for degradation. This results in loss of tight junctions and epithelial–mesenchymal transition [Ozdamar et al. 2005].

TGF-β contributes to the progression from papillomata via squamous cell carcinomata to undifferentiated spindle cell tumors. In late stage cancers, the autocrine production of TGF-β contributes to tumor progression.

Some polarized epithelial cells can be converted into migratory fibroblast-like cells by HGF (Hepatocyte Growth Factor) [Stocker and Perryman 1985; Nakamura et al. 1989]. HGF does not trigger any detectable decrease in E-Cadherin function, but it increases Integrin-mediated cell traction. Signaling through the receptor c-MET mediates epithelial–mesenchymal transition. c-MET activation occurs through the phosphorylation of the tyrosines Y1349 and Y1356. This recruits several SH2 containing adapters, including GRB-2, GAB, SHC-1, and CBL. It also recruits effector proteins, including PLC-γ, PI3-K, and STAT-3.

5.4 SIGNAL TRANSDUCTION LEADING TO INVASION

The engagement of homing receptors on the surface of transformed cells by their cognate ligands initiates the process of migration and invasion. The associated signal transduction pathways converge on the

rearrangements of the cytoskeleton that underlie locomotion. They constitute the alternate forming and breaking of adhesion contacts. Adhesions transmit propulsive forces and serve as traction points, over which the cell moves. Because cell migration involves the spatiotemporal organization of adhesion to and release from the substratum, the biochemical processes at the leading edge oscillate between those two states, and rear-end retraction involves similar oscillations shifted in phase.

All cell movements are manifestations of mechanical work that requires the conversion of the energy stored in ATP into motion. The cytoskeleton, a cytoplasmic system of fibers, is critical to cell motility. The initial response of a cell to migration promoting agents is the polarization and extension of protrusions in the direction of migration. During migration, lamellopodia (large protrusions), or filopodia (spike-like protrusions) attach to the extracellular matrix at the leading edge and contacts are broken at the trailing edge. The extensions are induced by the polymerization of Actin filaments, which form inside the leading edge of a cell. Actin filaments have two ends, described as barbed and pointed. Actin monomers bound to a molecule of ATP add to the barbed end.

– Actin filaments in lamellopodia grow in a branched fashion. Once a WASP (Wiscott-Aldrich Syndrome Protein, WAVE) family member is activated by a motility signal, it binds to the ARP2/3 (Actin-Related Protein 2/3) complex. This leads to the nucleation of branched Actin filament growth and lamellopodia formation (Figure 5.4.A). Cortactin (EMS-1) stabilizes individual branches, whereas Filamin A and α-Actinin stabilize the entire network by cross-linking filaments. A capping protein attaching to the barbed end terminates that growth. Through localized activation of the ARP2/3 complex, lamellopodia can be induced to grow in a particular direction, providing the basis for directional migration. In contrast to the ARP2/3 complex, Formins accelerate Actin nucleation at the barbed ends. The RHO effector mDIA1 belongs to the Formin family of gene products, which shares the

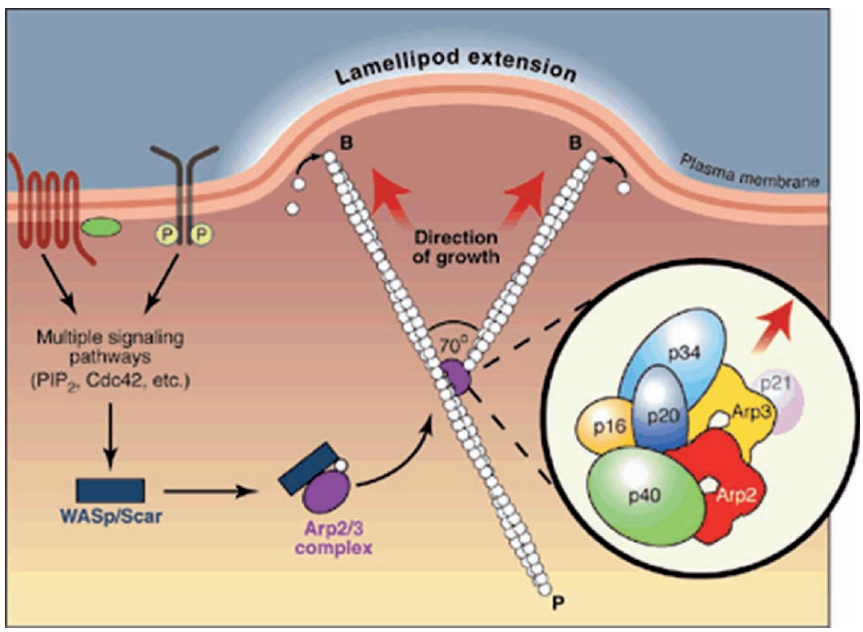


Figure 5.4.A. Branched Actin polymerization. Members of the WASP (SCAR) protein family (black) integrate signals from multiple pathways to activate the ARP2/3 complex (purple). This complex binds to the sides of Actin filaments (white) and nucleates the formation of new Actin filament branches, which elongate at their barbed ends (B). Red arrows indicate the direction of Actin filament growth. This expanding network of branching Actin filaments drives the protrusion of the plasma membrane and hence cell motility. (*Inset*) In the activated ARP2/3 complex, ARP2 and ARP3 are positioned with their five ancillary proteins such that they are able to direct barbed end growth of daughter Actin filaments. The clefts between the two lobes of ARP2 and ARP3 (partially obscured) point toward the bottom left of the inset. [Reproduced from Weeds and Yeoh 2001. With permission].

conserved tandem FH1-FH2 structure. It moves progressively along the growing end of Actin filaments.
– Actin bundles in filopodia elongate without branching at their barbed ends and release Actin monomers from their pointed ends. ENA {1} and VASP (Vasodilator-Stimulated Phosphoprotein) {19q13.2-q13.3} bind to the barbed ends of Actin filaments and antagonize both capping and branching, thereby allowing the continuous elongation [Ridley et al. 2003].

After conversion from ATP to ADP, Actin monomers are removed from the pointed end. Cofilin (ADF, Actin Depolymerizing Factor) severes the filaments at their pointed ends, releasing monomers that bind either Thymosin or Profilin. Profilin replaces the ADP on the monomers with ATP, which provides the initial step for another cycle of filament formation.

5.4.1 FAK-Dependent Pathways

Focal Adhesion Kinase (FAK, $P125^{FAK}$) plays a central role in Integrin dependent signal transduction. It contains no SH2 or SH3 regions, but it does contain phosphotyrosines and proline-rich regions that respectively bind to the SH2 or SH3 domains of other molecules. FAK is a cytoplasmic enzyme that colocalizes with Integrins at focal adhesions. Integrin binding by extracellular matrix ligands induces Integrin clustering and FAK activation. The binding of FAK to Talin may contribute to the indirect interaction among the β subunits of multiple Integrin receptors.

Integrin activation induces the auto-phosphorylation of FAK on Y397. This recruits SRC binding through its SH2 domain, an interaction that is stabilized through the SH3 domain of SRC. The resulting complex formation increases the catalytic activity of SRC to phosphorylate additional sites in FAK, including the regulatory loop tyrosines 576 and 577 in the FAK kinase domain [Cary et al. 2002]. SRC can phosphorylate FAK, CAS, Paxillin, Cortactin, and Tensin, and is required for Integrin mediated cell migration and spreading [Chellaiah et al. 1996]. Through COOH-terminal proline rich regions, activated FAK associates with the scaffolding molecule CAS, whose tyrosine phosphorylation is important for the regulation of cell migration. The phosphorylation of Y397 on FAK is essential for the phosphorylation of the associated molecule CAS. FAK and SRC phosphorylate CAS, which causes CAS to recruit CRK and locate to membrane ruffles at the leading edge of motile cells. The CAS/CRK complex may act as a molecular switch that induces cell motility by activating DOCK-180 (Dedicator of Cytokinesis 180), leading to the activation of the small GTP binding protein RAC. The cascade Integrin→FAK/c-SRC→CAS→CRK→DOCK 180→RAC→GEFs regulates adhesion disassembly and leads to lamellopodia formation. ERK and Calpain are also involved [Ridley et al. 2003].

Cell migration as well as Integrin-mediated spreading and formation of focal adhesions [Tamura et al. 1998] are inhibited by wild type PTEN but not by PTEN mutants with an inactive phosphatase domain. The 47 kD gene product PTEN (Phosphatase and Tensin Homologue Deleted from Chromosome 10) is a dual specific protein Tyrosine Phosphatase and Lipid Phosphatase. The PTEN protein is Tyrosine phosphorylated upon Integrin-dependent cell adhesion. The COOH-terminal three amino acids of PTEN, threonine, lysine, and valine (TKV), comprise a consensus motif for binding to proteins containing a PDZ domain. PTEN may dephosphorylate FAK leading to inhibition of the downstream target $P130^{CAS}$. Through the dephosphorylation of FAK, the phosphatase PTEN functions as a metastasis suppressor by negatively regulating cell interactions with the extracellular matrix. PTEN can also inhibit cell migration through its C2 domain independently of its lipid phosphatase activity, but dependent on the protein phosphatase activity and dephosphorylation of one specific threonine [Raftopoulou et al. 2004]. PTEN contains an approximately 175 amino acids region of homology to Auxillin and Tensin, a protein that interacts with Actin filaments at focal adhesions.

The members of the PRL (Phosphatase of Regenerating Liver) family of Tyrosine Phosphatases, PRL-1, PRL-2, and PRL-3, may regulate cell motility and invasion. PRL proteins share 76–87% sequence identity and are characterized by a unique COOH-terminal prenylation motif. PRL-1 encodes a 20 kD protein with an eight amino acid protein Tyrosine Phosphatase-active site. Outside the active site, PRL-1 has no homology to other Tyrosine Phosphatases. PRL-1 is able to dephosphorylate phosphotyrosine substrates, including $P130^{CAS}$, which may mediate deadhesion during cell migration. Although a fraction of the cellular PRL-1 is located in the cell nucleus [Diamond et al. 1994], the PRL family of phosphatases are mainly associated with the plasma membrane and endosomal structures in a prenylation dependent manner. The 22 kD Tyrosine Phosphatase PRL-3 (PTP4A3) is located at the cytoplasmic membrane when prenylated, and in the nucleus when not

conjugated to this lipid. Acccording to homology with PTEN in the phosphatase signature motif and its flanking regions, PRL-3 may act as a lipid phosphatase at the cytoplasmic face of the plasma membrane. PRL-2 is preferentially expressed in skeletal muscle, while PRL-3 is preferentially expressed in both skeletal muscle and heart, although both PRL-2 and PRL-3 are expressed at lower levels in other tissues.

The pathway Integrin→FAK/c-SRC→GRB→SOS→RAS, enhanced by FAK→Paxillin→CRK→C3G→RAS, leads to ERK (MAPK) activation and cytoskeletal changes. ERK signaling decreases Integrin-mediated adhesion through MLCK and through Calpain.

– ERK phosphorylates MLCK, a regulator of the contractile force within the cell. MLCK phosphorylates and activates the ATPase Myosin II, which breaks adhesive contacts at the trailing edge of the cell [Hood and Cheresh 2002].
– ERK regulates Calpain, a calcium activated protease that breaks rear contacts during migration.

JNK-1 is also required for cell movement. It phosphorylates the focal adhesion adaptor Paxillin on serine 178.

The SHC family of adapter proteins are recruited to activated Tyrosine Kinases in response to ligation of the Integrins $\alpha_1\beta_1$, $\alpha_6\beta_4$, $\alpha_5\beta_1$, or $\alpha_V\beta_3$. They enhance signaling through RAS. These proteins are composed of three interaction domains, a COOH-terminal SRC homology 2 (SH2) domain, a central Collagen homology (CH) domain, and an NH_2-terminal phospho-tyrosine binding (PTB) domain. SHC is essential for the reorganization of the cytoskeleton in response to extracellular matrix signaling. The PTB domain may be a crucial element in the induction of a metastatic phenotype.

The cascade Integrin→FAK/c-SRC→Phosphatidylinositol 3-Kinase, enhanced by a tri-molecular complex of Paxillin and CSK with FAK, leads to the rearrangement of Actin and Gelsolin [Hruska et al. 1995; Chellaiah and Hruska 1996]. It is important in cell spreading and migration. PI 3-Kinase can activate CDC42 and RAC-1 in a manner that is independent of AKT, because several GEFs are activated by PI 3-Kinase products. The phosphatidylinositol trisphosphates $PI(3,4,5)P_3$ and $PI(1,3,4)P_3$ are key signaling molecules that become rapidly and highly polarized in cells exposed to a gradient of chemoattractant. This process amplifies signals derived from shallow gradients and defines the location of RAC activation. It is driven by the localized accumulation and activation of PI 3-Kinase, which may account for the regulation of cell motility by PI 3-Kinase. MGAT5 products may be important in the activation of PI 3-Kinase. This is a mechanism through which carbohydrates regulate invasiveness.

- FAK is frequently expressed at higher levels in invasive tumors than in noninvasive lesions. Pancreatic carcinoma cells that undergo adhesion-dependent CAS phosphorylation are more motile and metastatic than tumor cells that do not.
- SRC expression and activity are often elevated in colon cancer. This causes the components of adherens junctions, including Vinculin, to be redistributed to SRC-induced Integrin adhesion complexes, and E-Cadherin remains internalized. Phosphorylation of the Integrin-regulated FAK on SRC specific sites is required for the SRC-induced deregulation of E-Cadherin [Avizienyte et al. 2002].
- PTEN may regulate tumor cell invasion and metastasis through interactions at focal adhesions.
- PTEN is often deleted in glioblastomata and prostate cancers. Deletion or mutation of PTEN may be a very early event in 30–50% of endometrial carcinomata. In Cowden disease patients, increased cancer risk correlates with germline mutations of PTEN.
- PRL-1 and PRL-3 promote cell migration, invasion, and metastasis [Zeng et al. 2003]. PRL-3 is expressed at elevated levels in colon cancer metastases compared to nonmetastatic colon tumors or in normal colon epithelium. This may be due to amplification of the PRL-3 gene on chromosome 8q24.12 [Saha et al. 2001].

5.4.2 Signaling Through Small GTPases

The signaling network associated with metastatic spread involves the RHO family of small GTPases, RHO, RAC, and CDC42, which control cytoskeletal reorganization and the formation of focal adhesion complexes. CDC42 activates RAC and RAC activates RHO. Their activation affects the formation of stress fibers (RHO), focal adhesions (RHO), lamellopodia (RAC), and filopodia (CDC42) through effects of these molecules on the Actin cytoskeleton. In the cell body, RHO promotes the assembly of Actin–Myosin filaments and cell contraction, while at the leading edge CDC42 and RAC-1 control cell polarity in response to external cues. CDC42 is a master regulator of cell polarity and is active toward the front of

migrating cells. Consistently, the subcellular location of activation of CDC42 and other guanosine triphosphatases is a critical determinant of cell behavior.

The small GTPases are major mediators of Integrin signaling. Integrins, through FAK, regulate the targeting of activated, GTP-bound RAC and RHO to the plasma membrane via lipid rafts. Lipid rafts are cholesterol and sphingolipid rich plasma membrane domains that contain glycosylphosphatidylinostol (GPI) anchored proteins. Integrins amy also activate small GTPases through other mediators. *p311 (PTZ17, C5ORF13)* is a 2036 bp mRNA encoding a 68 amino acid (8 kD) polypeptide with a PEST domain and a very short half-life. It associates with Integrin β_1 and activates RAC-1. In wounds P311 is expressed selectively in myofibroblasts and their activated precursors, possibly activating their migration.

RAC, RHO, and CDC42 have various downstream targets (Table 5.4.2.A) (Figure 5.4.2.A).

– CDC42 can affect polarity by localizing the microtubule organizing center (MTOC) and Golgi apparatus in front of the nucleus toward the leading edge. This facilitates microtubule growth and the movement of Golgi derived vesicles into the lamella. CDC42 mediates this process through a complex of PAR-3, PAR-6, and an atypical PKC.
– Active CDC42 at the front of the migrating cell recruits the HECT domain Ubiquitin E3 Ligase SMURF-1 via the PAR-6→PKCζ effector pathway. SMURF-1 binds to nucleotide free RHO and promotes RHO ubiquitination, leading to its proteasomal degradation. This process ensures that RHO is restricted to the rear of the cell, where it promotes tail retraction [Wang et al. 2003].
– The interaction of CDC42 with WASP (Wiscott-Aldrich Syndrome Protein) is essential for Actin polymerization. Binding of CDC42 to the GTPase binding domain of WASP induces the unfolding of WASP. The acidic domain and Cofilin homology region at the COOH-terminal end of WASP bind to the ARP2/3 complex, which initiates Actin polymerization. The proximal Verprolin homology region binds to monomers of un-polymerized Actin, which can be passed to the neighboring ARP2/3 complex for assembly into filaments. Phosphatidylinositol-4,5-bisphosphate is a weak activator of WASP. It may cooperate with CDC42 in the induction of Actin polymerization. The generation of phosphatidylinositol-4,5-bisphosphate leads to severing of Actin filaments, which provides nucleation sites for Actin monomer addition. Phosphatidylinositol-4-Phosphate 5-Kinase is a target for retinoic acid.
– Effectors of RAC, including the serine/threonine kinase PAK (P21-Activated Kinase, $P21^{PAK}$) and WASP, contain a CRIB (CDC42/RAC Interactive Binding) domain. Upon detachment, RAC and RHO are internalized. RHO synergizes with RAC

Table 5.4.2.A Effectors of RHO proteins. RHO proteins play central roles in regulating the complex process of cell motility. They exert their effects through multiple downstream targets.

RHO protein	Effector	Effector function	Biologic role
RHO-A, RHO-C	ROCK -I, -II	Kinase	Actomyosin contraction, Actin polymerization
	PRK1 (PKN)	Kinase	Endocytosis
	Citron	Kinase	Cytokinesis
RHO-B	PRK1	Kinase	Cell adhesion, EGFR trafficking
RHO-G	Kinectin	Scaffold	Microtubule binding
CHP	PAK	Kinase	JNK activation
RAC-1, -2, -3	PAK	Kinase	JNK activation
	PAR6	Kinase	Cell polarity
	IQGAP1	GTPase activation	Adherens junction
	IRS	Scaffold	Actin polymerization
CDC42	PAK	Kinase	JNK activation
	PAR6	Kinase	Cell polarity
	WASP	Scaffold	Actin polymerization
	MRCK-1, -2	Kinase	Actin organization
TCL, TC10	PAK	Kinase	JNK activation
	WASP	Scaffold	Actin polymerization

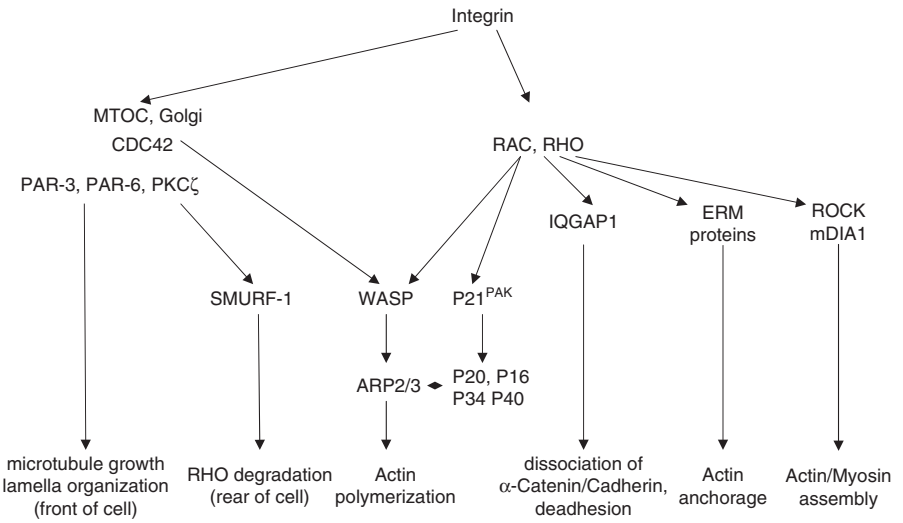


Figure 5.4.2.A. Induction of cell motility by RHO proteins. The ligation of Integrin receptors on the cell surface activates signaling through the RHO family proteins RAC, RHO, and CDC42. They lead to a multitude of downstream consequences that synergize to rearrange the cytoskeleton.

and its effector $P21^{PAK}$ to contribute to forming a complex of ARP2/3, P20, P16, P34, and P40. This complex mediates Actin assembly and induces the formation of membrane ruffling and lamellopodia. While the ARP2/3 complex tends to nucleate new Actin filaments at the sides of existing Actin filaments, resulting in a branching filament network, Formins nucleate the assembly of straight filaments. The ARP2/3 complex binds to the pointed (minus) end of the Actin filament, whereas Formins bind to the growing barbed (plus) end of the Actin filament. WASP regulates the ability of the ARP2/3 complex to nucleate Actin polymerization (Figure 5.4.2.B).

– IQGAP1 (P195, SAR1) is a target of CDC42 and RAC1 and may induce the dissociation of α-Catenin from Catenin/Cadherin complexes. This leads to a decrease in E-Cadherin mediated cell–cell adhesion [Kuroda et al. 1998]. IQGAP-1 interacts with CLIP170 (Restin, CYLN1) and regulates its association with microtubules. The anti-GAP IQGAP localizes to membrane ruffles, filopodia, and cell–cell connections.
– Important downstream links of RHO GTPase signaling, possibly through phosphatidylinositol-4,5-bisphosphate, are the ERM (Ezrin, Radixin, Moesin) family membrane-cytoskeleton linker proteins. The ERM proteins are phosphorylated and localized to the membrane in response to RHO activation and their COOH-terminal ends interact with filamentous Actin. They may therefore be regulatable scaffold proteins that anchor Actin filaments to the membrane. A fourth member of the ERM family is Merlin, which may interact with RAC-1 but not RHO-A. Merlin and the ERMs can interact with and regulate N-WASP, a critical regulator of Actin dynamics. RAC-1 promotes the phosphorylation and inhibition of the Ezrin antagonist NF2. Further contributing to invasion, RHO-A and RAC-1 can regulate the levels of MMP and TIMP expression.
– The serine-theronine kinase $P160^{ROCK}$ (RHO-Associated Kinase) is a mediator of RHO induced changes to the Actin cytoskeleton. The Plekstrin homology domain of $P160^{ROCK}$ interacts directly with the homing receptor CD44. Two substrates of this kinase are the Myosin Light Chain Phosphatase and the Myosin Light Chain, which regulate the assembly of the Actin–Myosin filament bundles and lead to the development of focal contacts. Another downstream target of RHO, Diaphanous (mDIA, Mamalian Homolog of Diaphanous), is also required for focal contacts. Microtubule stabilization near the leading edge is regulated by RHO and its effector mDIA1. Once the α-Tubulin in stabilized microtubules becomes de-tyrosinated, it may contribute to cell polarization.

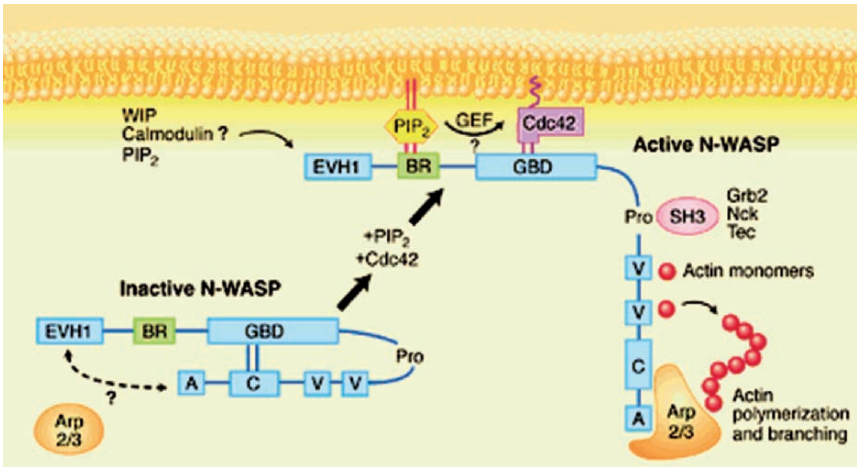


Figure 5.4.2.B. Regulation of WASP function. Protein–protein and protein–phospholipid interactions activate the N-WASP protein, which coordinates the initiation of Actin assembly. In its inactive state, N-WASP blocks its own activity by the folding of its GTPase binding domain (GBD) at the NH_2-terminus with its Cofilin homology domain (C) at the COOH-terminus. Cooperative binding of phosphatidylinositol bisphosphate (PIP_2) and activated CDC42 to the basic region (BR) and to the GBD of N-WASP, respectively, results in activation of the ARP2/3 complex. This complex binds to the acidic (A) and C regions of N-WASP and then initiates the assembly of Actin monomers into polymerized Actin filaments. [Reproduced from Fawcett and Pawson 2000. With permission.]

– Cytokine Receptor signals and stress signals may proceed via RHO-GTPases→MEKK→MKK→$p38^{JNK}$. The production of second messengers after RHO-GTPase family signaling or after G-protein signaling involves cAMP and calcium.
– The pathway RHO→CRIK→HGK leads to cell migration. HGK (Hepatocyte Progenitor Kinase-Like Kinase, Germinal Center Kinase-Like Kinase) is a member of the MAP4K (STE120) family of serine/threonine kinases. Members of the structural subclass of Germinal Center Kinases have a NH_2-terminal kinase domain and a COOH-terminal extension of variable length. The *hgk* gene {2q11.2} contains 33 exons, including nine alternatively spliced modules. HGK is physiologically expressed in macrophages. Alternative splicing modulates the length of the coiled–coil domain, the composition of the CNH domain, and the array of PXXP motifs. HGK contributes to anchorage independence without affecting the growth rate of cells. HGK activity supports epithelial cell invasion. This may be due to its ability to modulate *integrin* expression [Wright et al. 2003].

Four subfamilies of RHO proteins include those related to RHO-A, RAC-1, or CDC42, and those without GTPase activity (RND, RHO-H). RHO family proteins have lipid modifications that target them to the cell membrane. They are regulated by RHO-GAPs, RHO-GEFs, and RHO-GDIs. RHO-GDIs sequester GDP bound RHO proteins in the cytoplasm away from the GDP-GTP cycle [Sahai and Marshall 2002]. RHO-GDI2 is an inhibitor of guanine nucleotide dissociation for RHO proteins.

- RHO activity is required for full transformation induced by oncogenic RAS. In breast cancer and in testicular germ cell tumors, RHO-A expression levels correlate with progression. Furthermore, the overexpression of RHO-A induces metastatic potential in hepatoma cells. RHO-C is overexpressed in pancreatic ductal adenocarcinoma and in melanoma cells with high metastatic potential.
- RHO-H is altered in tumors of myeloid origin. It is frequently rearranged in multiple myeloma and in non-Hodgkin lymphoma. In B-cell lymphomata, the untranslated region of *rho-h* acquires point mutations that may affect its expression.
- The regulation of the Actin cytoskeleton by RHO-GTPases contributes to invasion and metastasis. Conversely, RHO-GDI2 is an invasion and metastasis suppressor gene, the expression of which correlates inversely with invasiveness in bladder cancer. It suppresses metastasis formation, but does not alter the growth rate [Gildea et al. 2002].

- A splice variant of RAC-1B is highly expressed in colon and breast cancers [Schnelzer et al. 2000; Jordan et al. 1999].
- Merlin (Neurofibromin-2, NF-2 Schwannomin, SCH) is a tumor suppressor for neurofibromatosis type 2 and meningioma. Neurofibromatosis type 2 is characterized by bilateral vestibular schwannomata, with predisposition to spinal and cranial schwannomata, meningiomata, and astrocytomata caused by loss-of-function mutations in the gene *nf2* {22q12.2} [Trofatter et al. 1993]. Ezrin is overexpressed in osteosarcoma cells [Khanna et al. 2001].
- The gene product IQGAP1 is upregulated in diffuse gastric cancer. This may reflect gene amplification at 15q26. Infection with *Helicobacter pylori*, a pathogenetic factor in gastric cancer, induces RHO-GTP and IQGAP-1 in gastric epithelial cells. In adherens junctions, E-Cadherin and IQGAP-1 are translocated from the plasma membrane to intracellular vesicles in response to *Helicobacter pylori*.
- While CLIP170 is rarely expressed in normal tissues, it is present in Reed–Sternberg cells, which are characteristic of Hodgkin disease. There, its overexpression may be a contributing factor in disease progression [Bilbe et al. 1992].
- *hgk* is frequently highly expressed and aberrantly spliced in tumor tissues, where typically at least five forms of the RNA message exist.
- P311 (PTZ17, C5ORF13) is expressed the highest in cerebellum, with lower expression in whole brain, liver, kidney, and heart. In invading glioblastoma cells, P311 is overexpressed. The protein localizes to focal adhesions and supports migration [Mariani et al. 2001].

5.4.3 Heterotrimeric G-Protein Signaling

Cell migration is often linked to signal transduction involving heterotrimeric Guanine Nucleotide Binding Proteins (G-Proteins) and small molecular weight Guanosine Triphosphatases (GTPases) (Figure 5.4.3.A). Such signals are activated in response to ligation of Chemokine Receptors. A main pathway proceeds through heterotrimeric G-Protein→GEF→small GTPase→ GAP, supported by heterotrimeric G-Protein→PI 3-K→small GTPase. The $G_{\beta\gamma}$ complex activates p110γ, the catalytic subunit of Phosphatidylinositol 3-Kinase. Phosphatidylinositol-3,4,5-trisphosphate may be essential and proximal to the activation of RAC, a critical regulator of migration, because it is a target of Plekstrin homology containing proteins that activate kinases and small GTPases. [Dekker and Segal 2000]

The activation of G-Proteins is important in the pathway of heterotrimeric G-Protein signaling. It requires the dissociation of protein bound GDP, an intrinsically slow process that is accelerated by Guanine Nucleotide Exchange Factors (GEFs). The GEFs comprise the DBL homology (DH) family.

– TIAM-1 (T-Lymphoma Invasion and Metastasis-1) {21q22.1} is a GEF for RAC-1. It also binds Ankyrin.
– VAV {19p13.3-p13.2} is a GEF for RAC-2. The *vav* gene is specifically expressed in cells of hematopoietic origin, including those of the erythroid, lymphoid, and myeloid lineages. In the absence of SHP, VAV is constitutively active.
– FGD-1 (FGDY) {Xp11.21} is a GEF for CDC42. It induces the downstream activation of JUN Kinase and $P70^{S6\ Kinase}$.
– DBL (MCF-2) {Xq27} is a GEF for RHO. Four splice variants of the *dbl* gene are expressed in a tissue specific manner.
– BCR {22q11.21} is a RHO-GEF family member. This 160 kD phosphoprotein also has serine/threonine kinase activity.
– LBC (Lymphoid Blast Crisis Oncogene, AKAP13, BRX) {15q24-q25} is a GEF for RHO. It also acts as an anchor protein for PKA and as a cofactor for Estrogen Receptor-mediated gene activation.
– ARHGEF1 ($P115^{RHO\text{-}GEF}$) {9q13.13}, ARHGEF2 (GEFH1) {1}, ARHGEF5 (TIM) {7q33-q35}, and ARHGEF12 (LARG) {11q23.3} are members of the RHO-GEF family.

The RHO-GEFs have a domain referred to as regulators of G-Protein signaling, which may bind to $G\alpha_{13}$-GTP or $G\alpha_{12}$-GTP and relay signal transduction from G-Proteins to GTPases, thus acting as GTPase Activating Proteins (GAPs). The GTPase reaction is slow so that it needs to be catalyzed by GAPs.

The pathway CD44→G-Protein→SHP-1→PI 3-K mediates chemotaxis. Enhanced SHP-1 activity leads to dephosphorylation of Phosphatidylinositol 3-Kinase, which may prevent adhesion and spreading of cancer cells, thus facilitating their dissemination. Through this mechanism, which is independent of

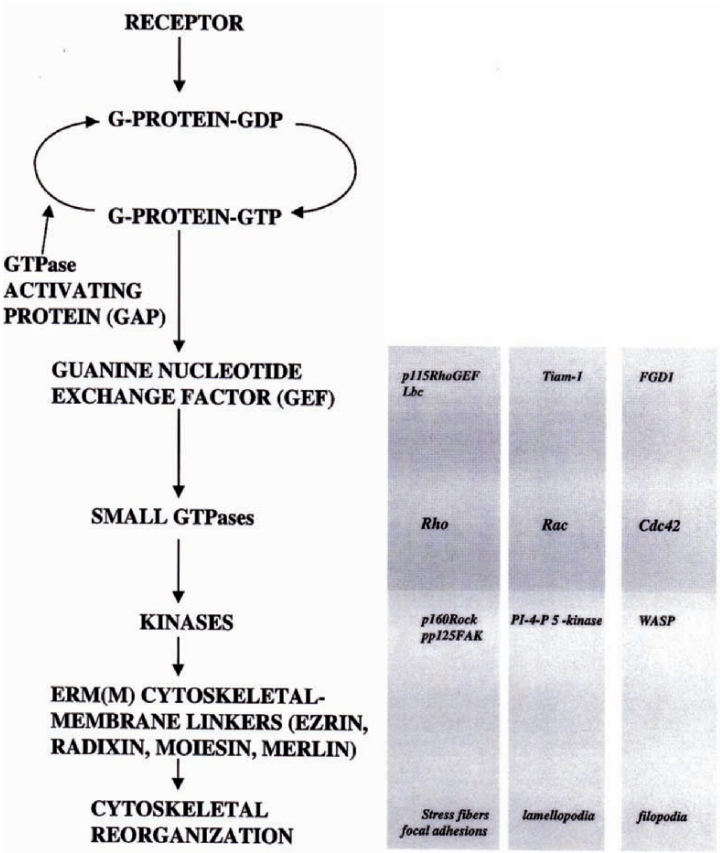


Figure 5.4.3.A. G-Protein signaling in cell migration. G-Protein coupled receptors that induce cells to migrate often signal through RHO family small GTPases. They reorganize the cytoskeleton by affecting focal adhesions, lamellopodia, and filopodia.

PKA, the engagement of CD44 by Osteopontin leads to tumor cell migration. The associated intracellular signals are specific for this interaction and are distinct from signaling induced by hyaluronate.

- In invasive T-cell lymphomata, proviral insertions may exist within coding exons of the *tiam-1* gene, which result in truncated protein fragments. Alternatively, amplification of the *tiam-1* locus may cause its overexpression. Affected cells have a higher potential to metastasize [Habets et al. 1994].
- Recombinations in the 5′ end of *dbl* result in the loss of NH_2-terminal codons, producing variants with oncogenic potential. This is associated with diffuse B-cell lymphoma [Srivastava et al. 1986].
- The *bcr* gene is the site of breakpoints in chronic myeloid leukemia and in acute lymphocytic leukemia that result in the generation of the two alternative forms of the Philadelphia chromosome translocation. They join distinct exons of *bcr* to a common subset of exons of *abl*, thus generating $P210^{BCR\text{-}ABL}$ and $P185^{BCR\text{-}ABL}$. The activation of ABL tyrosine kinase activity is necessary for the oncogenic potential of the chimeric oncogene. Sequences within the first exon of BCR appear to be essential for this activation and probably work through direct physical binding to the kinase regulatory domain of ABL.
- While normal and oncogenic forms of the *lbc* gene encode NH2-terminal DH and Pleckstrin homology domains, they differ in their COOH-termini. This is due to gene fusion consecutive to chromosome translocation. While LBC mostly locates to the cell membrane, the truncated oncogenic variant is distributed in the cytoplasm. LBC may be a pathogenetic factor in chronic myelogenous leukemia.
- Five alternative transcripts of *arhgef5* are specifically expressed in breast tumors. They encode modified or truncated proteins that may activate RAC-1 and CDC42 [Debily et al. 2004].

- LARG is a MLL fusion partner in acute myeloid leukemia. In this case, the 5′ end of *mll* at exon 6 may be fused in frame with the 3′ end of almost the entire open reading frame of *larg*. This fusion is the recult of an interstitial deletion [Kourlas et al. 2000].

5.4.4 Regulation of Migratory Signaling

Cell migration is characterized by the spatiotemporally coordinated interplay of adhesion and de-adhesion. It involves the establishment of a spatial and functional asymmetry among adhesion related and migration related molecules between the anterior (leading) and posterior (trailing) edges of the migrating cell. The generation of mechanical force is involved in coordinating cytoskeletal functions. In contrast, excessive adhesion or loss of traction prevent locomotion. Migration involves the generation of a net increase in Actin polymerization and adhesion formation at the front edge, while cell detachment and retraction occurs at the rear. The cells utilize two arrays of Actin filaments to produce protrusive and contractile elements that drive cell motility. The coupling between the arrays results from the unique properties of the lamellipodium. From the lamellipodium into the lamella behind, there is a transition from a fast retrograde flow of Actin polymer driven by polymerization to a slow flow driven by the interaction of antiparallel arrays of Actin with Myosin. In addition to driving protrusion, the lamellipodium may play a role in supplying filaments to the lamella for the assembly of the contractile network required for traction [Small and Resch 2005].

– Critical roles can be played by the alternate activation of the phosphatase SHP-1, which promotes de-adhesion, and its substrate Phosphatidylinositol 3-Kinase, the activation of which facilitates adhesion. Osteopontin induces PKC and Phosphatidylinositol 3-Kinase activity through ligation of Integrin $\alpha_V\beta_3$, while it activates SHP-1 through the engagement of CD44. This may suffice to induce a process of alternate adhesion and de-adhesion.
– Spatial asymmetry of signaling is associated with directional cell motility. PTEN is distributed unevenly in chemotaxing cells. By being recruited to the posterior edge, it compromises the activity of Phosphatidylinositol 3-Kinase at the trailing edge. This contributes to establishing an anterior-posterior gradient of the lipid messenger phosphatidylinositol 3,4,5-phosphate, which is required to impart directionality to the moving cell [Funamoto et al. 2002; Merlot and Firtel 2003].
– The phosphatase PRL-3 and its substrate CAS function in opposing ways. In response to Integrin ligation, FAK and SRC phosphorylate CAS, which then recruits CRK to membrane ruffles at the leading edge of motile cells. The CAS/CRK complex activates DOCK-180 and RAC, which leads to lamellopodia formation. The phosphatase PRL-1 is able to dephosphorylate phosphotyrosine substrates, including CAS, which may mediate de-adhesion and act in alternation with FAK and SRC during cell migration.
– Cell motion is among the processes regulated by intracellular calcium. Cyclic changes in traction stress, cell speed, and cell shape are associated with calcium transients. The opening of stretch-activated calcium channels in keratinocytes can induce spatially coordinated increases intraction stress that promote protrusion at the cell front, while simultaneously inducing retraction at the rear. Local increases in intracellular calcium occur within the filopodia of nerve growth cones and induce retraction in this region, while promoting outgrowth on the opposite side. In neutrophils, constitutive activity of the calcium dependent kinase Calpain functions as a negative regulator of protrusion and migration, whereas its localized activation supports chemotaxis.
– The migration of vascular endothelial cells occurs in a fluid dynamic environment due to blood flow. Shear stress enhances both the frontal pulling force and the tail retraction by increasing the traction force exerted by endothelial cells on the underlying substrate. It acts through the biochemical pathway of RHO→$P160^{ROCK}$.

The functional dichotomy of adhesion versus migration may reflect the regulation of the multistep process of tumor–endothelium recognition during homing, which involves initiation of contact, rolling, activation dependent arrest, and diapedesis. This process is highly orchestrated by variable expression of homing receptors and their endothelial counterreceptors thus constructing many specific homing pathways with relatively few molecular components [Butcher and Picker 1996].

Migratory signaling is tightly regulated by the domain structures of extracellular matrix molecules

and divisions of labor among their receptors. Homing receptors on metastasizing cells may mediate a two-step process of migration followed by adhesion, depending on their interaction with specific ligands. This sequence is determined by the presentation of a ligand cross-linked in the extracellular matrix or released from it, and by the presence or absence of a concentration gradient of the ligand.

- Osteopontin induces cellular chemotaxis but not homotypic aggregation of CD44 bearing cells, whereas the inverse is true for the interaction between CD44 and hyaluronate [Weber et al. 1996]. The different responses of cells after CD44 ligation by either Osteopontin or hyaluronate may account for independent effects on dissemination and implantation of neoplastic cells. While the interaction between Osteopontin and CD44 mediates the migration out of the bloodstream, additional interactions between CD44 and hyaluronate attach and organize these emigrant cells in their target organs.
- The heparin binding NH_2-terminus of Thrombospondin stimulates chemotaxis in a manner that is inhibitable by heparin and fucoidan, while the COOH-terminus mediates haptotaxis in a fashion that depends on the RGD motif.
- Extracellular proteolytic cleavage of Osteonectin has important consequences for its function. The domains I and IV of Osteonectin can be specifically cleaved and released by distinct serine proteases. These domains mediate the disruption of focal adhesions. Osteonectin derived domain II peptides regulate angiogenesis, while a NH_2-terminal peptide of Osteonectin induces MMP-2, possibly by inhibition of TIMP-2 secretion [Gilles et al. 1998].

Receptors may form complexes on the cell surface (Table 5.4.4.A). This enhances the plasticity of cellular responses, because the lateral recruitment of receptors can activate them in the absence of their cognate ligands and it can combine signaling modules in various ways.

- CD44 receptors may forms clusters on the cell surface in response to hyaluronate. This allows the trapping of MMP-9 on the cell surface and activates latent TGF-β [Yu and Stamenkovic 2000].
- CD44v3 binds MMP-7 and coordinates the cleavage of HB-EGF. After cleavage, HB-EGF ligates ERBB4. CD44 also associates with ERBB1, ERBB2, ERBB3, and ERBB4, and modulates their signaling.
- Clustering of Integrins triggers a cascade of intracellular signaling pathways leading to the phosphorylation of cytoplasmic and cytoskeletal substrates, including FAK and Paxillin.
- Proteins of the Immunoglobulin superfamily bind to Integrins and generally function in concert to mediate both cell–cell and cell–extracellular matrix interactions. These stimulate cytoskeletal reorganization, migration and invasion. The interaction between the PECAM-1 and Integrin $\alpha_V\beta_3$, VCAM-1 and Integrin α_4, MadCAM-1 and Integrin α_4, and ICAM and Integrin β_2 mediate leukocyte to endothelial cell adhesion, governing inflammatory responses and tumor metastases.

5.4.5 Survival During Invasion

The growth of most cells is anchorage dependent. Nontransformed cells require physical signals, derived from adhesion and mediated through adhesion receptors, to survive and divide. These requirements may assure that cells only proliferate in a suitable physical environment. Anchorage dependent cells that are deprived of the ability to bind

Table 5.4.4.A. Cross-talk among receptors for cell motility. The lateral recruitment of cell surface receptors enhances the plasticity of cellular responses to migratory stimuli. Integrins and CD44 play important roles in cell motility and both are involved in receptor–receptor interactions.

Ligand	Receptor	Crosstalk	Tissue/Cancer	Reference
HGF	MET	Integrin $\alpha_6\beta_4$	Colon	Trusolino et al. 2001
Osteopontin	CD44v	Integrin β_1	Breast Colon Ovaries	Katagiri et al. 1999; Weber 2001
hyaluronate	CD44	ERBB2	Breast	Wobus et al. 2002
hyaluronate, MMP-7, HB-EGF	CD44	ERBB4	Uterus Breast	Yu WH et al. 2002

immobilized extracellular matrix molecules undergo a form of apoptosis referred to as anoikis [Ruoslahti 1996]. The induction of apoptosis in nonadherent cells prevents their dissemination and aberrant localization. It is a default pathway that is activated unless survival signals are transduced.

Survival during the process of vascular transport of tumor cells is essential and it may select among all invasive cells the ones that will be able to grow distal metastases. Because disseminating cells stay in the vasculature for extended periods of time, their programmed cell death is the major limiting factor for metastasis formation. Once the released cells extravasate in the target organ, they form micrometastases, which grow into secondary tumors.

BCL-2 family members. Detachment of epithelial cells from their matrix triggers the release of the pro-apoptotic BCL-2 family members BIM and BMF from the Myosin V motor complex and the Actin cytoskeleton. In adherent cells, these proteins bind to the cytoskeleton, but after the cells have detached from the extracellular matrix, BIM and BMF are released and interact with the antiapoptotic protein BCL-2. This inhibits the anti-anoikis action of BCL-2 and initiates programmed cell death. Conversely, BCL-2 activity and expression are enhanced by Integrin ligation.

– Consecutive to the engagement by Fibronectin, the Integrin $\alpha_5\beta_1$ induces the expression of BCL-2, which protects cells from apoptosis.
– Engagement of the Integrin $\alpha_V\beta_3$ may increase the BCL-2:BAX ratio [Stromblad et al. 1996] and protect cells from programmed cell death. The ligand Osteopontin has antiapoptotic effects against multiple forms of stress [Weber et al. 1999].
– The engagement of Integrin $\alpha_V\beta_3$ on melanoma cells prevents apoptosis by inducing a fivefold increase in the relative BCL-2:BAX ratio. While little change occurs in the levels of BCL-2, BAX expression is suppressed [Petitclerc et al. 1999].

FAK. Many adhesion receptors negatively regulate cell death. When adherent cells express specific Integrin complexes in matrices that lack the cognate ligands they undergo apoptosis, whereas Integrin binding to cognate extracellular matrix ligands initiates survival signals. The downstream target of β-Integrins, ILK (Integrin-Linked Kinase), maintains the microtubule structure and sustains viability. ILK is essential for regulating PKB1 activity. The targets of FAK signaling, RAS, RAC, and ERK, are implicated in supporting cell survival. Furthermore, Integrin induced anti-anoikis often proceeds through the FAK→Phosphatidylinositol 3-Kinase→PKB pathway. PKB promotes survival, at least in part by phosphorylating and inactivating BAD and Caspase-9. The Integrin associated signaling molecule FAK has a prominent role as survival factor. P53 is involved in mediating the death signal under FAK deficiency, implying that its activity is also suppressed by the FAK pathway. SRC is frequently activated after Integrin engagement and can protect epithelial cells from anoikis via activation of Phosphatidylinositol 3-Kinase and PKB. The underlying pathway is Integrin→FAK/c-SRC→PI 3-K. Similar to the ligation of Integrins, the engagement of CD44v can protect from anoikis. Upon ligation of CD44v6, LYN is recruited to a signaling complex that activates Phosphatidylinositol 3-Kinase and PKB leading to the inhibition of apoptosis.

FAK cleavage by Caspases is an early event in anoikis that may eliminate late survival cues from the extracellular matrix. Similarly, the tumor suppressor and phosphatase PTEN de-phosphorylates phosphatidylinositol 3-phosphates, counteracting the FAK-associated survival pathway [Giancotti and Ruoslahti 1999]. Caspase-8 is recruited to unligated Integrins and activated [Stupack et al. 2001], which triggers de-adhesion induced cell death.

The mammary gland alveolar morphology depends on the deposition of a Laminin-rich extracellular matrix. The basement membrane matrix suppresses programmed cell death in mammary epithelial cells through the ligation of β_1 Integrins, whereas its loss induces the expression of Caspase-1 (Interleukin-1β Converting Enzyme, ICE) and anoikis [Boudreau et al. 1995].

FYN. Some β_1 and α_V Integrins may activate FYN via Caveolin-1 as a membrane adapter. This engages SHC→GRB-2→SOS→RAS→RAF and the MAPK cascade. Integrins that do not activate SHC are weak activators of ERK and of cell survival. The FAK and FYN pathways to ERK activation may cooperate. FAK is recruited and auto-phosphorylates on Tyrosine 397. This creates a binding site for the SH2 domain of SRC, which is activated through dephosphorylation by SHP-2. FAK is then phosphorylated on Tyrosine 925 by SRC and in that state can bind GRB-2 with the resulting activation of the SOS→RAS→RAF, and MAPK cascade.

RHO. The activity of RHO family proteins is regulated by Integrins and adherens junctions. RHO may thus mediate information about the physical environment that either supports the proliferation in response to growth factors or induces anoikis. The RHO-GTPases are mainly mediators of cell dissemination, however, they also regulate pathways that control the activation of *c-jun* and *c-fos* through JNK and $P38^{MAPK}$ cascades, which may affect programmed cell death. A mediator in the activation of JNK may be the serine–threonine kinase $P65^{PAK}$. RHO-GTPases can stimulate transcription from the *cyclin D* promoter and activate the Serum response transcription factor, possibly leading to G_1 progression [Hall 1998]. Active RAC1, a RHO family member, can provide a survival signal.

Cadherin. Cell–cell interactions through Cadherin and Catenin protect from anoikis through a pathway that depends on cytoskeletal integrity. During anoikis, β-Catenin is proteolytically cleaved by Caspase-3. The resulting β-Catenin cleavage products are still able to associate with E-Cadherin, but are unable to associate with α-Catenin, which is responsible for Actin filament binding and organization.

– Enterocytes are strikingly sensitive to the loss of anchorage due to rapid proteasomal degradation of E-Cadherin. This is important for the shedding of the enterocytes from the tips of the intestinal villi [Fouquet et al. 2004]. In colon cancer, this mechanism is often suppressed.
– In breast cancer, the loss of E-Cadherin induces resistance to anoikis [Derksen et al. 2006].

5.5 METASTASIS SUPPRESSOR GENES

Phenomena in biology typically have a counterbalance. This holds true for the regulation of cell dissemination. Like tumor suppressor genes inhibit cell cycle progression and serve as antagonistic controls for the effects of proto-oncogenes, the effects of genes that mediate metastatic spread are balanced by metastasis suppressor genes. Many of the derived gene products procure cell anchorage and inhibit cell migration. The receptors in this group may have ligands or counter-receptors that mediate cell attachment while lacking chemotactic ligands. Current evidence suggests that the expression of such adhesion molecules is functionally dominant over the expression of receptors that mediate metastatic behavior. Metastasis suppressors are usually transcriptionally silenced, rather than mutated, in invasive tumors.

5.5.1 Tissue Inhibitors of Metalloproteinases

Tissue Inhibitors of Metalloproteinases (TIMPs) are the endogenous modulators of the zinc dependent Matrix Metalloproteinases (MMPs) and their closely related proteinases, belonging to the ADAM (A Disintegrin and Metalloproteinase) and ADAM-TS (ADAM with Thrombospondin Repeats) families (Figures 5.5.1.A, B). TIMPs contain characteristic 12

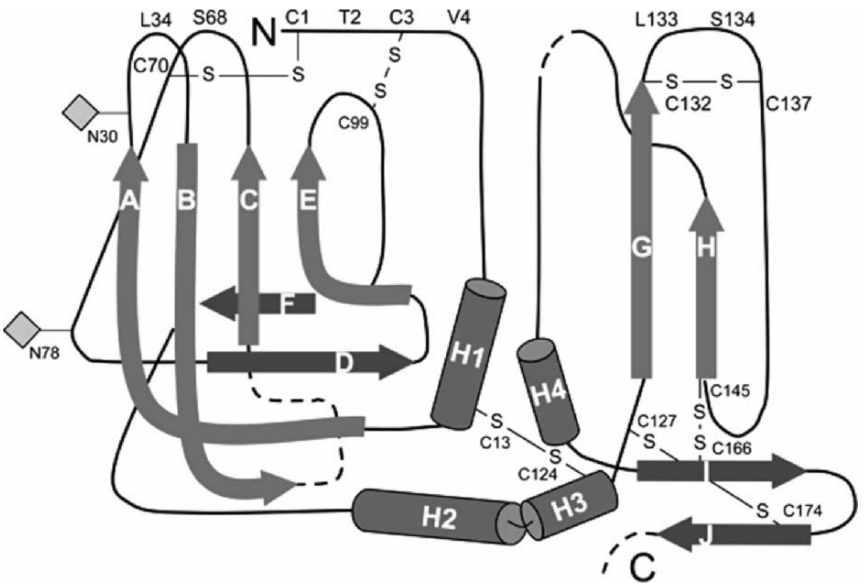


Figure 5.5.1.A. TIMP-1 ribbon structure. A schematic display of the secondary structure of Tissue Inhibitor of Metalloproteinases-1 (TIMP-1). Strands (A–J) and helices (H1–H4) are shown, two glycosylation sites are indicated by diamonds. [Reproduced from Nagase et al. 2002. With permission.]

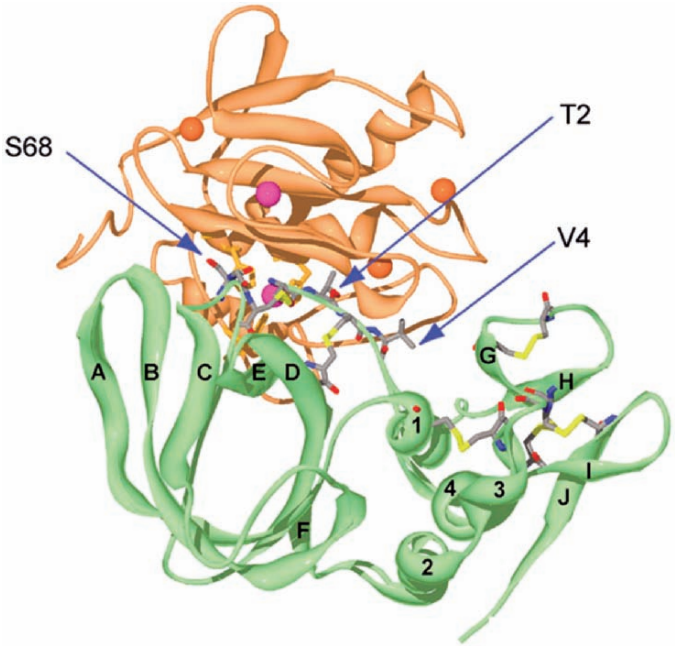


Figure 5.5.1.B. TIMP-1 ribbon structure. A ribbon diagram of Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) bound to the catalytic domain of Matrix Metalloproteinase-3. TIMP-1 is shown in green and the catalytic domain of MMP-3 is shown in light brown. Cystines, threonine 2, valine 4, and serine 68 in TIMP-1 are indicated: N = blue, O = red, C = grey, disulfide bonds = yellow. Strands and helices in TIMP-1 are labeled A-J and 1–4, respectively. The catalytic and structural zinc ions are shown in purple, and calcium ions are shown in orange. [Reproduced from Nagase et al. 2002. With permission.]

cysteine residues, which form six disulfide bonds, generating a tertiary structure with two major domains. There are four variants of TIMPs and each has its defined set of Metalloproteinase targets.

– TIMP-1 (Human Collagenase Inhibitor, HCI, Erythroid Potentiating Activity, EPA) {Xp11.3-p11.23} inhibits Metalloproteinases with Collagenase activity. It is inactive against several of the Membrane-Type MMPs (MT-MMPs), MMP-19, and the ADAM proteinase TACE (TNF-α Converting Enzyme, ADAM-17).
– TIMP-2 {17q25} binds to the type IV Collagenase pro-enzyme secreted by the same cells. This results in inhibition of the collagenolytic activity. TIMP-2 abrogates endothelial cell proliferation, induced by angiogenic factors, independently of MMP inhibition.
– TIMP-3 {22q12.1-q13.2} is expressed in many tissues, with highest expression in the placenta. TIMP-3 inhibits VEGF mediated angiogenesis through blocking the binding of VEGF to VEGFR2 and inhibiting its downstream signaling.
– TIMP-4 {3p25} binds with high affinity to pro-MMP-2 (pro-Gelatinase A) via the COOH-terminal Hemopexin domain (C domain) of pro-MMP-2.

While TIMPs play important roles in the regulation of MMP activity, other inhibitors of Metalloproteinases exist. The main inhibitor of MMPs in tissue fluids is α-Macroglobulin. It binds to MMPs and facilitates their clearance through endocytosis. Similarly, Thrombospondin-2 can form complexes with MMP-2 that are then cleared by endocytosis.

- Collagenases type IV are secreted in a latent form complexed with their TIMPs. Tissue inhibitors of Metalloproteinases negatively regulate invasion by preventing the matrix destroying actions of Metalloproteinases. Their overexpression reduces metastatic potential, whereas their reduction enhances the malignant phenotype [Stracke and Liotta 1995].
- A decrease in the level of TIMP-2, reflecting increased proteolytic activity and hence metastatic potential, can be a prognostic indicator that predicts metastasis of liver cancer.
- The expression of TIMP-4 is suppressive for the development of breast cancer metastases.

5.5.2 Serpins

The superfamily of serine protease inhibitors (Serpins) includes Maspin, Plasminogen Activator Inhibitor-1 (PAI-1), α1-Antitrypsin, α1-Antichymotrypsin, α2-Antiplasmin, Squamous Cell Carcinoma Antigen-2 (SCCA2), and C1-Esterase Inhibitor.

Maspin (Mammary Serpin, Serpin B5, Protease Inhibitor 5, PI5) {18q21.3} is a secreted Serine Protease Inhibitor [Zou et al. 1994]. It is expressed in epithelial cells and in corneal stromal cells. Maspin inhibits the cell surface-mediated Plasminogen activation by forming a complex with cell surface bound UPA and by reducing the release of active UPA. The inhibition of pericellular UPA by Maspin correlates with its inhibition of invasiveness [Biliran and Sheng 2001].

PAI-1 (PLANH1, Serpin E1) {7q21.3-q22} is a 402 amino acid glycoprotein that binds covalently to Plasminogen Activator and inhibits its activity. It also binds with high affinity and specificity to Vitronectin. This regulates motility that is mediated through the interaction of Vitronectin with its receptor Integrin $\alpha_V\beta_3$. PAI-1 is involved in inhibiting proteolysis and angiogenesis. There are at least 2 *pai-1* mRNA species, both encoded by a single gene, differing by 1 kb in the 3′ untranslated region. This suggests that these two transcripts arise by alternative poly-adenylation.

The *pai-2* (*planH2*, *serpin B2*) gene {18q21.3} encodes a protein containing 450 amino acids. High levels of PAI-2 arise in keratinocytes, monocytes, and the trophoblast.

Cleavage of the COOH-terminal loop of Antithrombin {1q23-q25} induces a conformational change in the molecule. The cleaved conformation has potent antiangiogenic and antitumor activity [O'Reilly et al. 1999]. The latent form of intact Antithrombin, which is similar in conformation to the cleaved molecule, also inhibits angiogenesis and tumor growth.

SCCA2 (Leupin, Serpin B4) {18q21.3} inhibits the Chymotrypsin-like proteinases Cathepsin G and Mast Cell Chymase.

C1-Esterase Inhibitor (Serpin G1) is a highly glycosylated serum protein of 105 kD, comprised of a single polypeptide chain of 478 amino acids with two disulfide bonds. C1-Esterase Inhibitor inhibits components of the complement and coagulation systems. It has two domains, the COOH-terminal Serpin homology domain comprising amino acids 114 through 478 and the NH_2-terminal domain comprising amino acids 1 through 113, which contains most of the carbohydrate.

- Maspin may block the migration of breast tumor cells [Zhang et al. 2000]. Maspin expression also correlates with good prognosis in prostate cancer.
- Antithrombin is widely expressed in prostate cancer but is gradually lost in tumors of high Gleason grade. Antithrombin may act as a local antiangiogenic factor, the effect of which is partially lost in poorly differentiated prostatic tumors [Cao et al. 2002].
- The *antithrombin III* familial variant AT Dublin occurs with increased frequency in children with cancer [Daly et al. 1987]. The variant does not compromise coagulation but is associated with acute lymphocytic leukemia (ALL). It is based on a V→G substitution at position 3. This causes the signal sequence to be cleaved two amino acids downstream into the mature protein, leading to abundance of a truncated Antithrombin protein that lacks the NH_2-terminal dipeptide.
- Overexpression of PAI-2 in melanoma cells causes them to grow as fully encapsulated tumors. This prevents their dissemination.
- In the plasma of patients with squamous cell carcinoma of the lung, C1-Esterase Inhibitor is significantly reduced [O-charoenrat et al. 2004].

5.5.3 Cystatins

The activity of cysteine proteinases is subject to control by members of the Cystatin superfamily, which comprises very tight, reversible, competitive inhibitors of the Papain family of cysteine proteases. The superfamily contains three families, Stefins, Cystatins, and Kininogens.

Family I Cystatins (Stefins A, B, and C) contain about 100 amino acid residues and lack disulfide bridges. They are mostly located intracellularly. Stefin A (Cystatin A, Acid Cysteine Proteinase Inhibitor) {3q21} is present in the cytosol of polymorphonuclear granulocytes. It contains a single polypeptide chain composed of 98 amino acid residues and has a molecular mass of 11 kD. It forms tight complexes with Papain and the Cathepsins B, H, and L. The *stfB* gene {21q22.3} is 2.5 kb in length and contains three small exons encoding the 98 amino acid protein. The role of Stefin B (Cystatin B, Neutral Cysteine Proteinase Inhibitor) may be as a protector against the proteinases leaking from lysosomes.

Family II Cystatins (Cystatin C, Cystatin D, Cystatin E/M, Cystatin F, Cystatin G, Cystatin S, Cystatin SA, Cystatin SN) are about 120 amino acids long and have two intrachain disulfide bonds.

Their location is extracellular. Salivary Cystatins, Cystatins S, SA, and SN, are mainly prevalent in saliva, tears, and seminal plasma, whereas Cystatin C is abundant in cerebrospinal fluid, seminal plasma, milk, synovial fluid, and urine and in the blood plasma of patients with uremia. Cystatin C is the most abundant extracellular inhibitor of cysteine proteases. It is a 13 kD protein constitutively secreted shortly after its synthesis. The *cst3* {20p11.2} gene, which encodes Cystatin C, contains three exons and two introns spanning 4.3 kb of genomic DNA.

Family III Cystatins (K-Kininogens, L-Kininogens) represent the most complex members of this protein superfamily and contain three Cystatin-like domains, each with two disulfide bonds at positions homologous to those in family II Cystatins. Kininogens are a group of proteinase inhibitors whose physiologic role is the control of cysteine proteinases, such as the Cathepsins. They are intravascular proteins. The *kininogen* gene {3q27} consists of 11 exons and is 27 kb long.

- Stefin A and Cystatin C are implicated in limiting the invasive behavior of squamous cell carcinoma of the head and neck. Their levels in primary tumor tissue and adjacent noncancerous mucosa predict survival [Strojan et al. 2004].
- Stefin A may inhibit Cathepsin B. Its expression correlates inversely with tumor progression of breast cancer, prostate cancer, and brain tumors. Furthermore, Stefin A is the major form of Cathepsin inhibitor in squamous epithelia. Progressive loss of its expression occurs during the progression of cervical intraepithelial neoplasia.
- Cystatin M is downregulated in metastatic breast tumor cells as compared to primary tumor cells [Sotiropoulou et al. 1997].

5.5.4 Cadherins

Cadherins are transmembrane glycoproteins of approximately 120 kD that promote cell–cell adhesion in solid tissues by a homophilic, calcium-dependent mechanism. The interaction of identical Cadherins on cells of the same tissue type implies a role for these molecules in defining tissue identity.

The extracellular domain of Cadherins contains four repeating calcium binding units, which explains the calcium dependence of Cadherin action. Cadherins show exon trans-splicing, a form of RNA shuffling that mixes and matches exons to create diversity in the resulting mRNA. This leads to the generation of a large variety of extracellular domains. The cytoplasmic domain is fairly large and preserved among Cadherins, presumably reflecting their function as linkers to the cytskeleton. The interaction between Cadherins and the Actin containing microfilaments of the cytoskeleton is accomplished through α-Catenin, β-Catenin, and γ-Catenin (Plakoglobin). This also localizes the Catenins to the plasma membrane and prevents them from redistributing to the cytoplasm or to the nucleus. The complex is regulated by tyrosine phosphorylation of the Catenins.

The Cadherin protein superfamily contains:

– Classical (type I) Cadherins that mediate adhesion at adherens junctions
– The higly related type II Cadherins
– The desmosomal Cadherins found in desmosome junctions
– Proto-Cadherins expressed primarily in the central nervous system
– Atypical Cadherin-like domain containing proteins

The type I, type II, and desmosomal Cadherins share a common domain organization comprising five tandem extracellular Cadherin domains, a single transmembrane domain, and a highly conserved cytoplasmic tail [Boggen et al. 2002]. According to their tissue distribution, four subtypes of Cadherins exist, E-Cadherin, N-Cadherin, P-Cadherin, and H-Cadherin. E-Cadherin (Cadherin-1, Uvomorulin, LCAM) {16q22.1} is an integral component of epithelial adherens junctions (Figure 5.5.4.A). β-Catenin is important in linking E-Cadherin to the Actin cytoskeleton through its interaction with α-Catenin and Vinculin. Retinoic acid upregulates the E-Cadherin/Catenin functional complex. N-Cadherin (Cadherin-2) {18q11.2} is a 907 amino acid protein that includes a 159 amino acid signal sequence. Its expression is predominantly neuronal. P-Cadherin (Cadherin-3, Placental Cadherin) {16q22.1} contains a signal sequence, a transmembrane region, four cysteine residues in the extracellular domain, and three *N*-linked glycosylation sites. H-Cadherin (Cadherin-13) {16q24.2-q24.3} is expressed most highly in the heart.

Migrating cells frequently lose Cadherin expression. This is the case for neural crest cells migrating to the neural ridge and for the migration of the tightly

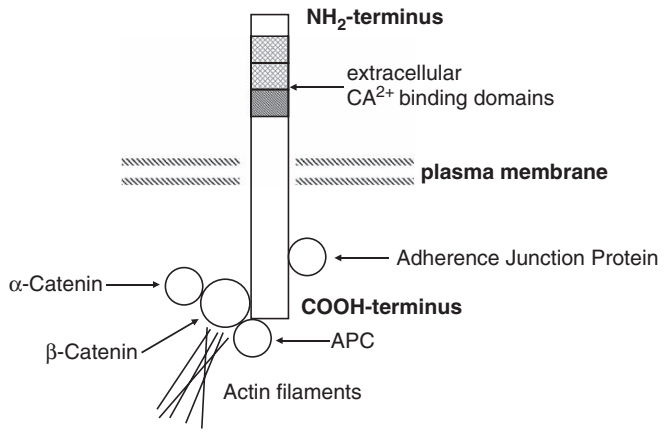


Figure 5.5.4.A. Structure of E-Cadherin. A schematic diagram of the transmembrane protein E-Cadherin and its interactions with the cytoskeleton and with APC. [Adapted from Sherbet and Lakshmi 1997].

compacted somites [Buck 1995]. When cells become incorporated into new tissues they express the Cadherin characteristic of those tissues. During morphogenic movement in embryogenesis, the cells of the epiblast initially express E-Cadherin but switch their expression to N-Cadherin when they become part of the mesoderm. Similarly, cells that form the neural plate switch from E-Cadherin to N-Cadherin when they separate from the overlying ectoderm.

Cadherin function may be compromised in cancer through various mechanisms, including downregulation (loss of heterozygosity, gene methylation), nonfunctionality (aberrant transcript processing, mutations), or lack of Catenin.

- Loss of heterozygosity for the long arm of chromosome 16 {16q}, which is the location of several *cadherins*, including *cdh1* and *cdh13*, occurs in breast carcinoma, lung carcinoma, prostate cancer, and hepatocellular cancer.
- In kidney, prostate, and ovarian carcinoma cells, loss of E-Cadherin function acquires invasive properties, whereas the expression of *E-cadherin* (*cdh1*) results in loss of this capability [Stracke and Liotta 1995]. Loss of Cadherin expression in squamous cell carcinomata of the head and neck is associated with poor differentiation and high invasiveness. Similar relationships arise in prostate cancer and cancers of the female reproductive tract [Buck 1995]. E-Cadherin is expressed in some of the metastatic lesions, however this may reflect reexpression after invasion.
- Aberrant methylation of the *cdh13* gene, which encodes H-Cadherin, occurs in breast and lung carcinomata. Expression of E-Cadherin may be downregulated in cancer by methylation of a CpG island in intron 1.
- The expression of *E-cadherin* is under the control of the strong repressor Snail. In epithelial–mesenchymal transitions, the expression levels of E-Cadherin and Snail are inversely correlated. High expression levels of Snail can contribute to epithelial tumor progression through suppression of *E-cadherin* expression. Beside loss of E-Cadherin expression, aberrant transcript processing may contribute to malignancy.

- Mutations in E-Cadherin predispose to gastric carcinomata [Guilford et al. 1998]. Diffuse type gastric carcinomata suffer from diminished cell–cell adhesion. About 50% of these carcinomata contain mutations in the *E-cadherin* gene, resulting in the destruction of the calcium binding sites of E-Cadherin. These alterations in E-Cadherin play a major role in the development of this cancer and the short survival of the patients [Birchmeier 1995]. Three distinct variants of E-Cadherin, with alterations in exons 8 or 9, are associated with these cancers. While the presence of wild type E-Cadherin leads to decreased levels of MMP-3, the mutant E-Cadherins do not downregulate MMP-3. Cell motility is enhanced by expression of the mutant E-Cadherins. This is consitent with a role for MMP-3 in the enhancement of cell motility [Fuchs et al. 2005].
- E-Cadherin can prevent the invasive phenotype in T-lymphoma cells. Because Cadherins contribute to the adhesion of epitheial cells, breakdown of their function may contribute to the spread of carcinomata.

- MMP-7 (Matrilysin) is expressed in the tumor cells of greater than 80% of intestinal adenomata, the majority of which are associated with the accumulation of β-Catenin in the nucleus. The *matrilysin* promoter is strongly upregulated by β-Catenin and is dependent upon a single optimal TCF-4 recognition site, consistent with abrogation of TCF mediated repression by β-Catenin. E-Cadherin blocks this induction of transcription by distributing β-Catenin to the membrane. In squamous cell carcinoma, the protein levels of MMP-7 are directly correlated to the degree of cell–cell contact. This implies that growth progression of squamous cell carcinoma increases its potential for invasion into the surrounding tissue.
- Expression of a Cadherin protein with a truncation in the cytoplasmic domain, leading to inability to bind β-Catenin, occurs in prostate cancer [Rashid et al. 2001].
- Increased invasiveness may be caused by the presence of normal levels of nonfunctional E-Cadherin. This is the case in certain forms of lung carcinomata and invasive gastric carcinoma, where α-Catenin is not expressed. Reduced expression of α-Catenin may also facilitate the invasiveness of breast cancer [Takayama et al. 1994].

5.5.5 Modulators of Integrin Function

TM4SF molecules. The TM4SF proteins (Tetraspan family, weave through the cell membrane four times) comprise CD9 (Motility-Related Protein-1, MRP-1, MIC-3, Tspan29) {12p13}, CD37, CD53, CD63 (ME491, MLA-1, Granulophysin) {12q12-q13}, CD81 (TAPA-1, Tspan28) {11p}, CD82 (SAR2, ST6, KAI-1, kang ai-1 – Chinese for Anti-Cancer-1) {11p11.2}, and CD151. Their structure yields a large and a small extracellular loop. A hallmark of the Tetraspanin superfamily is the presence of a CCG motif (the sequence cysteine–cysteine–glycine) within the large extracellular loop of the protein. In addition, the large extracellular loops of most Tetraspanins contain two or four additional cysteine residues, one of which is located 11 residues from the start of the fourth transmembrane domain. Tetraspanins associate with a wide variety of partner proteins in a Tetraspanin web. Besides interacting with each other, they also associate with many Immunoglobulin superfamily proteins, proteoglycans, Complement regulatory proteins, Integrins, growth factors, growth factor receptors, and signaling enzymes. For the CD9 and CD81 Tetraspanins, the most robust protein partners are the Immunoglobulin domain containing type 1 transmembrane proteins EWI-F (F2α Receptor Regulatory Protein, FPRP, PTGFRN, CD9P1) and EWI-2 (Glutamine–Tryptophan–Isoleucine-2, IGSF8, CD81P3). EWI proteins, through their direct interaction with ERM proteins, act as linkers to connect Tetraspanin associated micro-domains to the Actin cytoskeleton, thus regulating cell motility and polarity. CD9 and most other members of the TM4SF family associate with Integrins $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_6\beta_1$ [Hemler et al. 1996; Radford et al. 1996]. The TM4SF protein CD81 also interacts with Integrin $\alpha_4\beta_7$. At the plasma membrane, Integrin/Tetraspanin signaling complexes are partitioned into specific micro-domains proximal to cholesterol rich lipid rafts (Table 5.5.5.A). Various Tetraspanins are associated with Phosphatidylinositol 4-Kinase and PKC isoforms. They may facilitate the assembly of signaling complexes by tethering these enzymes to Integrin heterodimers.

- Several Tetraspanin family members are involved in metastasis suppression, an effect that may be related to their association with β_1 Integrins [Maecker et al. 1997; Hemler et al.1996]. The Integrin $\alpha_3\beta_1$ can suppress the malignant conversion of epidermal squamous cell papillomata to carcinomata through a mechanism that may entail CD81 [Owens and Watt 2001].
- The CD9 antigen is a 227 amino acid protein with four hydrophobic domains and one *N*-glycosylation site. It is a receptor for the pregnancy specific glycoprotein PSG-17. In squamous cell carcinoma of the head and neck, reduced CD9 expression is associated with high-grade and lower disease-free survival [Mhawech et al. 2004]. CD9 is also inversely correlated with metastasis in breast cancer.
- In pancreatic cancer, CD9 (MRP-1) expression is inversely associated with histopathological grading. CD82 (KAI1) gene expression is inversely associated with tumor status [Sho et al. 1998]. The reduction of CD9 and CD82 expression, and the increasing CD151 expression are indicators for a poor prognosis in patients with colon cancer [Hashida et al. 2003].
- CD82 inhibits metastasis formation by prostate cancer [Dong et al. 1995] and also by pancreas, hepatocellular, bladder, breast, esophagus, non-small cell lung cancer, and by squamous and lymphoid neoplasms. The downregulation of CD82

Table 5.5.5.A. TM4SF associated proteins. Tetraspanins bind to other membrane proteins. Most binding partners are Integrins, but some non-Integrin binding partners exist. These interactions play roles in cell physiology and in cancer patho-physiology.

Tetraspanin	Integrin	Cell types	Cancer
CD9	$\alpha_1\beta_1$	Cervical epithelial cells	
	$\alpha_2\beta_1$	Vascular smooth muscle cells, keratinocytes	
	$\alpha_3\beta_1$	Epithelia, trophoblast, endothelia, Schwann cells vascular smooth muscle cells	Fibrosarcoma, melanoma, bladder cancer, pancreas carcinoma, colon cancer, endometrial cancer
	$\alpha_4\beta_1$	B-lymphocytes, T-lymphocytes	
	$\alpha_5\beta_1$	B-lymphocytes, monocytes, trophoblast	
	$\alpha_6\beta_1$	Breast epithelium, endometrium, trophoblast	Fibrosarcoma
	$\alpha_7\beta_1$	Myocytes	
	$\alpha_6\beta_4$	Keratinocytes	
	$\alpha_{IIb}\beta_3$	Platelets	
CD53	$\alpha_4\beta_1$	T-lymphocytes	
CD63	$\alpha_3\beta_1$	Breast epithelium	Fibrosarcoma
	$\alpha_4\beta_1$	T-lymphocytes	
	$\alpha_6\beta_1$	Breast epithelium	Fibrosarcoma
	$\alpha_M\beta_2$	Neutrophils	
	$\alpha_{IIb}\beta_3$	Platelets	
CD81	$\alpha_3\beta_1$	Breast epithelium, cervical epithelium, neuritis, myocytes	Fibrosarcoma, pancreas carcinoma
	$\alpha_4\beta_1$	T-lymphocytes, B-lymphocytes, myocytes	Erythroleukemia, T-cell leukemia
	$\alpha_5\beta_1$	Myocytes	
	$\alpha6\beta_1$	Breast epithelium	Fibrosarcoma, rhabdomyosarcoma
	$\alpha_7\beta_1$	Myocytes	
CD82	$\alpha_3\beta_1$	Breast epithelium	Colon cancer
	$\alpha_4\beta_1$	T-lymphocytes, B-lymphocytes	Rhabdomyosarcoma
	$\alpha_5\beta_1$	Ovaries	
	$\alpha_6\beta_1$		Rhabdomyosarcoma
CD151	$\alpha_3\beta_1$	Epithelia, endothelia, neurites	Fibrosarcoma, pancreas carcinoma
	$\alpha_4\beta_1$	Megakaryocytes	Erythroleukemia
	$\alpha_5\beta_1$	Megakaryocytes, endothelia, T-lymphocytes	Erythroleukemia
	$\alpha_6\beta_1$	Cervical epithelium, colon epithelium, endothelia, B-lymphocytes,-megakaryocytes	Fibrosarcoma, erythroleukemia
	$\alpha_6\beta_4$	Keratinocytes, endothelia	Pancreas carcinoma
	$\alpha_{IIb}\beta_3$		Erythroleukemia

Tetraspanin	Non-Integrin binding partner	Cell types	Cancer
CD9	EWI-F		
	EWI-2	Kidney	
	CD44v		Pancreas carcinoma
CD81	EWI-F		
	EWI-2	Kidney, hepatocytes, B-lymphocytes, T-lynphocytes, NK cells	

expression in thyroid cancer cells reflects increased metastatic potential, and CD82 may serve as a prognostic marker of metastasis in thyroid cancer.

- Reduction or loss of CD63 expression is associated with increased malignancy of melanoma.

SHPS-1. Some Integrins and their associated proteins have anti-metastatic functions. SHPS-1 (CD172a, Signal Regulatory Protein α, SIRPα, Non-Receptor Type Protein-Tyrosine Phosphatase Substrate 1, PTPNS-1, Macrophage Fusion Receptor, MFR, MYD1) {20p13} contributes to

cell–cell communication through its association with its counterreceptor CD47 (IAP). It is a 120 kD transmembrane glycoprotein substrate for receptor Tyrosine Kinases, possessing three Immunoglobulin-like domains in the extracellular region, as well as four potential tyrosine phosphorylation sites and SH2 domain binding sites in the cytoplasmic region. The cytoplasmic domain of SHPS-1 also contains two immuno-receptor tyrosine-based inhibitory motifs (ITIMs) and a proline-rich region near the COOH-terminus, which represents a binding site for SH3 domain containing molecules. SHPS-1 binds to the phosphatases SHP-1 and SHP-2 and activates them, thereby promoting the Integrin-mediated reorganization of the cytoskeleton. The *shps-1* mRNA is ubiquitously expressed, being most abundant in the brain and the spleen. Multiple splice variants can be generated:

- The Integrin $\alpha_2\beta_1$, a receptor for Collagen and Laminin, abrogates the malignant phenotype of breast carcinoma [Zutter et al. 1995]. The Fibronectin Receptor Integrin $\alpha_5\beta_1$ may have similar properties [Stracke and Liotta 1995]. Loss of Integrin α_4 predisposes to metastasis formation in melanomata.
- The expression of *shps-1* {20p13} is downregulated by several oncogene products, including SRC, in fibroblasts and breast cancer cells. The overexpression of *shps-1* suppresses anchorage independence and dissemination. This may be due to the interaction of the extracellular domain of SHPS-1 with a polymeric form of Fibronectin [Oshima et al. 2002].
- SHPS-1 expression is absent or significantly reduced on the majority of myeloid blasts from patients with acute myeloid leukemia or chronic myeloid leukemia [Seiffert et al. 1999].

5.5.6 DCC and Semaphorins

Various adhesion molecules, when expressed on the surface of transformed cells, negatively regulate metastasis formation. One such adhesion molecule is DCC (Deleted in Colorectal Carcinoma) {18q21.3} [Enomoto et al. 1995; Younes et al. 1995]. The DCC gene product is a transmembrane protein of the Immunoglobulin superfamily that has structural features in common with various types of cell adhesion molecules, including NCAM (Neural Cell Adhesion Molecule), and its expression leads to increased cell–cell contact [Chuong et al. 1994]. The *dcc* gene is expressed in differentiated cell types of the intestine, in specific axonal populations projecting from the developing olfactory bulb, neocortex, hippocampus, and epithalamus. It is particularly strong during the targeting phase of axon outgrowth, whereafter it is rapidly downregulated. This reflects its function for DCC in axonal guidance systems [Hedrick et al. 1994; Shu et al. 2000]. In embryos, DCC is expressed in the epithelia of skin, gut, lung, and bladder, whereas in adults its expression is limited to the basal layer of stratified epithelium of skin, crypt regions of intestinal villi, and stem cells in mammary duct [Chuong et al. 1994].

– DCC regulates cell motility. The small GTPases RAC-1, CDC42, and RHO-A play a key role in the cytosolic signaling events induced by the Netrin-1 Receptor DCC. Several putative SH3 binding motifs, PXXP, are located in the cytoplasmic tail of DCC, reflecting possible interactions with SH3 containing adapter molecules to mediate Netrin-1 signaling to RHO-GTPases. DCC associates with the adapter protein NCK in embryonic spinal commissural neurons. The interaction of DCC and NCK is independent of Netrin-1 and involves the direct binding of DCC to two SH3 domains of NCK-1. DCC associates with the Actin cytoskeleton. In the presence of Netrin-1, NCK-1 bound to DCC is able to interact with downstream effectors via its SH2 domain to mediate changes in the Actin cytoskeleton through the activation of RAC-1.
– DCC induces apoptosis in the absence of ligand binding, but blocks apoptosis when engaged by Netrin-1. The DCC-induced apoptotic pathway proceeds via binding to DIP13α. The DIP13α protein has a Pleckstrin homology domain and a phosphotyrosine binding domain. It interacts with a region on the DCC cytoplasmic part of the molecule that is required for the induction of apoptosis. The DCC interacting domain on DIP13α is essential for its ability to enhance DCC-induced apoptosis. DCC is a Caspase substrate, and mutation of the site at which Caspase-3 cleaves DCC completely suppresses the pro-apoptotic effect of DCC.

The Semaphorins are a family of secreted, transmembrane, and membrane associated proteins that cause the repulsion of nerve growth cone guidance and can induce retraction in nonneural cells. Semaphorin 3A binds to endothelial cells and tumor cells. It also inhibits endothelial cells motility and

capillary sprouting. VEGF165 and SEMA3A are competitors for binding to the receptor NRP1 (Neuropilin-1, VEGF165R). This regulates NRP-1 mediated functions in endothelial cells and neurons.

The establishment of neuronal connections requires the accurate guidance of developing axons to their targets. This guidance process involves both attractive and repulsive cues in the extracellular environment. The Netrins and Semaphorins are proteins that can function as diffusible attractants or repellents for developing neurons. Netrins are chemoattractants for commissural axons in the vertebral spinal cord. DCC is expressed on spinal commissural axons and possesses Netrin-1 binding activity. Netrins are bifunctional molecules attracting and repelling various classes of axons. The DCC family of receptors mediates growth cone attraction by Netrins, whereas the UNC-5 proteins are required for the repulsive effect of the Netrins. The functionally related SLIT proteins exert repulsive action on axons through their receptor ROBO.

- The *dcc* gene spans approximately 1.4 mb and contains 29 exons. *dcc* is an alternatively spliced transcript, abnormal forms of which are expressed in colon cancer. The proximal and distal exons are present (exons 2 and 28–29), while exons located in the center of the molecule are absent (6–7 and 18–23). This correlates to DCC protein loss in the cells [Huerta et al. 2001].
- *dcc* is frequently deleted or its expression is reduced or absent in glioblastomata, gastrointestinal, and prostatic tumors. A decrease in DCC expression may also influence the prognosis of breast carcinoma patients [Koren et al. 2003]. Loss of heterozygosity of *dcc* in colorectal cancer is associated with both liver metastasis and lymph node metastasis [Kubo et al. 2004]. Loss of *dcc* gene expression is an important factor in the progress of pancreatic adenocarcinoma. A highly reduced or absent expression occurs in low or undifferentiated pancreatic tumor cells, whereas in the more differentiated tumors *dcc* expression tends to be conserved [Hohne et al. 1992].
- Overexpression of *dcc* induces apoptosis [Chen et al. 1999]. DCC may function as a metastasis suppressor protein by inducing apoptosis during metastasis. This likely occurs through functional Caspase cascades.
- Many tumor cell types express NRP1 and NRP2 and bind VEGF165. Deletions of chromosome 3p are common in small cell lung cancer. The genes for two related secreted Semaphorins, *sema3F* and *sema3B*, are located on 3p21.3. Because Semaphorins and VEGF bind antagonistically to Neuropilins, the loss of *semaphorin* genes is likely to facilitate angiogenesis. The levels of SEMA3F correlate inversely with stage and histologic subtypes, with more aggressive tumors showing increased VEGF and decreased SEMA3F.
- The upregulation of the mRNA levels of the neuroendocrine markers *neurotensin* (*nts*), *neuroendocrine-specific protein* (*nsp*), *neural cell adhesion molecule 1* (*ncam1*), and *γ-aminobutyric acid B-type receptor* (*gpr51*) is associated with increased metastatic potential in large cell lung carcinoma. In contrast, *semaphorin 3B* (*sema3B*) is dramatically downregulated.

5.5.7. Other Metastasis Suppressor Genes

KiSS-1. The *kiss-1* sequence {1q32} encodes a predominantly hydrophilic, 164 amino acid protein with a poly-proline-rich domain, indicative of a SH3 ligand, and a PKC α phosphorylation site. A 54 amino acid peptide of KiSS-1, which is COOH-termially amidated, is referred to as Metastin. It serves as a ligand for the G-Protein coupled receptor GPR54 (OT7T175, AXOR12) {19p13.3} [Ohtaki et al. 2001], which is expressed in the brain, pituitary gland, and placenta.

– KiSS-1 represses the expression of MMP-9 by effecting reduced NF-κB binding to the *mmp*-9 promoter.
– The metastasis suppressor properties of KiSS-1 signaling through GPR54 are mediated in part by the induction of apoptosis.

- KiSS-1 is a metastasis suppressor gene product that inhibits metastasis formation by melanomata and breast carcinomata without affecting tumorigenicity [Lee et al. 1996].
- Gastric cancers with low KiSS-1 have frequent venous invasion, distant metastasis, and tumor recurrence. This results in significantly worse survival. The expression of KiSS-1 is a strong prognostic factor for gastric cancer [Dhar 2004].

DMBT1. DMBT1 (Deleted in Malignant Brain Tumors 1, GP340) {10q25.3-q26.1} is a member of the scavenger receptor superfamily. DMBT1 binds in a calcium dependent manner to the lung surfactant

protein SFTPD. The interaction between SFTPD and DMBT1 plays a critical role in the suppression of alveolar macrophage activation, which may otherwise secrete MMP-2 and MMP-9 and contribute to the pathogenesis of chronic inflammation and emphysema [Wert et al. 2000]. DMBT1 may play a role in cellular and mucosal immune defense [Mollenhauer et al. 2001].

The transcript of *dmbt1* is subject to extensive alternative splicing. Epithelia express transcripts of 6 kb and larger, the esophagus expresses smaller transcripts of around 5 kb. The alternatively spliced form GP340 (Glycoprotein 340) likely is a truncated form of a receptor for SFTPD (Surfactants Pulmonary-Associated Protein D, Collectin-7) [Holmskov et al. 1999]. GP340 exists both in a soluble form and in association with the membranes of alveolar macrophages. Its 7,686 bp sequence encodes a polypeptide chain of 2,413 amino acids. The main sites of GP340 expression are lung, trachea, salivary gland, small intestine, and stomach.

- The structural and functional characteristics of DMBT1 are consistent with its function as a metastasis suppressor for brain, lung, and digestive tract cancer. Allelic loss on chromosome 10q is a genomic alteration common to approximately 80% of glioblastomata [Rasheed et al. 1995], but it is not seen in lower grade astrocytomata.
- *dmbt1* gene expression is down-regulated in oral squamous cell carcinoma through methylation of its promoter region [Imai et al. 2005].

BRMS-1. Gap junctions are membrane spanning channels composed of Connexins that allow the passage of small molecules between cells. Gap junctions are formed between like cell types (homo-specific) or different cell types (hetero-specific). BRMS-1 functions by restoring gap junction formation to transformed breast epithelial cells. The *brms1* (*breast cancer metastasis suppressor-1*) gene is organized over 10 exons spanning approximately 7 kb. Exon 1 is untranslated. The protein contains 246 amino acids, having a mass of around 28.5 kD. BRMS-1 contains phosphorylation sites for PKA (cAMP-Dependent Kinase), PKG (cGMP-Dependent Kinase), PKC, and CKII (Casein Kinase II). There are no canonical glycosylation sites. Two nuclear localization sequences are situated at amino acids 198–205 and 239–245 and the protein shuttles to the nucleus. BRMS-1 interacts with a SIN3/HDAC complex, which underlies its involvement with transcriptional regulation. Gene transcription is partially regulated at the level of chromatin structure. Histone Deacetylases (HDACs) remove acetyl groups from specific lysine residues and exist in diverse corepressor complexes. Due to the relative abundance and stability of SIN3 and HDACs, they may form core repressor complexes that are available for recruitment by gene specific transcription factors. BRMS-1 is a regulator of Histone deacetylation that modifies the expression of *connexins* [Hurst and Welch 2005].

- The loss of gap junctional intercellular communication correlates with tumor progression, invasion, and metastasis [Nicolson et al. 1988]. *brms-1* {11q13.1-q13.2} is a metastasis suppressor gene, which may inhibit the dissemination of breast cancer [Seraj et al. 2000]. Chromosome 11q is often lost in late stage breast cancers and regions near 11q13 are among the most common amplifications and deletions associated with breast cancer progression.
- BRMS-1 acts as a metastasis suppressor gene product in melanoma. *brms1* mRNA expression is high in melanocytes, reduced in early stage melanoma, and barely detectable in advanced or metastatic melanoma cells.
- BRMS-1 acts as a metastasis suppressor gene product in bladder cancer.

NM23. The Nucleoside Diphosphate Kinases are ubiquitous enzymes that catalyze the transfer of γ-phosphates, via a phospho-histidine intermediate, between nucleoside and dioxy-nucleoside tri- and di-phosphates. The smallest functional entity of Nucleoside Diphosphate Kinase is a heterodimeric protein, with NME-1 (Non-Metastatic Cells Protein 1, NM23, NM23-H1, GZMA-Activated DNAse; GAAD) {17q21.3}, and NME-2 (NM23B, NM23-H2, NDPKB) {17q21.3} constituting the A and B polypeptide chains of this enzyme. Each chain consists of 152 amino acids. The genes *nme-1* and *nme-2* encode two polypeptide chains that may be alternatively spliced and are responsible for the heterogeneity of the hexameric enzyme. A substrate for NM23 is KSR (Kinase Suppressor of RAS), a scaffold protein for the ERK pathway. NM23-H1 binding and phosphorylation of KSR may inactivate this scaffold protein, reducing ERK activation and metastatic colonization. The metastasis suppressors NME-1 and NME-2 are members of a family of gene products comprising NME-1 through NME-6.

- Loss of *nm23-H1* expression is associated with metastatic potential in many tumors, conversely, expression of this gene in cancer cells may reduce their metastatic ability [Steeg et al. 1988; Yoshida et al. 2000].
- *nme-1* RNA levels are differentially expressed in breast tumors and low levels are associated with histopathologic indications of high metastatic potential [Bevilacqua et al. 1989]. A S120G substitution, which does not represent a common polymorphism, in the NME-1 gene product is associated with advanced neuroblastomata but not with limited stage neuroblastomata [Chang et al. 1994].

AKAP-12. The SRC-Suppressed C Kinase Substrate (SSeCKS, Gravin, A-Kinase Anchor Protein 12, AKAP-12) is a scaffolding protein and a substrate for Protein Kinases A and C. It may act as a metastasis suppressor gene in prostate cancer [Xia et al. 2001]. Expression of SSeCKS is downregulated in *src* transformed or *ras* transformed cells [Lin et al. 1996].

MAP2K4. MAPK Kinase 4 (MKK4, c-JUN NH_2-Terminal Kinase-Activating Kinase JKK, Stress-Activated/ERK Kinase 1, SEK1) can suppress prostate cancer metastasis. The signal transduction pathway proceeds through the downstream activation of c-JUN. MKK4 may control the apoptotic response to stresses that occur in metastatic sites. [Kim et al. 2001].

REFERENCES

Agrawal D, Chen T, Irby R, Quackenbush J, Chambers AF, Szabo M, Cantor A, Coppola D, Yeatman TJ. 2002. Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. The Journal of the National Cancer Institute 94:513–521.

Ahmed N, Pansino F, Clyde R, Murthi P, Quinn MA, Rice GE, Agrez MV, Mok S, Baker MS. 2002. Overexpression of αvβ6 integrin in serous epithelial ovarian cancer regulates extracellular matrix degradation via the plasminogen activation cascade. Carcinogenesis 23:237–244.

Aigner S, Ramos CL, Hafezi-Moghadam A, Lawrence MB, Friederichs J, Altevogt P, Ley K. 1998. CD24 mediates rolling of breast carcinoma cells on P-selectin. FASEB Journal 12:1241–1251.

Aigner S, Sthoeger ZM, Fogel M, Weber E, Zarn J, Ruppert M, Zeller Y, Vestweber D, Stahel R, Sammar M, Altevogt P. 1997. CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. Blood 89:3385–3395.

Aishima S, Taguchi K, Terashi T, Matsuura S, Shimada M, Tsuneyoshi M. 2003. Tenascin expression at the invasive front is associated with poor prognosis in intrahepatic cholangiocarcinoma. Modern Pathology 16:1019–1027.

Albo D, Shinohara T, Tuszynski GP. 2002. Up-regulation of matrix metalloproteinase 9 by thrombospondin 1 in gastric cancer. Journal of Surgical Research 108:51–60.

Arch R, Wirth K, Hofmann M, Ponta H, Matzku S, Herrlich P, Zoeller M. 1992. Participation in normal immune responses of a metastasis-inducing splice variant of CD44. Science 257:682–685.

Aruffo A, Dietsch MT, Wan H, Hellstrom KE, Hellstrom I. 1992. Granule membrane protein 140 (GMP140) binds to carcinomas and carcinoma-derived cell lines. Proceedings of the National Academy of Sciences USA 89:2292–2296.

Arrufo A, Stamenkovich I, Melnick M, Underhill CB, Seed B. 1990. CD44 is the principal cell surface receptor for hyaluronate. Cell 61:1303–1311.

Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Janssen M, Zawaideh S, Rittling S, Denhardt DT, Glimcher MJ, Cantor H. 2000. The cytokine Eta-1/Opn is an essential molecular bridge to type 1 (cell-mediated) immunity. Science 287:860–864.

Avizienyte E, Wyke AW, Jones RJ, McLean GW, Westhoff MA, Brunton VG, Frame MC. 2002. Src-induced deregulation of E-cadherin in colon cancer cells requires integrin signalling. Nature Cell Biology 4:632–638.

Barbour AP, Reeder JA, Walsh MD, Fawcett J, Antalis TM, Gotley DC. 2003. Expression of the CD44v2–10 isoform confers a metastatic phenotype: importance of the heparan sulfate attachment site CD44v3. Cancer Research 63:887–892.

Bargatze RF, Jutila MA, Butcher EC. 1995. Distinct roles of L-selectin and integrins $\alpha_4\beta_7$ and LFA-1 in lymphocyte homing to Peyer's patch-HEV in situ: the multistep model confirmed and refined. Immunity 3:99–108.

Baumhueter S, Dybdal N, Kyle C, Lasky LA. 1994. Global vascular expression of murine CD34, a sialomucin-like endothelial ligand for L-selectin. Blood 84:2554–2565.

Behrend EI, Craig AM, Wilson SM, Denhardt DT, Chambers AF. 1994. Reduced malignancy of ras-transformed NIH 3T3 cells expressing antisense osteopontin RNA. Cancer Research 54:832–837.

Bein K, Simons M. 2000. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. The Journal of Biological Chemistry 275:32167–32173.

Beinke S, Ley SC. 2004. Functions of NF-kB1 and NF-kB2 in immune cell biology. Biochemical Journal 382:393–409.

Berx G, Cleton-Jansen AM, Nollet F, de Leeuw WJ, van de Vijver M, Cornelisse C, van Roy F. 1995. E-cadherin is a tumor/invasion suppressor gene mutated in human lobular breast cancers. EMBO Journal 14:6107–6115.

Berx G, Becker KF, Hofler H, van Roy H. 1998. Mutations of the human E-cadherin (*CDH1*) gene. Human Mutations 12:226–237.

Bevilacqua G, Sobel ME, Liotta LA, Steeg PS. 1989. Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological indicators of high metastatic potential. Cancer Research 49:5185–5190.

Bevilacqua M, Butcher E, Furie B, Furie B, Gallatin M, Gimbrone M, Harlan J, Kishimoto K, Lasky L, McEver R, Paulson J, Rosen S, Seed B, Siegelman M, Springer T,

Stoolman L, Tedder T, Varki A, Wagner D, Weissman I, Zimmerman G. 1991. Selectins: a family of adhesion receptors. Cell 67:233.

Bilbe G, Delabie J, Bruggen J, Richener H, Asselbergs FAM, Cerletti N, Sorg C, Odink K, Tarcsay L, Wiesendanger W, DeWolf-Peeters C, Shipman R. 1992. Restin: a novel intermediate filament-associated protein highly expressed in the Reed-Sternberg cells of Hodgkin's disease. EMBO Journal 11:2103–2113.

Biliran H, Sheng S. 2001. Pleiotrophic inhibition of pericellular urokinase-type plasminogen activator system by endogenous tumor suppressive maspin. Cancer Research 61:8676–8682.

Birchmeier W. 1995. E-cadherin as a tumor (invasion) suppressor gene. Bioessays 17:97–99.

Birchmeyer W, Behrens J. 1994. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochimica et Biophysica Acta 11898:11–26.

Bjornland K, Buo L, Scott H, Konttinen Y, Johansen HT, Aasen AO. 1998. Polymorphonuclear elastase in human colorectal carcinoma. International Journal of Oncology 12:535–540.

Blanco MJ, Moreno-Bueno G, Sarrio D, Locascio A, Cano A, Palacios J, Nieto MA. 2002. Correlation of Snail expression with histological grade and lymph node status in breast carcinomas. Oncogene 21:3241–3246.

Boggen TJ, Murray J, Chappuis-Flament S, Wong E, Gumbiner BM, Shapiro L. 2002. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science 296: 1308–1313.

Borgoño CA, Diamandis EP. 2004. The emerging roles of human tissue kallikreins in cancer. Nature Reviews Cancer 4:876–890.

Boudreau N, Sympson CJ, Werb Z, Bissell MJ. 1995. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science 267:891–893.

Bowen MA, Patel DD, Li X, Modrell B, Malacko AR, Wang W-C, Marquardt H, Neubauer M, Pesando JM, Francke U, Haynes BF, Aruffo A. 1995. Cloning, mapping, and characterization of activated leukocyte-cell adhesion molecule (ALCAM), a CD6 ligand. The Journal of Experimental Medicine 181:2213–2220.

Brooks PC, Silletti S, von Schalscha TL, Friedlander M, Cheresh DA. 1998. Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. Cell 92:391–400.

Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresh DA. 1996. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin αvβ3. Cell 85:683–693.

Buck CA. 1995. Adhesion mechanisms controlling cell–cell and cell–matrix interactions during the metastatic process. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 172–205.

Budd RC, Cerottini J-C, Horvath C, Bron C, Pedrazzini T, Howe RC, McDonald HR. 1987. Distinction of virgin and memory T-lymphocytes. Stable acquisition of Pgp-1 glycoprotein concomitant with antigenic stimulation. The Journal of Immunology 138:3120–3129.

Butcher EC, Picker LJ. 1996. Lymphocyte homing and homeostasis. Science 272:60–66.

Byrne GJ, Ghellal A, Iddon J, Blann AD, Venizelos V, Kumar S, Howell A, Bundred NJ. 2000. Serum soluble vascular cell adhesion molecule-1: role as a surrogate marker of angiogenesis. Journal of the National Cancer Institute 92:1329–1336.

Campbell IG, Freemont PS, Foulkes W, Trowsdale J. 1992. An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. Cancer Research 52:5416–5420.

Cao Y, Lundwall A, Gadaleanu V, Lilja H, Bjartell A. 2002. Antithrombin is expressed in the benign prostatic epithelium and in prostate cancer and is capable of forming complexes with prostate-specific antigen and human glandular kallikrein 2. American Journal of Pathology 161:2053–2063.

Carles-Kinch K, Kilpatrick KE, Stewart JC, Kinch MS. 2002. Antibody targeting of the EphA2 tyrosine kinase inhibits malignant cell behavior. Cancer Research 62:2840–2847.

Cary LA, Klinghoffer RA, Sachsenmaier C, Cooper JA. 2002. Src catalytic but not scaffolding function is needed for integrin-regulated tyrosine phosphorylation, cell migration, and cell spreading. Molecular and Cellular Biology 22:2427–2440.

Chang CL, Zhu XX, Thoraval DH, Ungar D, Rawwas J, Hora N, Strahler JR, Hanash SM, Radany E. 1994. nm23-H1 mutation in neuroblastoma. Nature 370:335–336.

Chauhan SS, Popescu NC, Ray D, Fleischmann R, Gottesman MM, Troen BR. 1993. Cloning, genomic organization, and chromosomal localization of human cathepsin L. The Journal of Biological Chemistry 268:1039–1045.

Chambers AF, Tuck AB. 1993. *Ras*-responsive genes and tumor metastasis. Critical Reviews of Oncogenesis 4:95–114.

Chan BM, Matsuura N, Takada Y, Zetter BR, Hemler ME. 1991. In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. Science 251:1600–1602.

Charpin C, Devictor B, Bergeret D, Andrac L, Boulat J, Horschowski N, Lavaut MN, Piana L. 1995. CD31 quantitative immunocytochemical assays in breast carcinomas. Correlation with current prognostic factors. American Journal of Clinical Pathology 103:443–448.

Chellaiah M, Fitzgerald C, Filardo EJ, Cheresh DA, Hruska KA. 1996. Osteopontin activation of *c-src* in human melanoma cells requires the cytoplasmic domain of the integrin α_v-subunit. Endocrinology 137:2432–2440.

Chellaiah M, Hruska K. 1996. Osteopontin stimulates gelsolin-associated phosphoinositide levels and phosphatidylinositol triphosphate-hydoxyl kinase. Molecular Biology of the Cell 7:743–753.

Chen JY, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett JE, Reczek P, Chambon P, Gronemeyer H. 1995. RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. EMBO Journal 14:1187–1197.

Chen YQ, Hsieh JT, Yao F, Fang B, Pong RC, Cipriano SC, Krepulat F. 1999. Induction of apoptosis and G2/M cell cycle arrest by DCC. Oncogene 18:2747–2754.

Cheung KL, Graves CR, Robertson JF. 2000. Tumour marker measurements in the diagnosis and monitoring of breast cancer. Cancer Treatment Reviews 26:91–102.

Chuong CM, Jiang TX, Yin E, Widelitz RB. 1994. cDCC (chicken homologue to a gene deleted in colorectal carcinoma) is an epithelial adhesion molecule expressed in the basal cells and involved in epithelial-mesenchymal interaction. Developmental Biology 164:383–397.

Craig AM, Bowden GT, Chambers AF, Spearman MA, Greenberg AH, Wright JA, McLeod M, Denhardt DT. 1990.

Secreted phosphoprotein mRNA is induced during multi-stage carcinogenesis in mouse skin and correlates with the metastatic potential of murine fibroblasts. International Journal of Cancer 46:133–137.

Crazzolara R, Kreczy A, Mann G, Heitger A, Eibl G, Fink FM, Mohle R, Meister B. 2001. High expression of the chemokine receptor CXCR4 predicts extramedullary organ infiltration in childhood acute lymphoblastic leukaemia. British Journal of Haematology 115:545–553.

Crnic I, Strittmatter K, Cavallaro U, Kopfstein L, Jussila L, Alitalo K, Christofori G. 2004. Loss of neural cell adhesion molecule induces tumor metastasis by up-regulating lymphangiogenesis. Cancer Research 64:8630–8638.

Crossin KL, Prieto AL, Hoffman S, Jones FS, Friedlander DR. 1990. Expression of adhesion molecules and the establishment of boundaries during embryonic and neural development. Experimental Neurology 109:6–18.

Cunningham BA. 1991. Cell adhesion molecules and the regulation of development. American Journal of Obstetrics and Gynecology 164:939–948.

Curran T, Teich NM. 1982. Candidate product of the FBJ murine osteosarcoma virus oncogene: characterization of a 55,000-dalton phosphoprotein. Journal of Virology 42:114–122.

Daly M, O'Meara A, Hallinan FM. 1987. Identification and characterization of a new antithrombin III familial variant (AT Dublin) with possible increased frequency in children with cancer. British Journal of Haematology 65:457–462.

De S, Chen J, Narizhneva NV, Heston W, Brainard J, Sage EH, Byzova TV. 2003. Molecular pathway for cancer metastasis to bone. The Journal of Biological Chemistry 278:39044–39050.

Debily M-A, Camarca A, Ciullo M, Mayer C, El Marhomy S, Ba I, Jalil A, Anzisi A, Guardiola J, Piatier-Tonneau D. 2004. Expression and molecular characterization of alternative transcripts of the ARHGEF5/TIM oncogene specific for human breast cancer. Human Molecular Genetics 13:323–334.

Degen WG, van Kempen LC, Gijzen EG, van Groningen JJ, van Kooyk Y, Bloemers HP, Swart GW. 1998. MEMD, a new cell adhesion molecule in metastasizing human melanoma cell lines, is identical to ALCAM (activated leukocyte cell adhesion molecule). American Journal of Pathology 152:805–813.

Dekker LV, Segal AW. 2000. Signals to move cells. Science 287:982–985.

de Launoit Y, Chotteau-Lelievre A, Beaudoin C, Coutte L, Netzer S, Brenner C, Huvent I, Baert JL. 2000. The PEA3 group of ETS-related transcription factors. Role in breast cancer metastasis. Advances in Experimental Medicine and Biology 480:107–116.

Denhardt DT, Guo X. 1993. Osteopontin: a protein with diverse functions. FASEB Journal 7:1475–1482.

Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS. 1987. Beta 1–6 branching of Asn-linked oligosaccharides is directly associated with metastasis. Science 236:582–985.

Derksen PW, Liu X, Saridin F, van der Gulden H, Zevenhoven J, Evers B, van Beijnum JR, Griffioen AW, Vink J, Krimpenfort P, Peterse JL, Cardiff RD, Berns A, Jonkers J. 2006. Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell 10:437–449.

Dhar DK, Naora H, Kubota H, Maruyama R, Yoshimura H, Tonomoto Y, Tachibana M, Ono T, Otani H, Nagasue N. 2004. Downregulation of KiSS-1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma. International Journal of Cancer 111:868–872.

Diamond RH, Cressman DE, Laz TM, Abrams CS, Taub R. 1994. PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth. Molecular and Cellular Biology 14:3752–3762.

Ding YB, Chen GY, Xia JG, Zang XW, Yang HY, Yang L. 2003. Association of VCAM-1 overexpression with oncogenesis, tumor angiogenesis and metastasis of gastric carcinoma. World Journal of Gastroenterology 9:1409–1414.

Dobner T, Wolf I, Emrich T, Lipp M. 1992. Differentiation-specific expression of a novel G protein-coupled receptor from Burkitt's lymphoma. European Journal of Immunology 22:2795–2799.

Dong J-T, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, Barrett JC. 1995. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. Science 268:884–886.

Donnini S, Morbidelli L, Taraboletti G, Ziche M. 2004. ERK1–2 and p38 MAPK regulate MMP/TIMP balance and function in response to thrombospondin-1 fragments in the microvascular endothelium. Life Science 74:2975–2985.

Ducut Sigala JL, Bottero V, Young DB, Shevchenko A, Mercurio F, Verma IM. 2004. Activation of transcription factor NF-κB requires ELKS, an IκB kinase regulatory subunit. Science 304:1963–1967.

Duffy MJ. 2001. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clinical Chemistry 47:624–630.

Egeblad M, Werb Z. 2002. New Functions for the matrix metalloproteinases in cancer progression. Nature Reviews Cancer 2:161–174.

El-Tanani M, Platt-Higgins A, Rudland PS, Campbell FC. 2004. Ets gene, PEA3 cooperates with β-Catenin-Lef-1 and c-Jun in regulation of Osteopontin transcription. The Journal of Biological Chemistry 279:20794–20806.

Enomoto T, Fujita M, Cheng C, Nakashima R, Ozaki M, Inoue M, Nomura T. 1995. Loss of expression and loss of heterozygocity in the DCC gene in neoplasms of the human female reproductive tract. British Journal of Cancer 71:462–467.

Erdmann I, Scheidegger EP, Koch FK, Heinzerling L, Odermatt B, Burg G, Lowe JB, Kundig TM. 2002. Fucosyltransferase VII-deficient mice with defective E-, P-, and L-selectin ligands show impaired $CD4^+$ and $CD8^+$ T cell migration into the skin, but normal extravasation into visceral organs. Journal of Immunology 168:2139–2146.

Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G, Scroggins BT, Neckers L, Ilag LL, Jay DG. 2004. Functional proteomic screens reveal an essential extracellular role for hsp90α in cancer cell invasiveness. Nature Cell Biology 6:507–514.

Even-Ram SC, Maoz M, Pokroy E, Reich R, Katz BZ, Gutwein P, Altevogt P, Bar-Shavit R. 2001. Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the $\alpha_v\beta_5$ integrin. The Journal of Biological Chemistry 276:10952–10962.

Fawcett JP, Pawson T. 2000. Signal transduction. N-WASP regulation–the sting in the tail. Science 290(5492):725–726.

Fischer GF, Majdic O, Gadd S, Knapp W. 1990. Signal transduction in lymphocytic and myeloid cells via CD24, a new member

of phosphoinositol-anchored membrane molecules. The Journal of Immunology 144:638–641.

Fishman DA, Liu Y, Ellerbroek SM, Stack MS. 2001. Lysophosphatidic acid promotes matrix metalloproteinase (MMP) activation and MMP-dependent invasion in ovarian cancer cells. Cancer Research 61:3194–3199.

Flannery T, Gibson D, Mirakhur M, McQuaid S, Greenan C, Trimble A, Walker B, McCormick D, Johnston PG. 2003. The clinical significance of cathepsin S expression in human astrocytomas. American Journal of Pathology 163:175–182.

Forsdyke DR. 1985. cDNA cloning of mRNAs which increase rapidly in human lymphocytes cultured with concanavalin-A and cycloheximide. Biochemical and Biophysical Research Communications 129:619–625.

Fouquet S, Lugo-Martinez VH, Faussat AM, Renaud F, Cardot P, Chambaz J, Pincon-Raymond M, Thenet S. 2004. Early loss of E-cadherin from cell–cell contacts is involved in the onset of Anoikis in enterocytes. The Journal of Biological Chemistry 279:43061–43069.

Fuchs M, Hermannstadter C, Specht K, Knyazev P, Ullrich A, Rosivatz E, Busch R, Hutzler P, Hofler H, Luber B. 2005. Effect of tumor-associated mutant E-cadherin variants with defects in exons 8 or 9 on matrix metalloproteinase 3. Journal of Cell Physiology 202:805–813.

Funamoto S, Meili R, Lee S, Parry L, Firtel RA. 2002. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. Cell 109:611–623.

Gardner HA, Berse B, Senger DR. 1994. Specific reduction in osteopontin synthesis by antisense RNA inhibits the tumorigenicity of transformed Rat1 fibroblasts. Oncogene 9:2321–2326.

Ghilardi G, Biondi ML, Mangoni J, Leviti S, DeMonti M, Guagnellini E, Scorza R. 2001. Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. Clinical Cancer Research 7:2344–2346.

Giancotti FG, Ruoslahti E. 1999. Integrin signaling. Science 285:1028–1032.

Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. 1997. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science 277:225–228.

Gildea JJ, Seraj MJ, Oxford G, Harding MA, Hampton GM, Moskaluk CA, Frierson HF, Conaway MR, Theodorescu D. 2002. RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. Cancer Research 62:6418–6423.

Gilles C, Bassuk JA, Pulyaeva H, Sage EH, Foidart J-M, Thompson EW. 1998. SPARC/osteonectin induces matrix metalloproteinase 2 activation in human breast cancer cell lines. Cancer Research 58:5529–5536.

Ginsburg D, Zeheb R, Yang AY, Rafferty UM, Andreasen PA, Nielsen L, Dano K, Lebo RV, Gelehrter TD. 1986. cDNA cloning of human plasminogen activator-inhibitor from endothelial cells. The Journal of Clinical Investigation 78:1673–1680.

Gladson CL, Pijuan-Thompson V, Olman MA, Gillespie GY, Yacoub IZ. 1995. Up-regulation of urokinase and urokinase receptor genes in malignant astrocytoma. American Journal of Pathology 146:1150–1160.

Granovsky M, Fata J, Pawling J, Muller WJ, Khokha R, Dennis JW. 2000. Suppression of tumor growth and metastasis in Mgat5-deficient mice. Nature Medicine 6:306–312.

Greenburg G, Hay ED. 1982. Epithelia suspended in collagen gels can loose polarity and express characteristics of migrating mesenchymal cells. The Journal of Cell Biology 85:3334–3339.

Gresham HD, Graham IL, Griffin GL, Hsieh JC, Dong LJ, Chung AE, Senior RM. 1996. Domain-specific interactions between entactin and neutrophil integrins. G2 domain ligation of integrin $\alpha_3\beta_1$ and E domain ligation of the leukocyte response integrin signal for different responses. The Journal of Biological Chemistry 271:30587–30594.

Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. 2004. IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118:285–296.

Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. 1991. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 65:13–24.

Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. 1998. E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405.

Habets GG, Scholtes EH, Zuydgeest D, van der Kammen RA, Stam JC, Berns A, Collard JG. 1994. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. Cell 77:537–549.

Hall A. 1998. Rho GTPases and the actin cytoskeleton. Science 279:509–514.

Hall JG, Pallister PD, Clarren SK, Beckwith JB, Wiglesworth FW, Fraser FC, Cho S, Benke PJ, Reed SD. 1980. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly–a new syndrome? Part I: clinical, causal, and pathogenetic considerations. American Journal of Medical Genetics 7:47–74.

Hashida H, Takabayashi A, Tokuhara T, Hattori N, Taki T, Hasegawa H, Satoh S, Kobayashi N, Yamaoka Y, Miyake M. 2003. Clinical significance of transmembrane 4 superfamily in colon cancer. British Journal of Cancer 89:158–67.

He B, Mirza M, Weber GF. 2006. An osteopontin splice variant induces anchorage independence in human breast cancer. Oncogene 25:2192–2202.

He B, Weber GF. 2003. Phosphorylation of NF-κB proteins by cyclic GMP-dependent kinase: A noncanonical pathway to NF-κB activation. The European Journal of Biochemistry 270:2174–2185.

He B, Weber GF. 2004. Synergistic activation of the CMV promoter by NF-κB P50 and cyclic GMP-dependent kinase. Biochemical and Biophysical Research Communications 321:13–20.

Hedrick L, Cho KR, Fearson ER, Wu TC, Kinzler KW, Vogelstein B. 1994. The DCC gene product in cellular differentiation and colorectal tumorigenesis. Genes and Development 8:1174–1183.

Hemler ME, Mannion BA, Berditchevski F. 1996. Association of TM4SF proteins with integrins: relevance to cancer. Biochimica et Biophysica Acta 1287:67–71.

Hirohashi S. 1998. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. American Journal of Pathology 153:333–339.

Hohne MW, Halatsch ME, Kahl GF, Weinel RJ. 1992. Frequent loss of expression of the potential tumor suppressor gene DCC in ductal pancreatic adenocarcinoma. Cancer Research 52:2616–2619.

Holmskov U, Mollenhauer J, Madsen J, Vitved L, Gronlund J, Tornoe I, Kliem A, Reid KB, Poustka A, Skjodt K. 1999.

Cloning of gp-340, a putative opsonin receptor for lung surfactant protein D. Proceedings of the National Academy of Sciences USA 96:10794–10799.

Hood JD, Cheresh DA. 2002. Role of integrins in cell invasion and migration. Nature Reviews Cancer 2:91–100.

Horak ER, Leek R, Klenk N, LeJeune S, Smith K, Stuart N, Greenall M, Stepniewska K, Harris AL. 1992. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. The Lancet 340:1120–1124.

Hornebeck W, Robert L. 1977. Elastase-like enzymes in aortas and human breast carcinomas: quantitative variations with age and pathology. Advances in Experimental Medicine and Biology 79:145–164.

Hruska KA, Rolnick F, Huskey M, Alvarez U, Cheresh D. 1995. Engagement of the osteoclast integrin αvβ3 by osteopontin stimulates phosphatidylinositol 3-hydroxyl kinase activity. Annals of the New York Academy of Science 760:151–165.

Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T. 2004. NF-κB is essential for epithelial–mesenchymal transition and metastasis in a model of breast cancer progression. Journal of Clinical Investigation 114:569–581.

Huerta S, Srivatsan ES, Venkatasan N, Livingston EH. 2001. Human colon cancer cells deficient in DCC produce abnormal transcripts in progression of carcinogenesis. Digestive Diseases and Sciences 46:1884–1891.

Hulett MD, Freeman C, Hamdorf BJ, Baker RT, Harris MJ, Parish CR. 1999. Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. Nature Medicine 5:803–809.

Hurst DR, Welch DR. 2005. Inhibition of tumor metastasis by targeting metastasis genes: BRMS1 as a prototype. In: Weber GF (ed.). Cancer therapy: molecular targets in tumor-Host interactions. Wymondham: Horizon Bioscience, pp. 83–92.

Hynes RO. 2003. Changing partners. Science 300:755–756.

Ibrahim S, Jilani I, O'Brien S, Rogers A, Manshouri T, Giles F, Faderl S, Thomas D, Kantarjian H, Keating M, Albitar M. 2003. Clinical relevance of the expression of the CD31 ligand for CD38 in patients with B-cell chronic lymphocytic leukemia. Cancer 97:1914–1919.

Imai MA, Moriya T, Imai FL, Shiiba M, Bukawa H, Yokoe H, Uzawa K, Tanzawa H. 2005. Down-regulation of DMBT1 gene expression in human oral squamous cell carcinoma. International Journal of Molecular Medicine 15:585–589.

Imhof BA, Piali L, Gisler RH, Dunon D. 1996. Involvement of α6 and αv integrins in metastasis. Current Topics in Microbiology and Immunology 213(I):195–203.

Jalkanen S, Bargatze RF, Herron LR, Butcher EC. 1986. A lymphoid cell surface protein involved in endothelial cell recognition and lymphocyte homing in man. European Journal of Immunology 16:1195–1202.

Jordan P, Brazao R, Boavida MG, Gespach C, Chastre E. 1999. Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. Oncogene 18:6835–6839.

Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M. 2001. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. Journal of Cell Biology 153:893–904.

Kanamori Y, Matsushima M, Minaguchi T, Kobayashi K, Sagae S, Kudo R, Terakawa N, Nakamura Y. 1999. Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Research 59:4225–4227.

Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT, Berman DM, Beachy PA. 2004. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 431:707–712.

Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annual Reviews of Immunology 8:621–663.

Katagiri YU, Sleeman J, Fujii H, Herrlich P, Hotta H, Tanaka K, Chikuma S, Yagita H, Okumura K, Murakami M, Saiki I, Chambers AF, Uede T. 1999. CD44 variants but not CD44s cooperate with β1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility and chemotaxis. Cancer Research 59:219–226.

Khanna C, Khan J, Nguyen P, Prehn J, Caylor J, Yeung C, Trepel J, Meltzer P, Helman L. 2001. Metastasis-associated differences in gene expression in a murine model of osteosarcoma. Cancer Research 61:3750–3759.

Kim HL, Griend DJ, Yang X, Benson DA, Dubauskas Z, Yoshida BA, Chekmareva MA, Ichikawa Y, Sokoloff MH, Zhan P, Karrison T, Lin A, Stadler WM, Ichikawa T, Rubin MA, Rinker-Schaeffer CW. 2001. Mitogen-activated protein kinase kinase 4 metastasis suppressor gene expression is inversely related to histological pattern in advancing human prostatic cancer. Cancer Research 61:2833–2837.

Kim JH, Skates SJ, Uede T, Wong KK, Schorge JO, Feltmate CM, Berkowitz RS, Cramer DW, Mok SC. 2002. Osteopontin as a potential diagnostic biomarker for ovarian cancer. The Journal of the American Medical Association 287:1671–1679.

Kishi T, Grass L, Soosaipillai A, Scorilas A, Harbeck N, Schmalfeldt B, Dorn J, Mysliwiec M, Schmitt M, Diamandis EP. 2003. Human kallikrein 8, a novel biomarker for ovarian carcinoma. Cancer Research 63:2771–2774.

Knight CG, Morton LF, Peachey AR, Tuckwell DS, Farndale RW, Barnes MJ. 2000. The collagen-binding A-domains of integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. The Journal of Biological Chemistry 275:35–40.

Kobayashi D, Yamada M, Kamagata C, Kaneko R, Tsuji N, Nakamura M, Yagihashi A, Watanabe N. 2002. Overexpression of early growth response-1 as a metastasis-regulatory factor in gastric cancer. Anticancer Research 22:3963–3970.

Koivunen E, Saksela O, Itkonen O, Osman S, Huhtala ML, Stenman UH. 1991. Human colon carcinoma, fibrosarcoma and leukemia cell lines produce tumor-associated trypsinogen. International Journal of Cancer 47:592–596.

Koliopanos A, Friess H, Kleeff J, Shi X, Liao Q, Pecker I, Vlodavsky I, Zimmermann A, Buchler MW. 2001. Heparanase expression in primary and metastatic pancreatic cancer. Cancer Research 61:4655–4659.

Koren R, Dekel Y, Sherman E, Weissman Y, Dreznik Z, Klein B, Gal R. 2003. The expression of DCC protein in female breast cancer. Breast Cancer Research and Treatment 80:215–220.

Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V. 2000. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. Journal of Cell Biology 148:615–624.

Kourlas PJ, Strout MP, Becknell B, Veronese ML, Croce CM, Theil KS, Krahe R, Ruutu T, Knuutila S, Bloomfield CD, Caligiuri MA. 2000. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proceedings of the National Academy of Sciences USA 97:2145–2150.

Kristiansen G, Pilarsky C, Wissmann C, Stephan C, Weissbach L, Loy V, Loening S, Dietel M, Rosenthal A. 2003. ALCAM/CD166 is up-regulated in low-grade prostate cancer and progressively lost in high-grade lesions. Prostate 54:34–43.

Kubo H, Miki C, Kusunoki M. 2004. Evaluation of genetic mutations of tumor suppresser genes in colorectal cancer patients. Hepatogastroenterology 51:114–117.

Kuniyasu H, Chihara Y, Kondo H. 2003a. Differential effects between amphoterin and advanced glycation end products on colon cancer cells. International Journal of Cancer 104:722–727.

Kuniyasu H, Chihara Y, Takahashi T. 2003b. Co-expression of receptor for advanced glycation end products and the ligand amphoterin associates closely with metastasis of colorectal cancer. Oncology Reports 10:445–448.

Kuniyasu H, Chihara Y, Kondo H, Ohmori H, Ukai R. 2003c. Amphoterin induction in prostatic stromal cells by androgen deprivation is associated with metastatic prostate cancer. Oncology Reports 10:1863–1868.

Kuniyasu H, Oue N, Wakikawa A, Shigeishi H, Matsutani N, Kuraoka K, Ito R, Yokozaki H, Yasui W. 2002. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. Journal of Pathology 196:163–170.

Kuroda S, Fukata M, Nakagawa M, Fujii K, Nakamura T, Ookubo T, Izawa I, Nagase T, Nomura N, Tani H, Shoji I, Matsuura Y, Yonehara S, Kaibuchi K. 1998. Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of F-cadherin-mediated cell–cell adhesion. Science 281:832–835.

Laferriere J, Houle F, Huot J. 2002. Regulation of the metastatic process by E-selectin and stress-activated protein kinase-2/p38. Annales of the New York Academy of Science 973:562–572.

Lauri D, Needham L, Martin-Padura I, Dejana E. 1991. Tumor cell adhesion to endothelial cells: endothelial leukocyte adhesion molecule-1 as an inducible adhesive receptor specific for colon carcinoma cells. Journal of the National Cancer Institute 83:1321–1324.

Ledda MF, Adris S, Bravo AI, Kairiyama C, Bover L, Chernajovsky Y, Mordoh J, Podhajcer OL. 1997. Suppression of SPARC expression by antisense RNA abrogates the tumorigenicity of human melanoma cells. Nature Medicine 3:171–176.

Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. 2000. Failure to regulate TNF-induced NF-κB and cell death responses in A20-deficient mice. Science 289:2350–2354.

Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. 1996. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. Journal of the National Cancer Institute 88:1731–1737.

Lee T, Esemuede N, Sumpio BE, Gahtan V. 2003. Thrombospondin-1 induces matrix metalloproteinase-2 activation in vascular smooth muscle cells. Journal of Vascular Surgery 38:147–154.

Lee W, Hasslinger A, Karin M, Tjian R. 1987. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. Nature 325:368–372.

Lehmann JM, Riethmueller G, Johnson JP. 1989. MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. Proceedings of the National Academy of Sciences USA 86:9891–9895.

Levicar N, Strojnik T, Kos J, Dewey RA, Pilkington GJ, Lah TT. 2002. Lysosomal enzymes, cathepsins in brain tumour invasion. Journal of Neurooncology 58:21–32.

Li R, Mitra N, Gratkowski H, Vilaire G, Litvinov R, Nagasami C, Weisel JW, Lear JD, DeGrado WF, Bennett JS. 2003. Activation of integrin αIIbβ3 by modulation of transmembrane helix associations. Science 300:795–798.

Lin X, Tombler E, Nelson PJ, Ross M, Gelman IH. 1996. A novel src- and ras-suppressed protein kinase C substrate associated with cytoskeletal architecture. The Journal of Biological Chemistry 271:28430–28438.

Lindeman JH, Hanemaaijer R, Mulder A, Dijkstra PD, Szuhai K, Bromme D, Verheijen JH, Hogendoorn PC. 2004. Cathepsin K is the principal protease in giant cell tumor of bone. American Journal of Pathology 165:593–600.

Lokeshwar VB, Bourguignon LY. 1992. The lymphoma transmembrane glycoprotein GP85 (CD44) is a novel guanine nucleotide-binding protein which regulates GP85 (CD44)-ankyrin interaction. The Journal of Biological Chemistry 267:22073–22078.

Lu TT, Cyster JG. 2002. Integrin-mediated long-term B cell retention in the splenic marginal zone. Science 297:409–412.

Maecker HT, Todd SC, Levy S. 1997. The tetraspanin superfamily: molecular facilitators. FASEB Journal 11:428–442.

Magklara A, Mellati AA, Wasney GA, Little SP, Sotiropoulou G, Becker GW, Diamandis EP. 2003. Characterization of the enzymatic activity of human kallikrein 6: Autoactivation, substrate specificity, and regulation by inhibitors. Biochemical and Biophysical Research Communications 307:948–955.

Mahoney MG, Simpson A, Jost M, Noe M, Kari C, Pepe D, Choi YW, Uitto J, Rodeck U. 2002. Metastasis-associated protein (MTA)1 enhances migration, invasion, and anchorage-independent survival of immortalized human keratinocytes. Oncogene 21:2161–2170.

Maki Y, Bos TJ, Davis C, Starbuck M, Vogt PK. 1987. Avian sarcoma virus 17 carries the Jun oncogene. Proceedings of the National Academy of Sciences USA 84:2848–2852.

Mariani L, McDonough WS, Hoelzinger DB, Beaudry C, Kaczmarek E, Coons SW, Giese A, Moghaddam M, Seiler RW, Berens ME. 2001. Identification and validation of P311 as a glioblastoma invasion gene using laser capture microdissection. Cancer Research 61:4190–4196.

Martin-Satue M, Blanco J. 1999. Identification of semaphorin E gene expression in metastatic human lung adenocarcinoma cells by mRNA differential display. Journal of Surgical Oncology 72:18–23.

Mashino K, Sadanaga N, Yamaguchi H, Tanaka F, Ohta M, Shibuta K, Inoue H, Mori M. 2002. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Research 62:2937–2941.

Matsumura Y, Tarin D. 1992. Significance of CD44 gene products for cancer diagnosis and disease evaluation. The Lancet 340:1053–1058.

Mawby WJ, Holmes CH, Anstee DJ, Spring FA, Tanner MJ. 1994. Isolation and characterization of CD47 glycoprotein: a multispanning membrane protein which is the same as integrin-associated protein (IAP) and the ovarian tumour marker OA3. Biochemical Journal 304:525–530.

McGary EC, Heimberger A, Mills L, Weber K, Thomas GW, Shtivelband M, Lev DC, Bar-Eli M. 2003. A fully human antimelanoma cellular adhesion molecule/MUC18 antibody inhibits spontaneous pulmonary metastasis of osteosarcoma cells in vivo. Clinical Cancer Research 9:6560–6566.

Merlot S, Firtel RA. 2003. Leading the way: directional sensing through phosphatidylinositol 3-kinase and other signaling pathways. The Journal of Cell Science 116:3471–3478.

Mhawech P, Dulguerov P, Tschanz E, Verdan C, Ares C, Allal AS. 2004. Motility-related protein-1 (MRP-1/CD9) expression can predict disease-free survival in patients with squamous cell carcinoma of the head and neck. British Journal of Cancer 90:471–475.

Miao X, Yu C, Tan W, Xiong P, Liang G, Lu W, Lin D. 2003. A functional polymorphism in the matrix metalloproteinase-2 gene promoter (-1306C/T) is associated with risk of development but not metastasis of gastric cardia adenocarcinoma. Cancer Research 63:3987–3990.

Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. 2005. Genes that mediate breast cancer metastasis to lung. Nature 436:518–524.

Mollenhauer J, Herbertz S, Helmke B, Kollender G, Krebs I, Madsen J, Holmskov U, Sorger K, Schmitt L, Wiemann S, Otto HF, Grone HJ, Poustka A. 2001. Deleted in malignant brain tumors 1 is a versatile mucin-like molecule likely to play a differential role in digestive tract cancer. Cancer Research 61:8880–8886.

Mueller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56.

Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D. 1999. The integrin αvβ6 binds and activates latent TGF β 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 96:319–328.

Nagase H, Brew K.2002. Engineering of tissue inhibitor of metalloproteinases mutants as potential therapeutics. Arthritis Research and therapy 4 (suppl3):S51–S56.

Nakabeppu Y, Ryder K, Nathans D. 1988. DNA binding activities of three murine Jun proteins: stimulation by Fos. Cell 55:907–915.

Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S. 1989. Molecular cloning and expression of human hepatocyte growth factor. Nature 34:440–443.

Nawa A, Nishimori K, Lin P, Maki Y, Moue K, Sawada H, Toh Y, Fumitaka K, Nicolson GL. 2000. Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides. Journal of Cellular Biochemistry 79:202–212.

Nicolson GL, Dulski KM, Trosko JE. 1988. Loss of intercellular junction communication correlates with metastatic potential in mammary adenocarcinoma cells. Proceedings of the National Academy of Sciences USA 85:473–476.

Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M. 2001. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. Journal of Cell Science 114:111–118.

O-charoenrat P, Rusch V, Talbot SG, Sarkaria I, Viale A, Socci N, Ngai I, Rao P, Singh B. 2004. Casein kinase II α subunit and C1-inhibitor are independent predictors of outcome in patients with squamous cell carcinoma of the lung. Clinical Cancer Research 10:5792–5803.

Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M. 2001. Metastasis suppressor gene *KiSS-1* encodes a peptide ligand of a G-protein coupled receptor. Nature 411:613–617.

Oinuma I, Ishikawa Y, Katoh H, Negishi M. 2004. The semaphorin 4D receptor plexin-B1 is a GTPase activating protein for R-Ras. Science 305:862–865.

Oldberg A, Ruoslahti E. 1986. Evolution of the fibronectin gene. Exon structure of cell attachment domain. The Journal of Biological Chemistry 261:2113–2116.

Oldberg A, Franzen A, Heinegard D. 1986. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. Proceedings of the National Academy of Sciences USA 83:8819–8823.

Olson TS, Ley K. 2002. Chemokines and chemokine receptors in leukocyte trafficking. American Journal of Physiology 283:R7–R28.

O'Reilly MS, Pirie-Shepherd S, Lane WS, Folkman J. 1999. Antiangiogenic activity of the cleaved conformation of the serpin antithrombin. Science 285:1926–1928.

Oshima K, Machida K, Ichigotani Y, Nimura Y, Shirafuji N, Hamaguchi M, Matsuda S. 2002. SHPS-1: A budding molecule against cancer dissemination. Cancer Research 62:3929–3933.

Owens DM, Watt FM. 2001. Influence of β1 integrins on epidermal squamous cell carcinoma formation in a transgenic mouse model: α3β1, but not α2β1, suppresses malignant conversion. Cancer Research 61:5248–5254.

Ozdamar B, Bose R, Barrios-Rodiles M, Wang H-R, Zhang Y, Wrana JL. 2005. Regulation of the polarity protein Par6 by TGFβ receptors controls epithelial cell plasticity. Science 307:1603–1609.

Parish CR, Freeman C, Hulett MD. 2001. Heparanase: a key enzyme involved in cell invasion. Biochimica et Biophysica Acta 1471:M99-M108.

Park BK, Zhang H, Zeng Q, Dai J, Keller ET, Giordano T, Gu K, Shah V, Pei L, Zarbo RJ, McCauley L, Shi S, Chen S, Wang CY. 2007. NF-κB in breast cancer cells promotes osteolytic bone metastasis by inducing osteoclastogenesis via GM-CSF. Nature Medicine 13:62–69.

Petitclerc E, Stroemblad S, von Schalscha TL, Mitjans F, Piulats J, Montgomery AMP, Cheresh DA, Brooks PC. 1999. Integrin $\alpha_V\beta_3$ promotes melanoma growth in human skin by regulating tumor cell survival. Cancer Research 59:2724–2730.

Pickl WF, Majdic O, Fischer GF, Petzelbauer P, Fae I, Waclavicek M, Stockl J, Scheinecker C, Vidicki T, Aschauer H, Johnson JP, Knapp W. 1997. MUC18/MCAM (CD146), an

activation antigen of human T-lymphocytes. The Journal of Immunology 158:2107–2115.

Pouliot N, Nice EC, Burgess AW. 2001. Laminin-10 mediates basal and EGF-stimulated motility of human colon carcinoma cells via $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins. Experimantal Cell Research 266:1–10.

Price SJ, Greaves DR, Watkins H. 2001. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. The Journal of Biological Chemistry 276:7549–7558.

Radford KJ, Thorne RF, Hersey P. 1996. CD63 associates with transmembrane 4 superfamily members, CD9 and CD81, and with β1 integrins in human melanoma. Biochemical and Biophysical Research Communications 222:13–18.

Raftopoulou M, Etienne-Manneville S, Self A, Nicholls A, Hall A. 2004. Regulation of cell migration by the C2 domain of the tumor suppressor PTEN. Science 303:1179–1181.

Rasheed BK, McLendon RE, Friedman HS, Friedman AH, Fuchs HE, Bigner DD, Bigner SH. 1995. Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. Oncogene 10:2243–2246.

Rashid MG, Sanda MG, Vallorosi CJ, Rios-Doria J, Rubin MA, Day ML. 2001. Posttranslational truncation and inactivation of human E-cadherin distinguishes prostate cancer from matched normal prostate. Cancer Research 61:489–492.

Rempel SA, Ge S, Gutierrez JA. 1999. SPARC: A potential diagnostic marker of invasive meningiomas. Clinical Cancer Research 5:237–241.

Rickman DS, Tyagi R, Zhu XX, Bobek MP, Song S, Blaivas M, Misek DE, Israel MA, Kurnit DM, Ross DA, Kish PE, Hanash SM. 2001. The gene for the axonal cell adhesion molecule TAX-1 is amplified and aberrantly expressed in malignant gliomas. Cancer Research 61:2162–2168.

Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. 2003. Cell migration: Integrating signals from front to back. Science 302:1704–1709.

Riemenschneider MJ, Knobbe CB, Reifenberger G. 2003. Refined mapping of 1q32 amplicons in malignant gliomas confirms MDM4 as the main amplification target. International Journal of Cancer 104:752–757.

Rivat C, Le Floch N, Sabbah M, Teyrol I, Redeuilh G, Bruyneel E, Mareel M, Matrisian LM, Crawford HC, Gespach C, Attoub S. 2003. Synergistic cooperation between the AP-1 and LEF-1 transcription factors in activation of the matrilysin promoter by the src oncogene: implications in cellular invasion. FASEB Journal 17:1721–1723.

Roos E. 1993. Adhesion molecules in lymphoma metastasis. Seminars in Cancer Biology 4:285–292.

Ruoslathi E. 1996. How cancer spreads. Scientific American 275:72–77.

Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinckerhoff CE. 1998. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Research 58:5321–5325.

Ryder K, Lau LF, Nathans D. 1988. A gene activated by growth factors is related to the oncogene *v-jun*. Proceedings of the National Academy of Sciences USA 85:1487–1491.

Saha S, Bardelli A, Buckhaults P, Velculescu VE, Rago C, St. Croix B, Romans KE, Choti MA, Lengauer C, Kinzler KW, Vogelstein B. 2001. A phosphatase associated with metastasis in colorectal cancer. Science 294:1343–1346.

Sahai E, Marshall CJ. 2002. RHO-GTPases and cancer. Nature Reviews Cancer 2:133–142.

Santamaria Babi LF, Perez Soler MT, Hauser C, Blaser K. 1995. Skin-homing T cells in human cutaneous allergic inflammation. Immunology Research 14:317–324.

Schnelzer A, Prechtel D, Knaus U, Dehne K, Gerhard M, Graeff H, Harbeck N, Schmitt M, Lengyel E. 2000. Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene 19:3013–3020.

Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. 2001. Epithelial cancer cell migration: a role for chemokine receptors? Cancer Research 61:4961–4965.

Seiffert M, Cant C, Chen Z, Rappold I, Brugger W, Kanz L, Brown EJ, Ullrich A, Bühring HJ. 1999. Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 94:3633–3643.

Seiter R, Arch R, Reber S, Komitowski D, Hofmann M, Ponta H, Herrlich P, Matzku S, Zoeller M. 1993. Prevention of tumor metastasis formation by anti-variant CD44. Journal of Experimental Medicine 177:443–455.

Senger DR, Peruzzi CA, Papadopoulos A. 1989. Elevated expression of secreted phosphoprotein I (osteopontin, 2ar) as a consequence of neoplastic transformation. Anticancer Research 48:1291–1299.

Seraj MJ, Samant RS, Verderame MF, Welch DR. 2000. Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. Cancer Research 60:2764–2769.

Sherbet GV, Lakshmi MS. 1997. The genetics of cancer: genes associated with cancer invasion, metastasis and cell proliferation. San Diego: Academic Press, p. 104.

Shih IM, Elder DE, Hsu MY, Herlyn M. 1994. Regulation of Mel-CAM/MUC18 expression on melanocytes of different stages of tumor progression by normal keratinocytes. American Journal of Pathology 145:837–845.

Sho M, Adachi M, Taki T, Hashida H, Konishi T, Huang CL, Ikeda N, Nakajima Y, Kanehiro H, Hisanaga M, Nakano H, Miyake M. 1998. Transmembrane 4 superfamily as a prognostic factor in pancreatic cancer. International Journal of Cancer 79:509–516.

Shu T, Valentino KM, Seaman C, Cooper HM, Richards LJ. 2000. Expression of the netrin-1 receptor, deleted in colorectal cancer (DCC), is largely confined to projecting neurons in the developing forebrain. Journal of Computational Neurology 416:201–212.

Small JV, Resch GP. 2005. The comings and goings of actin: coupling protrusion and retraction in cell motility. Current Opinions in Cell Biology 17:517–523.

Sotiropoulou G, Anisowicz A, Sager R. 1997. Identification, cloning, and characterization of cystatin M, a novel cysteine proteinase inhibitor, down-regulated in breast cancer. The Journal of Biological Chemistry 272:903–910.

Span PN, Manders P, Heuvel JJ, Thomas CM, Bosch RR, Beex LV, Sweep CG. 2002. Expression of the transcription factor Ets-1 is an independent prognostic marker for relapse-free survival in breast cancer. Oncogene 21:8506–8509.

Srivastava SK, Wheelock RHP, Aaronson SA, Eva A. 1986. Identification of the protein encoded by the human diffuse B-cell lymphoma (dbl) oncogene. Proceedings of the National Academy of Sciences USA 83:8868–8872.

Staunton DE, Dustin ML, Springer TA. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature 339:61–64.

Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, Sobel ME. 1988. Evidence for a novel gene associated with low tumor metastatic potential. Journal of the National Cancer Institute 80:200–204.

Stocker M, Perryman M. 1985. An epithelial scatter factor released by embryo fibroblasts. Journal of Cell Science 77:209–223.

Stracke ML, Liotta LA. 1995. Molecular mechanisms of tumor cell metastasis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 233–247.

Strobeck MW, DeCristofaro MF, Banine F, Weissman BE, Sherman LS, Knudsen ES. 2001. The BRG-1 subunit of the SWI/SNF complex regulates CD44 expression. The Journal of Biological Chemistry 276:9273–9278.

Strojan P, Oblak I, Svetic B, Smid L, Kos J. 2004. Cysteine proteinase inhibitor cystatin C in squamous cell carcinoma of the head and neck: relation to prognosis. British Journal of Cancer 90:1961–1968.

Strömblad S, Becker JC, Yebra M, Brooks PC, Cheresh DA. 1996. Suppression of p53 activity and $p21^{WAF1/CIP1}$ expression by vascular cell integrin $\alpha V\beta 3$ during angiogenesis. The Journal of Clinical Investigations 98:426–433.

Stupack DG, Puente XS, Boutsaboualoy S, Storgard CM, Cheresh DA. 2001. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. Journal of Cell Biology 155:459–470.

Suenaga N, Mori H, Itoh Y, Seiki M. 2005. CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase. Oncogene 24:859–868.

Sy MS, Liu D, Schiavone R, Ma J, Mori H, Guo Y. 1996. Interactions between CD44 and hyaluronic acid: Their role in tumor growth and metastasis. In: Guenthert U, Schlag PM, Birchmeier W (eds). Current Topics in Microbiology and Immunology 213(III):129–153.

Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM, Ginsberg MH, Calderwood DA. 2003. Talin binding to integrin β tails: A final common step in integrin activation. Science 302:103–106.

Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM, Schmidt AM. 2000. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature 405:354–360.

Takayama T, Shiozaki H, Inoue M, Tamura S, Oka H, Kadowaki T, Takatsuka V, Nagafuchi A, Tsukita S, Mori T. 1994. Expression of E-cadherin and α-catenin molecules in human breast cacner tissues and association with clinicopathological features. International Journal of Oncology 5:775–780.

Tamura M, Gu J, Matsumoto K, Aota S-i, Parsons R, Yamada KM. 1998. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 280:1614–1617.

Tanabe KK, Ellis LM, Saya H. 1993. Expression of CD44R1 adhesion molecule in colon carcinomas and metastases. The Lancet 341:725–726.

Tani K, Ogushi F, Shimizu T, Sone S. 2001. Protease-induced leukocyte chemotaxis and activation: roles in host defense and inflammation. Journal of Medical Investigations 48:133–141.

Thiery JP. 2002. Epithelial-mesenchymal transitions in tumour progression. Nature Reviews Cancer 2:442–454.

Toh Y, Kuninaka S, Endo K, Oshiro T, Ikeda Y, Nakashima H, Baba H, Kohnoe S, Okamura T, Nicolson GL, Sugimachi K. 2000. Molecular analysis of a candidate metastasis-associated gene, MTA1: possible interaction with histone deacetylase 1. Journal of Experimental and Clinical Cancer Research 19:105–111.

Toh Y, Pencil SD, Nicolson GL. 1994. A novel candidate metastasis-associated gene, mta1, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. The Journal of Biological Chemistry 269:22958–22963.

Toh Y, Pencil SD, Nicolson GL. 1995. Analysis of the complete sequence of the novel metastasis-associated candidate gene, mta1, differentially expressed in mammary adenocarcinoma and breast cancer cell lines. Gene 159:97–104.

Tomita Y, Nishiyama T, Watanabe H, Fujiwara M, Sato S. 1990. Expression of intercellular adhesion molecule-1 (ICAM-1) on renal-cell cancer: possible significance in host immune responses. International Journal of Cancer 46:1001–1006.

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K, Haase VH, Ambrose CM, Munroe D, Bove C, Haines JL, Martuza RL, MacDonald ME, Seizinger BR, Short MP, Buckler AJ, Gusella JF. 1993. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791–800.

Trusolino L, Bertotti A, Comoglio PM. 2001. A signaling adapter function for α6β4 integrin in the control of HGF-dependent invasive growth. Cell 107:643–654.

Tsai M-S, Hornby AE, Lakins J, Lupu R. 2000. Expression and function of CYR61, an angiogenic factor, in breast cancer cell lines and tumor biopsies. Cancer Research 60:5603–5607.

Tuck AB, Arsenault DM, O'Malley FP, Hota C, Ling MC, Wilson SM, Chambers AF. 1999. Osteopontin induces increased invasiveness and plasminogen activator expression in human mammary epithelial cells. Oncogene 18:4237–4246.

Van der Schoot CE, Huizinga TWJ, Gadd SK, Majdic O, Wijmans R, Knapp W, Von dem Borne AEG. 1989. Identification of three novel PI-linked proteins on granulocytes. In: Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, Von dem Borne AEGK (eds). Leukocyte typing IV: white cell differentiation antigens. Oxford: Oxford University Press, pp. 887–891.

van Kempen LC, van den Oord JJ, van Muijen GN, Weidle UH, Bloemers HP, Swart GW. 2000. Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. American Journal of Pathology 156:769–774.

Velasco G, Ferrando AA, Puente XS, Sanchez LM, Lopez-Otin C. 1994. Human cathepsin O: molecular cloning from a breast carcinoma, production of the active enzyme in *Escherichia coli*, and expression analysis in human tissues. The Journal of Biological Chemistry 269:27136–27142.

Vlodavsky I, Friedmann Y, Elkin M, Aingorn H, Atzmon R, Ishai-Michaeli R, Bitan M, Pappo O, Peretz T, Michal I, Spector L, Pecker I. 1999. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nature Medicine 5:793–802.

Waghray A, Keppler D, Sloane BF, Schuger L, Chen YQ. 2002. Analysis of a truncated form of cathepsin H in human prostate tumor cells. The Journal of Biological Chemistry 277:11533–11538.

Walz DA, Fenton JW. 1995. The role of thrombin in tumor cell metastasis. Invasion and Metastasis 14:303–308.

Wang H, Fu W, Im JH, Zhou Z, Santoro SA, Iyer V, DiPersio CM, Yu QC, Quaranta V, Al-Mehdi A, Muschel RJ. 2004. Tumor cell $\alpha_3\beta_1$ integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. The Journal of Cell Biology 164:935–941.

Wang H-R, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, Wrana JL. 2003. Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. Science 302:1775–1779.

Weber GF. 2001. The metastasis gene osteopontin: a candidate target for cancer therapy. Biochimica et Biophysica Acta 1552:61–85.

Weber GF, Adler B, Ashkar S. 1999. Osteopontin in oxidative stress responses. In: Ruffolo RR jr, Feuerstein GZ, Hunter AJ, Poste G, Metcalf BW (eds). Inflammatory cells and mediators in CNS diseases. Amsterdam: Harwood Academic Publishers, pp. 97–112.

Weber GF, Ashkar S, Glimcher MJ, Cantor H. 1996. Receptor-ligand interaction between CD44 and osteopontin (ETA-1). Science 271:509–512.

Weber GF, Bronson R, Ilagan J, Cantor H, Schmitz R, Mak TW. 2002. Absence of CD44 prevents sarcoma metastasis. Cancer Research 62:2281–2286.

Weeds A, Yeoh S. 2001. Action at the Y-branch. Science 294:1660–1661.

Wert SE, Yoshida M, LeVine AM, Ikegami M, Jones T, Ross GF, Fisher JH, Korfhagen TR, Whitsett JA. 2000. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. Proceedings of the National Academy of Sciences USA 97:5972–5977.

Wobus M, Rangwala R, Sheyn I, Hennigan R, Coila B, Lower EE, Yassin RS, Sherman LS. 2002. CD44 associates with EGFR and erbB2 in metastasizing mammary carcinoma cells. Applied Immunohistochemistry and Molecular Morphology 10:34–39.

Wright JH, Wang X, Manning G, LaMere BJ, Le P, Zhu S, Khatry D, Flanagan PM, Buckley SD, Whyte DB, Howlett AR, Bischoff JR, Lipson KE, Jallal B. 2003. The STE20 kinase HGK is broadly expressed in human tumor cells and can modulate cellular transformation, invasion, and adhesion. Molecular and Cellular Biology 23:2068–2082.

Xia W, Unger P, Miller L, Nelson J, Gelman I. 2001. The Src-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer. Cancer Research 61:5644–5651

Xie K, Huang S. 2003. Regulation of cancer metastasis by stress pathways. Clinical and Experimental Metastasis 20:31–43.

Yago K, Zenita K, Ginya H, Sawada M, Ohmori K, Okuma M, Kannagi R, Lowe JB. 1993. Expression of α-(1,3)-fucosyltransferases which synthesize sialyl Le(x) and sialyl Le(a), the carbohydrate ligands for E- and P-selectins, in human malignant cell lines. Cancer Research 53:5559–5565.

Yamada T, Endo R, Gotoh M, Hirohashi S. 1997. Identification of semaphorin E as a non-MDR drug resistance gene of human cancer. Proceedings of the National Academy of Sciences USA 94:14713–14718.

Yamada Y, Itano N, Narimatsu H, Kudo T, Morozumi K, Hirohashi S, Ochiai A, Ueda M, Kimata K. 2004. Elevated transcript level of hyaluronan synthase1 gene correlates with poor prognosis of human colon cancer. Clinical and Experimental Metastasis 21:57–63.

Yan Z, Robinson-Saddler A, Winawer S, Friedman E. 1993. Colon carcinoma cells blocked in polarization exhibit increased expression of carcinoembryonic antigen. Cell Growth and Differentiation 4:785–792.

Ye S, Dhillon S, Turner SJ, Bateman AC, Theaker JM, Pickering RM, Day I, Howell WM. 2001. Invasiveness of cutaneous malignant melanoma is influenced by matrix metalloproteinase 1 gene polymorphism. Cancer Research 61:1296–1298.

Yoshida BA, Sokoloff MM, Welch DR, Rinker-Schaeffer CW. 2000. Metastasis-suppressor genes: a review and perspective on an emerging field. Journal of the National Cancer Institute 92:1717–1730.

Yoshimasu T, Sakurai T, Oura S, Hirai I, Tanino H, Kokawa Y, Naito Y, Okamura Y, Ota I, Tani N, Matsuura N. 2004. Increased expression of integrin alpha3beta1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Science 95:142–148.

Younes A, Zhao S, Jendiroba D, Kleine HD, Cabanillas F, Andreeff M. 1995. Decreased expression of the deleted in colorectal carcinoma gene in non-Hodgkin's lymphoma. Blood 85:2813–2816.

Yousef GM, Polymeris ME, Grass L, Soosaipillai A, Chan PC, Scorilas A, Borgono C, Harbeck N, Schmalfeldt B, Dorn J, Schmitt M, Diamandis EP. 2003. Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. Cancer Research 63:3958–3965.

Yu C, Pan K, Xing D, Liang G, Tan W, Zhang L, Lin D. 2002. Correlation between a single nucleotide polymorphism in the matrix metalloproteinase-2 promoter and risk of lung cancer. Cancer Research 62:6430–6433.

Yu WH, Woessner JF Jr, McNeish JD, Stamenkovic I. 2002. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes and Development 16:307–323.

Yu Q, Stamenkovic I. 1999. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes and Development 13:35–48.

Yu Q, Stamenkovic I. 2000. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes and Development 14:163–176.

Yusa T, Blood CH, Zetter BR. 1989. Tumor cell interactions with elastin: implications for pulmonary metastasis. American Reviews of Respiratory Diseases 140:1458–1462.

Zeng Q, Dong JM, Guo K, Li J, Tan HX, Koh V, Pallen CJ, Manser E, Hong W. 2003. PRL-3 and PRL-1 promote cell migration, invasion, and metastasis. Cancer Research 63:2716–2722.

Zhang G, He B, Weber GF. 2003. Growth factor signaling induces metastasis genes in transformed cells. A molecular connection between Akt kinase and osteopontin in breast cancer. Molecular and Cellular Biology 23:6507–6519.

Zhang M, Volpert O, Shi YH, Bouck M. 2000. Maspin is an angiogenesis inhibitor. Nature Medicine 6:196–199.

Zhong H, May MJ, Jimi E, Ghosh S. 2002. The phosphorylation status of nuclear NF-κB determines its association with CBP/p300 or HDAC-1. Molecular Cell 9:625–636.

Zhong H, Voll RE, Ghosh S. 1998. Phosphorylation of NF-κB p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Molecular Cell 1:661–671.

Zhu D, Bourguignon LY. 2000. Interaction between CD44 and the repeat domain of ankyrin promotes hyaluronic acid-mediated ovarian tumor cell migration. Journal of Cell Physiology 183:182–195.

Zou Z, Anisowicz A, Hendrix MJ, Thor A, Neveu M, Sheng S, Rafidi K, Seftor E, Sager R. 1994. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. Science 263:526–529.

Zutter MM, Santoro SA, Staatz WD, Tsung YL. 1995. Re-expression of the $\alpha_2\beta_1$ integrin abrogates the malignant phenotype of breast carcinoma cells. Proceedings of the National Academy of Sciences USA 92:7411–7415.

SECTION III

MECHANISMS OF REGULATION OF CANCER-RELATED GENES

CHAPTER 6

DNA REPAIR

Although most cellular polymers, including RNA, proteins and polysaccharides, are regularly turned over and resynthesized based on the genetic blueprint, DNA cannot be turned over and requires repair when damaged. The genome is vulnerable to an array of damaging influences. More than 100 distinct forms of DNA damage are known, ranging from base modifications to DNA breaks and interstrand cross-links [Hoeijmakers 2001]. While the loss-of-sequence fidelity is a recessive trait, chromosomal instability may be dominant. Various DNA repair pathways exist: one group of mechanisms rectifies errors in the DNA sequence, another group checks the integrity of the chromosomes. Nucleotides affected by sequence errors are usually replaced by genetically encoded repair mechanisms after the removal of a short segment of a damaged strand and copying from the intact complementary strand. Alternatively, broken DNA can be repaired by recombination. Defects in the responsible mutator genes interfere with the proper execution of DNA repair, which can lead to damage in cancer-associated genes and result in transformation. While there is no repair mechanism for aneuploidy, protective processes exist to prevent chromosome instability during cell division. Their lack of function can predispose to transformation. Therefore, repair genes act as a second line of defense and are most accurately categorized as meta-suppressor genes rather than as tumor suppressor genes.

Due to the abundant use of the biradical oxygen in metabolism, oxidative damage to the DNA is a prominent risk factor for transformation.

– Chronic inflammation in the gastrointestinal tract increases the risk for the development of cancer. This may be caused in part by the accumulation of frameshift mutations in microsatellite DNA, secondary to the elevated generation of reactive oxygen intermediates [Gasche et al. 2001].
– The accumulation of reactive oxygen species can damage DNA, leading to the initiation or promotion of cancer. Mn-SOD, the only known superoxide scavenger in mitochondria, may be particularly important for antioxidant defense because mitochondria are the major sites for cellular metabolism, and hence production of reactive oxygen species. The signal sequence is essential for correct transport and processing of Mn-SOD by mitochondria. The polymorphism A16V in the signal sequence of Mn-SOD (the 9 position) produces a conformational change in the helical structure of the protein, which may decrease the efficiency of transport into mitochondria for the valine form of the protein. Individuals with the valine allele, associated with less efficient Mn-SOD enzyme transport into the mitochondria, have an increased susceptibility to lung cancer because of the more rapid accumulation of cancer causing reactive oxygen species [Wang et al. 2001].
– Zinc is an essential component of copper/zinc-superoxide dismutase. Dietary deficiencies in zinc can contribute to single and double strand DNA breaks and oxidative modifications to DNA that increase the risk for cancer development.

Besides reactive oxygen species, generated in an aerobic environment, the spontaneous hydrolysis of nucleotides at 37°C is an unavoidable source of DNA alterations. The exposure to UV light from the sun exerts DNA damage particularly in the skin.

Although genetic instability does not inevitably cause cancer, diseases based on defective repair of DNA damage often predispose to the formation of tumors.

– Hereditary nonpolyposis colorectal cancer (defect in mismatch repair) is characterized by microsatellite instability. Germline loss-of-function mutations of the mismatch repair genes *msh2*, *mlh1*, *msh6*, and *pms2* are frequently pathogenetic.
– Xeroderma pigmentosum (defect in nucleotide excision repair) is a rare, recessively inherited genodermatosis, which reflects some of the pathogenetic consequences of defects in nucleotide excision repair. Patients are prone to ultraviolet induced skin neoplasms from keratinocyte origin. The condition is associated with a high incidence of premalignant actinic keratoses, basal cell carcinomata, and squamous cell carcinomata. It may also include melanomata, keratoacanthomata, and angiomata. The diverse alterations of xeroderma pigmentosum gene products result in seven complementation groups (XPA through XPG) of the disease [Friedberg 2001]. The mutation rates for *p53* and *ptc* are increased in XP tumors. Patients with mutations in genes unique to nucleotide excision repair, such as *xpc* and *xpe*, and DNA reduplication, including *polη*, have few clinical symptoms beside cancer. Patients with mutations in components of transcription coupled repair, *csa* and *csb*, or in the common elements *xpa*, *xpb*, *xpd*, *xpf*, and *xpg* show very complex clinical symptoms involving the central nervous system and other organ systems. In contrast, trichothiodystrophy, where the XPD (ERCC2) and XPB (ERCC3) gene products are affected, does not display these dysregulations and is not associated with an increased risk of cancer [Yu et al. 1999]. Mutations in the genes *csa* (*group 8 excision repair cross-complementing protein*, *ercc8*) or *csb* (*ercc6*) underlie Cocayne syndrome. This condition is not prone to an increased incidence of cancer.
– Ataxia teleangiectasia (AT) (defect in repair of double-strand breaks) is caused by a loss-of-function mutation in the signaling protein kinase ATM that causes chromosome breakage and immune deficiency. It is inherited autosomally recessively. Patients with AT suffer from rare entities of pediatric non-Hodgkin lymphoma or lymphocytic leukemia. In general, lymphomata in AT patients tend to be of B-cell origin (B-CLL), whereas the leukemias tend to be of the T-CLL type. Other solid tumors, including mucinous adenocarcinoma of the stomach, medulloblastomata and gliomata, occur with increased frequency. The abnormal sensitivity of AT cells to killing by ionizing radiation and abnormal resistance to inhibition of DNA synthesis by ionizing radiation has revealed complementation groups for the classic form of the disease [Jaspers et al. 1988]. At least four of these (A, C, D, and E) map to chromosome 11q23 and are associated with mutations in the *atm* gene. In these patients, α-Fetoprotein tends to be elevated. The aneuploidy in AT affects predominantly the chromosomes 7 and 14.
– Nijmegen breakage syndrome [Weemaes et al. 1981] (defect in repair of double-strand breaks) is associated with microcephaly, stunted growth, mental retardation, café au lait spots, and immunodeficiency. The susceptibility to lymphomata and meningiomata is increased. Chromosomes 7 and 14 are preferentially affected. The Nijmegen breakage syndrome has been referred to as ataxia telangiectasia variant 1.
– Bloom syndrome (defect of a DNA Helicase) [Bloom 1966] is caused by a mutation in the REC-Q like Helicase BLM {15q26.1}. This Helicase likely plays a role in restarting DNA reduplication forks that are blocked at lesions, thereby promoting chromosome stability. Elevated generation of functional hemizygosity and homozygosity in somatic cells may play a role in the high cancer risk of persons with Bloom syndrome. Specifically, leukemia is frequent.
– Werner syndrome (defect of the REC-Q like DNA Helicase WRN) is a segmental progeric disorder with an autosomal recessive pattern of inheritance. The cells from Werner syndrome patients are characterized by slow growth rates, premature senescence, accelerated telomere shortening, and genome instability. Cells lacking WRN are afflicted by the deletion of telomeres. The Werner syndrome gene, *wrn* (*recq3*, *recql2*, *rec-q like 2*) {8p12-p11.2}, encodes a Helicase of the REC-Q subclass that acts as a tissue specific regulator of mismatch repair. WRN and the homologous recombination mediator protein RAD52 form a complex that colocalizes in foci associated with arrested replication forks. RAD52 modulates WRN Helicase activity in a DNA structure dependent manner, while WRN increases the efficiency of the RAD52-mediated strand annealing

between nonduplex DNA and homologous sequences. PARP-1 may be part of this complex involved in the processing of DNA breaks [Pieper et al. 2000]. Furthermore, WRN may be necessary for the replication of G-rich telomeric DNA, preventing telomere dysfunction and consequent genomic instability [Crabbe et al. 2004]. The cancer risk is increased in Werner syndrome patients. The proto-oncogene product MYC directly stimulates the transcription of *wrn*.

– Rothmund-Thompson syndrome (defect of a REC-Q like DNA Helicase) is autosomally recessively inherited. It is caused by mutations in the DNA Helicase REC-QL4 {8q24.3}. Malignancies, including osteogenic sarcomata and skin cancer, frequently occur.
– Fanconi anemia (FA) (chromosomal instability) is an autosomal recessive disorder. It is characterized by congenital abnormalities, defective hemopoiesis, and a high risk of developing acute myeloid leukemia or solid tumors, including head and neck cancer, cancers of the female genitals, esophageal cancer, and brain tumors. Symptoms include aplastic anemia, congenital anomalies, pancytopenia, and panmyelopathy. FA can be caused by mutations in at least 11 different genes, *fancA*, *fancB*, *fancC*, *fancD1* (*brca2*), *fancD2*, *fancE*, *fancF*, *fancG*, *fancI*, *fancJ*, *fancL*.
– Mosaic variegated aneuploidy [Warburton et al. 1991] (defect in chromosome segregation) is a genetic disorder, in which more than 25% of patients' cells are aneuploid and childhood cancers, such as rhabdomyosarcoma and leukemia, occur with increased frequency. It may be based on loss-of-function mutations (missense or truncations) in the repair gene *bub1B* {15q15}, which encodes BUBR1 (BUB Related 1), a key protein in the mitotic spindle checkpoint [Hanks et al. 2004].

Tumors with instability on the nucleotide sequence level (microsatellite instability) typically do not display chromosome abnormalities, conversely tumors with chromosome instability do not normally have instabilities on the nucleotide level.

6.1 DNA SEQUENCE FIDELITY

Microsatellite loci are inherently unstable entities in the genome and are present in numerous copies. They are widely dispersed, primarily within noncoding regions. Microsatellites are often copied incorrectly by DNA Polymerases because the template and daughter strands in these regions are particularly prone to misalignment or slippage during copying. These replication dependent events create loops of extra-helical bases, which would produce frameshift mutations unless reversed by the mismatch repair system (MMR) (Table 6.1.A). The mismatch repair system thus prevents expansions or contractions of simple repeat or iterated sequences. The primary role of this mechanism is to maintain DNA sequence fidelity by removing replication errors from DNA. The loss of function of genes that normally act in the maintenance of genetic stability can preclude the enzymatic correction of such inaccuracies and lead to microsatellite instability [Peinado et al. 1992]. This is reflected in a widespread expansion or contraction of these repeated sequences that affects the whole genome (microsatellite instability). Since the same pathway is also responsible for repairing base:base mismatches, defective cells also experience large increases in the frequency of spontaneous transition and transversion mutations. This instability can affect nonrepetitive sequences of the genome, leading to much higher rates of mutation in tumors than in nontransformed cells. This phenomenon has been referred to as a mutator phenotype [Loeb 1991, 1994]. Furthermore, loss of function of DNA repair genes gives rise to changes in oncogenes or tumor suppressor genes. DNA repair occurs mainly via two basic mechanisms, excision and recombination. Excision reactions are used when only one strand is affected, allowing the intact strand to be used as a template. This mechanism is employed by mismatch repair, base excision repair, and nucleotide excision repair. Recombination is primarily responsible for the repair of strand breaks and cross-links.

6.1.1 Direct Damage Reversal

Direct damage reversal undoes chemical modifications without replacing the affected DNA bases.

– Guanine residues are readily oxidized to 8-hydroxyguanine. Direct damage reversal can re-reduce this base to guanine.
– Alkylation of DNA bases, such as O^6-methylguanine, can be generated by cellular catabolites. Direct damage reversal can repair this lesion.

Repair occurs by transfer of the methyl group to a cysteine residue of the repair protein, such as MGMT. Because methylcysteine is chemically very stable the repair protein is not regenerated but is

Table 6.1.A. DNA repair genes. Specific subsets of DNA repair enzymes are active in the various forms of damage reversal. Together they protect the integrity of the DNA sequence and the chromosome structure

Protein	Characterization	Cancer
Mutator genes		
Direct damage reversal		
MGMT	Repair of O^6-methyl guanine by transfer of the alkyl group to the enzyme	Brain cancer, esophagus cancer
ABH2	1-Methyl Alanine Dioxygenase	
ABH3 (DEPC-1)	1-Methyl Alanine Dioxygenase	
DNA Polymerase κ	Low fidelity polymerase, spontaneous and damage induced mutagenesis	Lung cancer
Mismatch repair		
DUC-1	123 kD; recognition of a mismatch ATPase	
MSH2	100 kD; loop mismatch repair with MSH3, single mismatch repair with MSH6, recognition of a mismatch ATPase	Hereditary nonpolyposis colorectal cancer, endometrium cancer, ovarian cancer
MSH3	Loop mismatch repair with MSH2, recognition of a mismatch ATPase	
MSH6 (GTBP)	160 kD; single mismatch repair with MSH2, binds to G/T mismatch	Hereditary nonpolyposis colorectal cancer, glioma
MSH4	MutS homolog specialized for meiosis	
MSH5	MutS homolog specialized for meiosis	
T/G specific DNA Giycosylase	55 kD; removes the thymidine base in a G/T mismatch	Gastic cancer
A/G specific nicking enzyme	Simultaneously makes inclusion at the first phosphodiester bond both 5′ and 3′ to the mismatched adenine	
All-type mismatch nicking enzyme	Nicks the first phosphodiester bond both 5′ to any mismatch	
MLH1	80 kD; forms heterodimer with PMS2, transcription is silenced by promoter methylation	Hereditary nonpolyposis colorectal cancer
PMS1	Binds to MutS heteroduplex complex	Hereditary nonpolyposis colorectal cancer glioma endometrium cancer,overian cancer
PMS2	110 kD; forms heterodimer with MLH1, binds to MutS heteroduplex complex	Hereditary nonpolyposis colorectal cancer glioma
DNA polymerase α	2 subunits of 165 kD and 70 kD	
DNA polymerase δ	2 subunits of 125 kD and 50 kD	
POL η	Bypass of thymine–thymine dimers	Xeroderma pigmentosum V
Base excision repair		
UNG	Uracil DNA Glycosylase, repairs uracil and 5-hydroxyuracil, releases free uracil and initiates base excision repair, forms UDG/GAPDH which binds diadenosine tetraphosphate and regulates cell growth	Bloom syndrome
Hydroxymethyl Uracil DNA Glycosylase	Eliminates hydroxymethyl uracil	Werner syndrome
TDG	Thymine DNA Glycosylase, repairs U or T opposite G, Ethenocysteine	
SMUG1	Repairs U, preferentially from single-stranded DNA	
MBD4	Contains a methyl-CpG binding domain that deaminates 5-methylcytosine (m^5C) to thymine, repairs U or T opposite G at CpG sequences	
OGG1	Repairs 8-oxo guanine opposite C, formamidopyrimidine	Lung cancer, renal clear cell carcinoma
MYH (MUTYH)	Repairs A opposite 8-oxo guanine	

(continued)

Table 6.1.A. (continued)

NTH1	Repairs thymine glycol, cytosine glycol, dihydrouracil, formamidopyrimidine	
NEI1	Thymine Glycol DNA Glycosylase, release of thymine glycol from damaged DNA, associated with endonuclease activity that mediates phosphodiester bond cleavage	
NEI2 (NEH2, NEIL2)	Removes oxidative products of pyrimidines	
MPG	*N*-Methylpurine DNA Glycosylase, repairs 3-methyl adenine, ethenoadenine, hypoxanthine	
APE (REF-1, HAF1)	Apurinic/Apyrimidinic Endonuclease, repair of alkylating damage, redox regulator of JUN/FOS, stimulates DNA binding by JUN	
DNA Polymerase β	DNA gap filling enzyme, is induced by alkylating agents through phosphorylation of CRE and binding to the *pol β* promoter	
Nucleotide excision repair		
XPA	31 kD; structure-specific Endonuclease, damage recognition	Xeroderma pigmentosum
RPA	3 proteins $P70^{RPA}$ (RPA-1), $P32^{RPA}$ (RPA-2), and $P14^{RPA}$ (RPA-3); trimeric complex, DNA binding, damage recognition, interaction with XPA	
XPC	125 kD; initiator of nucleotide excision repair	Xeroderma pigmentosum
XPE-BF	125 kD; DNA binding	Xeroderma pigmentosum
TFII-H		
ERCC3 (XPB)	89 kD; component of transcription initiation factor TFII-H; 3′→ 5′ Helicase	Xeroderma pigmentosum, Cocayne syndrome
ERCC2 (XPD)	80 kD; component of transcription initiation factor TFII-H, possible DNA-dependent ATPase, 5′→3′ Helicase	Xeroderma pigmentosum, Cocayne syndrome, trichothiodystrophy
GTF2H1	62 kD; core TFII-H subunit, promotes bubble formation	
GTF2H4	52 kD; core TFII-H subunit	
GTF2H2	44 kD; zinc finger, core TFII-H subunit	Werdnig-Hoffmann disease
GTF2H3	34 kD; zinc finger, core TFII-H subunit	
GTF2H5 (TTDA)	8 kD; core TFII-H subunit	
CDK7	41 kD; serine/threonine kinase	
Cyclin H (CCNH)	38 kD; Cyclin	
MNAT1	CDK-7/Cyclin H Assembly Factor, RING finger protein	
XPD	135 kD; Endonuclease, makes 3′ incision	Xeroderma pigmentosum, Cocayne syndrome
ERCC5 (XPG)		
ERCC1	33 kD; structure specific Endonuclease	Xeroderma pigmentosum
ERCC4 (XPF)	112 kD; Endonuclease, makes 5′ incision	Xeroderma pigmentosum
RFC	Molecular complex of 5 proteins, P140, P40, P38, P37, P36	
ERCC6	Helicase	Cockayne syndrome
PCNA	Polymerase clamp	
RAD23A	HRD23A; binds to XPC and contributes to damage recognition	
RAD23B	HRD23B; binds to XPC and contributes to damage Recognition	
CENT2	Centrin-2, Caltacin-1; stabilizes XPC in the presence of HRD23	
DDB1	Forms a complex with DDB2	Xeroderma pigmentosum E
DDB2	Forms a complex with DDB1	Xeroderma pigmentosum E
RAD30A	DNA Polymerase	Xeroderma pigmentosum variant
DNA Polymerase δ	2 subunits of 125 kD and 50 kD	
DNA Polymerase ε	2 subunits of 261 kD and 55 kD	
LIG1 (DNA Ligase)	102 kD	

(continued)

Table 6.1.A. (continued)

Repair of single strand breaks		
DNA Ligase	Seals nicks with intact 5¢ phosphates and 3¢ hydroxyls	
Repair of double strand breaks		
XRCC1	Interacts with DNA Ligase III	
XRCC4	Interacts with DNA Ligase III, binds to COOH-terminus of DNA Ligase IV, double strand break rejoining and V(D)J recombination	
XRCC5	86 kD; regulatory subunit of DNA-PK (DNA-Dependent Protein Kinase), DNA end-binding protein, mediates nonhomologous end joining	
XRCC6	70 kD; regulatory subunit of DNA-Dependent Protein Kinase	T-cell lymphoma
XRCC7	350 kD; catalytic subunit of DNA-Dependent Protein Kinase, double-strand break repair and V(D)J recombination	
MRE11	3′→5′ Exonuclease	Colorectal cancer, prostate cancer, endometrial cancer
RAD50	Stimulates the Exonuclease MRE11	
RAD51	Recombinational repair, binds to RAD52 and RPA	Breast cancer
RAD52	46 kD; accessory factor for recombination	
RPA	3 proteins P70, P32, and P14; DNA binding, damage recognition	
NSB1	Interacts with RAD50	Nijmegen breakage syndrome
ATM	350 kD; phosphorylates BRCA1, repairs double-strand breaks caused by ionizing radiation, leads to dephosphorylation of Histone H1	Ataxia teleangiectasia, mature T-cell leukemia
BRCA1	Binds DNA repair protein RAD51, accessory factor for transcription and recombination, E3 Ubiquitin Ligase	Breast cancer, ovarian cancer
BRCA2	Binds DNA repair protein RAD51	Breast cancer, pancreas cancer
SHFM1 (DSS1)	BRCA2 associated	
G22P1 (KU70)	DNA end-binding protein, ATP-dependent DNA Helicase II, 70 kD subunit	
XRCC5 (KU80)	DNA end-binding protein, ATP-dependent DNA Helicase II, 80 kD subunit	
DNA ligase IV	96 kD; associates with XRCC4	
Repair of transcriptionally active regions		
CSA		Cocayne syndrome
CSB		Cocayne syndrome
XAB2	Interacts with XPA, CSA, and CSB	
ERCC3 (XPB)	89 kD; component of transcription initiation factor TFII-H; 3′→5′ Helicase	Xeroderma pigmentosum, Cocayne syndrome
ERCC2 (XPD)	80 kD; component of transcription initiation factor TFII-H, possible DNA-dependent ATPase, 5′→3′ Helicase	Xeroderma pigmentosum, Cocayne syndrome, trichothiodystrophy
ERCC5 (XPG)	135 kD; Endonuclease, makes 3′ incision	Xeroderma pigmentosum, Cocayne syndrome
MSH		
ATM	Phosphorylates BRCA1	
ATR	Protein kinase, homolog of ATM, phosphorylates BRCA1 after DNA damage	
BRCA1	Binds to RNA Polymerase II	Breast cancer, ovarian cancer
BRCA2		Breast cancer

(continued)

Table 6.1.A. (continued)

Chromosome stability genes		
BUB1	Mitotic spindle checkpoint kinase	Colorectal cancer
PTTG	Securin, regulates ESP1	Pituitary tumors
H2AFX (H2AX)	Histone, phosphorylated after DNA damage	
CHAF1A (CAF1)	Chromatin assembly factor	
DCLRE1A (SNM1)	DNA cross-link repair	
DCLRE1B (SNM1B)	Related to SNM1	
FANCA	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCB	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCC	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCD2	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCE	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCF	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCG (XRCC9)	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCLCB	Involved in tolerance or repair of DNA cross-links	Fanconi anemia
FANCM	DNA Helicase, possible nuclease in the XPF/HEF/MUS81 family	

degraded via the Ubiquitin pathway. In photoreactivation repair (light-induced repair), pyrimidine dimers are restored to their monomeric structure. A DNA Photolyase directly repairs this form of damage from UV light [Yu et al. 1999]. Light energy captured by the flavin and pterin chromophores in the enzyme breaks the cyclobutane ring.

- While *mgmt* {10q26} is rarely mutated in cancer, low levels of the MGMT protein are associated with various malignancies, such as testicular cancer and glioblastoma. This may reflect reduced transcription due to altered gene methylation.
- Upregulation of *mgmt* expression can be caused by binding of MEBP (MGMT Enhancer Binding Protein) to the *mgmt* enhancer, so that malfunction of the MEBP protein may contribute to low MGMT expression in certain malignancies [Lindahl and Wood 1999; Yu et al. 1999]. In some repair-deficient cells, cytoplasmic sequestration of MEBP accounts for a lack of *mgmt* expression [Chen et al. 1997].

6.1.2 Mismatch Repair

Cytotoxicity of methylating agents is caused mostly by methylation of the O^6 position of guanine in DNA to form O^6-methylguanine, which can direct the mis-incorporation of thymine during replication. Mismatched bases may be incorporated during the formation of hetero-duplexes or during DNA reduplication because of the limited fidelity of the DNA reduplication machinery. Neither direct damage reversal nor excision repair can fix them, because they have no mechanism to distinguish between the correct parental strand and the strand containing the misinformation. Mismatch repair removes a patch of nucleotides from one strand, followed by repair synthesis and ligation. Mismatch repair proteins usually function as heterodimers, which in the presence of ATP bind to heteroduplexes and initiate the repair (Figure 6.1.2.A). The recognition of mismatches is primarily accomplished by a heterodimer between MSH2 {2p22-p21} and MSH6 {2p16}, called MUTSα, or by a heterodimer between MSH2 and MSH3 {5q11-q12}, called MUTSβ [Friedberg 2001]. MSH2/MSH6 bind to mismatches and single base loops. MSH2/MSH3 focuses on insertion or deletion loops. PCNA is required to stabilize the heterodimers at mismatch sites on DNA and during the DNA synthesis step of mismatch repair. Mismatch repair may occur in two forms, long-patch repair has

large excision tracks but little sequence specificity, short-patch repair is specific for a defined sequence context and results in the excision of only a few nucleotides [Yu et al. 1999].

- The mismatch repair pathway is implicated in cancer through an association between microsatellite instability and colorectal tumors in HNPCC (hereditary nonpolyposis colon cancer) kindreds. Defects in mismatch repair genes underlie microsatellite instability. Hereditary nonpolyposis colon cancer is frequently caused by defects in mismatch repair genes, either by gene mutation (*msh2*, *mlh1*, or *msh6*) or by methylation and silencing of the *mlh1* gene.
- Beside genetic alterations in the coding regions, *msh2* and *mlh1* are subject to mutations in their promoter regions that compromise transcriptional efficiency and lead to colorectal cancer [Shin et al 2002].
- In endometrial carcinomata, *mlh1* methylation is common. It is significantly correlated with loss of MLH1 protein expression, microsatellite instability, diploid tumors, and lack of P53 overexpression. In contrast, *msh2* methylation is infrequent in endometrial carcinomata [Salvesen et al. 2000].
- *msh2* and *mlh1* can be defective in small cell lung cancer.
- Mismatch repair genes, including *msh2*, *mlh1*, *pms2*, and *pms1*, are often defective in prostate cancer [Chen et al. 2001].

6.1.3 Base Excision Repair

Base excision repair replaces just one nucleotide. It is estimated that this pathway repairs up to one million nucleotides/cell/day [Holmquist 1998]. The base excision repair mechanism typically removes

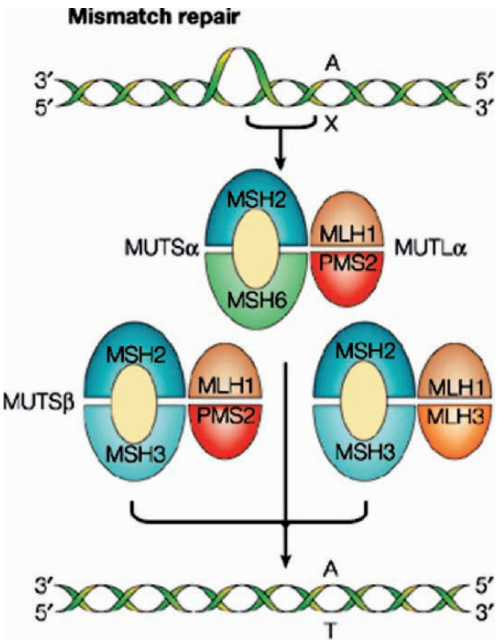


Figure 6.1.2.A. Mismatch repair. Among the DNA repair mechanisms that operate in response to the presence of base damage in DNA, three biochemical pathways result in the excision of damaged or inappropriate bases, comprising base excision repair, mismatch repair, and nucleotide excision repair. Although in general these pathways operate independently in response to specific types of base damage, there is evidence of overlap among them. Mismatch repair is dedicated primarily to the excision of nucleotides that are incorrectly paired with the nucleotide on the opposite DNA strand. Mispairing most frequently, but not exclusively, transpires during DNA reduplication because of the limited fidelity of the underlying biochemical mechanisms. Hence, the incorrect base occurs in the newly synthesized DNA strand. All cells have specific mechanisms by which they discriminate between newly reduplicated and parental DNA strands. The recognition of small loops generated by insertion or deletion of nucleotides, as well as single base mismatches, is primarily accomplished by a complex called MUTSα (a heterodimer of MSH2 and MSH6). Another heterodimer, MUTSβ (comprising MSH2 and MSH3) can also operate in the recognition of small loops during mismatch repair. Defects in mismatch repair predispose to cancer, primarily colon cancer, but also uterine, ovarian, and gastric cancers. [Reproduced from Friedberg 2001. With permission from Macmillan.]

– Oxidative damage to DNA bases
– DNA damage by alkylating agents
– The incorporation of uracil instead of thymine
– The occurrence of cytosine deamination, generating uracil or 5-methyluracil opposite to guanine

Base excision repair is initiated by the Glycosylase mediated removal of a damaged base or by the presence of an abasic site. This release of free bases from DNA, catalyzed by DNA Glycosylases, is the major feature of this form of repair. DNA Glycosylases recognize specific subsets of chemically altered or inappropriate bases and catalyze the hydrolysis of the *N*-glycosyl bond that links the base to the deoxyribose phosphate backbone of the DNA. Glycosylases have specificity to particular base lesions and may act as monofunctional (remove damaged base only) or bifunctional (remove damaged base and incise DNA backbone) enzymes. Most DNA *N*-Glycosylases are smaller than 30 kD, monomeric, with no cofactor requirement or divalent cation requirement.

– OGG1 (8-Oxoguanine DNA Glycosylase, 8-Hydroxyguanine DNA Glycosylase) {3p26.2} repairs 8-oxoguanine. OGG1 activity is inhibited by nitric oxide and by peroxynitrite. Inhibition is characterized by the formation of *S*-nitrosothiol adducts and loss of zinc ions.
– MPG (*N*-Methylpurine DNA Glycosylase, Methyladenine DNA Glycosylase, 3-Alkyladenine DNA Glycosylase) {16pter-p13.3} removes a diverse group of damaged bases from DNA, including cytotoxic and mutagenic alkylation adducts of purines.
– Thymine DNA Glycosylase (TDG) {12q24.1} and Methyl Binding DNA Glycosylase-4 (MBD4, Methyl-CpG-Binding Domain Protein 4, MED1) {3q21-q22} can specifically remove T from a T:G mismatch within a CpG context. Deamination of 5-methylcytosine is one of the most frequently occurring point mutations. Appropriate DNA repair must take into account that the incorrect base at T:G mismatches is invariably T. These two mismatch specific T Glycosylases accomplish this discrimination.
– UNG1 (Uracil DNA Glycosylase 1) {12q23-q24.1} removes uracil in DNA, resulting from deamination of cytosine or replicative incorporation of dUMP instead of dTMP.
– SMUG1 (Single Strand Selective Monofunctional Uracil DNA Glycosylase 1) {12q} is a Glycosylase that removes uracil from single-stranded and double-stranded DNA in nuclear chromatin, thus contributing to base excision repair. It acts equally on U:A and U:G.

DNA Glycosylases recruit an endonuclease such as APE1 (Apurinic/Apyrimidinic Endonuclease 1, Apex, HAP1, REF1) {14q12}, a class two endonuclease (cuts the 5′ bond), which cuts out the base leaving an apurinic site that is further processed by DNA Polymerase β and a DNA Ligase/XRCC1 complex. DNA Polymerase β performs the rate limiting polymerization step of deoxyribose phosphate removal in the monofunctional Glycosylase induced pathway. It is also essential for polymerization in the bi-functional Glycosylase-induced pathway. A third pathway that replaces a longer stretch of DNA (2–8 nucleotides) depends on Polymerase δ or ε with the cofactor PCNA, followed by DNAse IV (FEN-1), and repair by a DNA Ligase (I, II, III, or IV).

In addition to being absolutely required for base excision repair, APE1 (designated as REF-1 for Reducing Factor 1) is required for the redox activation of a number of spontaneously oxidized transcription factors. It reduces conserved cysteine residues in the DNA binding domains, thereby participating in protein repair after oxidative damage. APE also contributes to P53 activation.

- The polymorphic OGG1 variant S326C is associated with increased risk for various types of cancer. In clear cell renal cell carcinoma, a G445A transition in the *ogg1* gene, resulting in the R46Q amino acid change, causes drastically reduced DNA Glycosylase activity. This implies an impairment in its DNA repair capacity [Audebert et al. 2000]. Multiple forms of the OGG1 protein are produced in lung cells as a result of genetic polymorphisms and alternative splicing. There is frequent loss of heterozygosity at the *ogg1* locus in primary lung tumors.
- Deficiencies in DNA Ligase are associated with acute lymphoblastic leukemia and with Fanconi anemia.
- Haploinsufficiency of *DNA polymerase β* causes a reduction of *β-pol* mRNA and protein by about one half. It leads to a deficiency in base excision repair, resulting in accelerated DNA damage and increased mutational response to carcinogens [Cabelof et al. 2003].

6.1.4 Nucleotide Excision Repair

Nucleotide excision repair mainly corrects two types of damage

– The main function of the nucleotide excision pathway is the removal of UV induced DNA damage, such as cyclobutane pyrimidine dimers
– Lipid peroxidation can lead to the generation of malonedialdehyde–guanine adducts, which are too bulky to be removed by base excision repair and are therefore removed by nucleotide excision repair.

The recognition of all types of base damage during nucleotide excision repair may require two fundamental elements, the disruption of normal base pairing and an altered chemistry in the damaged strand, typically involving the bases [Friedberg 2001]. Nucleotide excision repair is initiated by a two-step recognition process (Figure 6.1.4.A), in the first step, a distortion of the DNA helix is recognized, in the second step, the damaged strand and chemical alteration are located. Recognition and binding to damaged DNA are accomplished by the XPA/P70^{RPA1} and XPC/HR23B complexes. XPC/HR23B is stabilized by the centrosomal protein Centrin-2 (Caltractin-1). These interactions may be the rate limiting step of nucleotide excision repair. A 9 protein complex, called TFII-H, consisting of XPB, XPD, P62, P52, P44, P34, MAT1, CDK7, and Cyclin H, forms an open bubble structure. The DNA Helicases XPB and XPD facilitate the partial unwinding of the DNA duplex. XPG and the complex XPF/ERCC1 are part of the endonucleases that induce incision. The 3′ incision is made by XPG, while the 5′ cut is executed by the XPF/ERCC1 hetero-dimer. Excision occurs in an ATP-dependent manner. The result is the introduction of two single-strand nicks into the damaged strand, 16–25 nucleotides 5′ to the damage and 2–9 nucleotides 3′ to the damage. The fragment is released by Helicase, and the resulting gap is filled in by the DNA Polymerase ε or δ, assisted by PCNA and DNA Ligase I.

DNA photoproducts are blocks to the DNA Polymerases α, δ, and ε, which cannot accommodate large distortions in their active sites. Reduplicative bypass of these photo-products is achieved by damage specific Y family DNA Polymerases that have larger active sites for nucleotide binding, which accommodate such adducts, and relaxed substrate specificity, albeit with a significant error rate that can be as high as 1–2%. There are three Y family Polymerases, POL η, POL λ, and POL κ.

- Tumor cells lacking functional P53 exhibit a partial deficiency in nucleotide excision repair. The role of P53 may be twofold. Firstly, it may bind TFII-H. Secondly, the P53 effect depends on the downstream involvement of GADD45α, which in turn may associate with PCNA. P33^{ING1} cooperates with P53 and binds to GADD45.
- Mutations in POL η are associated with a XP-V, a variant form of xeroderma pigmentosum.
- The testes are inherently repair-deficient, lacking XPA, both excision nucleases XPG and XPF/ERCC1, XRCC1, and Pol η. This may account for high sensitivity of testicular cancer to DNA damaging agents.

6.1.5 Repair of Single-Strand Breaks

Single-strand interruptions are processed and rejoined by the same enzymes that are responsible for the late stages of base excision repair, sometimes with the additional step of exonucleolytic removal of frayed ends and phosphorylation of the 5′ termini by DNA Kinase. Poly(ADP-Ribose) Polymerase-1 (PARP-1, ADPRT-1) {1q42} provides temporary protection of DNA single-strand breaks and consequently acts as an antirecombinogenic factor [Lindahl and Wood 1999]. PARP-1 catalyzes the transformation of β-nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and poly(ADP-ribose). Thus it participates in DNA repair. A lack of PARP-1 leads to persistence of DNA lesions that are normally repaired.

- Poly(ADP-ribose) metabolism mediated by PARP-1 and its catabolic counterpart, Poly(ADP-Ribose) Glycohydrolase (PARG), is important for the maintenance of genomic integrity by regulating cellular responses to genotoxic stress such as DNA repair and programmed cell death and thus it is protective against cancer development. PARP-1 deficiency leads to increased numbers of sister chromatid exchanges and sensitivity to ionizing radiation.
- A processed pseudogene of *parp-1* is located on chromosome 13q33-qter. An elevated frequency in allele B of this gene in the germline DNA of blacks is associated with an increased risk for multiple myeloma, prostate cancer, and colon cancer. The polymorphism reflects a 193 bp duplication within the processed pseudogene sequence, the absence of this duplicated region being characteristic of the B genotype [Lyn et al. 1993].

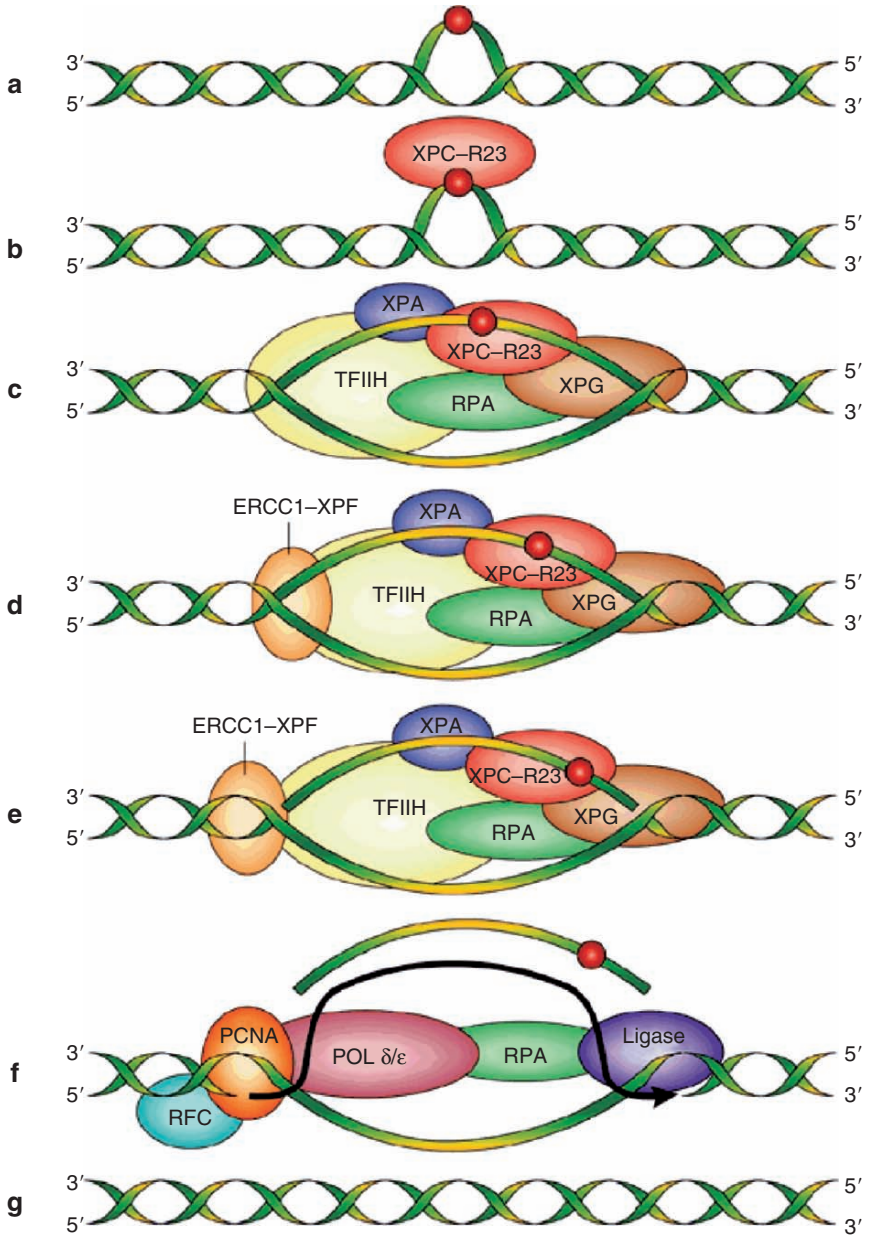


Figure 6.1.4.A. Nucleotide excision repair. (a) Nucleotide excision repair (NER) operates on base damage caused by exogenous agents, such as mutagenic and carcinogenic chemicals and photoproducts generated by sunlight exposure, that cause alterations in the chemistry and structure of the DNA duplex. (b) Such damage is recognized by XPC, which is stably bound to HHRAD23B (R23). (c) The binding of the XPC/HHRAD23 heterodimeric subcomplex is followed by the binding of several other proteins (XPA, RPA, TFII-H, and XPG). Of these, XPA and RPA facilitate specific recognition of base damage. TFII-H is a subcomplex of the RNA Polymerase II transcription initiation machinery which also operates during nucleotide excision repair. It consists of six subunits and contains two DNA Helicases (XPB and XPD) that unwind the DNA duplex in the immediate vicinity of the base damage. This local denaturation generates a bubble in the DNA, the ends of which comprise junctions between duplex and single-stranded DNA. (d) The subsequent binding of the ERCC1/XPF heterodimeric subcomplex generates a completely assembled nucleotide excision repair multiprotein complex. (e) XPG is a DNA Endonuclease that cuts the damaged strand at such junctions 3′ to the site of base damage. Conversely, the ERCC1/XPF heterodimeric protein is a DNA Endonuclease that cuts the damaged strand at such junctions 5′ to the site of base damage. This bimodal incision generates an oligonucleotide fragment of 27–30 nucleotides in length, which includes the damaged base. (f) This fragment is excised from the genome, concomitant with restoring the potential 27–30 nucleotide gap by repair synthesis. Repair synthesis requires DNA Polymerases δ or ε, as well as the accessory replication proteins PCNA, RPA, and RFC. The covalent integrity of the damaged strand is then restored by DNA Ligase. (g) Collectively, these biochemical events return the damaged DNA to its native chemistry and configuration. ERCC1 = Excision Repair Cross-Complementing 1, PCNA = Proliferating Cell Nuclear Antigen, POL = Polymerase, RFC = Reduplication Factor C, RPA = Replication Protein A, TFII-H = Transcription Factor IIH, XP = Xeroderma Pigmentosum. [Reproduced from Friedberg 2001. With permission from Macmillian.]

6.1.6 Repair of Double-Strand Breaks

DNA double-strand breaks arise physiologically during somatic recombination. They are also often generated after exposure to ionizing radiation and oxidative insults. Interactions between the ends of different DNA double-strand breaks can produce tumorigenic chromosome translocations.

In the repair of double-strand breaks, movement of the broken domains occurs within minutes, with clustering leading to the juxtaposition of broken chromosome ends occurring predominantly in G_1. This may reflect an adhesion process that involves the MRE11/RAD50/NBS1 (MRN) repair complex [Aten et al. 2004]. This complex is required for all the mechanisms of double-strand break repair. Its interactions may be important for the prevention of oncogenic translocations. The MRE11/RAD50/NBS1 comples tethers DNA ends together through its distinct architecture with a central globular domain and two protruding arms extending 40–50 nm. It also recruits the kinase ATM to broken DNA molecules. Phosphorylated NBS1 (Nijmegen Breakage Syndrome Protein 1) {8q21} stimulates inactive ATM dimers through multiple protein–protein contacts, and this interaction increases the affinity of ATM for its substrates. ATM responds to the presence of DNA double-strand breaks by phosphorylating proteins that initiate cell cycle arrest and DNA repair [Lee and Paull 2004]. This includes, among others, the phosphorylation of Histone H1. NBS1 is expressed at a low level in all tissues. High expression is focused on organs with physiologic DNA double strand breakage, such as testis, thymus, and spleen. Its expression is also enhanced at sites of high proliferative activity, including the subventricular layer of the telencephalon and the diencephalon, the liver, lung, kidney and gut, as well as striated and smooth muscle cells in various organs [Wilda et al. 2000].

Double-strand break repair must access nucleosomal DNA. This may occur by:

– Covalent modifications of Histones to create specific interaction sites for regulatory complexes
– By ATP-dependent hydrolysis to catalyze Histone mobilization
– By the incorporation of Histone variants, such as H2AX (Histone 2A Family Member X), into nucleosomes at the sites of double-strand breaks.

Sites of DNA damage are marked within minutes by the phosphorylation of the Histone species H2AX, which spreads over a region spanning thousands of bases around the lesion, suggesting that some chromatin remodeling may occur to facilitate access of the repair machinery. BRCA1 interacts with and activates the MRE11/RAD50/NBS1 protein complex, which is recruited to sites marked by the phosphorylated Histone H2AX.

There are two major mechanisms for the repair of double-strand breaks, homologous recombination and nonhomologous end joining (Figure 6.1.5.A). The balance between these principal repair pathways is apparently influenced by the relative amounts of RAD52 and KU (regulatory subunit of a Helicase and of DNA-Dependent Protein Kinase). Less frequently, the alternative mechanism of single-strand annealing takes place.

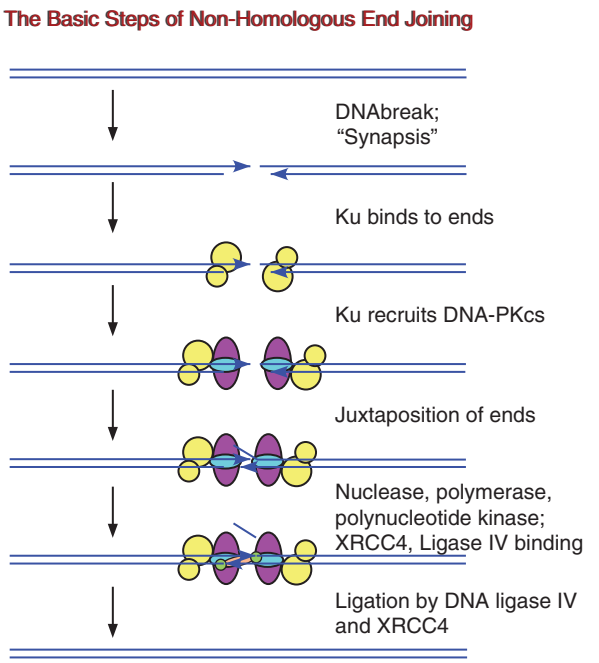


Figure 6.1.5.A. Repair of DNA strand breaks. DNA breaks are produced in numerous ways. In most cases, the ends of the break are not suitable for direct ligation because of the loss of some nucleotides. Nonhomologous end joining rejoins the correct ends, thus preventing chromosome rearrangements. To accomplish this, the two ends must be placed in proximity of each other until they can be ligated. This occurs through sister chromatid cohesion, constraints within the nucleosome structure, KU–KU interactions, DNA-PK binding, and MRN/MRX proteins. [Reproduced from http://asajj.roswellpark.org/huberman/DNA_Repair/dsbreak.html. With permission.]

Homologous recombination. Strand exchange between homologous DNA duplexes occurs after reduplication and mediates the repair of double-strand breaks. In this mechanism, the intact allele serves as a template in a quarternary DNA complex referred to as a Holiday Junction (Figure 6.1.5.B). One possible consequence of homologous (allelic) recombination is a loss of heterozygosity. In this regard, the loss of a wild-type allele after mutation of one allele of a tumor suppressor gene can contribute to the induction of malignant transformation.

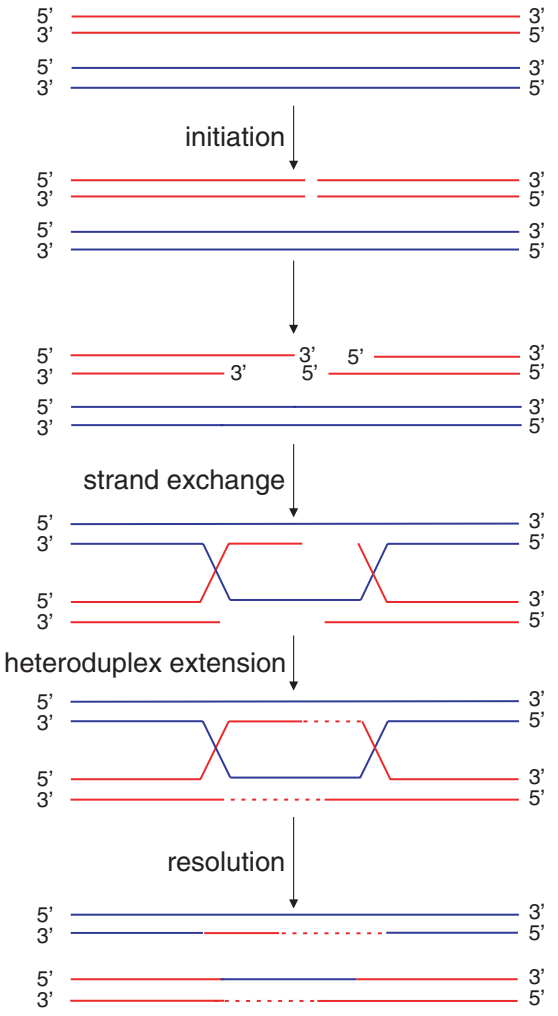


Figure 6.1.5.B. Homologous recombination. Homologous recombination is a form of double-strand break repair. A double-strand break is the initiation event. The pairing of the displaced complementary strands results in the formation of characteristic intermediates, called Holiday Junctions. The dashed lines indicate DNA that is newly synthesized by a DNA Polymerase in the process of homologous recombination.

RAD51 (REC-A) {15q15.1} is a key protein required for the occurrence of homologous recombination. It catalyzes the process through its homologous pairing and strand-exchange activities. RAD51 coats single-stranded DNA to form a nucleoprotein filament that invades and pairs with a homologous DNA duplex, initiating strand exchange between the paired DNA molecules. RAD51 activity is regulated in various ways.

– DNA strand breaks and ionizing radiation activate the kinases ATM and CHK2, which phosphorylate BRCA1 [Zhou and Elledge 2001]. Alternatively, BRCA1 may be phosphorylated by ATR in response to UV damage. Activated BRCA1 cooperates with BRCA2 and RAD51 to repair the double-strand break by homologous recombination.
– The major role of BRCA2 in double-strand break repair is through control of the RAD51 Recombinase, with which it interacts through its BRC repeats. BRCA2 works directly to regulate the availability and activity of RAD51 in this key reaction.
– RAD52 may modulate activities through its RAD51 interacting region, which spans the amino acids 291–330. The ability of RAD52 to induce homologous recombination requires its binding to the 34 kD subunit of RPA (Replication Protein A), amino acids 221–280.
– The c-ABL proto-oncogene product is a target of the ATM kinase after DNA double-strand breaks, and ABL binds to RAD51.

BRCA1 plays a critical role in this form of DNA repair by interacting with the essential components, RAD51, H2AX, and the MRE11/RAD50/NBS1 protein complex [Zhong et al. 1999a; Wang et al. 2000]. MRE11 {11q21} encodes a nuclease activity, which resects flush double strand break ends to generate single-stranded DNA tracts. BRCA1 can inhibit this activity of MRE11, regulating the length single-stranded DNA generation at sites of DNA breakage.

Nonhomologous end joining. An alternative mechanism to homologous recombination for the repair of double-strand breaks is nonhomologous end joining, which, however, has lower fidelity than homologous recombination and may result in lost or changed genetic information.

– After irradiation induced double-strand breaks nonhomologous end joining may set in
– Programmed double-strand breaks followed by nonhomologous end joining occur physiologically during V(D)J recombination

This form of repair begins with binding to the free DNA ends by DNA-Dependent Protein Kinase (DNA-PK). The DNA end-binding protein KU, consisting of two subunits of 70 kD and 80 kD, serves as the regulatory component of DNA-PK. Its effective nuclear substrate XRCC4 is tightly associated with the COOH-terminus of DNA Ligase IV. Thus, KU activity contributes to the regulation of DNA Ligase. BRCA1 is part of the large complex BASC (BRCA1-Associated Genome Surveillance Complex) that contains ATM, MRE11/RAD50/NBS1, MSH2/MSH6, MLH1, and BLM (Bloom's Helicase). Several proteins involved in the maintenance of genomic stability accumulate in the PML nuclear body, such as $P95^{Nibrin}$, MRE11, BLM, and Topoisomerase IIIα (a BLM interacting protein). PML can affect their function by regulating their localization into the PML nuclear body.

53BP1 (TP53BP1) {15q15-q21} is a mediator of the DNA damage checkpoint. It is phosphorylated in response to ionizing radiation in an ATM-dependent manner. 53BP1 is an adapter that recruits a subset of substrates to ATM and ATR. It binds to P53 through its COOH-terminal BRCT repeats (BRCA1 Carboxyl Terminus Repeats). 53BP1 is required for P53 accumulation, which leads to the interaction of P53 with CBP in PML nuclear bodies. It also binds to CHK2, BRCA1, and H2AX-γ [Lee and Paull 2004] and is essential for the formation of BRCA1 foci. 53BP1 contributes to G_2/M checkpoint arrest and to the S phase checkpoint in response to ionizing radiation. 53BP1 responds to DNA double-strand breaks by relocalization to discrete nuclear foci. These foci colocalize with those of the MRE11/RAD50/NBS1 complex and phosphorylate H2AX-γ. This complex facilitates the recruitment of repair factors. The loss of 53BP1 results in a partial decrease in the phosphorylation of key checkpoint target proteins [Wang et al. 2002]. The pathway to repair foci in response to DNA damage is branched and shows a regulatory hierarchy, in which H2AX-γ is required for NBS1 and 53BP1 foci, and 53BP1 controls the ability of BRCA1 but not NBS1 to form foci.

Single-strand annealing. The mechanism of single strand annealing repairs double strand breaks through homology between the ends of the joined sequence at staggered double strand breaks. Pairing precedes religation, not strand exchange. In contrast to homologous end joining, the homology is found not by invasion of the sister chromatid, but by the MRE11/RAD50/NBS1-mediated resection of the broken ends to create single-stranded DNA tails. When this resection reveals complementary sequences, the two DNA tails are annealed before being ligated by DNA Ligase IV. The overhanging tails are then trimmed by the ERCC1/XPF endonuclease. Single-strand annealing is error prone, because sequence information can be lost or rearranged when ends overlapping by as little as about 30 bp are unsuitably joined.

- BLM is delocalized in cells that lack PML as well as in acute promyelocytic leukemia blasts [Zhong et al. 1999b]. Moreover, the frequency of sister chromatid exchange, a distinctive molecular feature of genomic instability in Bloom cells, is greatly augmented in the absence of PML, suggesting that the localization of BLM in the PML nuclear body is required for its normal function [Salomoni and Pandolfi 2002].
- Nonhomologous end joining is an essential step in *antigen receptor* gene rearrangement. Two polymorphisms in *DNA ligase IV*, representing C→T transitions, result in the amino acid substitutions A3V and T9I. Inheritance of the CT or TT genotypes in these positions are associated with a reduction in the risk of developing multiple myeloma. This suggests a gene dosage effect for this polymorphism. Hence, genetic variants of *DNA ligase IV* may modulate the predisposition to multiple myeloma, a tumor characterized by aberrant *immunoglobulin* class switch recombination [Roddam et al. 2002].
- Two distinct DNA repair pathways converge on FANC D_2. Fanconi anemia (FA) and ataxia teleangiectasia (AT) cells are hypersensitive to DNA cross-linking. Patients with mutations in FANC D_2 have more severe clinical phenotypes than patients with other forms of Fanconi anemia or ataxia teleangiectasia. FA cells have defects in one of several proteins that form a nuclear complex (FANC A, C, E, F, and G) and, in cooperation with BRCA1, trigger the mono-ubiquitination of FANC D_2. This modification localizes FANC D_2 to nuclear foci that assemble at sites of DNA damage. Cells that lack FANC D_2 are defective in the S phase checkpoint induced by ionizing radiation. In response to ionizing radiation, FANC D_2 is phosphorylated by ATM on serine 222 in a manner that does not require the presence of other FA proteins or its mono-ubiquitination site lysine 651.
- Multiple endocrine neoplasia type 1 (MEN1) is an inherited tumor syndrome characterized by tumors

in multiple endocrine organs including the parathyroids, pancreatic islets, and the pituitary. Increased chromosome breakage occurs in the peripheral lymphocytes from the MEN1 patients. The gene mutated in MEN1 patients, *men1* (*menin1*) encodes a protein of 610 amino acids and has two nuclear localization signals in its COOH-terminus. Menin specifically interacts with FANC D_2. This interaction between Menin and FANC D_2 is enhanced by γ-irradiation. Menin is localized to chromatin and nuclear matrix, and the association with the nuclear matrix is enhanced by γ-irradiation. Therefore, Menin plays a role in the repair of DNA damage in concert with FANC D_2 [Jin et al. 2003]. The loss of *menin* expression leads to increased sensitivity to DNA damage.

6.1.7 Repair of Transcriptionally Active Regions

The nucleus contains three types of RNA Polymerase, differing among each other in template specificity and location in the nucleus. These Polymerases are large proteins, containing 8–14 subunits and having a total molecular mass greater than 500 kD.

– RNA Polymerase I is located in nucleoli, where it transcribes the tandem array of genes for 18S, 5.8S, and 28S ribosomal RNA (rRNA).
– RNA Polymerase II, which is located in the nucleoplasm, synthesizes the precursors of messenger RNA (mRNA), as well as small RNA molecules, including those of the splicing apparatus. Most promoters for RNA Polymerase II contain a TATA box between positions –30 and –100 from the start site. RNA Polymerase II, consisting of a 220 kD subunit A {17p13.1} and a 140 kD subunit B {4q12}, is guided to the start site by a set of transcription factors referred to as TFII (Transcription Factor for RNA Polymerase II). Initiation begins with the binding of TFII-D to the TATA box.
– The 5S ribosomal RNA and all the transfer RNA (tRNA) molecules are synthesized by RNA Polymerase III, which is located in the nucleoplasm.

In transcription coupled repair, RNA Polymerase is required for the recruitment of repair enzymes and repair is specific for the transcribed strand [Weinstein et al. 1995]. The arrest of RNA Polymerase II at a lesion in the template serves as a damage recognition signal. BARD-1 (BRCA-1 Associated RING Domain-1) {2q} interacts with RNA Polymerase II. Its association with phosphorylated BRCA1 leads to binding of the poly-adenylation factor CSTF-50. The inhibitory interaction of BARD1 with CSTF1 ensures that nascent RNAs are not erroneously poly-adenylated at sites of damage [Kleiman and Manley 1999]. The inhibition of poly-adenylation prevents inappropriate RNA processing and contributes to transcription coupled repair. BRCA1 also associates with the mismatch repair proteins MSH2 and MSH6, which are mediators of this process (Figure 6.1.7.A). The Cocayne Syndrome Proteins A (CSA) and B (CSB) recruit the TFII-H complex and the repair proceeds similar to nucleotide excision repair [Lindahl and Wood 1999; Friedberg 2001]. CSA is a cofactor for an SCF type Ubiquitin Ligase. CSB is a member of the ATP-dependent SWI2/SNF2 chromatin remodeling family and binds to DNA as a dimer. In the presence of ATP, CSB actively wraps the DNA around itself, and following ATP hydrolysis it releases the DNA. The interactions between CSA/CSB and RNA Polymerase II activate XPA/RPA, and the repair proceeds with the same mechanisms as nucleotide excision repair. In some differentiated cells of blood and neural origin, transcription coupled repair is the dominant mechanism of DNA repair, whereas it is secondary in keratinocytes.

- The disruption of *brca1* cripples the repair of oxidative base damage to the transcribed DNA strand. This may be a pathogenetic factor in hereditary breast cancer.
- Cells deficient in *mlh1* or *mlh2* are defective in transcription coupled repair. Mutations in the *mlh1* gene result in hereditary nonpolyposis colorectal cancer-2 (HNPCC2).

6.1.8 Postreplication Repair

The RAD6 group of repair proteins functions in postreplication repair. Proteins of the RAD6 group act on the stalled replication machinery that has encountered a damaged template, thereby accomplishing repair and allowing replication to resume. This repair can be achieved by two distinct RAD6-dependent mechanisms. One mechanism is error prone as it uses specialized trans-lesion Polymerases that insert correct or incorrect nucleotides across a damaged site, the other mode is error free because it uses the information of the undamaged sister duplex at the replication fork. The ubiquitination of proteins is pivotal for RAD6-dependent DNA repair. Furthermore, two other members of the RAD6 group, UBC13 and MMS2, form a hetero dimeric Ubiquitin conjugating enzyme, which

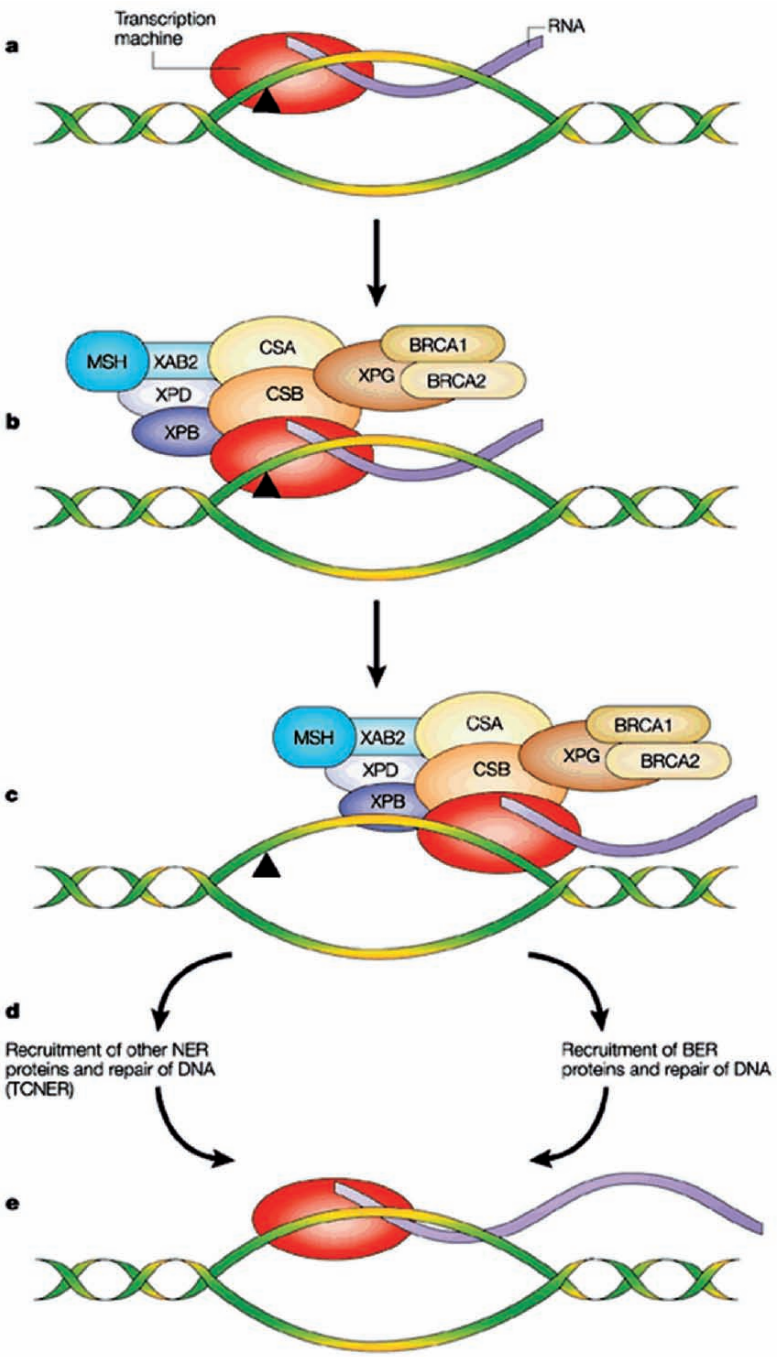


Figure 6.1.7.A. Transcription coupled repair. (a) Many types of base damage arrest normal transcription by presenting a block to the progress of the transcription machinery. (b) Arrested transcription by RNA Polymerase II may result in the recruitment of a large protein complex, which includes proteins involved in mismatch repair (represented generically as MSH), CSA, and CSB (Cockayne syndrome A and B), the NER proteins XPB, XPD, and XPG, BRCA1, and BRCA2, and XAB2, which binds to CSA. (c) This putative TCNER complex may dislocate the stalled transcription machinery from the site of base damage in the transcribed strand. (d) This provides access of the affected site to proteins required for the completion of nucleotide excision repair or base excision repair, depending on which of these repair modes is appropriate for the base damage. (e) Following these processes, normal transcription can again occur. BER = base excision repair, NER = nucleotide excision repair, TCNER = transcription coupled nucleotide excision repair. [Reproduced from Friedberg 2001. With permission from Macmillian.]

catalyzes the formation of noncanonical multi-Ubiquitin chains linked via K63 of Ubiquitin. RAD6 and UBC13/MMS2 are recruited to chromatin by their interaction with the RING finger containing, DNA binding proteins RAD18 and RAD5. PCNA is mono-ubiquitinated through RAD6 and RAD18, which additionally requires MMS2, UBC13, and RAD5 [Hoege et al. 2002].

6.2 CHROMOSOME STABILITY

During mitosis, the loss of proper attachment of the mitotic spindle to the kinetochore leads to nondisjunction and polyploidy. Defects of centrosome duplication, marked by interactions between Tubulin and the centrosome, result in mitotic cells with multipolar spindles that exert multidirectional forces on the kinetochore, resulting in chromosomal breakage and fragmentation. Chromosome breakage exposes unprotected, atelomeric ends and thus predisposes to chromosome fusions. During mitosis, the fused dicentric chromosomes are pulled to opposite poles, initially creating a bridge between the two poles. Eventually, these chromosomes break at their weakest point. This again exposes unprotected chromosome ends, creating a breakage-fusion-bridge cycle. Chromosome instability may also be reflected in translocations (Figure 6.2.A), sister chromatid exchanges, and double minute chromosomes (circular chromosomes lacking functional centromeres).

Genes whose products maintain chromosome stability, termed "caretakers," include those that are involved in chromosome metabolism, spindle assembly and dynamics, cell cycle regulation, and checkpoint control. In nontransformed cells, centrosomal clustering prevents the formation of multipolar spindles. The mitotic spindle assembly checkpoint pathway prevents the premature onset of mitotic anaphase prior to the proper attachment of all the chromosomes to the mitotic spindles, which would lead to mis-segregation. In pro-metaphase, the chromosomes establish bipolar attachments to the mitotic spindle. Unattached chromosomes generate a signal that delays the progress to anaphase. Several spindle checkpoint proteins localize to the kinetochores of sister chromatids that have not attached to the mitotic spindle. A number of the genes in the spindle checkpoint pathway encode protein kinases, implying a role for phosphorylation in the amplification of the checkpoint signal [Jallepalli and Lengauer 2001]. This signal is transduced by a relay of spindle checkpoint regulators, which include MPS1/TTK and CENP-E (Centromeric Protein E). The downstream target of CENP-E, BUB1 (Budding Uninhibited by Benomyl 1) {2q14} also participates in the spindle assembly checkpoint that can delay anaphase by blocking the activation of the anaphase promoting complex (Figure 6.2.B). The presence of an unattached kinetochore activates BUB1, MAD2 (MAD2L1) {4q27}, and MAD1 (Mitotic Arrest Deficient 1, MAD1L1, TXBP181, Tax Binding Protein 181) {7p22}, which together inhibit CDC20, an essential activator of the anaphase promoting complex [Adams and Kaelin 1998].

Following the attachment of all kinetochores to the mitotic spindle, CDC20 and the anaphase

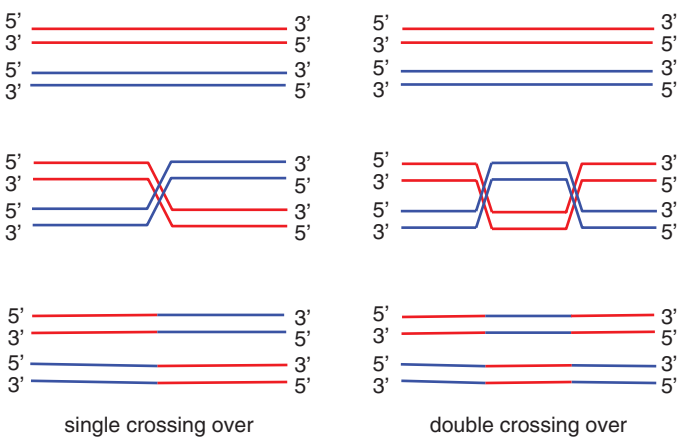


Figure 6.2.A. Principle of chromosome translocations. Two chromosomes are depicted in red and blue, respectively. (*Left panel*) A single crossing-over generates one break point, at which chimeric genes can be formed or regulatory elements can be placed in proximity of unrelated genes. (*Right panel*) In the case of double crossing-over, internal chromosome fragments are exchanged. There are two breakpoints.

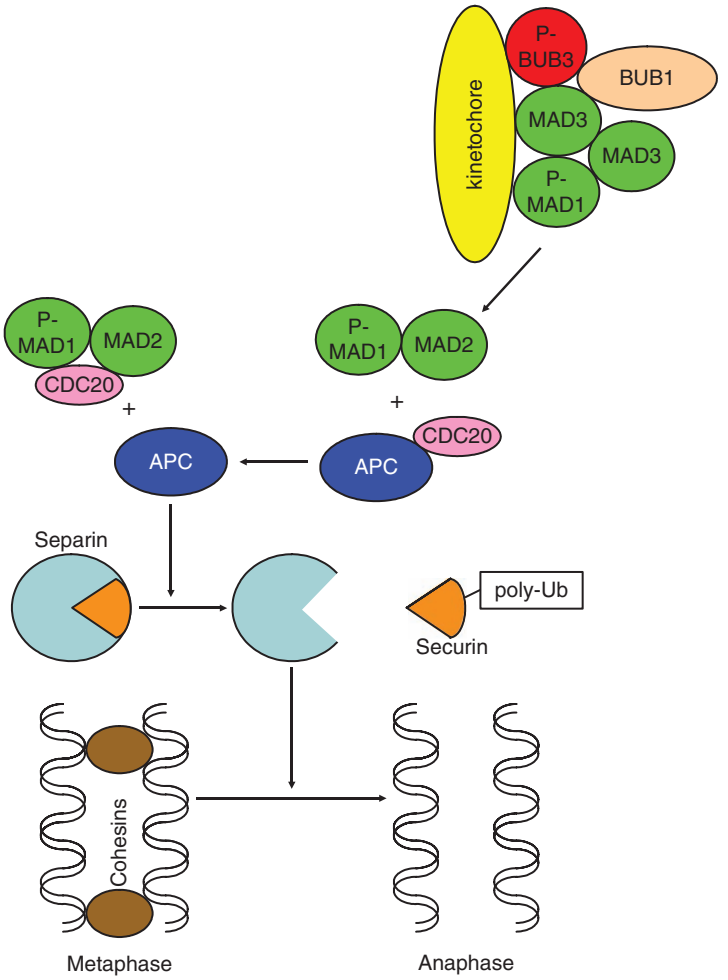


Figure 6.2.B. Spindle checkpoint. Once the mitotic spindle is assembled and all microtubules are bound, the kinase BUB1 phosphorylates BUB3. This causes the release of MAD2 and phosphorylated MAD1 (P-MAD1) from a protein complex associated with the kinetochore. P-MAD1 and MAD2 dissociate CDC20 from the anaphase promoting complex (APC), which allows it to activate Separin, which cleaves Cohesin and initiates the transition to anaphase. Securin (CUT1 or PDS1) needs to be targeted for degradation in the proteasome for Separin to become active. Separin (ESP1, ESPL1) breaks down sister chromatid cohesion. Cohesin subunits comprise SMC1, SMC3, SCC1, SA1, and SA2.

promoting complex become active. The anaphase promoting complex acts as a Ubiquitin Ligase. This results in the degradation of Securin (PTTG1, Pituitary Tumor Transforming Gene 1, EAP1, ESP1-Associated Protein 1) {5q33} in the proteasome and liberates the active protease Separin. The function of Separin (ESP1) is dependent on the degradation of Securin, a mechanism that prevents the separation of sister chromatids during metaphase. Following the activation of Separin, the separation of sister chromatids during mitosis is accomplished through the cleavage and delocalization of Cohesin by Separin. Sister chromatid cohesion is regulated very tightly. Both the premature release of cohesion in cells lacking *mad2* and the prolonged retention of cohesion in the absence of *securin* lead to aneuploidy [Jallepalli and Lengauer 2001].

Additional cancer related molecules are involved in maintaining chromosome stability at various stages of cytokinesis.

– The COOH-terminus of the APC proto-oncogene product is involved in maintaining chromosomal stability during mitosis. APC localizes to the kinetochore of metaphase chromosomes, where it interacts with the microtubule associated protein EB1. The COOH-terminal APC fragment normally becomes active in the metaphase– anaphase transition [Fodde et al. 2001].
– The microtubule motor cytoplasmic Dynein is a critical part of the centrosomal clustering.
– During cytokinesis, BRCA2 colocalizes with Aurora-B Kinase, a chromosome passenger protein required for cytokinesis. In cells lacking active BRCA2, cell separation is drastically delayed or does not occur at all. This may account, in part,

for the wide range of chromosome abnormalities that are associated with mutated *brca2*. BRCA2 deficient cells exhibit aneuploidy and centrosome amplification. This is due to impaired completion of cell division by cytokinesis. The impeded cell separation is accompanied by abnormalities in Myosin II organization during the late stages in cytokinesis. BRCA2 has a role in regulating these events, as it localizes to the cytokinetic mid-body. BRCA2 deficient cells experience considerable delays in cytokinesis, but many of them complete the cell cycle. Therefore, BRCA2 is not absolutely essential for the process of cell separation.

- Tumors often exhibit dramatic karyotypic changes, including gains and losses of chromosomes. Chromosome gain or loss occur if defects exist in sister chromatid cohesion and separation. Genomic instability and chromosome segregational defects in tumor cells are often associated with hyper-amplification of the centrosome and with the formation of multipolar spindles. However, centrosome amplification does not always lead to multipolar spindle formation. Spindle multipolarity arises in two distinct steps, an increase in centrosome numbers and an inhibition in centrosomal coalescence. Chromosome instability leads to frequent cytogenetic abnormalities and allelic losses (loss of heterozygosity).
- Many tumors with chromosomal instability have abnormalities in the cell cycle checkpoint that monitors the fidelity of mitosis [Lengauer et al. 1998]. Components of protein complexes responsibe for the attachment of the kinetochores to microtubules or for sister chromatid cohesion may be affected. This manifests as both qualitative and quantitative variations in chromosome numbers among cells from individual progenitors. The generation of aneuploidy (change in chromosome number resulting from unequal partitioning during mitosis) may be a mutagenic mechanism for driving tumor progression. Loss of hetereozygozity is also a possible consequence of chromosome instability. A large variety of solid tumors exhibit changes in DNA copy number. They include colon cancer, lung carcinoma, oropharyngeal cancers, prostate cancer, breast carcinoma, and ovarian carcinoma.
- Aneuploidy is associated with 50–70% of colorectal cancers. Mutations in APC may cause an inefficient attachment of spindle microtubule plus ends to the chromosome kinetochores and cell cortex, leading to defects in chromosome alignment in metaphase. A single truncating mutation in APC acts dominantly to interfere with microtubule attachments and to cause a dramatic increase in mitotic abnormalities [Green and Kaplan 2003].
- Loss-of-function mutations of *bub1* or *bubr1*, which encode kinases involved in mitotic spindle assembly checkpoint signaling, arise in a small subset of aneuploid tumors. They can disrupt mitotic checkpoint control [Cahill et al. 1998]. Expression of the NH_2-terminal region of the spindle checkpoint kinase BUB1, which contains the kinetochore association domain but lacks the checkpoint kinase domain, suppresses the spindle checkpoint. Such mutations in *bub1* cause dominant negative disruption of the spindle, leading to chromosome instability, which affects a portion of colorectal cancers.
- Thyroid follicular neoplasms commonly have aneuploidy. This accelerates the progression to a malignant state [Fagin 2002]. Oncogenic RAS may act as a mutator gene product in thyroid neoplasms by inducing centrosome amplification and chromosome misalignment. Sustained ERK activation, downstream of RAS, may lead to inappropriate phosphorylation of components of the kinetochore complex, presumably leading to chromosomal instability.
- The microtubule motor cytoplasmic Dynein is a critical part of the centrosomal clustering. The over-expression of the spindle protein NUMA in some tumors interferes with Dynein localization and promotes multipolarity. *numa-1* {11q13} maps to one of the most frequently amplified chromosomal segments in cancer cells [Quintyne et al. 2005]. The translocation t(11;17)(q13;q21) links exons encoding the retinoic acid binding and DNA binding domains of *rar*α to 5′ exons of *numa1*. The resulting fusion protein exists in sheetlike nuclear aggregates, partly colocalized with wild-type NUMA [Wells et al. 1997].
- Inherited mutations affecting *brca2* predispose to breast and ovarian cancer [Daniels et al. 2004].

6.3 INDUCTION OF DNA REPAIR

Cell cycle checkpoints are stages, in which the fidelity and stability of the cellular DNA is sensed. Cell cycle arrest and apoptosis are part of the DNA damage response repertoire. Cell cycle arrest needs to be established to provide enough time for

completeion of the reversal of DNA damage. Therefore, the signal transduction pathways leading to cell cycle checkpoints and the pathways leading to DNA repair are overlapping.

The class of signal transducers consisting of Phosphatidylinositol 3-Kinase related proteins includes ATM and ATR (ATM RAD3 Related). Their downstream targets are the checkpoint effector kinases CHK1 and CHK2, structurally unrelated serine/threonine kinases that share some overlapping substrate specificity. CHK2 phosphorylation on threonine 68 is required for its activation. CHK1 phosphorylation on serine 345, catalyzed by ATR, mediates its activation. BRCT repeat containing proteins, such as BRCA1 and P53BP1, may function by recruiting the substrates CHK1 and CHK2 to the kinases ATM or ATR [Zhou and Elledge 2001]. CHK2 targets CDC25A, CDC25C and BRCA1, implicating CHK2 in a network that controls G_1, S, and G_2/M checkpoints as well as DNA repair. An alternative pathway is induced by activation of $P38^{MAPK}$.

P53 is essential for the checkpoint control that arrests cells with damaged DNA in G_1. Phosphorylation by ATM and CHK2 stabilizes and activates P53, which directly inhibits DNA synthesis by interacting with PCNA. Members of the *gadd* gene family are regulated by P53, and GADD45 mediates BRCA1 activity. BRCA1, in turn, contributes essentially to DNA repair. If replication stalls and recombination between sister chromatids is induced, such as after exposure to hydroxyurea, BRCA1 redistributes into discrete nuclear sites. These sites contain:

– Proteins involved in fixing stalled replication, including MRE11/RAD50/NBS1
– Proteins involved in sister chromatid recombination, including BLM Helicase
– Proteins involved in DNA Polymerase loading, including PCNA and RFC

The complex MRE11/RAD50/NBS1 migrates to sites of DNA damage, marked by the phosphorylated Histone 2AX. Sites of DNA damage are marked within minutes by the phosphorylation of this Histone species, which spreads over a region spanning thousands of bases around the lesion. BRCA2 may also be recruited to such sites, where its role in RAD51 control could promote the recombinational events required for reduplication restart.

RFC (Replication Factor C, Activator 1) is a multimeric primer recognition protein consisting of five distinct subunits of 145 kD, 40 kD, 38 kD, 37 kD, and 36.5 kD. It contains RAD17. A direct regulatory linkage exists between RAD17 {5q13} and the checkpoint kinases ATM and ATR [Bao et al. 2001]. Genotoxic agents induce ATM-dependent phosphorylation or ATR-dependent phosphorylation of RAD17 on serines 635 and 645. This is important for the DNA damage induced G_2 checkpoint. In response to ionizing radiation, RAD17 associates with RAD1, a component of the RAD1/RAD9/HUS1 checkpoint complex. These PCNA-like proteins act as sensors of DNA damage. They may form doughnut-like hetero-dimers and could thus be loaded onto damaged DNA, akin to the loading of PCNA onto primed DNA. The toroidal RAD9/RAD1/HUS1 checkpoint complex stimulates components of the base excision repair pathway and activates DNA Ligase I.

- The complex MRE11/RAD50/NBS1 contributes to DNA repair. The expression of NBS1 and RAD50 is reduced in mismatch repair-deficient cancer cells. In these cancers, *mre11* may miss exon 5, which codes for a truncated protein [Giannini et al. 2003].
- RAD17 is over-expressed by colon carcinoma relative to normal colon. Whereas the increased expression of *rad17* by colon carcinomata may be related to their resistance to DNA damaging agents, the deletion of *rad17* in a variety of cancers may predispose them to increased rates of mutation [Bao et al. 1999].

6.4 MULTISTAGE CARCINOGENESIS

Carcinogenesis entails initiation, promotion, and progression [Boutwell 1974]. It is a multistep process [Nowell 1976], in which somatic cells acquire a series of stable genetic mutations in a specific clonal lineage. Because there are safeguards built into the system, more than one mutation must occur for cancer formation to be initiated [Knudson 1971; Knudson et al. 1975; Land et al. 1983; Levine 1995a,b]. This reflects, in part, the ability of nontransformed cells to repair DNA damage or to respond to it by terminating cell cycle progression.

Cancers typically arise from single cells that have undergone critical mutations, which release them from normal growth control. Their clonality is evidenced by molecular markers, such as unique chromosome rearrangements, the allelism of marker genes, or the clonality of antigen receptors on

transformed B-cell or T-cell tumors. This may mean that originally only a single cell acquires all the properties of transformation, or multiple cells originally transform but only the fastest growing line becomes dominant in tumor formation. During further growth, the initially homogeneous clones acquire additional mutations, thus making the resulting tumor heterogenous. Because most tumors have a clonal origin, tumorigenic cells must give rise to phenotypically diverse progeny, including cancer cells, with indefinite proliferative potential. This implies that cancer cells may undergo processes that are analogous to the proliferation and differentiation of normal stem cells.

Multistage carcinogenesis may be reflected in the cellular heterogeneity of tumors. Many types of tumors contain cancer cells with diverse phenotypes, reflecting aspects of the differentiation that normally occurs in the tissues from which the tumors arise. The variable expression of differentiation markers by cancer cells suggests that some of the heterogeneity in tumors originates as a result of the anomalous differentiation of tumor cells [Reya et al. 2001]. Like stem cells, tumorigenic cells have extensive proliferative potential and the ability to give rise to new tissues. Nevertheless, heterogeneity exists within tumor cell populations in the potential to proliferate, with only a fraction of the tumor cells expanding and forming colonies [Park et al. 1971; Bruce and Gaag 1963; Wodinsky et al. 1967; Fidler and Hart 1982; Hamburger and Salmon 1977]. Both tumors and normal tissues are composed of heterogenous combinations of cells with diverse phenotypic characteristics and diverse proliferative potentials.

In chronic myelogenous leukemia, the "Philadelphia chromosome" constitutes a translocation that places *c-abl* adjacent to *bcr* resulting in markedly elevated tyrosine kinase activity. The BCR-ABL fusion protein acts as a growth stimulus for B-lineage hematopoetic cells, but does not confer a fully transformed phenotype. During progression of the disease, additional karyotypic changes develop, including a second Philadelphia chromosome, trisomy 8, and an isochromosome for the long arm of chromosome 17 {17q}. Alterations of the short arm of chromosome 17 also arise and may include alterations of the *p53* gene.

The *bcl-2* gene creates growth advantage but needs interaction with another oncogene, such as *myc* to generate a fully transformed phenotype. The activation of *myc* in cells bearing the $P210^{BCR\text{-}ABL}$ fusion protein generates cells that are capable of forming aggressive tumors. In B-cell lymphomata, a translocation involving the *bcl-2* gene occurs in the majority of low grade lymphomata, often followed by a t(14;18) translocation, which places *c-myc* into the promoter region of *bcl-3* [Gauwerky and Croce 1995].

Colorectal cancer may be the result of 4–5 steps of gene damage, involving both the activation of oncogenes and the inactivation of tumor suppressor genes. The series of genetic events leading to the development of this cancer causes a progression of adenomatous polyps to invasive metastatic carcinomata. The multistep pathway to colorectal cancer entails consecutive defects in multiple genes (Figure 6.4.A) [Levine 1995a,b; Fodde et al. 2001].

Dysregulation of multiple pathways contributes to the development of anaplastic astrocytoma. The expression of TERT (Telomerase catalytic component), but not the inactivation of RB by HPV E7 or the inactivation of P53 and RB by HPV E6/E7, is sufficient for tumor initiation by circumventing cellular senescence. TERT in conjunction with E6/E7 cooperates with RAS pathway activation, but not with activation of the Phosphatidylinositol 3-Kinase pathway or activation of the Epidermal Growth Factor pathway, to generate grade III anaplastic astrocytomata. [Sonoda et al. 2001]

Some oncogene products can affect the expression or activity of other oncogenes, possibly leading to an acceleration of dysregulated growth. Thus, the trans-

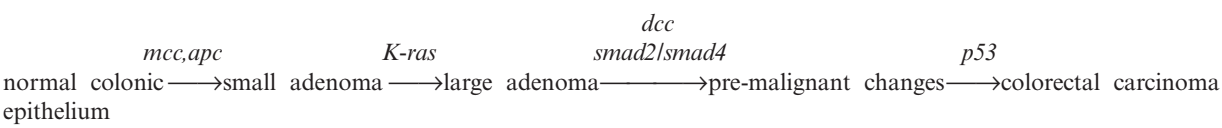


Figure 6.4.A. Multistep carcinogenesis. Consecutive steps of transformation are caused by cumulative genetic defects. The loss of tumor suppressor genes is more frequent than the gain or activation of proto-oncogene alleles.

formation of cells with *ras*, *src*, or *fms* tyrosine kinase oncogenes increases the phosphorylation status and activity of the proto-oncogenic serine/threonine kinase RAF. Stimulation through the PDGF Receptor rapidly induces the transcription of *myc*, *fos*, and *jun* family genes via phosphatidylinositol turnover, PLC activation, and consecutive PKC activation [Cooper 1990].

The multistage process of carcinogenesis is cumulative, with the activity of early stage mechanisms being continuously necessary through the late stages. Sustained over-activation of individual oncogenes is typically necessary for the initiation and growth of various tumors. The over-expression of the *myc* oncogene in hematopoietic cells leads to the development of malignant T-cell leukemias and myeloid leukemias. If *myc* expression is reversed, the leukemic cells undergo proliferative arrest, differentiation, and apoptosis [Felsher and Bishop 1999]. Similarly, the elevated expression of *c-myc* in the epidermis leads to angiogenic premalignant skin lesions, which regress when the c-MYC protein is inactivated [Pelengaris et al. 1999]. Sustained activation of c-MYC is required for maintaining invasive tumors of pancreatic β cells [Pelengaris et al. 2002]. Brief inactivation of *myc* results in the sustained regression of tumors and the differentiation of osteogenic sarcoma cells into mature osteocytes. Subsequent reactivation of *myc* does not restore the malignant properties, but instead induces apoptosis. Thus, brief inactivation of *myc* may cause epigenetic changes in tumor cells that render them insensitive to *myc* induced carcinogenesis [Jain et al. 2002]. H-RAS over-expression readily induces the development of melanomata. When the *ras* gene is inactivated the melanomata rapidly undergo apoptosis and regress [Chin et al. 1999a,b]. The expression of a *bcr-abl* fusion gene results in the development of leukemia. Its inactivation, even at advanced stages of disease, mediates rapid and extensive apoptosis of the leukemic cells [Huettner et al. 2000].

REFERENCES

Adams PD, Kaelin WG, Jr. 1998. Negative control elements of the cell cycle in human tumors. Current Opinion in Cell Biology 10:791–797.

Aten JA, Stap J, Krawczyk PM, van Oven CH, Hoebe RA, Essers J, Kanaar R. 2004. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science 303:92–95.

Audebert M, Chevillard S, Levalois C, Gyapay G, Vieillefond A, Klijanienko J, Vielh P, El Naggar AK, Oudard S, Boiteux S, Radicella JP. 2000. Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. Cancer Research 60:4740–4744.

Bao S, Chang M-S, Auclair D, Sun Y, Wang Y, Wong W-K, Zhang J, Liu Y, Qian X, Sutherland R, Magi-Galluzi C, Weisberg E, Cheng EYS, Hao L, Sasaki H, Campbell MS, Kraeft S-K, Loda M, Lo K-M, Chen LB. 1999. Hrad17, a human homologue of the Schizosaccharomyces pombe checkpoint gene rad17, is overexpressed in colon carcinoma. Cancer Research 59:2023–2028.

Bao S, Tibbetts RS, Brumbaugh KM, Fang Y, Richardson DA, Ali A, Chen SM, Abraham RT, Wang X-F. 2001. ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress responses. Nature 411:969–974.

Bloom D. 1966. The syndrome of congenital telangiectatic erythema and stunted growth. Journal of Pediatrics 68:103–113.

Boutwell RK. 1974. The function and mechanism of promoters of carcinogenesis. CRC Critical Reviews in Toxicology 2:419–443.

Bruce WR, Gaag HVD. 1963. A quantitative assay for the number of murine lymphoma cells capable of proliferation *in vivo*. Nature 199:79–80.

Cabelof DC, Guo Z, Raffoul JJ, Sobol RW, Wilson SH, Richardson A, Heydari AR. 2003. Base excision repair deficiency caused by polymerase β haploinsufficiency: Accelerated DNA damage and increased mutational response to carcinogens. Cancer Research 63:5799–5807.

Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B. 1998. Mutations of mitotic checkpoint genes in human cancers. Nature 392:300–303.

Chen FY, Harris LC, Remack JS, Brent TP. 1997. Cytoplasmic sequestration of an O^6-methylguanine-DNA methyltransferase enhancer binding protein in DNA repair-deficient human cells. Proceedings of the National Academy of Sciences USA 94:4348–4353.

Chen Y, Wang J, Fraig MM, Metcalf J, Turner WR, Bissada NK, Watson DK, Schweinfest CW. 2001. Defects of DNA mismatch repair in human prostate cancer. Cancer Research 61:4112–4121.

Chin L, Artandi SE, Shen Q, Tam A, Lee S-L, Gottlieb GJ, Greider CW, DePinho RA. 1999a. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 97:527–538.

Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner JW 2nd, Cordon-Cardo C, Yancopoulos GD, DePinho RA. 1999b. Essential role for oncogenic Ras in tumour maintenance. Nature 400:468–472.

Cooper GM. 1990. Oncogenes. Boston: Jones & Barlett.

Crabbe L, Verdun RE, Haggblom CI, Karlseder J. 2004. Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. Science 306:1951–1953.

Daniels MJ, Wang Y, Lee M, Venkitaraman AR. 2004. Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. Science 306:876–879.

Fagin JA. 2002. Branded from the start – distinct oncogenic initiating events may determine tumor fate in the thyroid. Molecular Endocrinology 16:903–911.

Felsher DW, Bishop JM. 1999. Reversible tumorigenesis by MYC in hematopoietic lineages. Molecular Cell 43:199–207.

Fidler IJ, Hart I. 1982. Biological diversity in metastatic neoplasms: origins and implications. Science 217:998–1003.

Fodde R, Smits R, Clevers H. 2001. *APC*, signal transduction and genetic instability in colorectal cancer. Nature Reviews Cancer 1:55–67.

Friedberg EC. 2001. How nucleotide excision repair protects against cancer. Nature Reviews Cancer 1:22–33.

Gasche C, Chang CL, Rhees J, Goel A, Boland CR. 2001. Oxidative stress increases frameshift mutations in human colorectal cancer cells. Cancer Research 61:7444–7448.

Gauwerky CE, Croce CM. 1995. Molecular genetics and cytogenetics of hematopoetic malignancies. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 18–37.

Giannini G, Ristori E, Cerignoli F, Rinaldi C, Zani M, Viel A, Ottini L, Crescenzi M, Martinotti S, Bignami M, Frati L, Screpanti I, Gulino A. 2003. Human MRE11 is inactivated in mismatch repair-deficient cancers. EMBO Reports 3:248–254.

Green RA, Kaplan KB. 2003. Chromosome instability in colorectal tumor cells is associated with defects in microtubule plus-end attachments caused by a dominant mutation in APC. The Journal of Cell Biology 163:949–961.

Hamburger AW, Salmon SE. 1977. Primary bioassay of human tumor stem cells. Science 197:461–463.

Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Mehes K, Nash R, Robin N, Shannon N, Tolmie J, Swansbury J, Irrthum A, Douglas J, Rahman N. 2004. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nature Genetics 36:1159–1161.

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419:135–141.

Hoeijmakers JH. 2001. Genome maintenance mechanisms for preventing cancer. Nature 411:366–374.

Holmquist FP. 1998. Endogenous lesions, S-phase-independent spontaneous mutations, and evolutionary strategies for base excision repair. Mutation Research 400:59–68.

Huettner CS, Zhang P, Van Etten RA, Tenen DG. 2000. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nature Genetics 24:57–60.

Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, Trinh M, Sundberg CD, Bishop JM, Felsher DW. 2002. Sustained loss of a neoplastic phenotype by brief inactivation of *MYC*. Sicence 297:102–104.

Jallepalli PV, Lengauer C. 2001. Chromosome segregation and cancer: Cutting through the mystery. Nature Reviews Cancer 1:109–117.

Jaspers NGJ, Gatti RA, Baan C, Linssen PCML, Bootsma D. 1988. Genetic complementation analysis of ataxia telangiectasia and Nijmegen breakage syndrome: a survey of 50 patients. Cytogenetics and Cell Genetics 49:259–263.

Jin S, Mao H, Schnepp RW, Sykes SM, Silva AC, D'Andrea AD, Hua X. 2003. Menin associates with FANCD2, a protein involved in repair of DNA damage. Cancer Research 63:4204–4210.

Kleiman FE, Manley JL. 1999. Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. Science 285:1576–1579.

Knudson AG Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proceedings of the National Academy of Sciences USA 68:820–823.

Knudson AG Jr, Hethcote HW, Brown BW. 1975. Mutation and childhood cancer: a probabilistic model for the incidence of retinoblastoma. Proceedings of the National Academy of Sciences USA 72:5116–5120.

Land H, Parada LF, Weinberg RA. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304:596–602.

Lee JH, Paull TT. 2004. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. Science 304:93–96.

Lengauer C, Kinzler KW, Vogelstein B. 1998. Genetic instabilities in human cancers. Nature 396:643–649.

Levine AJ. 1995a. Tumor suppressor genes. Scientific American Science and Medicine 2:28–37.

Levine AJ. 1995b. Tumor suppressor genes. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 86–104.

Lindahl T, Wood RD. 1999. Quality control by DNA repair. Science 286:1897–1905.

Loeb LA. 1991. Mutator phenotype may be required for multistage carcinogenesis. Cancer Research 51:3075–3079.

Loeb LA. 1994. Microsatellite instability: Marker of a mutator phenotype in cancer. Cancer Research 54:5059–5063.

Lyn D, Cherney BW, Lalande M, Berenson JR, Lichtenstein A, Lupold S, Bhatia KG, Smulson M. 1992. A duplicated region is responsible for the poly(ADP-ribose) polymerase polymorphism, on chromosome 13, associated with a predisposition to cancer. American Journal of Human Genetics 52:124–134.

Nowell PC. 1976. The clonal evolution of tumor cell populations. Science 194:23–28.

Park CH, Bergsagel BE, McCulloch EA. 1971. Mouse myeloma tumor stem cells: a primary cell culture assay. Journal of the National Cancer Institute 46:411–422.

Peinado MA, Malkhosyan S, Velazquez A, Perucho M. 1992. Isolation and characterization of allelic loss and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. Proceedings of the National Academy of Sciences USA 89:10065–120069.

Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. 1999. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. Molecular Cell 3:565–577.

Pelengaris S, Khan M, Evan GI. 2002. Suppression of Myc-induced apoptosis in β cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell 109:321–334.

Pieper AA, Blackshaw S, Clements EE, Brat DJ, Krug DK, White AJ, Pinto-Garcia P, Favit A, Conover JR, Snyder SH, Verma A. 2000. Poly(ADP-ribosyl)ation basally activated by DNA strand breaks reflects glutamate-nitric oxide neurotransmission. Proceedings of the National Academy of Sciences USA 97:1845–1850.

Quintyne NJ, Reing JE, Hoffelder DR, Gollin SM, Saunders WS. 2005. Spindle multipolarity is prevented by centrosomal clustering. Science 307:127–129.

Reya T, Morrison SJ, Clarke MF, Weissman IL. 2001. Stem cells, cancer, and cancer stem cells. Nature 414:105–111.

Roddam PL, Rollinson S, O'Driscoll M, Jeggo PA, Jack A, Morgan GJ. 2002. Genetic variants of NHEJ DNA ligase IV

can affect the risk of developing multiple myeloma, a tumour characterised by aberrant class switch recombination. The Journal of Medical Genetics 39:900–905.

Salomoni P, Pandolfi PP. 2002. The role of PML in tumor suppression. Cell 108:165–170.

Salvesen HB, MacDonald N, Ryan A, Iversen OE, Jacobs IJ, Akslen LA, Das S. 2000. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clinical Cancer Research 6:3607–3613.

Shin K-H, Shin J-H, Kim J-H, Park J-G. 2002. Mutational analysis of promoters of mismatch repair genes *hMSH2* and *hMLH1* in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: Identification of three novel germ-line mutations in promoter of the *hMSH2* gene. Cancer Research 62:38–42.

Sonoda Y, Ozawa T, Hirose Y, Aldape KD, McMahon M, Berger MS, Pieper RO. 2001. Formation of intracranial tumors by genetically modified human astrocytes defines four pathways critical in the development of human anaplastic astrocytomas. Cancer Research 61:4956–4960.

Wang B, Matsouka S, Carpenter PB, Elledge SJ. 2002. 53BP1, a mediator of the DNA damage checkpoint. Science 298:1435–1438.

Wang LI, Miller DP, Sai Y, Liu G, Su L, Wain JC, Lynch TJ, Christiani ZDC. 2001. Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. Journal of the National Cancer Institute 93:1818–1821.

Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes and Development 14:927–939.

Warburton D, Anyane-Yeboa K, Taterka P, Yu CY, Olsen D. 1991. Mosaic variegated aneuploidy with microcephaly: a new human mitotic mutant? Annales de genetique 34:287–292.

Weemaes CM, Hustinx TW, Scheres JM, van Munster PJ, Bakkeren JA, Taalman RD. 1981. A new chromosomal instability disorder: the Nijmegen breakage syndrome. Acta Paediatrica Scandinavia 70:557–564.

Weinstein IB, Carothers AM, Santella RM, Perera FP. 1995. Molecular mechansims of mutagenesis and multistage carcinogenesis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 59–85.

Wells RA, Catzavelos C, Kamel-Reid S. 1997. Fusion of retinoic acid receptor α to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. Nature Genetics 17:109–113.

Wilda M, Demuth I, Concannon P, Sperling K, Hameister H. 2000. Expression pattern of the Nijmegen breakage syndrome gene, Nbs1, during murine development. Human Molecular Genetics 9:1739–1744.

Wodinsky I, Swiniarski J, Kensler CJ. 1967. Spleen colony studies of leukemia L1210. I. Growth kinetics of lymphocytic L1210 cells *in vivo* as determined by spleen colony assay. Cancer Chemotherapy Reports 51:415–421.

Yu Z, Chen J, Ford BN, Brackley ME, Glickman BW. 1999. Human DNA repair systems: an overview. Environmental and Molecular Mutagenesis 33:3–20.

Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH. 1999a. Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science 285:747–750.

Zhong S, Hu P, Ye TZ, Stan R, Ellis NA, Pandolfi PP. 1999b. A role for PML and the nuclear body in genomic stability. Oncogene 18:7941–7947.

Zhou B-BS, Elledge S. 2001. The DNA damage response: putting checkpoints in perspective. Nature Reviews Cancer 1:S25–S31.

CHAPTER 7

EPIGENETIC REGULATION OF CANCER-ASSOCIATED GENE PRODUCTS

The coordination of the genetic programs for cell cycle progression and apoptosis, life span extension and senescence, cell motility, and adhesion requires stringent regulation of the molecules involved. There are various mechanisms to implement this control, including the regulation of protein synthesis and assembly by chaperones, the termination of function by ubiquitination and proteasome degradation, the modulation of function by sumolation, the control of gene expression by Histone modifications, and the transport and metabolism of compounds that affect cancer risk.

7.1 CHAPERONES

Misfolded proteins are inactive, can disturb cellular functions, and have the tendency to aggregate via hydrophobic interactions. The accumulation of misfolded proteins is a major consequence of free radical injury. Oxidation of amino acid residues results in conformational changes and exposure of hydrophobic residues at the protein surface. Heat Shock Proteins (HSPs) constitute a family of gene products induced in response to exposure to environmental stress. They play roles as chaperones in protein synthesis, folding, and transport. Heat Shock Proteins may contribute to cell cycle regulation by interacting with proto-oncogene products or tumor suppressor gene products. A number of multigene families encoding Heat Shock Proteins exist, with individual gene products varying in cellular expression, function, and localization. They are classified according to molecular weight, including HSP27, HSP70, and HSP90. Exceptions to this nomenclature are a small subset of chaperones that constitute glucose-regulated proteins, including GRP94 and GRP75. Chaperonins are HSP family members that are defined by a barrel-shaped double-ring conformation. Based on their characteristic structure, a central cavity is formed, which binds nonnative proteins via hydrophobic interactions. Conformational changes of the Chaperonin subunits induced through ATP hydrolysis change the inner lining of the cavity from a hydrophobic to a hydrophilic character, resulting in the release of the unfolded polypeptide into the central chamber and protein folding in a protected environment.

The unfolded protein response. The unfolded protein response (UPR) is induced by the overexpression of misfolded proteins in the endoplasmic reticulum. It is also induced in response to hypoxia. Molecular chaperones are essential effector proteins in this pathway. In the absence of endoplasmic reticulum stress, the resident chaperone GRP78 (BIP-8) binds to the effectors IRE-1 (Inositol Requiring Gene-1, ERN-1, Endoplasmic Reticulum-to-Nucleus Signaling-1), PERK, and ATF6, keeping them in an inactive state.

– During endoplasmic reticulum stress, unfolded proteins accumulate and GRP78 is released from IRE-1. This activates the unique endonuclease activity of IRE-1, the target of which is the *xbp-1* transcript that is not translated in the absence of stress. After cleavage by IRE-1 and religation by the transfer RNA ligase RLG1, the leucine zipper transcription factor XBP-1 (HAC1) [Liou et al. 1990] is synthesized and regulates the expression of certain stress-response genes by binding to UPR elements in their promoters. This splicing event removes a 26 base pair fragment, inducing a frameshift of the

mRNA transcript. Translation of the resulting reading frame causes the expression of a XBP-1 form of 371 amino acids that comprises the original NH_2-terminal DNA binding domain plus an additional transactivation domain in the COOH-terminus.

– A transient inhibition of protein synthesis occurs during the unfolded protein response, which is achieved by activation of the endoplasmic reticulum transmembrane component PERK (Protein Kinase R/ER-Related Kinase). PERK (HRI, eIF2AK3, eIF2α Kinase 3) {2p12} is a member of the eIF-2 family of kinases. Phosphorylation of eIF-2 (Eukaryotic Translation Initiation Factor 2) is involved in attenuating translation in response to endoplasmic reticulum stress. This leads to the loss of Cyclin D_1 from the affected cells, causing the arrest in G_1. PERK also induces ATF4 and GADD34 expression.
– ATF6 (Activating Transcription Factor 6) {1q22-q23} is a basic leucine zipper (bZIP) transcription factor, which is expressed constitutively in an inactive form in the membrane of the endoplasmic reticulum. Activation in response to endoplasmic reticulum stress results in the proteolytic cleavage of the NH_2-terminal cytoplasmic domain of ATF6 to produce a transcriptional activator that can induce genes involved in the unfolded protein response, such as *grp78*. ATF6 is cleaved by the Golgi-localized proteases S1P and S2P during activation of the unfolded protein response, thus liberating the cytosolic transcription factor domain. ATF6 induces the transcription of *xbp-1*, which is then spliced by the activated IRE-1 to produce a highly active transcription factor. This leads to the upregulation of endoplasmic reticulum chaperones.

The unfolded protein response may lead to apoptosis. The relevant inducer Caspase located in the endoplasmic reticulum is Caspase-12. TRAF2 interacts with pro-Caspase-12 and promotes its clustering with ensuing activation by cleavage. BAX and BAK undergo conformational changes and oligomerization, which leads to Caspase-12 cleavage. Downstream, Caspase-7 is activated and the transcription factor EIF2α is dephosphorylated and inactivated. Conversely, during endoplasmic reticulum stress, GSK3β is activated and phosphorylates P53, which accelerates its degradation.

- Targets of the unfolded protein response pathway, including CHOP (C/EBP Homologous Protein, GADD153) GRP94 (Glucose-Regulated Protein 94, BIP), and GRP170 (ORP150) are upregulated in breast tumors, hepatocellular carcinomata, gastric tumors, and esophageal adenocarcinomata [Ma and Hendershot 2004].
- XBP-1 is an essential survival factor for hypoxic stress and tumor growth. Loss of XBP-1 severely inhibits tumor growth due to a reduced capacity by the affected tumor cells to survive in a hypoxic microenvironment [Romero-Ramirez et al. 2004].

HSP27. In adults, HSP27 (HSPB1) {7q11.23} is expressed particularly in breast, uterus, cervix, placenta, skin, and platelets. HSP27 may function as a molecular chaperone and as a regulator in various signal transduction pathways. A stress-induced signal transduction pathway activates P38, P38 phosphorylates and activates MAPKAPK5, which can phosphorylate HSP27 [New et al. 1998]. The phosphorylation of HSP27 on serines 78 and 82 generates 2–3 isoforms with increased acidity. Small heat shock proteins are present in cells as large aggregates of about 500 kD. During heat stress, most HSP27 in the soluble fraction is phosphorylated and redistributed into the insoluble fraction.

- HSP27 is an Estrogen Receptor-associated protein [Ciocca and Luque 1991]. In endometrial carcinomata, the presence of HSP27 is correlated with the degree of tumor differentiation as well as with the presence of Estrogen and Progesterone Receptors. While there is a good correlation between HSP27 expression and Estrogen Receptor expression in breast cancer [Dunn et al. 1993], some, but not all estrogen sensitive breast cancers express HSP27.
- In patients with cervical cancer, HSP27 is predominantly expressed in well-differentiated and moderately differentiated squamous cell carcinomata. IgA antibodies to HSP27 may arise in the genital tracts of women with gynecologic cancers. In contrast, anti-HSP27 IgG is not associated with gynecologic malignancies. Cervical IgA to HSP90 is associated with ovarian cancer, while antibodies to HSP70 are not cancer associated [Korneeva et al. 2000].
- Distinct forms of HSP27 are expressed in lymphoid tissue of patients with acute lymphoblastic leukemia (ALL) [Ciocca et al. 1993]. In infant ALL, this is based on a unique pattern of phosphorylation of HSP27, expressed at a pre-B-cell stage of differentiation [Strahler et al. 1991].

HSP28. HSP28 may be associated with inhibition of cell proliferation. The protein is highly expressed in quiescent keratinocytes and downregulated during

proliferation. Steady state levels of HSP28 are elevated concomitantly with G_1 arrest. Phosphorylation activates HSP28 function, TGF-β_1 signaling increases the phosphorylation of HSP28 and suppresses growth.

- The expression of HSP28 in leukemic cells or in cervical cancer correlates with the state of differentiation [Sherbet and Lakshmi 1997].
- HSP28 expression in breast cancer is an indicator of a favorable prognosis [Sherbet and Lakshmi 1997].

HSP70. Denatured proteins activate heat shock factors within the cytosol by dissociating heat shock proteins that are normally bound to them. Once liberated, the heat shock factors are phosphorylated and form trimers, which then enter the nucleus and bind to heat shock elements within the promoters of various heat shock responsive genes, including *hsp70* {14q24.1}, leading to their transcription and translation. Once expressed, HSP70 binds to the denatured proteins in an ATP-dependent fashion. HSP70 consists of seven polypeptides (α, α′, β, γ, δ, ε, and ζ). The NH_2-terminal end contains an ATP-binding domain and the COOH-terminal part contains a substrate-binding domain. Substrate binding cannot occur in the absence of ATP binding, which is regulated by an EEVD motif. In contrast to the double-ring chaperonins, HSP70 acts as a monomer dedicated to the initial recognition and stabilization of nonnative polypeptides. Because HSP70 hydrolyzes ATP very inefficiently regulatory chaperone cofactors are required for its function. HSP70 proteins are among the first chaperones that bind newly synthesized polypeptides during folding and are intimately involved in the translocation of unfolded polypeptides across intracellular membranes. Accordingly, they are associated with ribosomes, mitochondria, and the endoplasmic reticulum. HSP70 serves structural functions in various cells by associating with Actin microfilaments and by being involved in the folding and dimerization of Tubulin. HSP70 binds to the under-phosphorylated form of RB, which may permit cell entry into S phase [Sherbet and Lakshmi 1997].

ST13 (Suppression of Tumorigenicity 13, P48, HOP) [Prapapanich et al. 1996] is an abundant, highly conserved protein that binds the major cytosolic chaperones HSP70 and HSP90 during an intermediate stage of Steroid Receptor assembly, but is absent from the mature receptor complex. A HSP90-binding domain is located on a central tetratricopeptide repeat, and a HSP70 binding domain maps to an NH_2-terminal tetratricopeptide repeat. ST13 acts as an adaptor that directs HSP90 to preexisting HSP70/Progesterone Receptor complexes.

- HSP70 suppresses transformation. This may be due to the formation of complexes between HSP70 and mutated oncogene products, including P53, RAS, or MYC.
- HSP70 expression in breast cancer correlates positively with Estrogen Receptor expression and negatively with EGF Receptor expression.

HSP90. The highly conserved HSP90 family consists of four gene products, namely the cytosolic HSP90α {1q21.2-q22} and HSP90β {6p12} forms, GRP94 (TRA-1, Tumor Rejection Antigen-1) {12q24.2-q24.3} in the endoplasmic reticulum, and HSP75 (TRAP1, TNFR-Associated Protein 1) {16} in the mitochondrial matrix. The monomer of HSP90 consists of conserved 25 kD NH_2-terminal and 55 kD COOH-terminal domains joined together by a charged linker region, which is not present in HSP75. Both the NH_2- and COOH-termini of HSP90 bind to substrate polypeptides including client proteins and co-chaperones. The NH_2-terminus contains an unusual ATP-binding site that has structural homology with the type II Topoisomerase Gyrase B, a NH_2-terminal fragment of the MUTL DNA mismatch repair protein, and a COOH-terminal fragment of the Histidine Kinase CHE A. Steroid Hormone Receptors form complexes with HSP90, a process that is involved in intracellular protein translocation.

- Association with the molecular chaperone HSP90 is required for the correct folding, stability, and function of multiple mutated, chimeric, and overexpressed signaling proteins that promote the growth or survival of cancer cells. The association with HSP90 stabilizes key regulatory proteins like HIF-1α, FAK, TERT, RAF-1, ERBB2, v-SRC, and BCR-ABL. HSP90 is required for the stability and dominant negative function of mutated P53. The disruption of these heterocomplexes by inhibition of HSP90 causes the rapid degradation of HSP90 client proteins in the proteasome.
- HSP90 is an essential, cytosolic protein, which is overexpressed in a wide variety of malignant tumors. High constitutive expression of HSP90α is common in acute leukemia cells. In contrast, the expression of HSP90β is very low in acute leukemia cells and in normal blood cells [Yufu et al. 1992].

7.2 UBIQUITINATION

Ubiquitin is an abundant, highly conserved 76 amino acid, 9 kD heat shock protein expressed in all cells. Two classes of *ubiquitin* genes comprise:

– In class I a *polyubiquitin* gene, either *ubiquitin B* {17p12-p11.1} or *ubiquitin C* {12q24.3}, encoding a poly-protein of tandemly repeated Ubiquitins.
– In class II the fusion products between a single *ubiquitin A* gene and one of two other possible sequences that encode either 52 or 76–80 amino acids. The 600 bp *uba* transcripts encode Ubiquitin–Ribosomal Protein fusions and represent products of the *uba52* gene {19p13.1-p12} and the *uba80* (*rps27A*, *ribosomal protein S27a*, *ubcep1*, *ubiquitin carboxyl extension protein 1*) gene.

Ubiquitination represents an enzymatically catalyzed formation of a covalent isopeptide bond between the COOH-terminus of Ubiquitin and the ε-amino group of lysines in the acceptor protein. The process of polyubiquitination plays a prominent role in regulating protein degradation [Hershko et al. 1982] via the 26S proteasome (Figure 7.2.A). This complex degrades the protein into small peptides and free poly-Ubiquitin. A target protein must be tagged with a multi-Ubiquitin chain composed of at least four ubiquityl moieties before it can be recognized and degraded by the proteasome. The 26S proteasome is composed of the 20S core catalytic complex flanked by 19S regulatory complexes on both sides. The 20S unit is arranged as a stack of four rings, two α and two β, organized as αββα. Proteasome degradation is adenosine 5′-trisphosphate (ATP) dependent. Ubiquitination is an essential pathway in the regulation of cell-cycle control. Ubiquitin-dependent degradation also regulates the functions of transcription factors that induce metastasis genes.

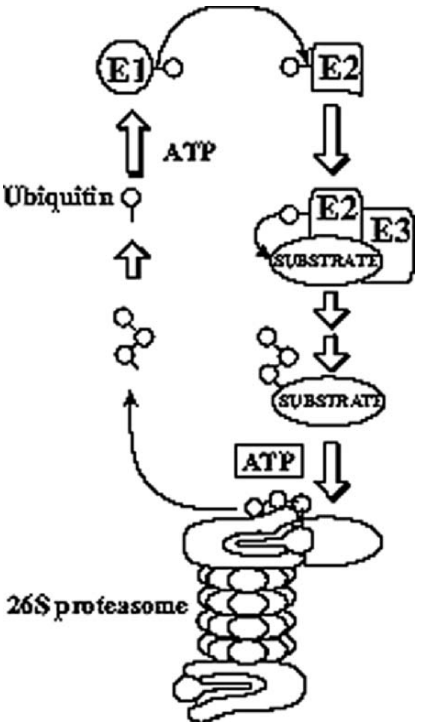


Figure 7.2.A. The Ubiquitin cycle. Ubiquitination involves three catalytic steps. Initially, an Ubiquitin activating enzyme (E1) induces the formation of a high-energy thiol ester bond with the COOH-terminal glycine of Ubiquitin in an ATP-dependent process. Then, an Ubiquitin conjugating enzyme (E2) accepts Ubiquitin from the E1/substrate/Ubiquitin intermediate by a transthiolation reaction. An Ubiquitin protein ligase (E3) catalyzes the transfer of Ubiquitin to the ε-amino group of a lysine residue on the substrate. In successive reactions, a poly-Ubiquitin chain is synthesized. Poly-ubiquitinated proteins are then recognized and degraded by the 26S proteasome complex and free Ubiquitin is released. [Reproduced from Alves dos Santos MCM 2001. With permission.]

Ubiquitination is a three-step process involving Ubiquitin activating (E1), conjugating (E2), and ligating (E3) enzymes.

– E1 enzymes generate a COOH-terminal high-energy thiol ester intermediate with activated Ubiquitin. This reaction is ATP consuming.
– Activated Ubiquitin is transferred to an active site cysteine residue of an Ubiquitin carrier protein, E2. More than 20 E2 proteins exist.
– An Ubiquitin–Protein Ligase, E3, binds to the target substrate and catalyzes the transfer of Ubiquitin from E2 to an ε-amino group of lysine within the target substrate or on a poly-Ubiquitin chain already attached to the substrate. In E3 Ubiquitin Ligase complexes, substrate recognition is accomplished by F-box proteins. In some cases, E3 accepts activated Ubiquitin from an E2 while creating a thiol ester intermediate before transferring it to the substrate. More commonly, an E3 assists in transferring Ubiquitin directly from E2 to the substrate, by bringing them into close proximity (Table 7.2.A; Figure 7.2.B). The catalytic function of E3 Ubiquitin Ligases depends on the presence of either a HECT domain or a RING finger domain. The R-box (a RING finger, small metal binding domain) motif is common to the homologous proteins APC2 (ANAPC2) {9} and ROC1 (Regulator of Cullins-1, Ring-Box 1, RBX1), and is shared among E3s that do not belong to the multi-subunit ligases, such as

Table 7.2.A. E3 Ubiquitin Ligases involved in the regulation of cancer-related gene products

E3	Class	Target
SMURF2	HECT	SMAD1,SMAD2
RSP5	HECT	RNA Polymerase II
WWP1	HECT	LKLF
MDM2	RING	P53
SIAH-1	RING	MYB
SIAH-2	RING	NCOR
CBL	RING	EGF Receptor PDGF Receptor
SCF^{MET30}	RING, SKP-1 based	MET4
CDC4	RING, SKP-1 based	Cyclin E, GCN4
SKP-1/Cullin-1/$SCF^{\beta\text{-}TRCP}$	RING, SKP-1 based	I-κB, $P105^{NF\text{-}\kappa B}$, β-Catenin, SMAD3
SIAH/SIP/SKP-1/EBI	RING, SKP-1 based variant	β-Catenin
VHL/Elongin BC/Cullin-2/RBX1	RING, ELO BC based	HIF-1α, HIF-2α
SKP-2		P27
SKP1/CUL1/CDC53		
BRCA1/BARD1		

the N-end rule UBR1 ligase and MDM2. In this respect, the only ligases devoid of an R-box are the HECT E3s. There are several major functional classes of E3s [Karin and Ben-Neriah 2000; Conaway et al. 2002].

- Specific targets for Ubiquitin-mediated degradation include the proto-oncogene products c-MYC, E1A, and c-FOS. Defects in ubiquitination are associated with certain cancers.

Anaphase promoting complex (APC/C). Anaphase-promoting complex (Cyclosome, APC/C) is a large multi-subunit complex, which is mainly responsible for the degradation of proteins that regulate the late events in mitosis. The anaphase-promoting complex is a 20S core complex with a minimum of eight subunits. It does not appear to have a catalytic function by itself, but operates in concert with a specific E2 in the ubiquitination of target proteins. APC/C recognizes and binds to its substrates. For the recognition of some of these substrates, including certain Cyclins, a nine-amino acid degenerate peptide motif in APC/C, called the destruction box (D-box), is essential. The APC/C complex is inactive during interphase and becomes activated at metaphase and early anaphase. The mechanisms by which APC/C is activated involve phosphorylation and dephosphorylation events, affecting two distinct regulatory subunits. One of these APC/C subunits, CDC20, is activated by mitotic CDKs to bind to the destruction box of APC/C. The other major APC/C regulator, HCDH1, which is necessary for the destruction of mitotic Cyclins, is inactivated by CDK phosphorylation, thereby allowing exit from mitosis. The activation of HCDH1 is mediated by the phosphatase CDC14, which reverses CDK-mediated phosphorylation, allowing HCDH1 to associate with APC/C and stimulate it.

SKP1/Cullin/F-Box or SKP1/Cullin/ROC1/F-Box system. Together with the anaphase-promoting complex, the F-box protein complex SCF (SKP1/CUL1/CDC53) constitutes the major Ubiquitin Ligase complex that regulates proteolysis during G_1/S and anaphase. Cyclin E is low in early G_1, rises to a peak in late G_1, and activates CDK2 around the G_1 to S transition, and then its levels decline again. The reduction of the Cyclin E levels is accomplished in the Ubiquitin–proteasome pathway. Phosphorylated Cyclin E bound to CDK2 and free Cyclin E are targeted for degradation by distinct pathways. Bound CDK2 phosphorylates Cyclin E in two places. The F-box protein CDC4 targets the phosphorylated Cyclin E for degradation. Ubiquitin-dependent proteolysis is mainly responsible for P27 degradation. The phosphorylation of P27 on threonine 187, typically by Cyclin E/CDK2, is required for this process.

The SCF and SCRF Ligase families are multi-subunit Ligase systems that use several common subunits and one variable component, an F-box protein, which functions as the substrate recognition module of the complex and mediates substrate specificity of the SCF complex. Genes encoding certain SCF subunits are essential to cell cycle

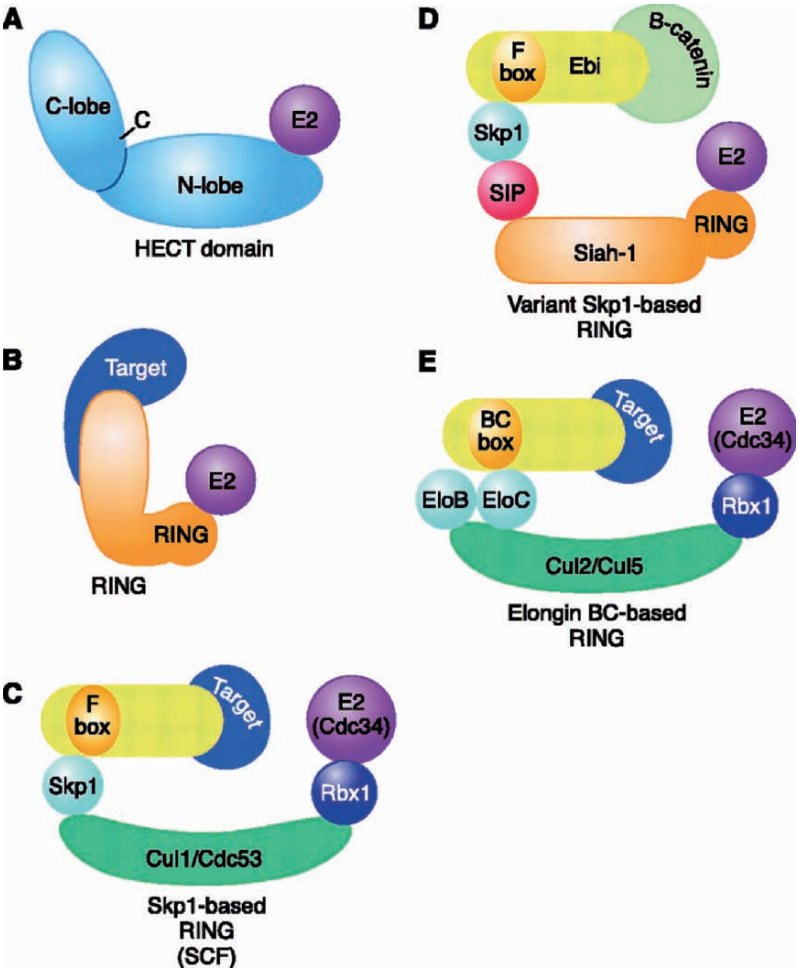


Figure 7.2.B. E3 Ubiquitin Ligases. E3 Ubiquitin Ligases function in the regulation of *POL II* transcription. These E3s include members of the families of HECT domain E3s, RING finger domain E3s, and the structurally related multiprotein SKP1 and Elongin BC based E3s. HECT and RING finger domains serve as docking sites for E2 Ubiquitin Conjugating Enzymes. Unlike RING finger domains, HECT domains contain a catalytic cysteine residue that accepts Ubiquitin from the E2 and transfers it to the target protein. (a) HECT domains are bilobal, with their NH_2-terminal lobes serving as the docking site for the E2 and their COOH-terminal lobes containing the catalytic cysteine. (b) One class of RING finger domain E3s include the RING domain and substrate-binding domain in the same polypeptide. (c) SKP1-based RING E3s include both SCF (SKP1/CULL/CDC53/F-box protein). (d) Variant SKP1-based complexes. SCF and Elongin BC-based E3s include a heterodimeric module composed of a member of the Cullin family and the RING finger protein RBX1 that activates ubiquitination of target proteins by the E2 Ubiquitin Conjugating Enzymes CCD34 and UBC5. SCF complexes include a member of the F-box family of proteins, which serve as substrate recognition subunits that bind specifically to and recruit target proteins for ubiquitination. F-box proteins are linked to a CULL(CDC53)/RBX1 module by the SKP1 adaptor protein, which binds to F-box proteins through a degenerate sequence motif, called the F-box. F-box proteins are modular and contain, in addition to an F-box, a protein–protein interaction domain that is responsible for binding selectively to target proteins. The VHL E3 Ubiquitin Ligase belongs to the family of Elongin BC-based E3s. In the context of the VHL E3, the VHL tumor suppressor protein functions analogously to F-box proteins in the SCF complex to recruit target proteins for ubiquitination. The VHL protein is linked to a CUL2/RBX1 module by the Ubiquitin-like Elongin-B and SKP1-like Elongin-C adaptor proteins, which bind to a degenerate BC-box proteins. [Reproduced from Conaway et al. 2002. With permission.]

progression, and their loss-of-function mutations result in cell cycle arrest. Commonly, the phosphorylation of many substrates of these E3 Ligases is required for being recognized. The SCF E3 Ligases have no apparent catalytic function of their own, but promote substrate ubiquitination through a recruited E2.

SKP1 (S-Phase Kinase Associated Protein 1) {5q31} likely serves as an adapter that links the F-box protein to the rest of the complex. The other

components, CUL1 (CDC53) and ROC1 (RBX1, HRT1), may serve as adapters for recruiting an E2 to the substrate. Because ROC1 acts as a common SCF subunit it is likely that it regulates the ubiquitination by SCF complexes. These subunits may also have other functions associated with the polymerization of the Ubiquitin chain, which require E2, but not the F-box protein. In SCF complexes, Cullin-1 is linked to one of a number of F-box proteins through the adapter protein SKP-1. F-box proteins interact with substrates for ubiquitination through COOH-terminal protein–protein interaction domains and with SKP-1 through the F-box motif. F-box proteins bind to particular phosphorylated substrates at defined time points during the cell cycle and link them to the SCF Ubiquitin Ligase.

Axin-2/Conductin forms a complex with β-Catenin, APC, and GSK3β. This multiprotein complex ("destruction complex") directs β-Catenin to degradation. After β-Catenin has been phosphorylated on four serine/threonine residues in the NH_2-terminus by the kinase GSK3β in the complex, it is transferred to the SCF complex (SKP/Cullin/F-Box complex), binds to the F-Box Protein β-TRCP, is ubiquitinated and degraded in the proteasome. In addition, β-Catenin is regulated by the RING finger E3 Ligase SIAH-1 in conjunction with SIP/SKP-1/EBI. SIAH-1 functions as a single subunit RING finger E3 Ligase, which also targets the oncogenic transcription factor c-MYB.

DET1 promotes the ubiquitination and degradation of the proto-oncogenic transcription factor c-JUN by assembling a multi-subunit Ubiquitin Ligase containing DDB1 (DNA Damage Binging Protein 1), CUL4A (Cullin 4A), ROC1 (Regulator of Cullins 1), and CPM1 (Constitutively Photomorphogenic 1). Phosphorylation of the AP-1 transcription factor c-JUN, at multiple sites within its transactivation domain, is required for JNK induced neuronal apoptosis. The Ubiquitin Ligase SCF^{FBW7} antagonizes apoptotic JNK signaling by ubiquitinating phosphorylated c-JUN and facilitating its degradation [Wertz et al. 2004; Nateri et al. 2004].

- The F-box protein CDC4 (FBW7, SCF^{FBW7}) mediates the ubiquitination of Cyclin E. Some forms of breast and ovarian cancer have elevated Cyclin E protein levels in the absence of increased gene expression. Mutations in the tumor suppressor CDC4 implicate this F-box protein in their pathogenesis, because they prevent CDC4 from targeting Cyclin E for degradation, resulting in uncontrolled cell proliferation. Loss-of-function mutations of CDC4 also occur in endometrial adenocarcinomata.
- Stabilization of the oncogenic transcription factor β-Catenin plays a critical role in cancer, particularly in colon cancer and melanoma. β-Catenin levels are regulated by two E3 Ubiquitin Ligases; one responsive to WNT signaling and the other responsive to activation of P53. Phosphorylation of β-Catenin by Glycogen Synthetase Kinase 3β mediates its ubiquitination by the SKP-1/Cullin-1/β-TRCP complex. Upon WNT signaling, this phosphorylation and degradation are blocked.
- The F-box protein SKP-2 is part of an E3 Ligase, SKP-1/SKP-2/NEDD-8 modified CUL1/ROC1 that regulates the ubiquitination of P27. The putative tumor suppressor Connexin-43 inhibits the expression of SKP-2, resulting in elevated levels of P27 and inhibition of proliferation. SKP-2 is overproduced in lymphomata, breast cancer, prostate cancer, and oral cancers. This leads to lower levels of P27 and facilitates cell cycle progression.
- Forkhead transcription factors play a pivotal role in tumor suppression by inducing growth arrest and apoptosis. Their loss of function due to phosphorylation and proteasomal degradation is implicated in cell transformation. SKP-2 directs the ubiquitination and subsequent degradation of FOXO1. This effect of SKP-2 requires the PKB dependent phosphorylation of FOXO1 at serine 256. By this mechanism, SKP-2 may favor tumorigenesis [Huang et al. 2005].
- The activity of the stress inducible, metastasis associated transcription factor NF-κB is regulated by ubiquitination. In response to activating signals, the inhibitor protein I-κB is phosphorylated and thus targeted for degradation. The recognition component of the phosphorylated I-κB specific E3 Ubiquitin Ligase is the F-box/WD Protein β-TrCP (Transducin Repeat Containing Protein, $E3RS^{I\text{-}\kappa B}$, E3 Receptor Subunit of I-κB). SKP1 and CUL1 associate with $E3RS^{I\text{-}\kappa B}$ and contribute to the ubiquitination of I-κB. The E3 recognition motif of the I-κBs is DpSGXXpS (p denotes phosphorylation). Genetic abnormalities in the chromosomal region of the *e3rs*$^{I\text{-}\kappa B}$ gene are frequent in glioblastoma, prostate cancer, and small cell lung cancer [Karin and Ben-Neriah 2000].

Von Hippel-Lindau Associated Elongins C and B. The VCB (von Hippel-Lindau Associated Elongins C

and B) complex is related to the SCF Ligases and is likely to function biochemically as an E3 Ubiquitin Ligase. Similarly to SCF, it is composed of several subunits, one of which, ROC1 (RBX1), is shared with SCF. Its substrate recognition subunit is the tumor suppressor gene product VHL. A substrate for VHL is Hypoxia-Inducible Factor 1α (HIF1α), which binds VHL and is degraded in the presence of the VHL complex, but escapes degradation in VHL deficient cells or in hypoxia. VHL associates with an adapter system that is similar to that of SCF Ligases, where the VHL-associated proteins Elongin C and Elongin B are homologous to SKP1 and Ubiquitin, respectively, and the Elongin B/Elongin C partner CUL2 is homologous to CUL1. ROC1 also associates with CUL2 and VHL. In this setting, VHL enters a multi-protein complex with Elongin B, Elongin C, Cullin-2, and ROC1, all proteins that are associated with ubiquitination. Elongin C and Cullin-2 share sequence similarity with SKP1 and CDC53. ROC1 contains a RING-H2 finger-like motif and interacts with Cullins. HIF-1 is tightly regulated by Ubiquitin-dependent proteolysis through the VHL/Elongin B/Elongin C complex. In abundant oxygen supply, cellular HIF-1α is rapidly ubiquitinated. The Elongin B/Elongin C complex interacts with the BC-box motif in VHL and bridges its interactions with Cullin-2.

- The *vhl* tumor suppressor gene {3p25.5} is mutated in most sporadic clear-cell renal carcinomata and in von Hippel–Lindau syndrome. A large fraction of the known VHL mutations alters the BC-box and disrupts the VHL complex.

Homologous with E6-AP Carboxyl Terminus Domain E3s (HECT). A large protein family of at least 30 members contain the 350 amino acid HECT domain (homologous to the E6-AP COOH-terminus) and a conserved active cysteine near the COOH-terminus. These E3 proteins have a variable NH_2-terminal domain that, with the exception of E6-AP, anchors directly to the substrate. In some cases, the recognition function of a HECT protein is attributable to a protein–protein interaction module, called the WW domain. It is a 38–40 amino acid stretch containing a hydrophobic binding pocket for a PPXY peptide (the PY motif), which is prevalent within several HECT E3 substrates. E6-AP (Human Papillomavirus E6-Associated Protein, Ubiquitin–Protein Ligase E3A, UBE3A) {15q11-q13} interacts with one of its targets, P53, indirectly through the papillomavirus oncoprotein E6. In contrast to most other E3 Ligases, the action of E6-AP involves an intermediary thiol ester transfer reaction in which the E3 protein first accepts Ubiquitin from E2 and then transfers it to the substrate by facilitating an amide linkage between Ubiquitin and the substrate protein. The conserved COOH-terminal cysteine forms the Ubiquitin acceptor site. Its substitution abolishes the thiol ester transfer of Ubiquitin from the E2, and consequently abrogates the ubiquitination activity of the HECT E3 Ligase.

The WW domain containing E3 Ligase NEDD4 ubiquitinates plasma membrane proteins, such as the epithelial sodium channel complex. CBL proteins include sites of interaction with WW domains, and they are substrates for NEDD4 and ITCH. Their ubiquitination targets them for proteasomal degradation. Consequently, NEDD4 inhibits CBL-B-mediated ubiquitination and downregulation of EGFR, thus reversing effects on proximal events in signaling through this receptor. Similarly, NEDD4 reverses the CBL mediated downregulation of activated SRC. This reflects a negative regulation of RING finger E3 Ligases by HECT family E3s resulting in a tight control of protein tyrosine kinases [Magnifico et al. 2003].

The abundance of SMAD proteins is regulated by the Ubiquitin–proteasome pathway. SMADs can associate with E3 Ubiquitin Ligases, such as JAB-1, ROC-1, or SMURFs. SMURFs belong to the HECT domain containing E3 enzymes, which interact through their WW domains with a specific PY motif in certain SMADs. SMURF-1 (SMAD Ubiquitination Regulatory Factor-1) {7q21.1–31.1} is a HECT domain E3 Ubiquitin Ligase that binds to SMAD-1 or SMAD-5 via their PY motifs and mediates the ubiquitination and degradation of these SMAD proteins.

- The E3 Ubiquitin–Protein Ligase E6-AP mediates the HPV (human papillomavirus) induced degradation of the tumor suppressor P53 in cervical cancer.
- EDD (E3 Isolated by Differential Display, HYD) {8q22.3} is a HECT domain E3 Ligase. Allelic imbalance at the *edd* locus is common in ovarian cancer, breast cancer, hepatocellular carcinoma, squamous cell carcinoma of the tongue, and metastatic melanoma. It is likely to represent amplification of the *edd* gene locus rather than loss of heterozygosity. The *edd* gene is frequently overexpressed in breast and ovarian cancer, implying a

potential role in cancer progression [Clancy et al. 2003].

- RNF11 (RING Finger Protein 11) is a 154 amino acid protein that has a RING-H2 finger domain, a PY motif, an Ubiquitin interacting motif, a 14–3–3 binding sequence, and a PKB phosphorylation site. RNF11 interacts with the HECT type E3 Ubiquitin Ligases NEDD4, AIP4, SMURF1, and SMURF2. It can enhance TGF-β signaling through a direct association with SMAD4, the common signal transducer in the TGF-β, BMP, and Activin pathways. RNF11 is highly expressed in breast cancer and in prostate cancer [Azmi and Seth 2005].

MDM2. The tumor suppressor P53 is a target for Ubiquitin-dependent degradation. The proto-oncogene product MDM2 is a P53-specific RING finger E3 Ubiquitin–Protein Ligase. It binds to the transactivation domain of P53 and targets it for degradation by facilitating its ubiquitination. MDM2 catalyzes P53 monoubiquitination on a cluster of six COOH-terminal lysine residues. When autoubiquitinated, the Ubiquitin Ligase activity of MDM2 for P53 is impaired. Upon SUMO1 conjugation, MDM2 is protected from ubiquitination and elicits increased Ubiquitin Ligase activity, as reflected in increased ubiquitination and degradation of P53. While P300 with MDM2 catalyzes the polyubiquitination of P53, P53 is stabilized by the binding of P300 to the oncoprotein E1A [Grossman et al. 2003].

- The proto-oncogene product MDM2 binds a NH_2-terminal region of P53 and targets it for degradation through the Ubiquitin pathway. Enhanced MDM2 levels in tumor cells can cause a decrease in the concentration of functional P53 and abolish the ability of P53 to arrest a cell in response to damage. MDM2 levels are high in many sarcomata, including common bone and soft tissue forms. Overexpression of the *mdm2* oncogene occurs in leukemias.
- Point mutations in the zinc-finger encoding region of *mdm2* are associated with non-Hodgkin lymphomata, leukemias, and hepatocellular carcinomata. Point mutations in other domains occur in liposarcomata.

CBL. Many E3 Ligases, such as the 120 kD proto-oncogene product CBL {11q23.3}, are RNF. The RING finger domain of CBL is adjacent to the NH_2-terminal tyrosine kinase binding/transforming domain. This transforming region contains a phosphotyrosine binding domain that interacts with autophosphorylated tyrosine kinases via a D(N,D)XpY motif (p denotes phosphorylation). The tyrosine kinase-binding domain is composed of a four-helix bundle, a calcium binding EF hand, and a divergent SH2 domain. The protein also contains a proline rich region (amino acids 481–690) capable of mediating interactions with SH3 domain containing proteins, and a COOH-terminal leucine zipper that may mediate intermolecular oligomerization. The ubiquitination of protein tyrosine kinases terminates their signaling by marking them for degradation. CBL is an adapter protein for tyrosine kinases, which positively regulates their ubiquitination dependent on its variant SH2 and RING finger domains.

– c-CBL suppresses the signaling of activated Growth Factor Receptor tyrosine kinases, including EGF Receptors, PDGF Receptors, and CSF-1 Receptors, by inducing their ubiquitination.
– The NH2-terminal and RING finger domains of CBL are essential for the negative regulation of the tyrosine kinases ZAP-70 and SYK.
– CBL is associated with SRC family protein tyrosine kinases, which also phosphorylate it on three consensus SH2 domain binding sites, Y700, Y731, and Y774.
– CBL-l interacts with the SH2 domains of the CRK-I or CRK-II adaptor proteins, which link it to the RAP-1 family Guanine Nucleotide Exchange Factor C3G (GRF2). Phosphorylated CBL recruits the CRK-II/C3G complex to lipid rafts, where C3G specifically activates the small GTP-binding protein TC10.
– CBL associates with the P85 subunit of Phosphatidylinositol 3-Kinase via pY731 binding to the SH2 domain of P85 [Lupher et al. 1999].

Insulin ligates its cognate tyrosine kinase receptor to stimulate the transport of glucose into fat and muscle cells. A receptor substrate in this pathway is CBL, which is recruited to the Insulin Receptor by interaction with the adaptor protein CAP (CBL Associated Protein, SH3D5, SORBS1, Sorbin, and SH3 Domain Containing 1). Upon phosphorylation of CBL, the CAP/CBL complex dissociates from the Insulin Receptor and moves to a Caveolin enriched membrane domain. There, Flotillin forms a ternary complex with CAP and CBL, directing the localization of the CAP/CBL complex to a lipid raft

subdomain of the plasma membrane. This localization generates a pathway that is crucial in the regulation of glucose uptake [Mastick et al. 1995; Baumann et al. 2000].

After internalization from the plasma membrane, receptor molecules are rapidly delivered to early endosomes (sorting endosomes). Most of the soluble content of sorting endosomes is delivered to the lysosomes for degradation in the Ubiquitin–proteasome pathway, whereas the majority of proteins associated with the endosomal membrane recycle back to the plasma membrane. Cell membrane proteins destined for lysosomal degradation are segregated into intraendosomal vesicles, which results in the formation of late endosomes or multivesicular bodies (MVBs), thus providing a mechanism to target them for degradation. Certain receptors, including the Epidermal Growth Factor Receptor (EGFR) and the Growth Hormone Receptor (GHR) are transported together with their ligand into lysosomes for degradation. This form of downregulation is important for cellular regulation, and disrupted internalization or degradation often results in the loss of cell growth control [van Kerkhof et al. 2001]. CDC42 and c-CBL are critical components involved in the regulation of EGFR protein levels. CBL binds to the EGF Receptor and induces its degradation, thus preventing excessive EGFR signaling. Activation of CDC42 protects the EGF Receptor from the negative regulatory activity of c-CBL. Activated CDC42 binds to $P85^{COOL-1}$ (β-PIX), a protein that directly associates with c-CBL. This inhibits the binding of CBL to the EGF Receptor [Wu et al. 2003].

Gene products homologous to CBL are CBL-B {3q} and CBL-C {19q13.2}.

- Mutations within the α-helical structure that links the SH2 and RING finger domains render CBL proteins oncogenic. Mutants of c-CBL that function as dominant oncogenic forms induce the upregulation of signaling downstream of tyrosine kinase receptors, which leads to transformation. In a pre-B-cell lymphoma, a deletion of 17 amino acids abolishes the ability of c-CBL to promote the ubiquitination of receptor tyrosine kinases [Lupher et al. 1999].
- *cbl* is located on chromosome 11q23.3 telomeric to *mll*, which is frequently fused to other loci by translocations. Interstitial deletion can fuse *mll* exon 6 in-frame to *cbl* exon 8 in adult AML (acute myeloid leukemia). The genomic junction region involves the fusion of the 3' portion of an Alu element in intron 6 of *mll* with the 5' portion of an Alu element in intron 7 of *cbl*. The transcriptional orientation of both genes is from centromere to telomere [Fu et al. 2003].

BRCA1/BARD1. Many of the major pathways of the DNA damage response involve protein modification and degradation by ubiquitination. BARD1 (BRCA1-Associated RING Domain-1) {2q} [Wu et al. 1996] interacts with the NH_2-terminal region of BRCA1. BARD1 shares homology with the two most conserved regions of BRCA1, the NH_2-terminal RING motif and the COOH-terminal BRCT domain. The BARD1 protein also contains three tandem Ankyrin repeats. Progression to S phase in the cell cycle is accompanied by the aggregation of nuclear BARD1 into BRCA1 nuclear dots. BRCA1 has E3 Ubiquitin Ligase activity [Lorick et al. 1999; Ruffner et al. 2001].

- The Ubiquitin Ligase activity of the RING heterodimer BRCA1/BARD1 is inactivated by the BRCA1 mutation C61G, which predisposes to breast cancer [Hashizume et al. 2001]. In the presence of BRCA1, BARD1 acts synergistically in DNA repair. In the absence of BRCA1, BARD1 elevates the levels of P53 and promotes apoptosis.

UBR1. Short half-lives are characteristic of damaged or otherwise abnormal proteins. Degradation signals (degrons) are features of such proteins that confer metabolic instability. The essential component of a particular degradation signal, N-degron, is a destabilizing NH_2-terminal residue of a protein. The set of amino acids that are destabilizing in a given cell type yields the "N-end rule," which relates the half-life of a protein to the identity of its NH_2-terminal residue. The N-end rule pathway is a proteolytic pathway of the Ubiquitin system. UBR1 (Ubiquitin-Protein Ligase E3 Component N-Recognin 1, E3α) {15q15-q21.1} and UBR2 (C6ORF133) act in this pathway. It recognizes proteins with basic or bulky hydrophobic residues at their NH_2-terminus.

Cell cycle progression is extensively regulated by ubiquitination. Together with APC/C, the F-box protein complex SCF constitutes the major Ubiquitin Ligase complex that regulates proteolysis during G_1/S and anaphase. Genes encoding certain

SCF subunits are essential to cell cycle progression, and their loss-of-function mutations result in cell cycle arrest. Cyclin E is low in early G_1, rises to a peak in late G_1, activates CDK2 around the G_1 to S transition, and then its levels decline again. The reduction of the Cyclin E levels is accomplished in the Ubiquitin–proteasome pathway. The phosphatase CDC25A is important during the initiation of S phase. CDC25A dephosphorylates and activates the CDK2/Cyclin E complex. Two Ubiquitin Ligases, SCF and APC/C, are involved in the regulation of CDC25A. SCF regulates the abundance of CDC25A in S phase and G_2. In response to DNA strand breaks, CHK1 catalyzes the tyrosine phosphorylation of CDC25A that leads to ubiquitination and degradation by SCF/β-TRCP. Anaphase-promoting complex (Cyclosome, APC/C) is mainly responsible for the degradation of proteins that regulate the late events in mitosis. APC/C controls the activity of CDC25A at the exit of mitosis.

Molecules of cell cycle control may be regulated through the Ubiquitin–proteasome pathway. This facilitates cell cycle progression. The tumor suppressor P53 is a target for Ubiquitin-dependent degradation. The proto-oncogene product MDM2 is a P53-specific RING finger E3 Ubiquitin–Protein Ligase. It binds to the transactivation domain of P53 and targets it for degradation by facilitating its ubiquitination. E6-AP interacts with P53 indirectly, through the papillomavirus oncoprotein E6, and targets it for degradation.

Ubiquitination is reversible because the Ubiquitin moiety can be enzymatically removed. Ubiquitin is synthesized in a variety of functionally distinct forms, including a linear head to tail poly-Ubiquitin precursor. The terminal Ubiquitin moiety in many of these precursors has extra COOH-terminal residues, which are removed by deubiquitinating thiol proteases to expose glycine–glycine residues. Deubiquitination plays an essential role in various processes. During degradation, it is important to release the Ubiquitin from the lysine residues of the proteolytic end products.

A number of proteins related to Ubiquitin exist. Despite their low homology to Ubiquitin these Ubiquitin-Like Proteins (UBLs, Ubiquitin-Like Protein Processing Enzymes, ULPs) share homology to Ubiquitin. They fall into two categories.

– Proteins that are not available for conjugation (RAD23, DSK2p, Elongin B).
– Proteins that, like Ubiquitin, are attached to other proteins. To this group belong the Interferon inducible Ubiquitin cross-reactive proteins UCRP (ISG15, IFI15, G1P2), NEDD8 (which targets CDC53), and SUMO1.

7.3 SUMOLATION

SUMO proteins posttranslationally modify numerous cellular proteins to affect their metabolism and function. There is no evidence that sumolation targets its substrates for degradation. Instead, it has more diverse effects including directing cellular localization or functional activity. SUMO E3 Ligases are located at nuclear pores. Frequently, the sumolation of transcription factors results in their reduced activity. Three members of the SUMO (Small Ubiquitin-Like Modifier, Sentrin, SMT3C, PIC1) [Mahajan et al. 1997; Matunis et al. 1997] family include SUMO1 {2q32.2-q33}, and closely related SUMO2 and SUMO3 {21q22.3}. SUMO1, a 101 amino acid polypeptide of 11 kD, shares about 50% sequence identity with SUMO2 and SUMO3. It has a COOH-terminal tail of four residues that is cleaved off by cysteine proteases called ULP to generate the active form of the protein. These enzymes expose the COOH-terminal glycine–glycine residues. Due to the absence of suitable lysines in SUMO, it cannot be conjugated to generate poly-SUMO chains.

SUMO1 is synthesized as a precursor. After endo-proteolytic cleavage of the precursor SUMO1 molecule, SUMO is first activated in an ATP-dependent reaction by formation of a thiolester bond of its COOH-terminal glycine with E1. The SUMO activating enzyme E1 is heterodimer, which consists of the 38 kD AOS1 and the 71 kD UBA2. In the second step, activated SUMO is transferred to the SUMO conjugating (E2) enzyme UBC9 (UBE21) {16p13.3}. UBC9 forms a thiol ester linkage with SUMO. This E2 enzyme is specific for SUMO and does not act on Ubiquitin. In the last step, transfer of SUMO to the ε-amino group of a lysine in the target protein takes place, catalyzed by an E3 Ligase. Sumo E3 Ligases include:

– PIAS1 (DDX-BP1, GBP) contains a zinc binding motif and a highly acidic region. PIAS1 targets STAT-1 for sumolation
– PIAS2 (PIASX) has high homology to PIAS1
– PIAS3 is an inhibitor of activated STAT3
– PIAS4 (PIASY) preferentially enhances the conjugation of SUMO2 to GATA-2, resulting in the suppression of GATA-2-dependent transcription

– RAN-BP2 (NUP358) acts as an E3 by binding to SUMO and UBC9 to position the SUMO-E2 thiolester in an orientation that enhances conjugation. It catalyzes the sumolation of SP100 and HDAC4. RAN-BP2 localizes sumolated RAN-GAP1 to the nuclear pore complex
– CBX4 (PC2) is a SUMO E3 for the transcriptional corepressor CTBP

PEST sequences are defined as a stretch of at least 12 amino acids rich in proline, glutamate, aspartate, serine, and threonine residues without any positively charged amino acids. Most of the SUMO targets contain one or more strong PEST sequences.

Sumolation is a dynamic, reversible process. Desumolation is catalyzed by ULP Proteases, members of the cysteine protease category. The ULP proteases ULP1 (SENP8, Sentrin-Specific Protease Family Member 8, DEN1) and ULP2 (SMT4) [Li and Hochstrasser 1999; Li and Hochstrasser 2000] share a homology in a 200 residue region, termed ULP domain (UD), which harbors the catalytically active region.

Nuclear bodies. PML (Promyelocytic Leukemia Protein, MYL) {15q22} is an Interferon-inducible RING finger containing nuclear phosphoprotein that is essential for the formation of nuclear bodies (nuclear dots, PML oncogenic domains, PODs, nuclear domain 10, ND10, subnuclear speckles). More than 30 proteins colocalize to these structures, including SP100, DAXX, ISG20, BLM, and CBP. PML undergoes sumolation at three lysine residues. This is catalyzed by SUMO1, which is covalently linked to RAN-GAP1 in the nuclear pore complex. Sumolation of PML regulates the assembly and stability of the PML nuclear bodies. Sumolation of PML also directs P53 to the nuclear bodies and leads to a stimulation of the transcriptional and pro-apoptotic activity of P53. Similarly, another component of nuclear bodies, SP100 (Speckled), is sumolated by SUMO1. The interaction of SP100 with chromosomal non-Histone proteins points to its role in chromatin organization. SP100 sumolation enhances its binding to HP1α (Heterochromatin Protein 1α), suggesting that the communication between nuclear bodies and chromatin is regulated in part by sumolation. PIASY (Protein Inhibitor of Activated STAT Y), a nuclear matrix associated SUMO E3 Ligase, represses the activity of the WNT-responsive transcription factor LEF1 by sequestration into the PML nuclear body [Sachdev et al. 2001]. HIPK2, a kinase cofactor of homeodomain transcription factors, localizes to nuclear bodies when modified by SUMO [Kim et al. 1999].

Chromatin structure. Histone Deacetylase 4 (HDAC4) allows DNA to condense by removing acetyl groups from Histones in the chromatin. This has the effect of repressing the transcription of the affected genes. Sumoylation of HDAC4, facilitated by the nuclear pore protein RAN-BP2, is necessary for the full gene suppressing activity of HDAC4. Due to the localization of RAN-BP2, it is likely that sumolation of HDAC4 occurs upon nuclear entry. SUMO modification of Topoisomerase moves it away from mitotic chromatin. Topoisomerase I and Topoisomerase II, when un-sumolated, help maintain chromosome cohesion, perhaps through their effects on chromosome structure. When tagged with SUMO [Mao et al. 2000], Topoisomerase rapidly moves to the nucleolus. It can no longer sustain chromosome cohesion, thus allowing the chromosomes to separate. UBC9 (Ubiquitin Carrier Protein 9, UBE21) is an E2 conjugating enzyme that is essential for the sumolation of Topoisomerase.

DNA repair. The sumolation site in P53 is located within the COOH-terminus, at lysine 386. Modification of this residue moderately stimulates the transcriptional and pro-apoptotic activity of P53. Sumolation of the P53 family member P73 does not notably alter its transcriptional properties but rather contributes to regulating its subcellular localization. MDM2 is sumolated at lysine 446, which is located within the RING finger domain. Sumolation of MDM2 can protect it from ubiquitination. Under normal growth conditions, SUMO1 keeps MDM2 in a stable and active mode with the consequence that P53 is efficiently degraded. DNA damage induces the de-sumolation of MDM2 followed by its ubiquitination and results in P53 accumulation.

The RAD6 (UBE2, HHR6) pathway is central to postreplicative DNA repair. Two principal elements of this pathway are the Ubiquitin E2 conjugating enzymes RAD6 and the MMS2/UBC13 heterodimer, which are recruited to chromatin by the RING finger proteins RAD18 and RAD5, respectively. RAD6 and MMS2/UBC13 catalyze the ubiquitination of PCNA on lysine 63. The SUMO E2 UBC9 regulates this pathway modifying PCNA on the same lysine. These modifications differentially affect the resistance of cells to DNA damage [Hoege et al. 2002]. This reflects

a role for SUMO in regulating DNA repair. In S phase, PCNA can be modified by SUMO. Sumolated PCNA functionally cooperates with SRS2, a helicase that blocks recombinational repair by disrupting RAD51 nucleoprotein filaments. The recruitment of SRS2 by modified PCNA in S phase prevents aberrant recombination events of reduplicating chromosomes [Pfander et al. 2005]. The SUMO conjugating enzyme UBE21 specifically interacts with RAD52, RAD51, P53, and UBL1. The interaction is mediated by the self-association region of RAD52 [Shen et al. 1996]. Through this interaction, cell cycle control, apoptosis, DNA repair, and ubiquitination are connected.

BLM, encodes a REC-Q DNA Helicase, the absence of which results in genomic instability and predisposition to cancer. BLM is a substrate for SUMO modification, with K317, K331, K334 and K347 being the preferred lysines of modification. SUMO modification is a negative regulator of the DNA damage sensing function of BLM [Eladad et al. 2005].

Gene transcription. I-κB function as an inhibitor relies on the cytoplasmic sequestration of the NF-κB transcription factor. I-κB is sumolated on the same acceptor lysine as that targeted by Ubiquitin [Desterro et al. 1998]. Hence, sumolation antagonizes ubiquitination, resulting in the stabilization of I-κB and the consequent reduction in NF-κB transcriptional activity. A similar competition between sumolation and ubiquitination exists for MDM2, the E3 Ubiquitin Ligase for P53 [Buschmann et al. 2000]. In preventing the attachment of Ubiquitin to the same acceptor, SUMO stabilizes MDM2 and thus enhances the Ubiquitin-mediated degradation of P53.

Apoptosis. CD95 (FAS) is sumolated. DAXX can bind to and undergo covalent modification by SUMO-1, an Ubiquitin-like protein that associates with the death domain of CD95. Modification of PML by SUMO-1 sequesters DAXX in nuclear domains (ND-10 domains) and may inhibit the pro-apoptotic function associated with cytoplasmic DAXX. A distinct mechanism involves Sentrin, an Ubiquitin-like protein that can covalently modify cellular proteins. Sentrin binds CD95 and protects cells from CD95L induced cell death. This is accomplished by interaction of DAXX, but not FADD, with Sentrin and with the conjugating enzyme UBC9 [Ryu et al. 2000].

Growth Factor signaling. The steroid hormone 17β-estradiol (estrogen) plays a significant role in the normal physiology and in transformation of the mammary gland through binding to its nuclear receptor ERα. ERα undergoes various posttranslational modifications, which regulate its transcriptional activity and its stability. It is a target for SUMO-1 modification, which occurs strictly in the presence of hormone and leads to increased transcriptional activity. ERα is sumolated at conserved lysine residues within the hinge region. PIAS1 (GBP, DDXBP1) {15q22} and PIAS3 {1q21} are E3 Ligases for ERα. PIAS1 and PIAS3, as well as UBC9, also modulate ERα-dependent transcription, independently of their SUMO-1 conjugation activity [Sentis et al. 2005].

- Nuclear bodies form dynamically during the phases of the cell cycle. They are also sensitive to external stimuli, such as stress and virus infection. Nuclear bodies are disrupted in malignant promyelocytic leukemia cells. Their proper formation requires SUMO modified PML. In healthy cells, SP100 and the tumor suppressor PML are conjugated to SUMO-1 within nuclear bodies during interphase, but they become deconjugated during mitosis. In addition, phosphorylation is an important factor in the differential modification of PML nuclear bodies during the cell cycle.
- The PAX3-FKHR fusion protein leads to rhabdomyosarcoma. DAXX drastically represses gene transcription, likely through the recruitment of Histone Deacetylases. The transcriptional activity of PAX3 is repressed by DAXX, whereas the oncogenic fusion protein PAX3-FKHR is unresponsive to this repressive action. SUMO-1 modified PML, but not its oncogenic fusion PML-RARα, can derepress the transcriptional activity of PAX3 through sequestering the repressor DAXX into the nuclear bodies [Li et al. 2000; Lehembre et al. 2001].
- The transcriptional activation of the Androgen Receptor is regulated through its interactions with various cofactors. The cofactor ZIMP10 associates through its central region with the transactivation domain of the Androgen Receptor. In prostate cancer cells, ZIMP10 augments the transcriptional activity of the Androgen Receptor. It colocalizes with AR and SUMO-1 at replication foci throughout S phase and is capable of enhancing the sumolation of AR [Sharma et al. 2003].

7.4 NUCLEOSOME MODIFICATIONS

Histone proteins organize the DNA into nucleosomes, which are regular repeating structures of chromatin. Nucleosomes consist of eight Histone proteins and DNA wrapped around them (Figure 7.4.A). Nucleosomes contain 146 bp of DNA and a core Histone octamer, which is composed of two copies of each of H2A, H2B, H3, and H4. The linker Histone H1 stabilizes the assembly of the octameric core to higher order structures of chromatin. Because chromatin structure limits the access of DNA binding proteins to the DNA, the regulation of gene transcription is controlled, in part, by distinct modifications to Histones that result in structural changes to the nucleosomes. This chromatin-mediated repression is counteracted by acetylation, methylation, or phosphorylation of the NH_2-terminal tails of the Histones, which are likely to interact with DNA regulatory proteins. Histone Acetyl Transferases (HATs) and Histone Deacetylases (HDACs) determine Histone conformation, and thus the access of the transcriptional machinery to DNA (Table 7.4.A). This is further modulated by Histone Methyl Transferases and Histone Demethylases as well as Histone Kinases and Histone Phosphatases. Histone acetylation, Histone methylation, and DNA methylation are orchestrated coordinately. Additionally, complexes such as SWI/SNF alter the association of Histones with DNA by use of ATP hydrolysis. Inositol polyphosphates can modulate the activities of several chromatin remodeling complexes.

Histone acetylation. The association of Histones with DNA is modulated by alterations of the charge interactions between the NH_2-terminal Histone tails and the DNA. Acetylation of lysine residues on the NH_2-terminal tails of Histones [Vidali et al. 1968] neutralizes the positive charge of the Histone tail and decreases its affinity to

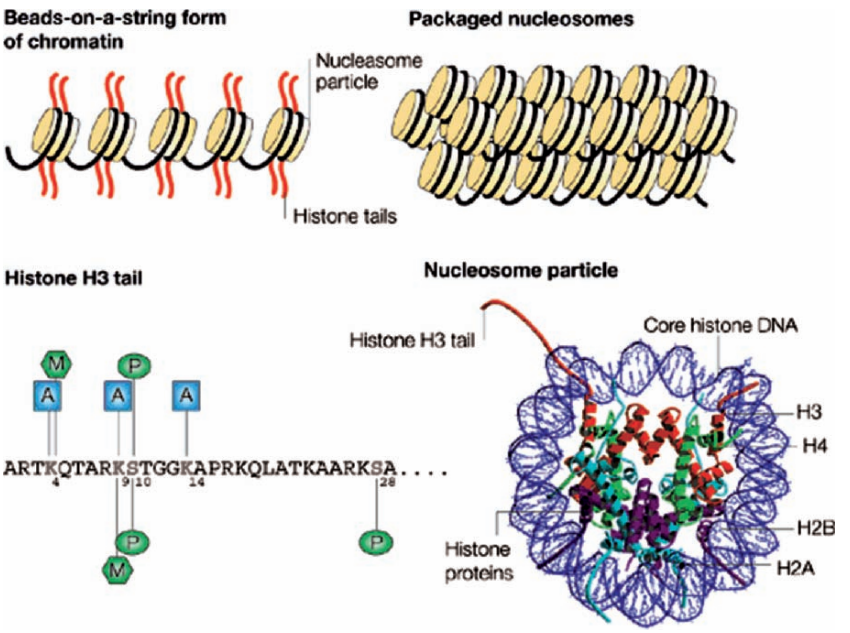


Figure 7.4.A. Nucleosome structure. Chromatin organization and the Histone H3 NH_2-terminal tail. The nucleosome particles that make up chromatin are depicted as yellow cylinders. The DNA is shown as black strands and the NH_2-terminal Histone tails are displayed as red squiggles. Higher order chromatin, characteristic of condensed chromatin or heterochromatin, is to the right of the chromatin schematic. Below and to the right is the high-resolution structure of the nucleosome core particle, in which the DNA double helix is in blue and the Histone H3 dimer is in red, H4 is in green, H2A is in aqua, and H2B is in purple. Shown below and to the left is the Histone H3 tail region from yeast with the modifications that regulate gene activity. Acetylation is represented by A, phosphorylation is represented by P, and methylation is represented by M. Modifications that promote transcriptional activation are shown above the sequence and modifications that induce transcriptional silencing or chromosome condensation are shown below the sequence. [Reproduced from Marmorstein 2001. With permission from Macmillan.]

Table 7.4.A. Histone Acetyl Transferases and Histone Deacetylases. The Histone acetylation status determines the accessibility of DNA for transcription factors. This mechanism contributes to the regulation of gene expression. Histone acetylation opens the nucleosome structure and facilitates transcription factor binding, whereas Histone deacetylation closes it and silences the affected DNA regions

Group	Enzyme	Substrate	Interactions
HATs			
GNAT	GCN5	H2B, H3	SPT/ADA/GCN5 Acetylase
			P300/CBP
	PCAF		
	HAT1	H3, H4	P300/CBP
MYST	MYST1	H2A, H3, H4	ATM
	MYST3	H3	NUA3
	MORF		
	TIP60	H2A, H3, H4	ATM
			PLIP/PLA2
	HBO1	H3, H4	ORCL1
SRC	SRC-1		TBP
			TFII-B
	SRC-2		
	NCOA3		PCAF
	TIF-2		
	GRIP1		
ATF-2	ATF-2	H2B, H4	JUN
			ATM
$TAF_{II}250$	TAF1		TBP
P300/CBP	P300		CBP
			PCAF, GCN5
	CBP		P300
			PCAF, GCN5
HDACs			
Class I	HDAC1		RB1
			SIN3/SAP18/HDAC2
	HDAC2		SIN3/SAP18/HDAC1
	HDAC3		NCOR/TAB2
	HDAC8	H3, H4	
Class II	HDAC4	all 4 core Histones	MEF2
			Calmodulin
	HDAC5		MEF2
			SMRT/HDAC7
	HDAC6		Tubulin
	HDAC7		MEF2D
			SMRT/HDAC5
	HDAC9		MEF2
Class III	SIRT1	H3, H4	P53
			FOXO3
	SIRT2		Tubulin
	SIRT3		
	SIRT4		
	SIRT5		
	SIRT6		
	SIRT7		

negatively charged DNA (Figure 7.4.B). This mitigates their interaction. As a consequence, the affected DNA changes its nucleosomal conformation and becomes more accessible to transcription factors. This mechanism modulates the regulation of transcription, and certain HAT enzymes correspond to key transcriptional coactivators. They include P300/CBP and PCAF.

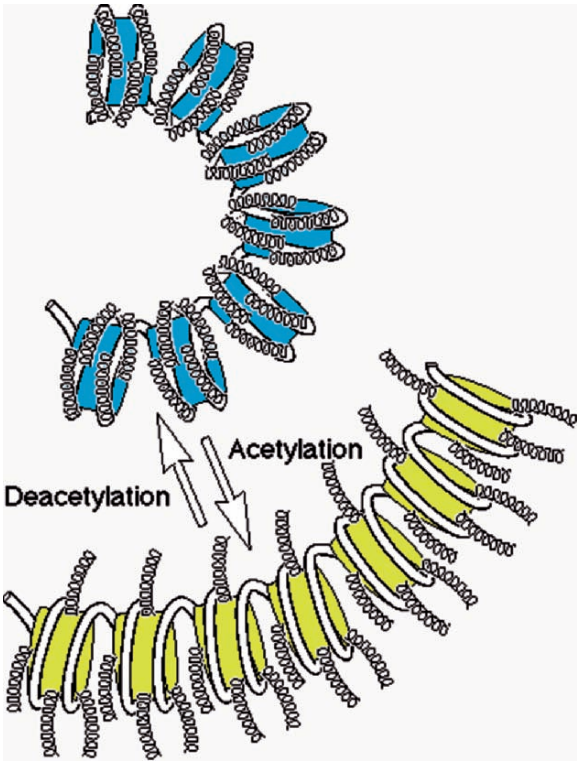


Figure 7.4.B. Histone acetylation and deacetylation. The Histone acetylation level determines DNA accessibility and transcription. In a state of hypoacetylation (blue), there are strong internucleosomal interactions. The Histone tails constrain wrapping of the DNA on the nucleosome surface. In a state of hyperacetylation (yellow), weak internucleosomal interactions are prevalent. The Histone tails do not constrain the DNA, which thus becomes accessible to transcription factors. [Reproduced from http://www.average.org/~pruss/Nucleosomes/Ac/ acetyl.html. There are instances where we have been unable to trace or contact the copyright holder. If notified the publisher will be pleased to rectify any errors or ommissions at the earliest opportunity.]

All Acetyl Transferases use Acetyl Coenzyme A as the common acetyl donor, but they exhibit high specificity for their acetyl acceptors. All HAT proteins are associated with large multiprotein complexes. HATs are divided into several families on the basis of highly conserved structural motifs. These include:

– The GNAT (GCN5-Related *N*-Acetyl Transferases) family comprising GCN5 and PCAF
– The MYST (Monocytic Leukemia Zinc-Finger Protein) family comprising TIP60, HBO1, MOZ, and MORF
– The SRC family of Steroid Receptor Coactivators comprising SRC-1, SRC-3, NCOA3 (AIB1), TIF-2, and GRIP1
– The ATF-2 family comprising ATF-2 (CREBP1, CREB-2)
– The $TAF_{II}250$ family
– The P300/CBP family

HAT enzymes also target non-Histone protein substrates, including transcription factors such as E2F, P53, or GATA1, and are sometimes referred to as FATs (Factor Acetyl Transferases). Besides being characterized by their HAT domains, many HATs have a 110 amino acid bromo-domain, which is characteristic of transcriptional regulatory proteins. The bromodomain recognizes and interacts with acetylated lysine on the target protein. The HATs P300/CBP and PCAF have transcriptional coactivator activity.

HDACs form multiprotein complexes that are primarily involved in the repression of gene transcription by virtue of the compaction of the chromatin structure that accompanies the removal of charge neutralizing acetyl groups from the Histone lysine tails. There are four classes grouping the 18 known HDACs:

– Class I comprises HDAC-1, -2, -3, and -8
– Class II includes HDAC-4, -5, -6, -7, -9, and -10
– Class III entails SIRT-1, -2, -3, -4, -5, -6, and -7
– Class IV has HDAC-11 as the only member

Class I HDACs display some sequence homology to members of the classes II and IV, but not to those of class III. Class I, II, and IV HDACs are zinc-dependent enzymes, whereas the Deacetylase activity of class III members is NAD^+ dependent.

Like HATs, HDACs also have targets distinct from Histones, including the transcription factors P53, E2F, GATA1, TFII-E, and TFII-F.

Transcriptional repression by nuclear hormone receptor corepressors occurs through the recruitment of NCOR (Nuclear Receptor Co-Repressor) and SMRT (Silencing Mediator of Retinoic X Receptor and Thyroid Receptor). These proteins associate with HDAC-1 and HDAC-2 for transcriptional repression. RB recruits HDAC 1 to the E2F regulated *cyclin E* promoter.

- Aberrant Histone acetylation, caused by the disruption of HAT or HDAC activity, may be associated with the development of cancer through the regulation of oncogene expression or tumor suppressor gene silencing. Genes that encode HAT enzymes are translocated, amplified, overexpressed, or mutated in various hematologic and epithelial cancers. Two closely related HATs, CBP and P300, are altered in some tumors by either

mutation or translocation. Missense mutations in P300, and mutations encoding truncated P300, arise in colorectal and gastric primary tumors and in other epithelial cancers. In these cases, the second allele is frequently deleted generating a loss of heterozygosity. Loss of heterozygosity of *p300* is also associated with 80% of glioblastomata and loss of heterozygosity around the *cbp* locus occurs in hepatocellular carcinomata.

- Translocations of *cbp* and *p300*, resulting in in-frame fusion with a number of genes, may underlie several hematologic malignancies. Individuals with the developmental disorder Rubinstein–Taybi syndrome carry a mutation in CBP that inactivates its HAT activity, and increases the risk of cancer [Petrij et al. 1995; Murata et al. 2001], particularly malignant tumors of the head [Miller and Rubinstein 1995].
- HDACs are involved in mediating the function of oncogenic translocation products in specific forms of leukemia and lymphoma. *moz* (*myst3*, *znf220*) may be fused to *tif2* (*transcriptional mediator/intermediary factor 2*) in the forms of leukemia associated with chromosome 8 inversion inv(8)(p11;q13). The translocation t(8;16)(p11;p13) is a cytogenetic hallmark for the M4/M5 subtype of acute myeloid leukemia (AML). This form of AML displays monocytic differentiation, erythrophagocytosis by the leukemic cells, and a poor response to chemotherapy. The chromosome fusion generates a MOZ-CBP fusion protein.
- The oncoprotein that is encoded by one of the translocation-generated fusion genes in acute promyelocytic leukemia, PML-RARα, represses transcription by associating with a corepressor complex that contains HDAC activity [Di Croce et al. 2002].
- In non-Hodgkin lymphoma, the transcriptional repressor BCL-6 (B-Cell Lymphoma 6, LAZ3, Lymphoma-Associated Zinc Finger-3, ZNF51) is overexpressed. This causes aberrant transcriptional repression through the recruitment of HDACs, leading to lymphoid oncogenic transformation.
- AML subtype M2 is associated with the t(8;21) chromosomal translocation, which produces an AML1-ETO fusion protein, a potent dominant transcriptional repressor through its recruitment of HDAC activity. Imbalance in Histone acetylation can lead to changes in chromatin structure and transcriptional dysregulation of genes that are involved in the control of cell cycle progression, differentiation, or apoptosis [Marks et al. 2001].
- Increased expression of the coactivator proteins that mediate Estrogen Receptor activity leads to estrogen independence. NCOA3 (AIB1) may be overexpressed due to amplification in breast cancer.

Histone methylation. Histones are frequently methylated on lysine or arginine residues. Histone Methyl Transferases (HMTs) are enzymes that catalyze the transfer of 1–3 methyl groups from the cofactor S-adenosylmethionine to lysine or arginine residues of Histone proteins. SET (Su(var)3–9, Enhancer of Zeste, Trithorax) domains and PR (PRDI/BF1/RIZ homology region) domains are catalytic core motifs characteristic of Lysine Methyl Transferases. The SET domain comprises 120–150 amino acids, while the PR domain has a length of about 130 amino acids. SET and PR domains share sequence homology. HMT may act as tumor suppressors.

– RIZ1 (RB-Binding Zinc Finger Protein, PRDM2) is a member of the S-adenosylmethionine dependent Methyl Transferases, which contains a PR domain. RIZ1 binds to RB and acts as a tumor suppressor. It also contributes to B-lymphocyte differentiation. Due to alternative promoter usage, *riz* {1p36} produces 2 mRNA messages, only the full-length gene product RIZ1 contains the PR domain and has tumor suppressor function [Derunes et al. 2005].
– PRDM1 (BLIMP1, PRDI-BF1) {6q21-q22.1} is a transcriptional repressor of *c-myc*. Its expression drives the terminal differentiation of B-lymphocytes.
– MLL1 (Myeloid or Lymphoid Leukemia, Mixed Lineage Leukemia, ALL1, TRX1, CXXC7) is a 431 kD protein with zinc finger-like domains, AT hook motifs, and a Methyl Transferase homology domain. The oncogenic function of *mll1* {11q23} is activated in acute leukemia by chromosomal translocations. The resulting fusion proteins lack the MLL1 SET domain. This may have a dominant negative effect on wild-type MLL1.
– SUV39H1 (Suppressor of Variegation 3–9 Homolog 1) {Xp11.23} and SUV29H2 {10} methylate Histone H3 on lysine 9 and create a binding site for HP1 (Heterochromatin Protein 1). The catalytic motif is contained in the SET domain, which requires adjacent cysteine rich regions to confer Histone Methyl Transferase activity. SUV39H1 is a RB binding protein that can be recruited by the RB/E2F complex for the transcriptional repression of E2F responsive promoters. Histone methylation may regulate genome stability [Peters et al. 2001].

SUV39H1 and SUV29H2 are required for correct chromosome segregation. Loss of SUV39H function impairs heterochromatin and genome stability. This may lead to B-cell lymphomata.
– NSD1 (ARA267) acts as a transcriptional coregulator in conjunction with the Androgen Receptor.
– The serine/threonine kinase MDS1 {3q26} and the transcriptional repressor EVI1 {3q26} may be fused. *mds1* exists in normal tissues both as a unique transcript and as a normal fusion transcript with *evi1*, with an additional 188 codons at the 5′ end of the *evi1* open reading frame. This additional region has about 40% homology at the amino acid level with the PR domain of RIZ [Fears et al. 1996].
– HRMT1L2 (PRMT1, IR1B4) {19q13} is a Protein Arginine Methyl Transferase that functions as a Histone Methyl Transferase that is specific for H4. PRMT1 also methylates nRNPA1. Three splice variants of *prmt1*, encode polypeptides of 343 amino acids (variant 1), 361 amino acids (variant 2), and 347 amino acids (variant 3). The full-length protein (variant 3) contains an in-frame stop codon in the middle of exon 3, and a downstream start codon that resumes transcription. There is ubiquitous expression of all three splice variants, with the highest levels in cerebellum, mammary gland, prostate, brain, and thyroid.

- Gene silencing of the HMT gene *riz1* is common in carcinomata of the breast, liver, colon, and lung, as well as in melanoma, osteosarcoma, and neuroblastoma. RIZ1 is also subject to mutations in cancer.
- In cancer, the *mds1-evi1* gene may be subject to viral integrations or translocations, generating a short gene product that lacks the PR domain. The short protein may act in a dominant negative fashion and is oncogenic in myeloid cells.
- Mutation in the SET gene *nsd1* {5q35} causes Sotos syndrome (cerebral gigantism), which is characterized by the overgrowth of neural tissues, heart defects, and an increased risk of cancer. A fraction of Beckwith–Wiedemann syndromes is also due to mutations in *nsd1*. In childhood AML, the translocation t(5;11)(q35;p15.5) juxtaposes and fuses *nsd1* with *nup98*.
- Variants 1 and 2 of the H4-specific HMT HRMT1L2 are frequently downregulated in breast cancers in comparison to normal breast tissue [Scorilas et al. 2000].

Histone phosphorylation. The phosphorylation of Histone H3 on serine 10 in nucleosomes containing JUN and FOS is correlated with the activation of gene expression. Like acetylation, phosphorylation occurs in the Histone tail. The condensation of chromatin during cell division involves Histone modifications, specifically the phosphorylation of serine 10 on Histone H3.

Apoptosis is regulated, in part, by phosphorylation of serine 14 in the tail of Histone H2B. This event may trigger the chromatin condensation that is followed by DNA fragmentation. The active kinase in this process is MST1, which is induced by Caspase-3.

Chromatin plasticity. SWI/SNF complexes are ATP-dependent chromatin remodeling enzymes that have global functions in transcription. They are implicated in the regulation of gene expression and cell cycle control and act by unwinding the chromatin in the vicinity of the promoters they activate.

The SWI/SNF complex acts in concert with other mechanisms of chromatin modeling, such as Histone acetylation. The SWI-2 subunit contains a bromo-domain. This implies a mechanism for its recruitment to acetylated Histone.

SMARCA4 (SWI/SNF-Related Matrix-Associated Actin-Dependent Regulator of Chromatin A4, BRG-1, Brahma Related Gene-1, SNF2β) [Khavari et al. 1993] is a 205 kD nuclear protein that contains a proline-rich domain, six sequence motifs characteristic of DNA-dependent ATPases, and a bromo-domain. BRCA1 can directly interact with the SMARCA4 subunit of the SWI/SNF complex and mediate its coactivator function on *p53* transcription through this complex. SMARCA4 is also required for RB signaling to specific cell cycle targets. Furthermore, FANC-A associates with SMARCA4. This interaction may recruit the SWI/SNF complex to target genes, thereby enabling coupled nuclear functions, such as transcription and DNA repair [Otsuki et al. 2001].

- The *smarcB1* (*ini1*, *integrase interactor 1*, *snf5*) gene {22q11} encodes a protein component of the SWI/SNF chromatin remodeling complex. The *ini1* gene is often mutated or deleted in malignant rhabdoid tumors [Reincke et al. 2003]. Two forms of INI1, that differ by the variable inclusion of nine amino acids, potentially are produced by differential RNA splicing. Either form of INI1 induces a dramatic change in morphology, growth suppression, and cell cycle arrest in rhabdoid tumor cells. Senescence-associated proteins are upregulated, while levels of proteins implicated in cell cycle progression are downregulated.

- SMARCA4 (BRG-1) plays a role in familial breast cancer. Germline mutations in the tumor suppressor gene *brca1* predispose individuals to breast and ovarian cancers. BRCA1 is associated with the chromatin remodeling complex SWI/SNF through a direct interaction with the SMARCA4 (BRG-1) subunit. The activation of transcription by P53 completely depends on the integrity of this complex. This implies a link between chromatin remodeling and hereditary breast cancer [Bochar et al. 2000].

7.5 INTERMEDIARY METABOLISM

Enzymes for the transport and disposition of xenobiotics are essential in metabolizing and detoxifying environmental and chemical carcinogens. Changes in their activities affect the exposure to genotoxic compounds and hence the risk of transformation. Genetic variation in these enzymes is a major cause of interindividual differences in the susceptibility to cellular transformation and in the response to anticancer drugs.

7.5.1 Transport

Membrane transporters play important roles in the absorption, distribution, and elimination of numerous compounds (Table 7.5.1.A).

– Efflux transporters typically contain two nucleotide binding domains and at least two transmembrane domains. Their transmembrane domains define the substrate specificity. Efflux transporters prevent toxins from entering vital organs at the blood–brain barrier, the blood–testis barrier, the placenta, and the ovaries. They contribute to multidrug resistance and belong to the ABC (ATP-binding cassette) superfamily. ATP hydrolysis provides the energy for substrate

Table 7.5.1.A. Drug transporters

Efflux transporters

ABCA1 (ABC-Binding Cassette Protein A1, CERP): cholesterol efflux pump

ABCB1 (MDR1, P-Glycoprotein, PGY1): 2 transmembrane domains with 6 membrane spanning domains each

ABCCs (MRPs, MDR-Associated Proteins)
- ABCC1 (MRP1) 3 transmembrane domains, transport of organic anions
- ABCC2 (MRP2, cMOAT) 3 transmembrane domains, transport of anionic conjugates
- ABCC3 (MRP3) 3 transmembrane domains, preference for the transport of glucuronide conjugates
- ABCC4 (MRP4) 2 transmembrane domains, transport of cAMP and cGMP
- ABCC5 (MRP5) 2 transmembrane domains, transports nucleotide analogs and glutathione conjugates
- ABCC6 (MRP6) 3 transmembrane domains, transports glutathione conjugates
- ABCC7 (MRP7, CFTR) transports 17β-estradiol glucuronide
- ABCC11 (MRP8)
- ABCC12 (MRP9)

ABCDs: peroxisomal transporters
- ABCD1
- ABCD2
- ABCD3
- ABCD4

ABCGs: efflux of cellular lipids, including cholesterol and phospholipids
- ABCG1, macrophages
- ABCG2 (MXR, Mitoxantrone Resistance Protein; Breast Cancer Resistance Protein, BCRP): a half-transporter that needs to dimerize to form a functional transporter
- ABCG5 (Sterolin-1) intestines and liver
- ABCG8 (Sterolin-2) intestines and liver

Uptake transporters (Solute Carrier Family, SLC)

Organic cation transporters: 2 families, OCT and OCTN, contain 12 transmembrane domains – only 11 in OCTN1, contain a nucleotide-binding motif
- SLC22A1 (OCT1)
- SLC22A2 (OCT2)
- SLC22A3 (OCT3)
- SLC22A4 (OCTN1)
- SLC22A5 (OCTN2)

(continued)

Table 7.5.1.A. (continued)

Organic anion transporters: 2 families, OAT and OATP, contain 8–12 transmembrane domains
– SLC22A6 (OAT1)
– SLCC22A7 (OAT2)
– SLC22A8 (OAT3)
– SLC22A11 (OAT4)
– SLC21A3 (OATP1, OATP-A)
– SLC21A6 (OATP2, OATP-C, LST1)
– SLC21A8 (OATP8)
– SLC21A9 (OATP-B)
– SLC21A11 (OATP-D)
– SLC21A12 (OATP-E)
– SLC21A14 (OATP-F)
Nucleoside transporters: uptake of purine and pyrimidine nucleosides, equilibrative and concentrative transporters
– SLC29A1 (ENT1, Equilibrative Nucleoside Transporter 1)
– SLC29A2 (ENT2)
– SLC28A1 (CNT1, Concentrative Nucleoside Transporter 1)
– SLC28A2 (CNT2)
– SLC28A3 (CNT3)
Glucose transporters
– SLC2A1 (GLUT1)
– SLC2A2 (GLUT2)
– SLC2A3 (GLUT3)
Peptide transporters (hydrogen ion/peptide cotransporters): contain 12 transmembrane domains
– SLC15A1 (PEPT1)
– SLC15A2 (PEPT2)
Neurotransmitter transporters
– SLC6A1 (γ-amino butyric acid transporter)
– SLC6A2 (norepinephrine transporter)
– SLC6A3 (dopamine transporter)
– SLC6A4 (serotonin transporter)
– SLC6A5 (glycine transporter)
– SLC6A6 (taurine transporter)
– SLC6A7 (L-proline transporter)
– SLC6A8 (creatine transporter)
Cationic amino acid transporters: principal transporter of the cationic amino acids, arginine, lysine, and ornithine
– SLC7A1 (ATRC1, HCAT1)
– SLC7A2 (ATRC2, HCAT2)
– SLC7A3

translocation. In the intestines, efflux transporters mediate the ejection of resorbed molecules back into the lumen, thus limiting their bioavailability.

– Solute carrier uptake transporters (SLC) facilitate the cellular uptake of molecules that cannot diffuse through the cell membrane. In the intestines, they regulate food absorption. Organic ion transporters are abundantly expressed in liver and kidney, where they take up organic molecules from the blood. This is an early step for drug metabolism in the liver and for excretion in the kidneys. In the liver, organic ion transporters are also important to maintain bile flow.

ABCB1, ABCG2, and ABCC1 are promiscuous efflux transporters of both hydrophobic and hydrophilic compounds. Stem cells often express high levels of specific ABC membrane transporters. Whereas hematopoietic stem cells are characterized by high levels of ABCG2, the *abcg2* gene is silenced in most committed progenitor cells and mature blood cells [Scharenberg et al. 2002].

- ABCB1 (MDR1, P-Glycoprotein, PGP) {7q21.1} is an efflux transporter frequently highly expressed in cancer cells. ABCB1 transports a wide range of hydrophobic neutral or cationic compounds. It constitutes an important mechanism for resistance to cancer chemotherapeutics.
- *abcb1* influences the susceptibility to develop renal epithelial tumors. The polymorphism C3435T is associated with expression levels and modulates

disease risk. Especially T and TT carriers are at risk for developing nonclear cell renal cell carcinoma, including papillary and chromophobe renal cell carcinoma as well as oncocytic adenomata [Siegsmund et al. 2002].

- The C3435T *abcb1* polymorphism may involve both the susceptibility to and the clinical outcome of childhood ALL. Carriers of the TT genotype are more at risk of developing ALL than other individuals, whereas CC genotype carriers may have worse prognosis [Jamroziak et al. 2004].
- The expression of the nucleoside transporter SLC29A1 (ENT1) {6p21.2-p21.1} in various tumors suggests a role in the cellular response to nucleosides and their analogs.
- Increased glucose uptake and utilization is exhibited by malignant cells. The principal mechanism, by which transformed cells achieve this, is the overexpression of the Glucose Transporter protein family. The expression levels of Glucose Transporters may correlate with tumor grade. The possible relationship between GLUT1 (Glucose Transporter-1, SLC2A1) expression and tumor blood supply suggests that malignant cells may have an adaptive ability to compensate for a compromised microenvironment [Mendez et al. 2002].

7.5.2 Disposition

Biotransformation renders lipophilic agents (mostly xenobiotics) more hydrophilic to facilitate their elimination. Phase I drug metabolizing enzymes introduce polar functional moieties (amino-, carboxyl-, sulfhydryl-, hydroxyl-) and typically decrease chemical reactivity. Phase II drug metabolizing enzymes catalyze the addition of small endogenous molecules (glucuronidation, sulfation, glutathione conjugation, acetylation) to facilitate excretion [Williams 1949]. Genetic variations in these metabolizing enzymes can be associated with substantial clinical consequences. They may affect the susceptibility to transformation through modulating the concentrations of carcinogens or of protective compounds.

Cytochrome P450. Cytochromes P450 [Omura and Sato 1962] (CYPs, Mixed Function Oxidases, MFOs, Heme-Thiolate Monooxygenases) constitute a superfamily of enzymes with an iron/protoporphyrin prosthetic group that is central to the function of these enzymes. The iron/protoporphyrin is bound to the sulfur of cysteine and can form a complex with carbon monoxide (the heme/carbon monoxide complex conveys maximum light absorbance near 450 nm). The common motif of all CYP enzymes is the sequence $FX_{6-9}CXG$ near the COOH-terminus. This catalytic center transfers one atom of atmospheric oxygen to the substrate, while the other is reduced to water. Cytochromes P450 have an absolute requirement for molecular oxygen and NADPH. A complete functional catalytic complex requires Cytochrome P450, NADPH–Cytochrome P450 Reductase, and phosphatidylcholine. The phospholipid contributes to a negatively charged environment at a neutral pH. Cytochrome P450s are membrane bound in the smooth endoplasmic reticulum. They are most abundant in the liver and hepatic metabolism is the most important route for xenobiotics (first pass metabolism).

The wide range of functions for Cytochrome P450s necessitates many distinct CYP450 molecules, comprising 18 gene families that encode 57 enzymes (Figure 7.5.2.A). Furthermore, genetic variation exists for all major *cyp* genes. For some of them, allelic variants are the results of single nucleotide polymorphisms (SNPs) that create altered splice sites, frameshifts, premature stop codons, or missense mutations, which result in loss-of-function alleles. In other cases, SNPs cause changes in the amino acid sequences of the encoded enzymes that lead to changes in the catalytic activity. In some cases, up to 12 additional copies of a *cyp* gene can exist in tandem, their gene products generating an ultrahigh metabolism phenotype. The *cyp* genes are highly inducible by various influences, which are mediated predominantly through the Aryl Hydrocarbon Receptor and members of the nuclear receptor superfamily. The prolonged exposure to drugs or xenobiotics may cause the transcriptional induction of the relevant *cyp* genes and lead to enhanced metabolism of these and other agents. Inducers of *cytochrome P450* genes may also stimulate the hydroxylation of androgens, estrogens, progestagens, glucocorticoids, bilirubin, and vitamin D, decreasing their biological activity.

CYP enzymes catalyze the metabolism of a wide range of endogenous and exogenous substrates. They may be involved in the inactivation of carcinogens or in the activation of procarcinogens. During their oxidative metabolism, procarcinogens form reactive metabolites capable of binding

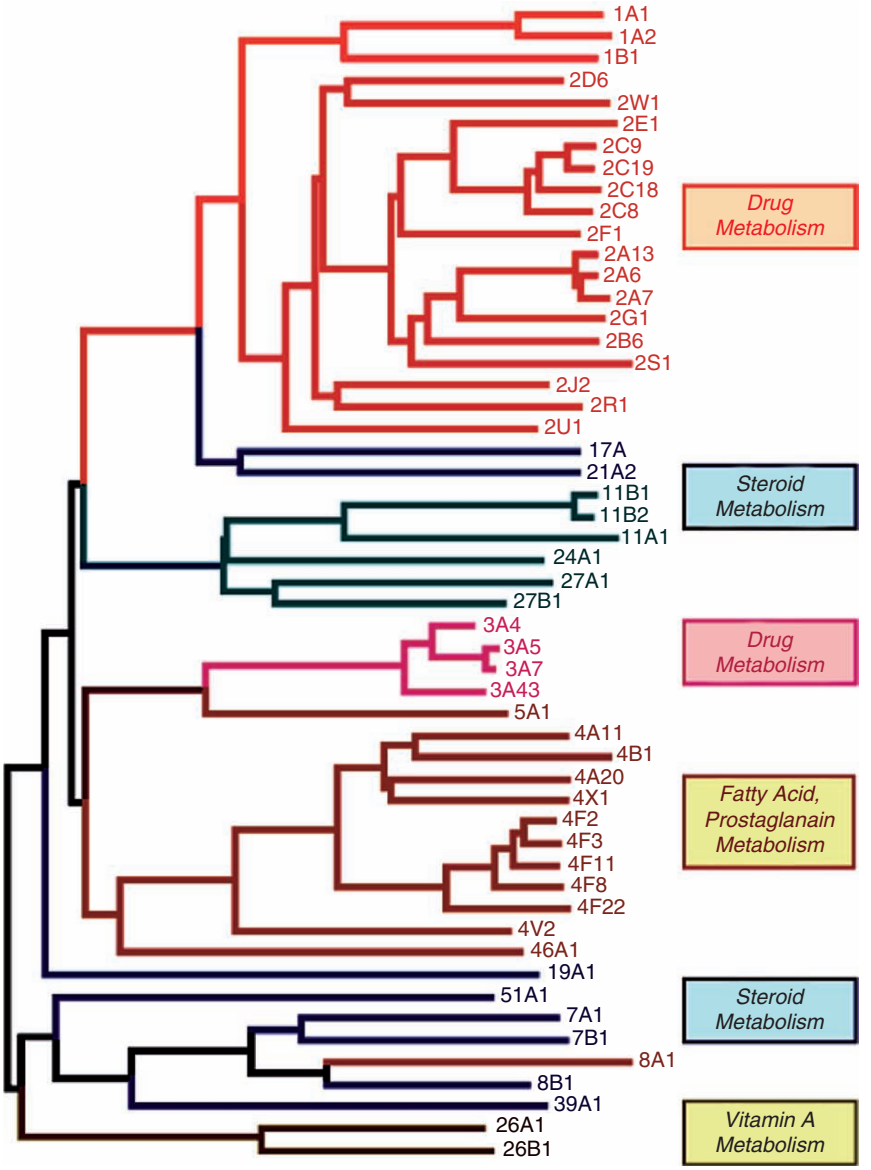


Figure 7.5.2.A. Gene family of Cytochrome P450s. A phylogenetic tree of human Cytochrome P450 genes and their associated biological functions. [Reproduced from http://www.aist.go.jp/aist_e/ aist_today/2002_04/hot_line/hot_line_23.html. With permission.]

to biopolymers, such as proteins and nucleic acids [Grover and Sims 1968]. This can lead to mutagenicity and carcinogenicity. Activating functions include the conversion of

– aryl amines, benzo-*a*-pyrene, and other polycyclic aromatic hydrocarbons by CYP1A1; polycyclic aromatic hydrocarbons (PAHs) are present in the environment from industrial combustion and tobacco products
– toluene and heterocyclic amines by CYP1A2; CYP1A2 in the stomach activates aryl amines from cigarette smoke to mutagens, which may cause gastric cancer
– nitropyrenes by CYP1B1
– benzene, butadiene, chloroform, and vinyl chloride by CYP2E1
– aflatoxin by CYP1A2 and CYP3A4.

• Polymorphisms in *cyp1A1*, the product of which is distributed extrahepatically including the lungs, are associated with modifications in the risk for lung and prostate cancer.

- Chemical carcinogens often require metabolic activation in order to be able to bind to DNA and contribute to cancer causation. The Cytochrome P450 1A2 (CYP1A2) *F allele is involved in the metabolic activation of polycyclic aromatic hydrocarbons and may be associated with an increased risk of colon cancer. A positive association exists between the development of colorectal cancer and the mutant homozygous genotype in *msp1* polymorphism of *cyp1A1* gene in a Japanese population.
- The expression of CYP1B1 is predominantly extrahepatic with high amounts in the endometrium. CYP1B1 is important in steroid metabolism because it catalyzes the 4′ hydroxylation of estradiol. The *cyp1B1* gene has 24 allelic variants. Polymorphisms in *cyp1B1* modulate the risk for endometrial cancer [Sasaki et al. 2003].
- Specific variant alleles of *cyp2C8*, *cyp2C9*, *cyp2C19*, *cyp2D6*, *cyp3A4*, *cyp2A6*, and *cyp2B6* have been correlated with increased cancer risk in epidemiological studies. However, the responsible environmental procarcinogens remain to be identified.

Flavin Monooxygenases. Flavin-Containing Monooxygenases (FMOs, Dimethylaniline Monooxygenases, Dimethylaniline N-Oxidases) are microsomal enzymes that catalyze the oxygenation of nucleophilic heteroatom containing xenobiotics through a mechanism that requires NADPH and oxygen. This serves to increase water solubility and generally to decrease toxic potential. Of the diverse nitrogen functional groups in xenobiotics, only secondary and tertiary acyclic amines, cyclic amines, arylamines, hydroxylamines, and hydrazines are oxidized by FMO. S-oxidation occurs almost exclusively by FMOs. These enzymes have at least 80% homology to each other and include:

– FMO1 {1q23-q25} predominantly expressed in fetal liver
– FMO2 {1q} most abundant in the lungs
– FMO3 {1q23-q25} in adult liver and brain
– FMO4 {1q} in adult liver
– FMO5 {1q21.1} in the liver

Nitric oxide ($NO^{\cdot}$) modifies the functions of a variety of proteins containing cysteine thiols or transition metal centers by S-nitrosylation. In inflamed liver, which may be associated with tumors, nitric oxide is overproduced and hepatic FMO are rigorously suppressed. Nitric Oxide-mediated S-nitrosylation results in the suppression of FMO based drug metabolism or detoxification [Ryu et al. 2004].

- Secondary *N*-alkylarylamines can be *N*-oxygenated to reactive *N*-hydroxylated metabolites that are responsible for the mutagenic and carcinogenic activities of the aromatic amines. Chemically unstable hydroxylamine intermediates of aromatic amines degrade into bladder carcinogens.
- The hydroxamic acid intermediates of *N*-arylacetamides are bioactivated into liver carcinogens.

UDP–Glucuronosyl Transferases. UDP–Glucuronosyl Transferases are Phase II enzymes that are located in the endoplasmic reticulum of the liver and the intestinal epithelial cells. Two subfamilies comprise UGT1 and UGT2. They utilize the activated form of glucuronic acid, uridine diphosphate glucuronic acid (UDPGA). Glucuronide formation is one of the most common routes of phase II metabolism. The glucuronide conjugates are excreted into the kidneys or the intestinal tract.

- The induction of bladder carcinogenesis may occur as a result of the glucuronidation of *N*-hydroxylarylamine. The resulting *O*-glucuronides become concentrated in the urine, where they are hydrolyzed by the acidic pH. They may further react to electrophilic arylnitrenium species, which can bind covalently to nucleic acids and proteins, thus initiating carcinogenesis.

Glutathione conjugating enzymes. Glutathione S-Transferases are Phase II enzymes that catalyze the conjugation of reduced glutathione to various substrates. Cytosolic Glutathione S-Transferases form a superfamily consisting of four distinct families, named α, μ, π, ο, κ, and θ. They may detoxify solvents and pesticides. Specifically, the Glutathione Transferases μ1, θ1, and π1 are involved in the detoxification of polycyclic aromatic hydrocarbons. A member of the μ class gene family (*gstm1*) {1p13.3} is polymorphic and is only expressed in 55–60% of individuals.

- The risk for cancer of the proximal colon is increased about twofold in carriers of the *gstm1* null allele [Zhong et al. 1993]. Furthermore the age of onset of colon cancer may be affected by the genotypes of *gstm1* and *gstt1* [Chenevix-Trench et al. 1995].
- Null genotypes of *gstm1* and *gstt1* are associated with an increased risk of bladder cancer.
- A polymorphism of Glutathione Peroxidase places either a leucine or a proline at codon 198 of GPX1 {3p21.3}. The GPX1 proline/leucine

genotype, compared to the GPX1 proline/proline genotype, may significantly increase the risk of bladder cancer and may influence its disease status [Ichimura et al. 2004].

- Cysteine, the redox active amino acid in glutathione, can protect from the carcinogenic effects of acetaldehyde, which reaches high levels in smokers and alcoholics.

Acetyl Transferases. Acetylator phenotype is a common genetic trait. It results from the presence of specific alleles of the genes encoding for Arylamine N-Acetyl Transferase. The highly homologous genes *nat1* {8p23.1-p21.3} and *nat2* {8p23.1-p21.3} code for the genetically invariant and variant *N*-Acetyl Transferase proteins, respectively. NAT1, which is responsible for the *N*-acetylation of certain arylamines, displays no genetic variation, whereas the rapid or slow acetylation of therapeutic and carcinogenic agents is due to variation of NAT2. NAT2 polymorphisms are associated with several disease states, including some cancers.

- Aromatic and heterocyclic amines require metabolic activation to electrophilic intermediates that initiate carcinogenesis. For cancers in which *N*-acetylation is negligible and *O*-acetylation is an activation step, such as for colorectal cancer induced by heterocyclic amines, NAT2 rapid acetylator phenotype is at higher risk [Lang et al. 1986]. Individuals that are both rapid acetylators and exhibit a high Cytochrome P450 1A2 activity may have an even higher risk of colorectal cancer.
- *N*-acetylation is a detoxification step for aromatic amines. The slow *N*-acetylation phenotype is a susceptibility factor in occupational and smoking related urinary bladder cancer. The cancer risk is particularly high in the slowest NAT2 acetylator phenotype or genotype.

Other enzymes. Sulfate conjugation causes increased water solubility and a reduced pKa. It is important in the biotransformation of steroid hormones, catecholamine neurotransmitters, Thyroxine, and bile acids. Sulfotransferases (SULT) catalyze either the bioactivation or the detoxification of a wide range of promutagens and procarcinogens. There are two SULT families, SULT1 and SULT2. The *sult1A1* gene {16p12.1-p11.2} possesses a G/A polymorphism that results in an arginine to histidine amino acid substitution in position 213. The histidine allele has low activity and low thermal stability. Reduced bladder cancer risk is associated with this allele [Zheng et al. 2003].

The enzyme Cystathionine β-Synthase reduces homocysteine levels and thus may protect against colorectal cancers. The *cbs* gene {21q22.3} has a variant, 844ins68, which is linked with increased activity. This variant may be involved in the development of colorectal cancers with aberrant DNA methylation and microsatellite instability [Shannon et al. 2002].

REFERENCES

Alves dos Santos MCM. 2001. Ubiquitin system-dependent regulation of growth hormone receptor signal transduction and effects of oxidative stress. Utrecht, The Netherlands.

Azmi P, Seth A. 2005. RNF11 is a multifunctional modulator of growth factor receptor signalling and transcriptional regulation. European Journal of Cancer 41:2549–2560.

Baumann CA, Ribon V, Kanzaki M, Thurmond DC, Mora S, Shigematsu S, Bickel PE, Pessin JE, Saltiel AR. 2000. CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 407:202–207.

Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F, Shiekhattar R. 2000. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. Cell 102:257–265.

Buschmann T, Fuchs SY, Lee CG, Pan ZQ, Ronai Z. 2000. SUMO-1 modification of Mdm2 prevents its self-ubiquitination and increases Mdm2 ability to ubiquitinate p53. Cell 101:753–762.

Chenevix-Trench G, Young J, Coggan M, Board P. 1995. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. Carcinogenesis 16:1655–1657.

Ciocca DR, Luque EH. 1991. Immunological evidence for the identity between the hsp27 estrogen-regulated heat shock protein and the p29 estrogen receptor-associated protein in breast and endometrial cancer. Breast Cancer Research and Treatment 20:33–42.

Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA. 1993. Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. Journal of the National Cancer Institute 85:1558–1570.

Clancy JL, Henderson MJ, Russell AJ, Anderson DW, Bova RJ, Campbell IG, Choong DY, Macdonald GA, Mann GJ, Nolan T, Brady G, Olopade OI, Woollatt E, Davies MJ, Segara D, Hacker NF, Henshall SM, Sutherland RL, Watts CK. 2003. EDD, the human orthologue of the hyperplastic discs tumour suppressor gene, is amplified and overexpressed in cancer. Oncogene 22:5070–5081.

Conaway RC, Brower CS, Conaway JW. 2002. Emerging roles of ubiquitin in transcription regulation. Science 296:1254–1258.

Derunes C, Briknarova K, Geng L, Li S, Gessner CR, Hewitt K, Wu S, Huang S, Woods VI Jr, Ely KR. 2005. Characterization of the PR domain of RIZ1 histone methyltransferase. Biochemical and Biophysical Research Communications 333:925–934.

Desterro JM, Rodriguez MS, Hay RT. 1998. SUMO-1 modification of IκBα inhibits NF-κB activation. Oncogene 20:7243–7249.

Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG. 2002. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295:1079–1082.

Dunn DK, Whelan RD, Hill B, King RJ. 1993. Relationship of HSP27 and oestrogen receptor in hormone sensitive and insensitive cell lines. Journal of Steroid Biochemistry and Molecular Biology 46:469–479.

Eladad S, Ye TZ, Hu P, Leversha M, Beresten S, Matunis MJ, Ellis NA. 2005. Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is regulated by SUMO modification. Human Molecular Genetics 14:1351–1365.

Fears S, Mathieu C, Zeleznik-Le N, Huang S, Rowley JD, Nucifora G. 1996. Intergenic splicing of MDS1 and EVI1 occurs in normal tissues as well as in myeloid leukemia and produces a new member of the PR domain family. Proceedings of the National Academy of Sciences USA 93:1642–1647.

Fu J-F, Hsu J-J, Tang T-C, Shih L-Y. 2003. Identification of CBL, a proto-oncogene at 11q23.3, as a novel MLL fusion partner in a patient with de novo acute myeloid leukemia. Genes, Chromosomes and Cancer 37:214–219.

Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM. 2003. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 300:342–344.

Grover PL, Sims P. 1968. Enzyme-catalysed reactions of polycyclic hydrocarbons with deoxyribonucleic acid and protein in vitro. The Biochemical Journal 110:159–160.

Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T. 2001. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. The Journal of Biological Chemistry 276:14537–14540.

Hershko A, Eytan A, Ciechanover A, Haas AL. 1982. Immunochemical analysis of the turnover of ubiquitin-protein conjugates in intact cells. Relationship to the breakdown of abnormal proteins. The Journal of Biological Chemistry 257:13964–13970.

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419:135–141.

Huang H, Regan KM, Wang F, Wang D, Smith DI, van Deursen JM, Tindall DJ. 2005. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. Proceedings of the National Academy of Sciences USA 102:1649–1654.

Ichimura Y, Habuchi T, Tsuchiya N, Wang L, Oyama C, Sato K, Nishiyama H, Ogawa O, Kato T. 2004. Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. Journal of Urology 172:728–732.

Jamroziak K, Mlynarski W, Balcerczak E, Mistygacz M, Trelinska J, Mirowski M, Bodalski J, Robak T. 2004. Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. European Journal of Haematology 72:314–321.

Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annual Review of Immunology 18:621–663.

Khavari PA, Peterson CL, Tamkun JW, Mendel DB, Crabtree GR. 1993. BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. Nature 366:170–174.

Kim YH, Choi CY, Kim Y. 1999. Covalent modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. Proceedings of the National Academy of Sciences USA 96:12350–12355.

Korneeva I, Bongiovanni AM, Girotra M, Caputo TA, Witkin SS. 2000. IgA antibodies to the 27-kDa heat-shock protein in the genital tracts of women with gynecologic cancers. International Journal of Cancer 87:824–828.

Lang NP, Chu DZ, Hunter CF, Kendall DC, Flammang TJ, Kadlubar FF. 1986. Role of aromatic amine acetyltransferase in human colorectal cancer. Archives of Surgery 121:1259–1261.

Lehembre F, Muller S, Pandolfi PP, Dejean A. 2001. Regulation of Pax3 transcriptional activity by SUMO-1-modified PML. Oncogene 20:1–9.

Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ, Chen JD. 2000. Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Molecular and Cellular Biology 20:1784–1796.

Li SJ, Hochstrasser M. 1999. A new protease required for cell-cycle progression in yeast. Nature 398:246–251.

Li SJ, Hochstrasser M. 2000. The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. Molecular and Cellular Biology 20:2367–2377.

Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, Ting JP, Glimcher LH. 1990. A new member of the leucine zipper class of proteins that binds to the HLA DR α promoter. Science 247:1581–1584.

Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proceedings of the National Academy of Sciences USA 96:11364–11369.

Lupher ML Jr, Rao N, Eck MJ, Band H. 1999. The Cbl protooncoprotein: a negative regulator of immune receptor signal transduction. Immunology Today 20:375–382.

Ma Y, Hendershot LM. 2004. The role of the unfolded protein response in tumour development: friend or foe? Nature Reviews Cancer 4:966–977.

Magnifico A, Ettenberg S, Yang C, Mariano J, Tiwari S, Fang S, Lipkowitz S, Weissman AM. 2003. WW domain HECT E3s target Cbl RING finger E3s for proteasomal degradation. The Journal of Biological Chemistry 278:43169–43177.

Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell 88:97–107.

Mao Y, Sun M, Desai SD, Liu LF. 2000. SUMO-1 conjugation to topoisomerase I: A possible repair response to topoisomerase-mediated DNA damage. Proceedings of the National Academy of Sciences USA 97:4046–4051.

Marmorstein R. 2001. Protein modules that manipulate histone tails for chromatin regulation. Nature Reviews Molecular Cell Biology 2:423.

Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. 2001. Histone deacetylases and cancer: causes and therapies. Nature Reviews Cancer 1:194–202.

Mastick CC, Brady MJ, Saltiel AR. 1995. Insulin stimulates the tyrosine phosphorylation of caveolin. The Journal of Cell Biology 129:1523–1531.

Matunis MJ, Coutavas E, Blobel G. 1997. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. The Journal of Cell Biology 135:1457–1470.

Mendez LE, Manci N, Cantuaria G, Gomez-Marin O, Penalver M, Braunschweiger P, Nadji M. 2002. Expression of glucose transporter-1 in cervical cancer and its precursors. Gynecologic Oncology 86:138–143.

Miller RW, Rubinstein JH. 1995. Tumors in Rubinstein-Taybi syndrome. American Journal of Medical Genetics 56:112–115.

Murata T, Kurokawa R, Krones A, Tatsumi K, Ishii M, Taki T, Masuno M, Ohashi H, Yanagisawa M, Rosenfeld MG, Glass CK, Hayashi Y. 2001. Defect of histone acetyltransferase activity of the nuclear transcriptional coactivator CBP in Rubinstein-Taybi syndrome. Human Molecular Genetics 10:1071–1076.

Nateri AS, Riera-Sans L, Da Costa C, Behrens A. 2004. The ubiquitin ligase SCF^{Fbw7} antagonizes apoptotic JNK signaling. Science 303:1374–1378.

New L, Jiang Y, Zhao M, Liu K, Zhu W, Flood LJ, Kato Y, Parry GC, Han J. 1998. PRAK, a novel protein kinase regulated by the p38 MAP kinase. EMBO Journal 17:3372–3384.

Omura T, Sato R. 1962. A new cytochrome in liver microsomes. The Journal of Biological Chemistry 237:1375–1376.

Otsuki T, Furukawa Y, Ikeda K, Endo H, Yamashita T, Shinohara A, Iwamatsu A, Ozawa K, Liu JM. 2001. Fanconi anemia protein, FANCA, associates with BRG1, a component of the human SWI/SNF complex. Human Molecular Genetics 10:2651–2660.

Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A, Opravil S, Doyle M, Sibilia M, Jenuwein T. 2001. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 107:323–337.

Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ, Breuning MH. 1995. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376:348–351.

Pfander B, Moldovan GL, Sacher M, Hoege C, Jentsch S. 2005. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. Nature 436:428–33.

Prapapanich V, Chen S, Nair SC, Rimerman RA, Smith DF. 1996. Molecular cloning of human p48, a transient component of progesterone receptor complexes and an Hsp70-binding protein. Molecular Endocrinology 10:420–431.

Reincke BS, Rosson GB, Oswald BW, Wright CF. 2003. INI1 expression induces cell cycle arrest and markers of senescence in malignant rhabdoid tumor cells. Journal of Cell Physiology 194:303–313.

Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, Mori K, Glimcher LH, Denko NC, Giaccia AJ, Le QT, Koong AC. 2004. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Research 64:5943–5947.

Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM. 2001. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proceedings of the National Academy of Sciences USA 98:5134–5139.

Ryu SD, Yi HG, Cha YN, Kang JH, Kang JS, Jeon YC, Park HK, Yu TM, Lee JN, Park CS. 2004. Flavin-containing monooxygenase activity can be inhibited by nitric oxide-mediated S-nitrosylation. Life Sciences 75:2559–2572.

Ryu SW, Chae SK, Kim E. 2000. Interaction of Daxx, a Fas binding protein, with sentrin and Ubc9. Biochemical and Biophysical Research Communications 279:6–10.

Sachdev S, Bruhn L, Sieber H, Pichler A, Melchior F, Grosschedl R. 2001. PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes and Development 15:3088–3103.

Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N, Dahiya R. 2003. CYP1B1 Gene polymorphisms have higher risk for endometrial cancer, and positive correlations with estrogen receptor α and estrogen receptor β expressions. Cancer Research 63:3913–3918.

Scharenberg CW, Harkey MA, Torok-Storb B. 2002. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood 99:507–512.

Scorilas A, Black MH, Talieri M, Diamandis EP. 2000. Genomic organization, physical mapping, and expression analysis of the human protein arginine methyltransferase 1 gene. Biochemical and Biophysical Research Communications 278:349–359.

Sentis S, Le Romancer M, Bianchin C, Rostan MC, Corbo L. 2005. SUMOylation of the Estrogen receptor α hinge region by SUMO-E3 ligases PIAS1 and PIAS3 regulates ERα transcriptional activity. Molecular Endocrinology 19:2671–2684.

Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. 2002. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 50:520–524.

Sharma M, Li X, Wang Y, Zarnegar M, Huang CY, Palvimo JJ, Lim B, Sun Z. 2003. hZimp10 is an androgen receptor co-activator and forms a complex with SUMO-1 at replication foci. EMBO Journal 22:6101–6114.

Shen Z, Pardington-Purtymun PE, Comeaux JC, Moyzis RK, Chen DJ. 1996. Associations of UBE2I with RAD52, UBL1, p53, and RAD51 proteins in a yeast two-hybrid system. Genomics 37:183–186.

Sherbet GV, Lakshmi MS. 1997. The genetics of cancer. Genes associated with cancer invasion, metastasis and cell proliferation. San Diego/London/Boston/New York/Sydney/ Tokyo/Toronto: Academic Press.

Siegsmund M, Brinkmann U, Schaffeler E, Weirich G, Schwab M, Eichelbaum M, Fritz P, Burk O, Decker J, Alken P, Rothenpieler U, Kerb R, Hoffmeyer S, Brauch H. 2002. Association of the P-glycoprotein transporter MDR1(C3435T) polymorphism with the susceptibility to renal epithelial tumors. The Journal of the American Society of Nephrology 13:1847–1854.

Strahler JR, Kuick R, Hanash SM. 1991. Diminished phosphorylation of a heat shock protein (HSP 27) in infant acute lymphoblastic leukemia. Biochemical and Biophysical Research Communications 175:134–142.

van Kerkhof P, Alves dos Santos CM, Sachse M, Klumperman J, Bu G, Strous GJ. 2001. Proteasome inhibitors block a late step in lysosomal transport of selected membrane but not soluble proteins. Molecular Biology of the Cell 12:2556–2566.

Vidali G, Gershey E, Allfrey VG. 1968. Chemical studies of histone acetylation. The distribution of ε-N-acetyllysine in calf thymus histones. The Journal of Biological Chemistry 243:6361–6366.

Wertz IE, O'Rourke KM, Zhang Z, Dornan D, Arnott D, Deshaies RJ, Dixit VM. 2004. Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. Science 303:1371–1374.

Williams RT. 1949. Detoxication Mechanisms: The Metabolism of Drugs and Allied Organic Compounds. London: Chapman & Hall.

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MC, Hwang LY, Bowcock AM, Baer R. 1996. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nature Genetics 14:430–440.

Wu WJ, Tu S, Cerione RA. 2003. Activated Cdc42 sequesters c-Cbl and prevents EGF receptor degradation. Cell 114:715–725.

Yufu Y, Nishimura J, Nawata H. 1992. High constitutive expression of heat shock protein 90 α in human acute leukemia cells. Leukemia Research 16:597–605.

Zheng L, Wang Y, Schabath MB, Grossman HB, Wu X. 2003. Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case-control study. Cancer Letters 202:61–69.

Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK. 1993. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 14:1821–1824.

CHAPTER 8

INTERACTION OF THE GROUPS OF CANCER-RELATED GENE PRODUCTS

The three main components of molecular carcinogenesis, dysregulated growth, overcoming of replicative senescence, and metastasis formation, are functionally connected. The interdependence of these mechanisms underlies the consistent patterns of epidemiology, histology, and topology of metastasis formation (Table 8.A).

8.1 CROSS-COMMUNICATION AMONG GROWTH FACTOR PATHWAYS

Connections of the RAS pathway. The EGF Receptor can act as a nodal point for the cross-communication among several classes of signal, including G-Protein Coupled Receptors and Integrins, the RAS and PI3-K pathways, and it can also be activated indirectly by IGF-1. IGF-1 partially mediates its survival effect on mammary epithelial cells through the transactivation of the *EGF receptor* and the consequent stimulation of MAPK. Whereas IGF-1 or EGF can induce the expression of early G_1 type Cyclins in mammary cells, IGF-1 is specifically required to traverse the G_1/S checkpoint [Stull et al. 2002].

EGF signaling induces the expression of the inhibitory SMADs -6 and -7, which induce intracellular antagonism to Transforming Growth Factor-β (TGF-β) superfamily signals. In addition, activation of MAP Kinases (ERKs), which may act downstream of the EGF Receptor, can modify TGF-β signals through the direct phosphorylation of SMADs. Activation of MAP Kinase leads to the phosphorylation of R-SMADs in the linker region, which inhibits their nuclear translocation. Conversely, MAP Kinases phosphorylate STATs on serine residues, thus potentiating the primary STAT-activating stimulus. MAP Kinase can also enhance nuclear receptor activation. The coactivator functions of AIB1 and SRC-1 are increased by MAPK-mediated phosphorylation.

The RAF→MEK→ERK and PI 3-K→PKB signaling pathways are often simultaneously activated in response to growth factors. In some cell types, the small guanine nucleotide-binding protein RAS acts as an upstream positive effector of both the RAF and the Phosphatidylinositol 3-Kinase pathways. However, activation of the protein kinase RAF may lead to opposing cellular responses, such as proliferation, growth arrest, or apoptosis. In a differentiation stage-specific manner, PKB can form a complex with RAF and directly phosphorylate it on serine 259 within the regulatory domain. This modification creates a binding site for a 14-3-3 protein, supports the sequestration of RAF, and inhibits the activation of the RAF→MEK→ERK pathway. The PKB-dependent inhibition of RAF occurs in differentiated myotubes, but not in their myoblast precursors. Excessive PKB activity or inhibition of the RAF pathway in myotubes induces a hypertrophic phenotype. In breast cancer cells, this mechanism may shift the cellular response from cell cycle arrest to proliferation [Rommel et al. 1999; Zimmermann and Moelling 1999].

The nonreceptor protein tyrosine kinase SRC is critical for cell proliferation. Certain mutations in SRC cause uncontrolled cell proliferation and transformation There are differences in downstream signaling between SRC and its oncogenic mutant. Ligand-induced SRC signaling is mediated by the GTPase RAP-1 (KREV-1), which activates ERK-1.

Table 8.A. Genes associated with malignancies. The classical cancer genes (oncogenes and tumor suppressor genes) control cell replication. For cancer to occur, additional functions need to be dysregulated: genes that cause cellular senescence have to be inactivated and expression of gene products that mediate metastasis formation is essential. For cell cycle progression and cell dissemination alike, there is a physiologic balance which may be disturbed by excessive activity of promoters or by diminished function of suppressors. Defects in mutator genes give rise to alterations in other cancer associated genes.

Indicative of basic mechanisms of homeostasis in human biology, all groups of genes involved in malignancies consist of promoting and suppressing components. Loss of function in one group or gain of function in the counterbalancing group may each affect the equilibrium of forces and constitute a predisposing factor for malignant growth. Thus, mutations that enhance the function of oncogenes and mutations that inhibit the function of tumor suppressor genes equally pose a risk for uncontrolled growth of the affected cells and similar relationships hold for senescence genes and metastasis genes and their respective suppressors.

Genes	Function	Examples
Oncogenes		
Cell cycle	*Growth factors	EGF, PDGF
	*Growth factor receptors	HER-2, ERBB
	*Signal transduction molecules associated with growth factor receptors	PKB, ABL, RAS
Cell death	*Inhibitors of apoptosis	MORF4, Survivin
Tumor suppressor genes		
Cell cycle	*Receptors	DCC, PTC
	*Signal transduction molecules	P53, RB, APC
Cell death	*Mediators of apoptosis	CD95,TNF
Senescence genes	*Cell cycle regulators	P53, RB, $P21^{CIP1/WAF1}$, FOS
Senescence suppressor genes	*Regulators of telomere length	Telomerase
Metastasis genes	*Homing receptors and their ligands	CD44, Selectins, Osteopontin
	*Proteinases	MMPs
Metastasis	*Adhesion receptors	Cadherins, LCAM, KAI1
suppressor genes	*Proteinase inhibitors	TIMPs
Mutator genes		
DNA sequence fidelity	*Mismatch repair	MSH,PMS
	*Base excision repair	Uracil DNA Glycosylase
	*Nucleotide excision repair	ERCC
	*Repair of double strand breaks	XRCC,RAD50,NSB1
	*Direct damage reversal	Photolyase
	*Repair of transcriptionally active regions	RAD51,BRCA1
Chromosome stability	*Mitosis control	BUB1,PTTA

In addition, the oncogenic SRC mutant Y527F transduces signals through RAS [Xing et al. 2000].

Notch and SHH pathways. Notch family members act as tumor suppressors in the skin, partially through inducing the expression of $p21^{CIP1/WAF1}$ and repression of *c-fos*. SHH signal transduction may counteract this effect. Basal cell carcinomata are frequently caused by aberrant signaling through SHH. These tumors have reduced expression levels of Notch-1, Notch-2, and JAG-1. The absence of Notch-1 leads to aberrant expression of GLI-2, a target transcription factor in the SHH signaling pathway.

Connections of the SMAD pathway. SMADs may regulate STAT signaling through their interactions with the cofactor P300. Alternatively, SMADs may negatively regulate transcription by recruiting corepressors such as TGIF (TGF-β Induced Factor) {18p11.3} or the proto-oncoproteins SKI {1p36.3} and SNO-N, which can all associate with Histone Deacetylase. The nuclear oncoprotein SKI binds directly to SMAD-2, SMAD-3, and SMAD-4 on a TGF-β responsive promoter element. This leads to the recruitment of the nuclear transcriptional corepressor NCOR, and possibly its associated Histone Deacetylase complex, and represses the

SMAD-mediated activation of transcription. This ability to overcome TGF-β induced growth arrest may be responsible for the transforming activity of SKI [Luo et al. 1999].

Connections of the VHL pathway. Solid tumors often have deficiencies in oxygenation. HIF-1 is a transcription factor responsible for the oxygen-dependent regulation of genes that respond to hypoxic challenge. In VHL defective cells, HIF-1 α subunits are stabilized, HIF-1 is constitutively active, and certain hypoxia regulated genes are constitutively expressed. HIF-1 is involved in the cross-regulation of various oncogenic signaling pathways.

– Phosphatidylinositol 3-Kinase activity is often increased under hypoxic conditions. The Phosphatidylinositol 3-Kinase→PKB pathway is inhibited by the phosphatase PTEN. PKB activation leads to the stabilization of HIF-1α, whereas PTEN attenuates its stabilization in response to hypoxia. Mutations in *pten* enhance HIF-1 activated responses.
– HIF-1 interacts with the oncogenic RAS pathway. RAS upregulates the expression of the transmembrane glucose transporter *slc2A1* (*glucose transporter 1*, *glut1*). This occurs in part by a RAS-dependent increase in HIF-1α protein levels that lead to transactivation of the *glut1* promoter. This supports the metabolic requirements of tumor cells. A loss of HIF-1α suppresses tumor growth in RAS-transformed cells.

8.2 CELL CYCLE CONTROL AND SENESCENCE

Cellular senescence is genetically dominantly controlled by senescence genes, which act in part by inducing cell cycle arrest. Hence, many tumor suppressor genes also act as senescence genes. Genetically determined signaling pathways that contribute to senescence and act as tumor suppressors involve P53, RB1, down-modulators of c-FOS (Stress-Induced Kinases), and PTEN.

Telomere dysfunction may result in chromosome breaks and trigger apoptosis. This occurs through P53-dependent as well as P53-independent pathways [Harrington and Robinson 2002]. Various DNA damage stimuli as well as critical telomere shortening stabilize and activate P53 and $P21^{CIP1/WAF1}$. Consequently, P53 mediates some of the adverse effects of shortened telomeres, culminating in cell cycle arrest or apoptosis.

Interleukin-6 (IL-6) and Insulin-Like Growth Factor-1 (IGF-1) are proliferative and survival factors for multiple myeloma cells. These cytokines also upregulate Telomerase activity without alteration in the protein expression levels of TERT (Telomere Reverse Transcriptase). This is mediated by signal transduction through Phosphatidylinositol 3-Kinase and PKB, followed by phosphorylation of TERT. In addition, transcriptional activation of *tert* following the signal by Phosphatidylinositol 3-Kinase, PKB, and NF-κB plays a role [Akiyama et al. 2002].

The FOXO (Forkhead Group Box Gene O) family of winged helix transcription factors are activated in response to oxidative stress and can increase cellular resistance to it. This occurs, in part, through the activation of the expression of *superoxide dismutase*. β-Catenin binds to FOXO and augments its transcriptional activity. This molecular interaction is enhanced in cells exposed to oxidative stress [Essers et al. 2005].

The FOXO transcription factors are regulated by SIRT, a mediator of cellular longevity, and they are crucial in the downstream suppression of the age accelerating effects by Insulin or IGF-1 signaling pathways. Because FOXO transcription factors are also active in and TGF-β signaling, they connect multiple oncogenic pathways to the regulation of senescence.

8.3 CELL CYCLE CONTROL AND DISSEMINATION

Tumorigenesis originates in gain-of-function mutations of oncogenes (growth factors, receptors, and signal transduction molecules regulating the cell division cycle) or loss-of-function mutations of tumor suppressor genes (cell cycle checkpoint mediators, apoptosis inducers) or DNA repair genes. Commonly, they lead to upregulation of cell division. The defining characteristics of both benign and malignant tumors are excessive growth and immortalization. In contrast, only malignant tumors express gene products that mediate invasiveness. Metastatic dissemination is a consequence of aberrant expression or splicing of stress response genes [Weber and Ashkar 2000]. The consistent topology of metastasis formation by specific cancers, such as the high frequency of colony formation in bone and brain by malignant

breast tumors, implies that metastasis gene expression is a necessary consequence of gain of function by specific oncogenes [Zhang et al. 2003]. Cells, in which oncogenic signaling also activates the aberrant expression or splicing of metastasis genes, become malignant (Figure 8.3.A). Cells, in which the genetic program of stress response genes has been terminally silenced can only form benign tumors.

APC pathway. The dissolution of tight junctions is an early event in epithelial–mesenchymal transition. PAR-6 (PARD6A, TAX40) is a regulator of epithelial cell polarity and tight junction assembly. Beside stimulating cell proliferation, the WNT pathway can regulate PAR-6 activity. In astrocytes, WNT1 activates RHO-A, while RHO-GEF associates with APC. APC and GSK-3β link PAR-6 to microtubules and cell polarity. A complex of PAR-6 and PKCζ activates the E3 Ubiquitin Ligase SMURF1. This contributes to cell polarity by ubiquitination and degradation of RHO-A [Wang et al. 2003].

ASEF (APC-Stimulated Guanine Nucleotide Exchange Factor) is a guanine nucleotide exchange factor for RAC, but not for RHO or CDC42. ASEF contains DBL homology, Pleckstrin homology, and SH3 (SRC homology 3) domains. ASEF activity is negatively regulated by its NH_2-terminus. APC binding to this NH_2-terminal region relieves the negative regulation. The Armadillo repeat domain of the tumor suppressor APC constitutes the binding site for ASEF. The APC/ASEF interaction mediates cell flattening, membrane ruffling, and lamellopodia formation [Kawasaki et al. 2000].

The overexpression of cell surface glycoproteins of the CD44 family is an early event in the colorectal adenoma-to-carcinoma sequence. Deregulated CD44 expression is already present in aberrant crypt foci with dysplasia, the earliest detectable lesions of colorectal neoplasia. The WNT pathway may

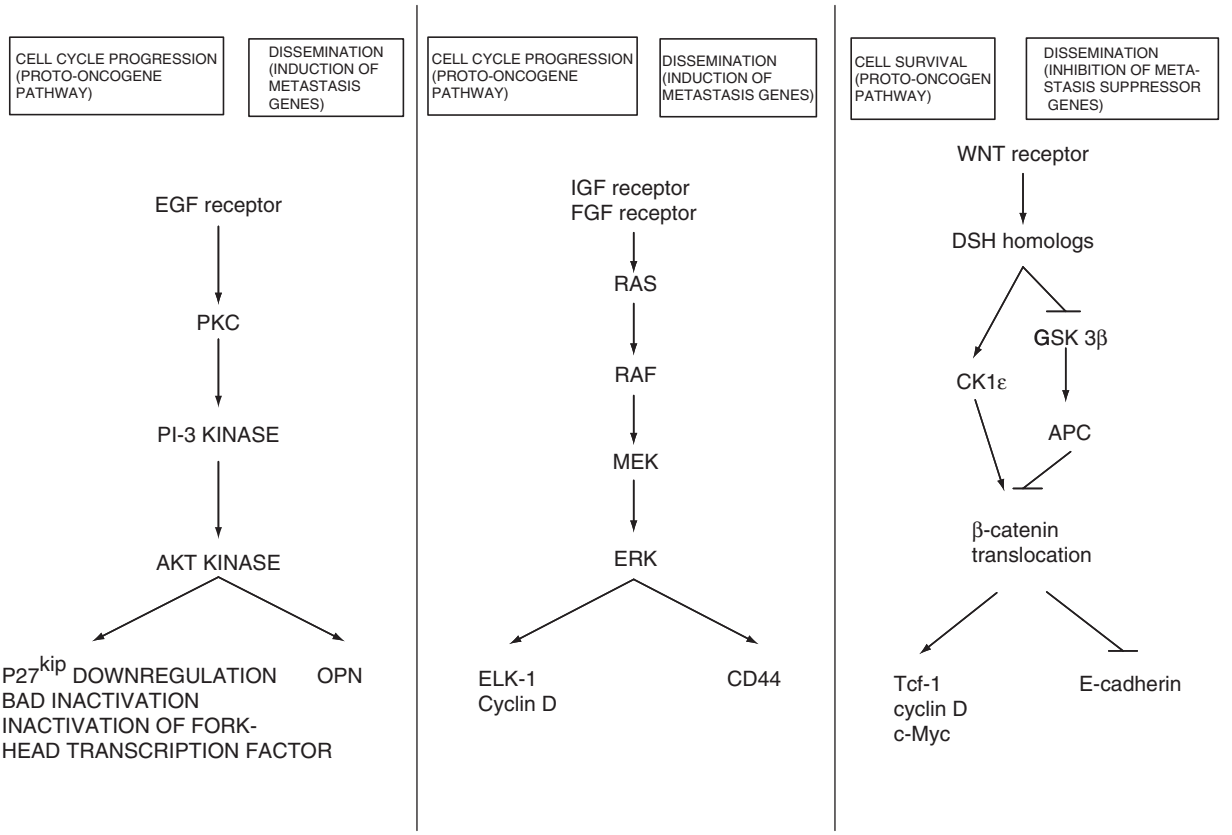


Figure 8.3.A. Molecular connections between growth and dissemination. In benign tumor cells, the signal transduction pathways that lead to excessive growth do not activate genes that convey invasiveness to the cells. In contrast, growth factor signal transduction in malignant tumor cells branches and leads to growth as well as induction of metastasis genes.

regulate the expression of CD44 and its variants [Wielenga et al. 1999], in part through the activity of β-Catenin. Furthermore, *osteopontin* is a target gene for the APC pathway component TCF-4. The sequence CAAAG in the *osteopontin* promoter can sequester TCF-4 in a complex with β-Catenin and E-Cadherin. This leads to *osteopontin* gene expression and to metastasis formation in breast cancer cells [El-Tanani et al. 2001]. The proto-oncoprotein β-Catenin also activates the expression of the *laminin-5 γ2* gene through two TCF-binding elements, the transcription of *matrilysin* and of *upar*.

HGF triggers the destabilization of adherens junctions by transcriptional downregulation of *cadherins*, redistribution of the Cadherin/Catenin complexes from the Actin cytoskeleton to the soluble membrane fraction, MMP-mediated proteolytic cleavage of Cadherins from the cell surface, or disruption of the structural integrity of adherens junctions by tyrosine phosphorylation of Catenin [Trusolino and Comoglio 2002]. In colorectal carcinomata, amplification of the HGF Receptor gene *met* provides a selective advantage that potentiates the ability of the cells to produce hepatic secondary lesions [Di Renzo et al. 1995]. Two somatic mutations of *met* are selected for during the metastatic spread of head and neck squamous cell carcinomata [Di Renzo et al. 2000].

RAS pathway. *ras* overexpression confers a metastatic phenotype, that may be mediated by type IV Collagenases or motility associated cytokines [Stracke and Liotta 1995]. The activated oncogene products H-RAS or K-RAS induce *cd44* transcription [Hofmann et al. 1993; Kogerman et al. 1996]. Increased transcript levels for the standard form of CD44 are accompanied by the appearance of alternatively spliced RNAs and the synthesis of CD44 variants. *ras* is a proto-oncogene whose gain-of-function mutations also induce upregulated transcription of *osteopontin* [Craig et al. 1988] through an AP-1-binding site in the promoter. H-RAS causes transformation and expression of *osteopontin* and *metalloproteinases*. The protein synthesized by the tumor suppressor gene *Krev-1* (*rap1A*, *ras related protein 1A*) reverses the cellular phenotype and delays metastasis formation in a manner that is correlated with reduced *osteopontin* expression [Su et al. 1993].

The R-RAS branch of the RAS superfamily of small GTPases consists of R-RAS, R-RAS2 (TC21, Teratocarcinoma Oncogene 21), and R-RAS3 (M-RAS, Muscle RAS Viral Gene Homolog). R-RAS, R-RAS2, and R-RAS3 possess transforming activities similar to those of RAS. Posttranslational lipid modification anchors the R-RAS proteins to the plasma membrane. Activation of the Integrin→FAK→$P130^{CAS}$→ CRK cascade recruits R-RAS guanine nucleotide exchange factors (GEFs), AND-34 and C3G (GRF-2), resulting in GTP loading and R-RAS activation. Active GTP-bound R-RAS associates with downstream effector molecules to modulate JNK activation, cell migration, and invasion. R-RAS and H-RAS modulate the adhesiveness of Integrins for their substrates. R-RAS increases the affinity of Integrins $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$, and $\alpha_V\beta_3$ for the extracellular matrix. In contrast, constitutively active H-RAS signals a decrease in affinity to Integrins $\alpha_5\beta_1$, $\alpha_{6A}\beta_1$, $\alpha_{6A}\beta_3$, and $\alpha_{6B}\beta_3$ [Oertli et al. 2000; Furuhjelm and Peranen 2003]. This may reflect their differential subcellular localization. Whereas H-RAS dissociates from lipid rafts upon activation, activated R-RAS remains localized to lipid rafts.

Elevated expression of Matrix Metalloproteinases is associated with increased metastatic potential in many tumor cells. In addition to its proliferative effects, the ERK pathway supports metastatic activity in tumor cells [Tanimura et al. 2003]. The induction of the ERK pathway correlates with the acquisition of an invasive phenotype. This is based on the elevated expression of MMP-9, MMP-3, and MMP-14, as well as CD44 in tumor cells.

The ETS family comprises more than 30 members, which regulate multiple genes-associated with angiogenesis, adhesion, and invasion. ETS-binding sites are contained in the promoters of various *metalloproteinases* and *integrin* subunits. ETS transcription factors may be activated through the RAS pathway. In malignant gliomata, ETS-1 induces the overexpression of *upa* and of *integrin* α_5. The Fibronectin Receptor Integrin $\alpha_5\beta_1$ is important for metastasis formation by these tumors. Conversely, the ETS family member TEL (Translocation ETS Leukemia, ETV6, ETS Variant 6) is a tumor suppressor that interacts with the corepressors SIN3 and SMRT. TEL is bi-allelically disrupted in acute leukemia and loss of heterozygosity occurs in various cancers. TEL inhibits RAS-induced cell growth and represses the transcription of *stromelysin-1*. In leukemia, tyrosine kinases may fuse with TEL. These fusion partner tyrosine kinases are constitutively activated by homo-dimerization through the pointed

domain of the TEL protein. This is followed by activation of MAP Kinases and STAT-5. In fusions of TEL with AML-1, chimeric fusion proteins act as dominant negative mutants against the wild type AML-1. The remaining normal *tel* allele is often deleted in pre-B cell leukemia that harbors *tel-aml1*, reflecting the role of *tel* as a tumor suppressor gene.

In tumors, many growth factors activate RHO proteins. They include EGF, HGF, PDGF, and TGF-β. Two families of GTPases have been associated with transformation (RAS) or metastasis formation (RHO). RHO GTPases and RAS signaling may be linked through $P190^{RHO\text{-}GAP}$, which interacts with $P120^{RAS\text{-}GAP}$, a negative regulator of RAS activity. Furthermore, RHO GTPase activity may be required for efficient *ras* induced transformation and RAS can activate RAC. MAP Kinase, which acts downstream of RAS, is important for cellular proliferation as well as for anchorage independence.

Lipid Kinase pathway. In epithelial cells, HGF (Hepatocyte Growth Factor) activates a genetic program that involves cell–cell dissociation, growth, and invasiveness. This program is aberrantly activated during cancer progression, and its effect is exerted through PI 3-K. *osteopontin* is a major transcriptional target for HGF [Ariztia et al. 2003]. Furthermore, HGF promotes the CD44-dependent adhesion to Osteopontin. This interaction is functionally important for invasiveness [Medico et al. 2001].

In squamous cell carcinoma, PKB activation causes epithelial–mesenchymal transition characterized by downregulation of *desmoplakin*, *E-cadherin* and *β-catenin* and upregulation of the mesenchymal cell specific protein Vimentin. This is associated with reduced cell–cell adhesion, increased motility, and invasiveness [Grille et al. 2003]. PKB increases the secretion of MMP-2 and MMP-9 from immortalized mammary epithelial cells and from ovarian carcinomata [Park et al. 2001; Thant et al. 2000]. PKB enhances the invasiveness of pancreatic carcinoma cells via upregulation of IGF-1.

Like in the regulation of cell growth, the ERK and PKB pathways may coalesce in the regulation of migration. The Insulin-Like Growth Factor Receptor 1 (IGF-1R) may be expressed at elevated levels on the surface of breast cancer cells. Its ligation by IGF induces the expression of Urokinase Plasminogen Activator-1 (UPA). This induction proceeds through pathways that involve Phosphatidylinositol 3-Kinase and MEK (Mitogen Activated Protein Kinase Kinase) [Dunn et al. 2001]. The activation of the Epidermal Growth Factor Receptor stimulates migration and MMP-9 dependent invasion of ovarian cancer cells. The process depends on the activation of MAP Kinases and of Phosphatidylinositol 3-Kinase [Ellerbroek et al. 2001].

In colorectal cancer, Bombesin activates Phospholipase C, MAPK, Focal Adhesion Kinase, and PI 3-K. While several of these pathways support proliferation and migration, Phosphatidylinositol 3-Kinase is selectively involved in the cell migration signaling pathway [Patel et al. 2004].

PKC and SRC pathways. Bombesin induces the chemotaxis of small cell lung carcinoma [Stracke and Liotta 1995]. It also enhances the migration of prostate cancer cells while not affecting their adhesion to extracellular matrix proteins. This is mediated through the activation of PKC to phosphorylate $P125^{FAK}$ on tyrosine. Bombesin also increases tyrosine phosphorylation of a 90 kD protein associated with Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$, which may mediate the association of these Integrins with $P125^{FAK}$ [Aprikian et al. 1997].

In breast cancer cells, motility and invasiveness may correlate with PKC activity [Kiley et al. 1999]. Invasive breast cancer cells have the ability to extend membrane protrusions (invadopodia) into the extracellular matrix. These structures are associated with sites of active matrix degradation. Cortactin, Paxillin, and PKCμ form a complex in these protrusions. This complex of proteins is not formed in non-invasive cells [Bowden et al. 1999].

Malignant gliomata express higher levels of PKCα and lower levels of PKCδ than low grade astrocytomata. PKCα decreases the expression of the differentiation marker GFAP (Glial Fibrillary Acidic Protein). Glioblastoma multiforme exhibits increased levels of PKCα compared to normal brain tissue and this relates to its proliferative and invasive potential [Benzil et al. 1992].

Cells expressing high levels of ERBB2 have increased invasive and metastatic potential, possibly due to the altered expression of homing receptors. Epidermal Growth Factor (EGF) stimulated signaling promotes the migration of carcinoma cells in a manner that depends on FAK and induces complex formation between $P130^{CAS}$ and SRC as well as JNK activation. EGF can induce *osteopontin* gene expression [Atkins et al. 1997] through signal transduction via PKC and tyrosine kinases [Chackalaparampil et al. 1996].

SRC mediates the disruption of focal adhesions and Actin stress fibers. This increases cell motility. Several SRC substrates are either cytoskeletal proteins or are associated with the cytoskeleton. They include FAK, Tensin, Caveolin, and Paxillin.

– The SRC substrate FAK regulates cellular motility. SRC can form a trimeric complex with FAK and CAS, which induces tyrosine phosphorylation and activation of FAK. Through its interactions with FAK, SRC activates $P190^{RHO\text{-}GAP}$. This blocks downstream signaling by RHO-A.
– Signaling through SRC and FAK to JNK may promote the expression of *mmp-2* and *mmp-9*.
– SRC can form a complex with R-RAS that suppresses Integrin activity and reduces cell–matrix interactions.
– SRC disrupts adherens junctions by suppressing E-Cadherin localization and function. It tyrosine phosphorylates E-Cadherin complexes, which targets them for ubiquitination.
– SRC is a nonreceptor tyrosine kinase that may link FGFR signaling to Cortactin, a focal adhesion associated protein with the capacity to bind filamentous Actin.

Fibroblast Growth Factors (FGF) are osteoblast mitogens. FGFR ligation mediates cell growth through activation of PLC-γ and ERK pathways, and it leads to cell motility through CAS and SRC. Besides inducing cell cycle progression, these signaling intermediates cause the induction of the metastasis gene *osteopontin* [Tang et al. 1996; Iseki et al. 1997; Iseki et al. 1999].

SMAD pathway. The cytokine Osteopontin contributes importantly to the dissemination of various cancers. Among the growth factors that signal to activate *osteopontin* gene expression is TGF-β [Noda et al. 1988]. Upon ligation and phosphorylation of its type I receptor, SMAD-2 or SMAD-3 forms a complex with SMAD-4 and translocates to the nucleus. SMAD-3 binds directly to the *osteopontin* promoter (bases -180 to -229). In contrast, SMAD-4 interacts with the repressor HOXA-9, which binds the *osteopontin* promoter adjacent to the SMAD-3-binding site, to dislodge it and facilitate gene transcription [Shi et al. 2001].

Signaling by arachidonic acid metabolites. COX-2 (Cyclooxygenase-2) increases breast cancer invasiveness through elevated levels of several Matrix Metalloproteinases and through enhanced angiogenesis, mediated by the increased production of angiogenic factors. The COX-2 derived prostaglandins E_2 and I_2 have essential functions in endothelial cell spreading and migration mediated by Integrin $\alpha_V\beta_3$. Both prostaglandins may be important in the activation of CDC42 and RAC, which occurs following ligation of this Integrin.

Platelet-Type 12-Lipoxygenase enhances the expression of Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$. This causes a more spread morphology and resistance to apoptosis [Pidgeon et al. 2003]. The overexpression of 12-Lipoxygenase by prostate cancer cells increases angiogenesis and metastatic growth.

Induction of migration by various growth factors. Some growth factors can mediate the chemotaxis of tumor cells. However, factor-dependent tumor growth and chemotaxis may be transduced through distinct receptors. Specific growth factors attract the migration of individual tumor cells [Stracke and Liotta 1995], including:

• FGF-1 (acidic FGF) – bladder carcinoma
• FGF-2 (basic FGF) – prostate carcinoma and teratocarcinoma
• HGF (SF) – carcinoma
• histamine – melanoma and carcinoma
• IGF-1 – melanoma
• IGF-2 – rhabdomyosarcoma
• IL-6 – ductal breast carcinoma
• IL-8 – melanoma
• NGF – embryonal carcinoma
• PDGF – teratocarcinoma
• $TGF\text{-}\beta_1$ – lung adenocarcinoma

Various oncogenes confer a metastatic phenotype, including *v-mos*, *H-ras*, *v-raf*, *v-src*, *v-fes*, *v-fms*, *jun*, *fos*, and mutant *p53*.

Motility associated cytokine genes, including *type IV collagenases* and *osteopontin*, can also be induced by *ras*, *v-mos*, *v-raf*, *v-fes* and *v-src* [Greenberg et al. 1989]. *osteopontin* is inducible by tumor promoters [Craig et al. 1989; Craig et al. 1990] and growth factors [Gadeau et al. 1993], but it can be silenced by the tumor suppressor $P33^{ING1}$ [Takahashi et al. 2002].

Homing receptors are downstream targets of growth factor signaling. Growth factors and oncogenes can regulate *cd44* expression. TNF-α may induce the expression of CD44 variants v3, v6, and v9 [Haegel-Kronenberger et al. 1997]. IL-6 (Interleukin-6), the main survival and growth factor for multiple myeloma cells, strongly increases *cd44* gene expression, *cd44* RNA alternative splicing, and polarized

membrane distribution of CD44 [Vincent and Mechti 2004].

8.4 CELL CYCLE CONTROL AND DNA REPAIR

There is a close relationship between cell cycle regulation and DNA repair. The expression of some repair genes fluctuates during the cell cycle. Cells containing damaged genomic DNA arrest in the G_1/S or G_2/M transitions. Likewise, high level expression of certain repair enzymes can cause arrest in the G_1/S transition. Cell cycle arrest is frequently a prerequisite for DNA repair.

Overwhelming DNA damage leads to apoptosis. In such cases, many of the repair enzymes are subject to proteolytic degradation, often by Caspases.

P53 is an essential mediator of G_1 cell cycle arrest and of some forms of apoptosis. P53 also plays roles in several mechanisms of DNA repair.

The promoter for the mismatch repair gene *msh2* contains a P53-binding site [Warnick et al. 2001]. Upon UV irradiation, P53 activates the transcription of *msh2*. This upregulation depends critically on the functional interaction of P53 with c-JUN [Scherer et al. 2000].

In addition to being absolutely essential for base excision repair, APE1 (REF-1, Reducing Factor 1) is required for the redox activation of a number of spontaneously oxidized transcription factors. It reduces conserved cysteine residues in the DNA-binding domains, thereby participating in protein repair after oxidative damage. APE1 also contributes to P53 activation.

Tumor cells lacking functional P53 exhibit a partial deficiency in nucleotide excision repair. The role of P53 may be twofold:

- P53 can bind TFII-H. In nucleotide excision repair, the nine protein complex TFII-H facilitates the partial unwinding of the DNA duplex and the formation of an open bubble structure. These are early steps in the repair process.
- P53 induces *p21* and *gadd45* expression. P21 inhibits DNA synthesis by interacting with the DNA Polymerase δ subunit PCNA (Proliferating Cell Nuclear Antigen). The P53 effect also depends on the involvement of GADD45α, a nuclear protein with high expression in quiescent cells in G_1. GADD45 may associate with PCNA and P21. This complex supports cell cycle arrest and DNA repair.

53BP1 (TP53BP1) is a mediator of the DNA damage checkpoint. It is phosphorylated in response to ionizing radiation in an ATM-dependent manner. 53BP1 acts as an adapter that recruits a subset of substrates to ATM and ATR. It binds to P53 through its COOH-terminal BRCT repeats (BRCA1 Carboxyl Terminus repeats). 53BP1 is required for P53 accumulation, which leads to the interaction of P53 with CBP in PML nuclear bodies.

The ATM (Ataxia Teleangiectasia Mutated) protein is a member of the Phosphatidylinositol 3-Kinase family of proteins that respond to DNA damage by phosphorylating key substrates involved in DNA repair or cell cycle control.

ATM acts as an upstream kinase in cell cycle checkpoint regulation. ATM phosphorylates P53, resulting in its stabilization, and thus in a marked increase in its concentration. This is followed by the nuclear accumulation of P53, which induces the transcription of the CDK2 Inhibitor $p21^{CIP1/WAF1}$ and leads to cell cycle arrest in G_1. ATM also phosphorylates CHK2, which then phosphorylates CDC25A on serine 123, leading to its degradation in the Ubiquitin pathway [Mailand et al. 2000]. The phosphatase CDC25A is important during the initiation and progression of S phase. Mechanisms dependent on CHK2 and P53 are activated after DNA double-strand breaks, but not single-strand breaks.

In response to DNA double-strand breaks, phosphorylated NBS1 stimulates inactive ATM dimers through multiple protein–protein contacts, and this interaction increases the affinity of ATM for its substrates. ATM thus responds to the presence of DNA double-strand breaks by phosphorylating proteins that initiate DNA repair. Among others, activation of ATM leads to the phosphorylation of BRCA1 in the BASC repair complex and to the phosphorylation of Histone H1. Ataxia teleangiectasia (AT) represents a defect in the repair of double-strand breaks, which is caused by a loss-of-function mutation in *atm*. It is inherited autosomally recessively. Patients with this disease suffer from rare entities of pediatric non-Hodgkin lymphoma or lymphocytic leukemia. Solid tumors, including mucinous adenocarcinomata of the stomach, medulloblastomata and gliomata, occur with increased frequency. AT cells have abnormal sensitivity of killing by ionizing radiation and abnormal resistance to inhibition of DNA synthesis by ionizing radiation.

Nickel, cadmium, cobalt, and arsenic compounds are carcinogens due to their interference with the nucleotide and base excision repair mechanisms. They act, in part, by inactivation of the XPA (Xeroderma Pigmentosum group A) protein and PARP (Poly(ADP-Ribose) Polymerase).

Poly(ADP-ribosyl)ation is a posttranslational modification that alters the functions of the acceptor proteins. Upon heat shock, PARP-1 rapidly accumulates at heat shock gene loci. It provides temporary protection of DNA single-strand breaks and consequently acts as an antirecombinogenic factor. During DNA repair, PARP binds to single-strand nicks in the DNA together with other components of the base excision repair complex.

Stress, including heat shock, can induce the transcription of acute response genes. Activated by binding specifically to nicked DNA, PARP uses nicotinamide adenine dinucleotide (NAD) to add long chains of ADP-ribose to target proteins, including Histones, transcription factors (including OCT-1, P53, AP-2, NF-κB, YY1), and DNA repair proteins. It thus facilitates chromatin decondensation, which enables RNA Polymerase to transcribe these genes. PARP then poly-ADP-ribosylates the transcription factors, leading to their inactivation and preventing repeated cycles of transcription. The transient nature and modulation of poly (ADP-ribosyl)ation depend on the activity of Poly(ADP-ribose) Glycohydrolase, which hydrolyzes poly-ADP bound to acceptor proteins in free ADP-ribose residues. PARP can also play a role in limiting cell cycle progression by facilitating apoptosis. In response to stresses, PARP-1 activity in the cell increases substantially. The accumulation of poly(ADP ribose) and the depletion of NAD^+ and ATP induce mitochondrial depolarization and the release of AIF, ultimately leading to energy failure and cell death.

Transcription requires five basal factors, TFII-B, TFII-D, TFII-E, TFII-F, and TFII-H. A sixth factor, TFII-A, potentiates the magnitude of transcription. The initial step of transcription is promoter recognition by TFII-D, a multi-subunit complex containing TBP (TATA-Binding Protein) and at least 14 tightly associated factors. At a later stage, TFII-F and TFII-H are required for progression into the elongation phase of transcription. Transcription Factor II-H (TFII-H) possesses nine subunits. They are divided into two subcomplexes, the core complex (ATP-Dependent DNA Helicase, XPB, P34, P52, P62) and the Cyclin Activating Kinase complex (CDK7, Cyclin H, MAT1). TFII-H facilitates the partial unwinding of the DNA duplex, assists in nucleotide excision repair, and is required for transcription initiation by RNA Polymerase. Transcriptional elongation depends on RNA Polymerase II and on elongation factors, including TFII-F, SII, Elongin (SIII), and ELL.

The TFII-H components XPD (ERCC2) and XPB (ERCC3) are Helicases involved in nucleotide excision repair and in transcription coupled repair. They unwind the affected DNA and allow for the recruitment of repair proteins. TFII-H contains a kinase capable of phosphorylating the COOH-terminal domain of the largest subunit of RNA Polymerase II (RNAPII). The Cyclin Activating Kinase (CAK) complex is part of the TFII-H complex required for nucleotide excision repair. In addition, TFII-H interacts with P53 to regulate its activity, and P53 modulates TFII-H activity in DNA repair.

CDK7, Cyclin H, and MAT1 participate in cell cycle regulation at the transition from the G_2 phase to the M phase. MAT1 is strongly associated with CDK7 and Cyclin H and stimulates the kinase activity of the complex. TFII-H and CAK are both able to phosphorylate CDC2 and CDK2 [Shiekhattar et al. 1995; Serizawa et al. 1995]. The kinase activity of TFII-H, which is constant during the cell cycle, is reduced after UV light irradiation.

Proteins that bind methylated DNA (methylated DNA-binding proteins, MBDs) link DNA methylation to the Histone code. These proteins recognize and bind to methylated CpG islands, relatively independently of their specific DNA sequence. They target gene-silencing protein complexes, including Histone Deacetylases and Histone Methyl Transferases, to their cognate sites and thus mediate Histone modifications. Methylated DNA-binding proteins also contribute to DNA repair.

– MBD-1 (PCM1, CXXC3) {18q21.1} binds to a variety of methylated sequences, provided they contain at least 12 symmetrically methylated CpGs, and inhibits transcription.
– MBD-2 {18q21} localizes to foci of heavily methylated satellite DNA. It can act as a Demethylase. In colon cancer, MBD-2 (NY-CO-41) is a potential tumor antigen [Scanlan et al. 1998].

– MBD-3 {19p13.3} is a subunit of the NURD (Nucleosome Remodeling and Deacetylase) complex that accumulates in nuclear foci. The assembly of an active Histone Deacetylase complex requires the interaction between the NURD components MBD-3 and MTA-2.
– MBD-4 (Methyl-CpG Binding Domain Protein-4, Methyl-CpG-Binding Endonuclease 1, MED1) {3q21-q22} is a DNA repair protein that is active in DNA mismatch repair by binding to MLH-1. The methyl-CpG-binding domain of MBD4 preferentially targets 5-methylcytosine CpG/TpG mismatches, which result from deamination at methyl-CpG. MBD-4 may function to repair mutations at methyl-CpG.
– The distribution of MECP2 (Methyl-CpG-Binding Protein 2) {Xq28} along chromosomes parallels that of methyl-CpG. It is concentrated in pericentromeric heterochromatin, which contains about 40% of all genomic 5-methyl cytosine. MECP2 is able to bind a single methyl-CpG pair.
– KAISO {Xq23} contains a NH_2-terminal POZ/BTB domain and 3 COOH-terminal zinc finger motifs. It is a likely effector of Cadherin signaling.

8.5 SENESCENCE AND DNA REPAIR

Certain genes influence variations in life span. Single genes, that can increase life expectancy when activated, are associated specifically with cell functions other than DNA repair, but when the pathways that they influence are followed to their final destination, they mediate
– Increasing the rate of DNA repair
– Increasing the rate of antioxidant production
– Decreasing the rate of oxidant production
Therefore, the common pattern across most life span influencing genes is in their downstream effect of altering the rate of DNA damage.

DNA repair and telomere synthesis have common features. The chromosome ends are protected by telomeres, which prevent them from being recognized as DNA strand breaks. However, the length of the telomeres reduces with replicative senescence, potentially exposing the chromosomes to destabilizing influences. The disruption of telomere integrity induces genomic instability and the activation of DNA damage responses. Loss of protection of the chromosome ends by the telomeres leads to the state of crisis.

Telomere-binding proteins regulate telomere length, positioning, and function. Many of them are also involved in DNA strand break repair. This dual function may be based on the biologic necessity to protect the chromosome ends from being recognized as chromosome breaks.

Telomere function requires two telomere-specific DNA-binding proteins, TRF1 and TRF2. TRF1 regulates telomere length, presumably by blocking the access of Telomerase to DNA. Tankyrase ADP-ribosylates TRF1, which diminishes its ability to bind telomeric DNA. However, Tankyrase does not interact with TRF2 [Smith et al. 1998]. TRF2 protects the chromosome ends, and it also associates with DNA strand breaks of nontelomeric DNA. DNA damage surveillance networks are not activated by telomeres, in part because TRF2 prevents the recognition of telomeric ends as double-strand breaks by facilitating their organization into T loops. Unlike H2AXγ, TRF2 forms transient foci in early stages of damage recognition and processing, which occur before the association of ATM with the lesions [Bradshaw et al. 2005]. The abrogation of TRF2 or DNA-PK activity causes end-to-end chromosomal fusion.

The structure-specific endonucleases XPG and XPF/ERCC1 are directed to damaged DNA sites by XPA and make the 3′ and 5′ incisions either side of the damaged site. XPF/ERCC1 is also a component of the telomeric TRF2 complex and removes the 3′ overhang from uncapped telomeres.

The DNA repair protein KU, which is required for double-strand break repair, may bind to the telomeres. While located there, KU regulates telomere addition, protects telomeres from recombination and nucleolytic degradation, promotes transcriptional silencing of genes proximal to the telomeres, and positions the telomeres within the nucleus [Fisher and Zakian 2005]. The subunit KU70 also interacts with the telomere-associated proteins TRF2 and HP-1α (Heterochromatin Protein 1α) [Song et al. 2001].

Various progeric syndromes are caused by genome maintenance defects, suggesting a direct link between senescence and DNA repair. In this setting, DNA strand breaks, mediated by reactive oxygen species, may accelerate metabolic aging. As a consequence of the elevated rate of mutagenesis, some of these conditions are associated with an increased incidence of malignancies [Hasty et al. 2003].

(a) Werner syndrome is a segmental progeric disorder (it displays a subset of symptoms associated with normal aging) with an autosomal recessive pattern of inheritance. The defect causes accelerated replicative senescence, accumulation of DNA damage foci in cells, and increased chromosomal instability. Patients with Werner syndrome exhibit a number of symptoms resembling a premature aging phenotype, including alopecia, osteoporosis, arteriosclerosis, diabetes, cataract, teleangiectasia, and skin atrophy. The mean life span is about 47 years. The cancer risk is increased, predominantly for nonepithelial malignancies, such as soft tissue sarcomata and benign meningiomata. There is also a high frequency of thyroid cancer and melanoma in Japanese patients [Goto et al. 1996]. The Werner syndrome gene, *wrn* (*recql2*) {8p12-p11.2}, encodes a Helicase of the REC-Q subclass. The gene product is a tissue-specific regulator of mismatch repair in fibroblastoid, but not in lymphoblastoid, cells [Bennett et al. 1997]. WRN and the homologous recombination mediator protein RAD52 form a complex that colocalizes in foci associated with arrested replication forks. RAD52 either inhibits or enhances WRN Helicase activity in a DNA structure-dependent manner, while WRN increases the efficiency of RAD52-mediated strand annealing between nonduplex DNA and homologous sequences [Baynton et al. 2003]. Furthermore, WRN and PARP-1 are part of a complex involved in the processing of DNA breaks. In response to cellular stress, PARP-1 becomes activated, but the poly(ADP-ribosyl)ation of other cellular proteins is severely impaired in Werner syndrome cells [von Kobbe et al. 2003]. The proto-oncogene product MYC directly stimulates the transcription of *wrn*.

(b) Rothmund–Thompson syndrome (poikiloderma congenitale) [Rothmund 1868; Thomson 1923] is autosomally recessively inherited. It is caused by mutations in a REC-Q like DNA Helicase, RECQL4 {8q24.3} [Kitao et al. 1999]. It presents with alopecia, poikiloderma, telangiectasia and is frequently accompanied by juvenile cataract, saddle nose, congenital bone defects, disturbances of hair growth, and hypogonadism. Malignancies, including osteogenic sarcomata and skin cancer, frequently occur.

(c) Bloom syndrome [Bloom 1966] is caused by a mutation in a REC-Q like Helicase {15q26.1}. It leads to butterfly erythema, light sensitivity, hypo-gammaglobulinemia, proportionate pre- and postnatal growth deficiency, telangiectatic hypo- and hyperpigmented skin. The BLM Helicase may play a role in restarting DNA replication forks that are blocked at lesions, thereby promoting chromosome stability. Elevated generation of functional hemizygosity and homozygosity in somatic cells may play a role in the high cancer risk of persons with Bloom syndrome. Leukemia is frequent.

(d) Ataxia telangiectasia (AT) is caused by a defect in the signaling protein kinase ATM and is inherited autosomally recessively. It causes skin atrophy, sclerosis, teleangiectasia, immune deficiencies, poikilodermia, and neurodegeneration. The risk of malignancies is increased. Patients with this condition suffer from rare entities of pediatric non-Hodgkin lymphoma. Lymphocytic leukemia is associated with ataxia telangiectasia [Hecht et al. 1966]. In general, lymphomata in ataxia telangiectasia patients tend to be of B-cell origin (B-CLL), whereas the leukemias tend to be of the T-CLL type. Solid tumors, including mucinous adenocarcinoma of the stomach [Haerer et al. 1969], medulloblastomata and gliomata, occur with increased frequency in this disease [Gatti et al. 1991]. The abnormal sensitivity of AT cells to killing by ionizing radiation and abnormal resistance to inhibition of DNA synthesis by ionizing radiation has led to the identification of complementation groups for the classic form of the disease [Jaspers et al. 1988]. At least four of these (A, C, D, and E) map to chromosome 11q23 and are associated with mutations in the *atm* gene. The disease shows signs of accelerated aging and has a mean life span of around 20 years.

(e) Hutchinson-Gilford progeria [Hutchinson 1886; Gilford 1904] is caused by mutations in the *lamin A* gene {1q21.2} [Eriksson et al. 2003]. Its mode of inheritance is autosomal. The disease leads to alopecia, sclerosis, wrinkling, cachexia, and arteriosclerosis. Osteosarcoma is associated with the condition. The outcome is death at an average age of 12.

(f) Down syndrome is caused by trisomy of the chromosome 21. This is reflected in progeria, cataracts, alopecia, loss of vision, neurodegeneration,

and thyroid dysfunction. Down syndrome cells are highly susceptible to the induction of chromosomal aberrations due to asymmetrical chromatid interchanges. The repair of DNA damage is more rapid in Down syndrome cells than in normal cells. The rapid repair results in a higher probability of producing chromosome aberrations, and hence higher aberration frequencies in Down syndrome cells than normal cells [Preston 1981]. Down syndrome may be associated with an increased occurrence of testicular cancer [Hsiung Stripp et al. 2003], retinoblastoma [Brichard et al. 2003], acute lymphoblastic leukemia (ALL) [Hasle 2001], and distinct forms of myeloid leukemia [Ohsaka et al. 2002; Hasle et al. 2003].

- ALL in Down syndrome typically has a favorable prognosis.
- Primary lymphoma of the thyroid gland is rare. The histopathology of most low grade thyroid lymphomata is of a MALT (mucosa-associated lymphoid tissue) type. A typical feature of this type of lymphoma is a close lymphocyte–epithelium interaction. This form of lymphoma can arise in Down syndrome.
- Transient abnormal myelopoiesis almost never arises in children who do not have Down syndrome. In this condition with a typical onset soon after birth, the blood and bone marrow are subject to changes that appear typical of leukemia. About 20–30% of these cases progress to leukemia, most frequently AML. Myelodysplastic syndrome may also be a precursor to AML. AML in Down syndrome is usually of the FAB M7 subtype, which affects mainly the platelet producing cells in the bone marrow.

(g) Forms of xeroderma pigmentosum display age related phenotypes [Nakura et al. 2000]. XPF forms a tight complex with ERCC1 and acts as a structure-specific endonuclease, responsible for the 5′ incision during nucleotide excision repair. A mutation in the *xpf* gene leads to premature aging, progressive liver and kidney dysfunction, cachexia, hypertension, neuronal degeneration, and skin atrophy. Cockayne syndrome [Cockayne 1933] is related to some forms of xeroderma pigmentosum. It is an autosomal recessive disorder that is characterized by thin hair, cachexia, pigmentary retinal degeneration, hearing loss, cataract, and neurodegeneration with mental retardation, dwarfism, precociously senile appearance, optic atrophy, deafness, marble epiphyses in some digits, and photosensitivity. Disproportionately long limbs with large hands and feet and flexion contractures of joints are usual skeletal features. The clinical presentation ranges from the very mild UV-sensitive syndrome to a range classified as CS types I, II, and III and the severe, neonatally lethal cerebro-oculofacial-skeletal syndrome. There are two complementation groups in Cockayne syndrome. The disease is caused by a defect in transcription-coupled DNA repair due to loss-of-function mutations in CS-A (ERCC8) or CS-B (ERCC6), and has a life expectancy of around 20 years. The defects in Cockayne syndrome are confined to actively transcribed genes. The risk for cancer is not elevated. Trichothiodystrophy (TTD) occurs in an autosomal recessive fashion through a defect in nucleotide excision repair. It leads to cachexia, osteoporosis, cataracts, sulfur deficient fragile hair, and neurodegeneration. Trichothiodystrophy is caused by a subset of mutations in *xpb* (*ercc3*), *xpd* (*ercc2*), or *tfb5* (*gtf2H5*). All the mutations in trichothiodystrophy cases, irrespective of whether they are homozygotes, hemizygotes, or compound heterozygotes, cause a substantial and specific reduction, by up to 70%, in the cellular concentration of TFII-H [Botta et al. 2002]. The sensitivity to skin cancer is elevated.

(h) The premature aging syndrome, Okamoto type causes severe growth and developmental abnormalities, including cataracts, diabetes mellitus, osteoporosis, and erythroid macrocytosis. The risk for osteosarcoma may be increased [Okamoto et al. 1997].

REFERENCES

Akiyama M, Hideshima T, Hayashi T, Tai YT, Mitsiades CS, Mitsiades N, Chauhan D, Richardson P, Munshi NC, Anderson KC. 2002. Cytokines modulate telomerase activity in a human multiple myeloma cell line. Cancer Research 62:3876–3882.

Aprikian AG, Tremblay L, Han K, Chevalier S. 1997. Bombesin stimulates the motility of human prostate-carcinoma cells through tyrosine phosphorylation of focal adhesion kinase and of integrin-associated proteins. International Journal of Cancer 29:498–504.

Ariztia EV, Subbarao V, Solt DB, Rademaker AW, Iyer AP, Oltvai ZN. 2003. Osteopontin contributes to hepatocyte growth factor-induced tumor growth and metastasis formation. Experimental Cell Research 288:257–267.

Atkins KB, Simpson RU, Somerman MJ. 1997. Stimulation of osteopontin mRNA expression in HL-60 cells is independent

of differentiation. Archives of Biochemistry and Biophysics 343:157–163.

Baynton K, Otterlei M, Bjoras M, Von Kobbe C, Bohr VA, Seeberg E. 2003. WRN interacts physically and functionally with the recombination mediator protein RAD52. The Journal of Biological Chemistry 278:36476–36486.

Bennett SE, Umar A, Oshima J, Monnat RJ Jr, Kunkel TA. 1997. Mismatch repair in extracts of Werner syndrome cell lines. Cancer Research 57:2956–2960.

Benzil DL, Finkelstein SD, Epstein MH, Finch PW. 1992. Expression pattern of α-protein kinase C in human astrocytomas indicates a role in malignant progression. Cancer Research 52:2951–2956.

Bloom D. 1966. The syndrome of congenital telangiectatic erythema and stunted growth. Journal of Pediatrics 68:103–113.

Botta E, Nardo T, Lehmann AR, Egly JM, Pedrini AM, Stefanini M. 2002. Reduced level of the repair/transcription factor TFIIH in trichothiodystrophy. Human Molecular Genetics 11:2919–2928.

Bowden ET, Barth M, Thomas D, Glazer RI, Mueller SC. 1999. An invasion-related complex of cortactin, paxillin and PKCμ associates with invadopodia at sites of extracellular matrix degradation. Oncogene 18:4440–4449.

Bradshaw PS, Stavropoulos DJ, Meyn MS. 2005. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. Nature Genetics 37:193–197.

Brichard B, Vermylen C, De Potter P, Casteels I. 2003. Down syndrome: possible predisposition to retinoblastoma. Medical and Pediatric Oncology 41:73–74.

Chackalaparampil I, Peri A, Nemir M, Mckee MD, Lin PH, Mukherjee BB, Mukherjee AB. 1996. Cells in vivo and in vitro from osteopetrotic mice homozygous for c-src disruption show suppression of synthesis of osteopontin, a multifunctional extracellular matrix protein. Oncogene 12:1457–1467.

Cockayne EA. 1933. Inherited abnormalities of the skin and its appendages. London: Oxford University Press.

Craig AM, Bowden GT, Chambers AF, Spearman MA, Greenberg AH, Wright JA, McLeod M, Denhardt DT. 1990. Secreted phosphoprotein mRNA is induced during multi-stage carcinogenesis in mouse skin and correlates with the metastatic potential of murine fibroblasts. International Journal of Cancer 46:133–137.

Craig AM, Nemir M, Mukherjee BB, Chambers AF, Denhardt DT. 1988. Identification of the major phosphoprotein secreted by many rodent cell lines as 2ar/osteopontin: enhanced expression in H-ras-transformed 3T3 cells. Biochemical and Biophysical Research Communications 157:166–173.

Craig AM, Smith JH, Denhardt DT. 1989. Osteopontin, a transformation-associated cell adhesion phosphoprotein, is induced by 12-O-tetradecanoylphorbol 13-acetate in mouse epidermis. Journal of Biological Chemistry 264:9682–9689.

Di Renzo MF, Olivero M, Giacomini A, Porte H, Chastre E, Mirossay L, Nordlinger B, Bretti S, Bottardi S, Giordano S, Plebani M, Gespach C, Comoglio PM. 1995. Overexpression and amplification of the MET/HGF receptor gene during the progression of colorectal cancer. Clinical Cancer Research 1:147–154.

Di Renzo MF, Olivero M, Martone T, Maffe A, Maggiora P, Stefani AD, Valente G, Giordano S, Cortesina G, Comoglio PM. 2000. Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. Oncogene 19:1547–1555.

Dunn SE, Torres JV, Oh JS, Cykert DM, Barrett JC. 2001. Upregulation of urokinase-type plasminogen activator by insulin-like growth factor-I depends upon phosphatidylinositol 3-kinase and mitogen-activated protein kinase kinase. Cancer Research 61:1367–1374.

Ellerbroek SM, Halbleib JM, Benavidez M, Warmka JK, Wattenberg EV, Starck MS, Hudson LG. 2001. Phosphatidylinositol 3-kinase activity in epidermal growth factor-stimulated matrix metalloproteinase-9 production and cell surface association. Cancer Research 61:1855–1861.

El-Tanani MK, Barraclough R, Wilkinson MC, Rudland PS. 2001. Regulatory region of metastasis-inducing DNA is the binding site for T-cell factor-4. Oncogene 20:1793–1797.

Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS. 2003. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423:293–298.

Essers MAG, de Vries-Smits LMM, Barker N, Polderman PE, Burgering BMT, Korswagen HC. 2005. Functional interaction between β-catenin and FOXO in oxidative stress signaling. Science 308:1181–1184.

Fisher TS, Zakian VA. 2005. Ku: A multifunctional protein involved in telomere maintenance. DNA Repair 4:1215–1226.

Furuhjelm J, Peranen J. 2003. The C-terminal end of R-Ras contains a focal adhesion targeting signal. The Journal of Cell Science 116:3729–3738.

Gadeau AP, Campan M, Millet D, Candresse T, Desgranges C. 1993. Osteopontin overexpression is associated with arterial smooth muscle cell proliferation in vitro. Arteriosclerosis and Thrombosis 13:120–125.

Gatti RA, Boder E, Vinters HV, Sparkes RS, Norman A, Lange K. 1991. Ataxia-telangiectasia: an interdisciplinary approach to pathogenesis. Medicine 70:99–117.

Gilford H. 1904. Ateleiosis and progeria: continuous youth and premature old age. British Medical Journal 2:914–918.

Goto M, Miller RW, Ishikawa Y, Sugano H. 1996. Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiology, Biomarkers and Prevention 5:239–246.

Greenberg AH, Egan SE, Wright JA. 1989. Oncogenes and metastatic progression. Invasion and Metastasis 9:360–378.

Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, Donowitz M, Tsichlis PN, Larue L. 2003. The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Research 63:2172–2178.

Haegel-Kronenberger H, de la Salle H, Bohbot A, Galon J, Tartour E, Cazenave JP, Hanau D. 1997. Regulation of CD44 isoform expression and CD44-mediated signaling in human dendritic cells. Advances in Experimental Medicine and Biology 417:83–90.

Haerer AF, Jackson JF, Evers CG. 1969. Ataxia-telangiectasia with gastric adenocarcinoma. Journal of the American Medical Association 210:1884–1887.

Harrington L, Robinson MO. 2002. Telomere dysfunction: multiple paths to the same end. Oncogene 21:592–597.

Hasle H. 2001. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncology 2:429–436.

Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, Head DR. 2003. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia 17:277–282.

Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. 2003. Aging and genome maintenance: Lessons from the mouse? Science 299:1355–1359.

Hecht F, Koler RD, Rigas DA, Dahnke GS, Case MP, Tisdale V, Miller RW. 1966. Leukemia and lymphocytes in ataxia-telangiectasia. The Lancet 2:1193.

Hofmann M, Rudy W, Günthert U, Zimmer SG, Zawadzki V, Zoeller M, Lichtner RB, Herrlich P, Ponta H. 1993. A link between *ras* and metastatic behavior in tumor cells: *ras* induces CD44 promoter activity and leads to low-level expression of metastasis-specific variants of CD44 in CREF cells. Cancer Research 53:1516–1521.

Hsiung Stripp DC, Vaughn D, Van Arsdalen K, Whittington R. 2003. Three cases of advanced seminoma and Down's syndrome: a possible association. American Journal of Clinical Oncology 26:197–199.

Hutchinson J. 1886. Case of congenital absence of hair, with atrophic condition of the skin and its appendages, in a boy whose mother had been almost wholly bald from alopecia areata from the age of six. The Lancet I:923.

Iseki S, Wilkie AO, Morriss-Kay GM. 1999. Fgfr1 and Fgfr2 have distinct differentiation- and proliferation-related roles in the developing mouse skull vault. Development 126:5611–5620.

Iseki S, Wilkie AO, Heath JK, Ishimaru T, Eto K, Morriss-Kay GM. 1997. Fgfr2 and osteopontin domains in the developing skull vault are mutually exclusive and can be altered by locally applied FGF2. Development 124:3375–3384.

Jaspers NGJ, Gatti RA, Baan C, Linssen PCML, Bootsma D. 1988. Genetic complementation analysis of ataxia telangiectasia and Nijmegen breakage syndrome: a survey of 50 patients. Cytogenetics and Cell Genetics 49:259–263.

Kawasaki Y, Senda T, Ishidate T, Koyama R, Morishita T, Iwayama Y, Higuchi O, Akiyama T. 2000. Asef, a link between the tumor suppressor APC and G-protein signaling. Science 289:1194–1197.

Kiley SC, Clark KJ, Duddy SK, Welch DR, Jaken S. 1999. Increased protein kinase Cδ in mammary tumor cells: relationship to transformtion and metastatic progression. Oncogene 18:6748–6757.

Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, Furuichi Y. 1999. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. Nature Genetics 22:82–84.

Kogerman P, Sy MS, Culp LA. 1996. Oncogene-dependent expression of CD44 in Balb/c 3T3 derivatives: correlation with metastatic competence. Clinical and Experimental Metastasis 14:73–82.

Luo K, Stroschein SL, Wang W, Chen D, Martens E, Zhou S, Zhou Q. 1999. The Ski oncoprotein interacts with the Smad proteins to repress TGFβ signaling. Genes and Development 13:2196–2206.

Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J, Lukas J. 2000. Rapid destruction of human Cdc25A in response to DNA damage. Science 288:1425–1429.

Medico E, Gentile A, Lo Celso C, Williams TA, Gambarotta G, Trusolino L, Comoglio PM. 2001. Osteopontin is an autocrine mediator of hepatocyte growth factor-induced invasive growth. Cancer Research 61:5861–5868.

Nakura J, Ye L, Morishima A, Kohara K, Miki T. 2000. Helicases and aging. Cellular and Molecular Life Sciences 57:716–730.

Noda M, Yoon K, Prince CW, Butler WT, Rodan GA. 1988. Transcriptional regulation of osteopontin production in rat osteosarcoma cells by type β transforming growth factor. The Journal of Biological Chemistry 263:13916–13921.

Oertli B, Han J, Marte BM, Sethi T, Downward J, Ginsberg M, Hughes PE. 2000. The effector loop and prenylation site of R-Ras are involved in the regulation of integrin function. Oncogene 19:4961–3969.

Ohsaka A, Hisa T, Watanabe N, Kojima H, Nagasawa T. 2002. Tetrasomy 21 as a sole chromosome abnormality in acute myeloid leukemia. fluorescence in situ hybridization and spectral karyotyping analyses. Cancer Genetics and Cytogenetics 134:60–64.

Okamoto N, Satomura K, Hatsukawa Y, Hayashida M, Saijo K, Ohno T, Goto M. 1997. Premature aging syndrome with osteosarcoma, cataracts, diabetes mellitus, osteoporosis, erythroid macrocytosis, severe growth and developmental deficiency. American Journal of Medical Genetics 69:169–170.

Park BK, Zeng X, Glazer RI. 2001. Akt1 induces extracellular matrix invasion and matrix metalloproteinase-2 activity in mouse mammary epithelial cells. Cancer Research 61:7647–7653.

Patel O, Dumesny C, Giraud AS, Baldwin GS, Shulkes A. 2004. Stimulation of proliferation and migration of a colorectal cancer cell line by amidated and glycine-extended gastrin-releasing peptide via the same receptor. Biochemical Pharmacology 68:2129–2142.

Preston RJ. 1981. X-ray-induced chromosome aberrations in Down lymphocytes: an explanation of their increased sensitivity. Environmental Mutagenesis 3:85–89.

Rommel C, Clarke BA, Zimmermann S, Nunez L, Rossman R, Reid K, Moelling K, Yancopoulos GD, Glass DJ. 1999. Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. Science 286:1738–1741.

Rothmund A. 1868. Ueber Cataracte in Verbindung mit einer eigenthuemlichen Hautdegeneration. Albrecht von Graefes Archiv fuer Klinische und Experimentelle Ophthalmologie 14:159–182.

Scanlan MJ, Chen Y-T, Williamson B, Gure AO, Stockert E, Gordan JD, Tureci O, Sahin U, Pfreundschuh M, Old LJ. 1998. Characterization of human colon cancer antigens recognized by autologous antibodies. International Journal of Cancer 76:652–658.

Scherer SJ, Maier SM, Seifert M, Hanselmann RG, Zang KD, Mueller-Hermelink HK, Angel P, Welter C, Schartl M. 2000. p53 and c-Jun functionally synergize in the regulation of the DNA repair gene hMSH2 in response to UV. The Journal of Biological Chemistry 275:37469–37473.

Serizawa H, Makela TP, Conaway JW, Conaway RC, Weinberg RA, Young RA. 1995. Association of Cdk-activating kinase subunits with transcription factor TFIIH. Nature 374:280–282.

Shi X, Bai S, Li L, Cao X. 2001. Hoxa-9 represses TGF-β-induced osteopontin gene transcription. The Journal of Biological Chemistry 276:850–855.

Shiekhattar R, Mermelstein F, Fisher RP, Drapkin R, Dynlacht B, Wessling HC, Morgan DO, Reinberg D. 1995. Cdk-activating kinase complex is a component of human transcription factor TFIIH. Nature 1995 374:283–287.

Smith S, Giriat I, Schmitt A, de Lange T. 1998. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 282:1484–1487.

Song K, Jung Y, Jung D, Lee I. 2001. Human Ku70 interacts with heterochromatin protein 1α. The Journal of Biological Chemistry 276:8321–8327.

Stracke ML, Liotta LA. 1995. Molecular mechanisms of tumor cell metastasis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 233–247.

Stull MA, Richert MM, Loladze AV, Wood TL. 2002. Requirement for IGF-I in epidermal growth factor-mediated cell cycle progression of mammary epithelial cells. Endocrinology 143:1872–1879.

Su ZZ, Austin VN, Zimmer SG, Fisher PB. 1993. Defining the critical gene expression changes associated with expression and suppression of the tumorigenic and metastatic phenotype in Ha-ras-transformed cloned rat embryo fibroblast cells. Oncogene 8:1211–1219.

Takahashi M, Seki N, Ozaki T, Kato M, Kuno T, Nakagawa T, Watanabe K, Miyazaki K, Ohira M, Hayashi S, Hosoda M, Tokita H, Mizuguchi H, Hayakawa T, Todo S, Nakagawara A. 2002. Identification of the $p33^{ING1}$-regulated genes that include *cyclin B1* and proto-oncogene *DEK* by using cDNA microarray in a mouse mammary epithelial cell line NMuMG. Cancer Research 62:2203–2209.

Tang KT, Capparelli C, Stein JL, Stein GS, Lian JB, Huber AC, Braverman LE, DeVito WJ. 1996. Acidic fibroblast growth factor inhibits osteoblast differentiation in vitro: altered expression of collagenase, cell growth-related, and mineralization-associated genes. The Journal of Cellular Biochemistry 61:152–166.

Tanimura S, Asato K, Fujishiro SH, Kohno M. 2003. Specific blockade of the ERK pathway inhibits the invasiveness of tumor cells: down-regulation of matrix metalloproteinase-3/-9/-14 and CD44. Biochemical and Biophysical Research Communications 304:801–806.

Thant AA, Nawa A, Kikkawa F, Ichigotani Y, Zhang Y, Sein TT, Amin AR, Hamaguchi M. 2000. Fibronectin activates matrix metalloproteinase-9 secretion via the MEK1-MAPK and the PI3K-Akt pathways in ovarian cancer cells. Clinical and Experimantal Metastasis 18:423–428.

Thomson MS. 1923. An hitherto undescribed familial disease. British Journal of Dermatology and Syphilis 35:455–462.

Trusolino L, Comoglio PM. 2002. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nature Reviews Cancer 2:289–300.

Vincent T, Mechti N. 2004. IL-6 regulates CD44 cell surface expression on human myeloma cells. Leukemia 18:967–975.

von Kobbe C, Harrigan JA, May A, Opresko PL, Dawut L, Cheng WH, Bohr VA. 2003. Central role for the Werner syndrome protein/poly(ADP-ribose) polymerase 1 complex in the poly (ADP-ribosyl)ation pathway after DNA damage. Molecular and Cellular Biology 23:8601–8613.

Wang H-R, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, Wrana JL. 2003. Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. Science 302:1775–1779.

Warnick CT, Dabbas B, Ford CD, Strait KA. 2001. Identification of a p53 response element in the promoter region of the hMSH2 gene required for expression in A2780 ovarian cancer cells. The Journal of Biological Chemistry 276:27363–27370.

Weber GF, Ashkar S. 2000. Stress response genes: the genes that make cancer metastasize. The Journal of Molecular Medicine 78:404–408.

Wielenga VJ, Smits R, Korinek V, Smit L, Kielman M, Fodde R, Clevers H, Pals ST. 1999. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the Wnt pathway. American Journal of Pathology 154:515–523.

Xing L, Ge C, Zeltser R, Maskevitch G, Mayer BJ, Alexandropoulos K. 2000. c-Src signaling induced by the adapters Sin and Cas is mediated by Rap1 GTPase. Molecular and Cellular Biology 20:7363–7377.

Zhang G, He B, Weber GF. 2003. Growth factor signaling induces metastasis genes in transformed cells: molecular connection between Akt kinase and osteopontin in breast cancer. Molecular and Cellular Biology 23:6507–6519.

Zimmermann S, Moelling K. 1999. Phosphorylation and regulation of Raf by Akt (protein kinase B). Science 286:1741–1744.

SECTION IV

INTERACTIONS BETWEEN HOST AND TUMOR

Interactions between tumor cells and their surrounding tissue are not a consequence of transformation, rather they may be caused by basic processes of homeostasis or due to the particular differentiation of the organ of origin for the cancer. However, these interactions can bear profoundly on the ability of transformed cells to grow into tumors. This may be mediated by the transduction of signals that either support or restrict tumor promotion, or it may be through the provision of nutrients by the blood supply. The recognition of a tumor by the immune system is also an important determinant. Clinically, those interactions may cause prominent symptoms.

CHAPTER 9

STRUCTURAL GROWTH CONTROL

The complexity of multicellular organisms necessitates coordination among a diverse range of specialized cell types through intercellular communication. This communication is refined by the three-dimensional organ structure. It is formed by the extracellular matrix, a network of macromolecules that provides contextual information and an architectural scaffold for cell adhesion and migration. The communication between cells and the extracellular matrix is accomplished mostly through adhesion receptors, such as Integrins. Signaling pathways associated with growth factor receptors and hormone receptors also play an important part in maintaining the proper tissue architecture. The disruption of this form of signaling results in dramatic changes of cell–cell and cell–matrix interactions. The influences occur on three aspects of cell physiology, cell cycle progression, cell shape, and cell polarity.

- Cell binding to the extracellular matrix of the substratum ensures normal tissue patterning by exerting spatial and temporal control of cell cycle progression [Huang and Ingber 1999]. In epithelial and other nontransformed cells, homotypic and heterotypic cell–cell contacts can induce the inhibition of cell cycle progression. In this process, called contact inhibition, a signal leads to the termination of cell division once a continuum of cells has been formed. Tumor cells are not subject to this mechanism. Malignancy results when cells grow at times and in locations, where proliferation is normally suppressed, that is, when they become autonomous of the normal controls that spatially constrain growth within living tissue.
- The influence of the extracellular matrix on cell shape is determined by the molecular complexity (composition), pliability (mechanical rigidity), and topography of the matrix. These factors need to synergize for adhesion-mediated signaling. It is likely that matrices derived from distinct tissues have unique composition/rigidity/topography signatures that determine their capacity to affect cell shape [Cukierman et al. 2001]. These interactions also inhibit cell motility. A loss of this spatial control leads to a disorganization of the normal tissue architecture that is the hallmark of neoplastic transformation.
- Epithelial cell polarity, comprising apical and basal surfaces, is an important feature of normal stromal context. Orientational cues are received from the microenvironment, particularly the basement membrane. In addition, epithelial cell–cell contact through adherence junctions, gap junctions, tight junctions, and desmosomes generates epithelial cell polarity. Encircling the apical ends of epithelial cells are adherens junctions that link to the intracellular Actin cytoskeleton. The even distribution of adherens junctions is an active process that depends on the small guanosine triphosphatase (GTPase) RAP1 [Knox and Brown 2002]. The loss of epithelial cell polarity may lead to increased proliferation and tumorigenesis. Hence, aberrations in stroma can precede and stimulate the development of parenchymal cancers [Bissell and Radisky 2001].

9.1 INTEGRIN SIGNALING

Signaling to induce cell cycle progression is initiated primarily through the ligation of growth factor receptors. In contrast, the ligation of Integrins does

not normally suffice to promote cell divisions. Nevertheless, Integrin signaling may be critical for growth through preventing anoikis and coalescing with growth factor signal transduction. Integrins often associate with growth factor receptors and enhance their signals. Integrin $\alpha_v\beta_3$ associates with the Insulin Receptor, PDGF Receptor, and VEGF Receptor. Integrin $\alpha_5\beta_1$ associates with the EGF Receptor, possibly via CD9 [Giancotti and Ruoslahti 1999]. Integrins are necessary for the optimal activation of specific growth factor receptors by facilitating the induction of MAPK, which in the absence of attachment is blocked in some cells at the level of RAF or MEK activation. JNK activity regulates the progression through the G_1 phase of the cell cycle. Because most growth factors are poor activators of JNK, the ability of Integrins to activate this kinase may account for the frequent requirement of Integrin-mediated adhesion in cell proliferation. The dependence on communication with the microenvironment via Integrins protects the body from cell growth in places where this could be harmful.

Most Integrins signal through Focal Adhesion Kinase (FAK). The cytoplasmic tail of Integrin β chains binds Talin, Paxillin, and Vinculin. FAK is recruited and autophosphorylates on tyrosine 397. This creates a binding site for the SH2 domain of SRC.

– FAK is phosphorylated on tyrosine 925 by SRC and then can bind GRB with activation of the SOS→RAS→RAF→MAP kinase cascade. A downstream target of this pathway is c-FOS.
– SRC is activated through dephosphorylation by SHP-2. SRC phosphorylates the cytoskeletal proteins Paxillin and Tensin, and the docking protein $P130^{CAS}$. $P130^{CAS}$ then recruits the adapter proteins CRK and NCK. CRK may activate JNK, which enters the nucleus and phosphorylates c-JUN.

The integrin-induced pathways of JNK and MAPK activation may synergize. c-JUN combines with c-FOS to form AP-1, which regulates genes that are important for the progression through the G_1 phase of the cell cycle. The expression of Cyclin D_1 is essential for cell cycle progression because it triggers a series of nuclear events that mediate passage through the late G_1 restriction point and entry into S phase. The *cyclin* D_1 promoter is coordinately regulated by JNK and MAPK.

Some β_1 and α_V Integrins may activate FYN via Caveolin-1 as a membrane adapter. This engages the SHC→GRB2→SOS→RAS→RAF→MAP Kinase cascade. MAPK is required for cell growth in part because it phosphorylates the transcription factor TCF, resulting in the transcription of *c-fos*. Integrins that do not activate SHC are weak activators of MAPK and of cell proliferation. The FAK and FYN pathways to MAPK activation may cooperate.

- Tumors are stiffer than normal tissue because they have a stiff stroma. Matrix stiffness perturbs epithelial morphogenesis by clustering Integrins to enhance ERK activation, which signals in a critical growth pathway, and to increase ROCK-generated contractility. Elevated RHO-dependent cytoskeletal tension drives focal adhesions, disrupts adherens junctions, perturbs tissue polarity, enhances growth, and hinders lumen formation. ERK and RHO constitute part of an integrated mechanoregulatory circuit, linking matrix stiffness to cytoskeletal tension through Integrins. This regulates tumor growth [Paszek et al. 2005].
- In glioblastomata, promigratory signaling through the Epidermal Growth Factor Receptor depends on FAK. The COOH-terminal region of FAK, which includes the FAT domain, is essential for targeting and activating FAK at the focal adhesions. The NH_2-terminal domain of FAK recognizes the cytoplasmic domain of Integrin β_1 and is required for the regulation of FAK in EGF-dependent migration. The loss of FAK from focal adhesions inhibits EGF Receptor signal transduction and transmits a proapoptotic signal to an NH_2-terminal variant of FAK present in the nucleus [Jones et al. 2001].

9.2 CELL–CELL COMMUNICATION

Most normal cells within solid tissues have functional gap junctional intercellular communication. The family of Connexins forms hexameric units in the cell membranes, which couple with corresponding Connexins in contiguous cells. This serves to synchronize either the metabolic or the electrotonic functions of the cells within a tissue. Gap junction channels have a diameter of approximately 1.5–2 nm and are large enough to permit the direct diffusion of small molecules and ions, but not proteins, complex lipids, polysaccharides, or RNA. Channel passage does not require ATP and results from passive diffusion. Connexins are expressed in a tissue and development

specific manner. Pluripotent stem cells have no expression of *connexin* genes or functional gap junctions [Loewenstein 1966; Trosko and Ruch 1998].

Cell–cell communication can take place through soluble mediators released in a paracrine function. This does not require cell–cell contact. Rather, it conveys microenvironmental cues over longer distances. Typically, this form of communication is not bidirectional, but has a sender (often neuroendocrine cells) and a recipient (often parenchymal cells).

- Many neoplastic cells have fewer and smaller gap junctions, express reduced levels of Connexins, and have lower gap junctional communication than their nonneoplastic counterparts [Cesen-Cummings et al. 1998].
- The proto-oncogene products RAS, SRC, and ERBB block gap junctional intercellular communication [Yamasaki 1990; Trosko et al. 1990]. Cancers have dysfunctional homologous or heterologous gap junctional intercellular communication and are not subject to contact inhibition. The disruption of gap junctional intercellular communication plays a role during the tumor promotion phase of carcinogenesis. Stable downregulation of gap junctional intercellular communication leads to the conversion of premalignant cells to invasive and metastatic cancer cells [Trosko and Ruch 1998].
- Neuroendocrine cells provide paracrine stimuli for the propagation of local carcinoma cells. Neuroendocrine differentiation is associated with the progression of prostate cancer toward an androgen-independent state. Apoptosis comprises a critical defense mechanism against transformation. The neuropeptides Bombesin and Calcitonin inhibit apoptosis in androgen-dependent and androgen-independent prostate carcinoma cells. Hence, neuropeptides confer antiapoptotic capabilities on nonneuroendocrine cells in close proximity to the neuroendocrine cells, and thereby contribute to the aggressive clinical course of prostate tumors containing neuroendocrine elements [Vilches et al. 2004].

9.3 STROMAL EFFECTS

Transformed stroma can induce malignancy in lung and mammary epithelia [Nakamura et al. 1997; Barcellos-Hoff and Ravani 2000]. Two important mediators of this effect are TGF-β and HGF.

Defective stromal cells, which are unable to respond to TGF-β, stimulate the development of epithelial cancers derived from the forestomach and the prostate epithelium. These tumors are associated with an increased abundance of stromal cells and activation of paracrine HGF signaling as a possible cause for epithelial cell proliferation [Bhowmick et al. 2004]. Similar mechanisms may play a role in the stromally induced carcinomata that occur in heritable hamartomatous polyposis diseases. Juvenile polyposis syndrome is caused by defects in the genes encoding BMPR-IA or SMAD4, while Cowden disease is caused by inactivation of the gene encoding PTEN. All of these gene products are associated with TGF-β signaling [Radisky and Bissel 2004].

HGF is present at high concentrations in the stroma of tumors [Yao et al. 1996] and may contribute to structural growth control (referred to as "landscaper factor") in a paracrine fashion in several neoplastic settings. Mutant versions of the HGF Receptor (MET), despite their enhanced catalytic efficiency, are not sufficient to cause cell transformation per se, but require the presence of HGF. This indicates that the availability of active HGF is crucial for MET-dependent tumorigenicity.

Senescent fibroblasts stimulate the growth of preneoplastic and neoplastic epithelial cells, but not of normal cells. This is caused, to a large extent, by secreted factors. Cellular senescence alters the microenvironment around the cell in two ways that may contribute to both aging and carcinogenesis.

- Senescent cells overproduce growth factors and cytokines that can stimulate the growth of precancerous cells. This derangement of homeostasis can synergize with accumulating mutations to favor the early stages of tumorigenesis [Campisi 1997; Campisi 2000].
- Senescent cells may impair the structural integrity of the microenvironment, allowing cells that harbor oncogenic mutations to transform fully. Senescent tissues express proteases and inflammatory cytokines. By secreting damaging molecules, senescent cells can disrupt the surrounding tissue architecture. Senescent dermal cells generate more Metalloproteinases that break down proteins in the surrounding extracellular matrix than young dermal cells. They also generate more of the proinflammatory cytokine Interleukin 1α. The enzymes secreted by senescent dermal fibroblasts may be able to destroy the basement membrane and underlying stroma that keep potentially

cancerous cells in check. Relatively few cells could in this way exert far-reaching deleterious effects upon tissue integrity and organ function [Campisi 1997].

The tumor-promoting functions of senescent cells are determined to a large extent by the expression of P21 and P16. P21 is responsible, in part, for the paracrine tumor-promoting functions of stromal fibroblasts. During fibroblast senescence, P21 may play a role not only in the inhibition, but also in the induction of gene expression. Responsive genes include those for mitogenic and antiapoptotic secreted factors, such as CYR61, CTGF, Epithelin (Granulin), pro-Saposin, TGF-α, and APPβ. Their induction induces paracrine growth promoting activities, and they may account for the tumor-promoting activities of senescent fibroblasts. In breast cancer and prostate cancer, the expression of P16 constitutes an unfavorable prognostic marker, which likely reflects its tumor-promoting role in senescent stroma. In prostate cancer, P21 expression is associated with early relapse and with progression to androgen independence [Roninson 2003].

- The breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation. Tumor-derived breast fibroblasts support ductal alveolar morphogenesis, whereas normal organ-specific fibroblasts retard the morphological conversion of epithelial cells [Shekar et al. 2001]. Mammary epithelial cells in contact with the basement membrane undergo growth arrest and respond to MMP-3 with apoptosis, while in the absence of this contact they continue to proliferate and respond to MMP-3 with epithelial–mesenchymal transition and transformation [Sternlicht et al. 1999]. Fibrotic breast disease predisposes to breast cancer. The presence of a fibrotic focus may be a prognostic marker in ductal carcinoma in situ [Colpaert et al. 2001; Hasebe et al. 2002].
- Environmentally induced fibrotic disorders of the lung can increase the incidence of lung cancer. This is the case for exposure to asbestos, which causes parenchymal and pleural fibrosis and increased risk for lung cancer and mesothelioma [Kannerstein et al. 1977].
- Hepatocellular carcinoma is accompanied by a fibrotic stromal reaction, in which hepatic stellate cells show increased fibrogenesis, proliferation, and matrix degradation. At the same time, their retinoid production and cytokine release are reduced [Bissell and Radisky 2001].

9.4 OTHER FACTORS

Increased levels of the chondroitin sulfate proteoglycan Versican in the peritumoral stroma of prostate cancer or breast cancer are predictive of the risk of relapse. Versican may facilitate the local spread of tumor cells, potentially via destabilization of focal adhesions. Versican can affect the cell adhesion and migration on extracellular matrix by osteosarcoma cells, astrocytoma cells, and melanoma cells. Versican also inhibits the attachment of cells to Fibronectin by binding to the RGD sequence in Fibronectin [Sakko et al. 2003].

Not all cells that leave the site of a primary tumor also form metastases. Most of the cells that reach secondary organs succumb to apoptosis [Wong et al. 2001]. Molecular factors present in specific organs can affect metastasis formation by influencing the survival of various cancer cells, their gene expression, and growth ability. Thus, bone metastases of breast cancers, but not their primary tumor cells, may receive growth signals from IGF-1 or TGF-β [Yoneda et al. 2001]. The organ environment can markedly change the gene expression patterns of cancer cells, resulting in alteration of their characteristics. One type of cancer cells, growing in two distinct sites, can express different levels of various proteolytic enzymes [Nakajima et al. 1990; Gohji et al. 1997].

REFERENCES

Barcellos-Hoff MH, Ravani SA. 2000. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Research 60:1254–1260.

Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL. 2004. TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303:848–851.

Bissell MJ, Radisky D. 2001. Putting tumors in context. Nature Reviews Cancer 1:46–54.

Campisi J. 1997. Aging and cancer: the double-edged sword of replicative senescence. Journal of the American Geriatric Society 45:482–488.

Campisi J. 2000. Cancer, aging and cellular senescence. In Vivo 14:183–188.

Cesen-Cummings K, Fernstrom MJ, Malkinson AM, Ruch RJ. 1998. Frequent reduction of gap junctional intercellular communication and connexin 43 expression in human and mouse lung carcinoma cells. Carcinogenesis 19:61–67.

Colpaert C, Vermeulen P, Van Marck E, Dirix L. 2001. The presence of a fibrotic focus is an independent predictor of early metastasis in lymph node-negative breast cancer patients. American Journal of Surgical Pathology 25:1557–1558.

Cukierman E, Pankov R, Stevens DR, Yamada KM. 2001. Taking cell-matrix adhesions to the third dimension. Science 294:1708–1712.

Giancotti FG, Ruoslahti E. 1999. Integrin signaling. Science 285:1028–1032.

Gohji K, Nakajima M, Boyd D, Dinney CP, Bucana CD, Kitazana S, Kamidono S, Fidler IJ. 1997. Organ-site dependence for the production of urokinase-type plasminogen activator and metastasis by human renal cell carcinoma cells. American Journal of Pathology 151:1655–1661.

Hasebe T, Sasaki S, Imoto S, Mukai K, Yokose T, Ochiai A. 2002. Prognostic significance of fibrotic focus in invasive ductal carcinoma of the breast: a prospective observational study. Modern Pathology 15:502–516.

Huang S, Ingber DE. 1999. The structural and mechanical complexity of cell-growth control. Nature Cell Biology 1:E131–E138.

Jones G, Machado J Jr, Merlo A. 2001. Loss of focal adhesion kinase (FAK) inhibits epidermal growth factor receptor-dependent migration and induces aggregation of NH_2-terminal FAK in the nuclei of apoptotic glioblastoma cells. Cancer Research 61:4978–4981.

Kannerstein M, Churg J, McCaughey E, Selikoff IJ. 1977. Pathogenic effects of asbestos. Archive of Pathology and Laboratory Medicine 101:623–628.

Knox AL, Brown NH. 2002. Rap1 GTPase regulation of adherens junction positioning and cell adhesion. Science 295:1285–1288.

Loewenstein WR. 1966. Permeability of membrane junctions. Annals of the New York Academy of Science 137:441–472.

Nakajima M, Morikawa K, Fabra A, Bucana DC, Fidler I. 1990. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. Journal of the National Cancer Institute 82:1890–1898.

Nakamura T, Matsumoto K, Kiritoshi A, Tano Y, Nakamura T. 1997. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor-stromal interactions. Cancer Research 57:3305–3313.

Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, Weaver VM. 2005. Tensional homeostasis and the malignant phenotype. Cancer Cell 8:241–254.

Radisky DC, Bissel MJ. 2004. Respect thy neighbor! Science 303:775–777.

Roninson IB. 2003. Tumor cell senescence in cancer treatment. Cancer Research 63:2705–2715.

Sakko AJ, Ricciardelli C, Mayne K, Suwiwat S, LeBaron RG, Marshall VR, Tilley WD, Horsfall DJ. 2003. Modulation of prostate cancer cell attachment to matrix by versican. Cancer Research 63:4786–4791.

Shekar MPV, Werdell J, Santer SJ, Pauley RJ, Tait L. 2001. Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: Implications for tumor development and progression. Cancer Research 61:1320–1326.

Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98:137–146.

Trosko JE, Chang CC, Madhukar BV, Klaunig JE. 1990. Chemical, oncogene and growth factor inhibition of gap junctional intercellular communication: an integrative hypothesis of carcinogenesis. Pathobiology 58:265–278.

Trosko JE, Ruch RJ. 1998. Cell-cell communication in carcinogenesis. Frontiers in Bioscience 3:208–236.

Vilches J, Salido M, Fernandez-Segura E, Roomans GM. 2004. Neuropeptides, apoptosis and ion changes in prostate cancer: methods of study and recent developments. Histology and Histopathology 19:951–961.

Wong CW, Lee A, Shientag L, Yu J, Dong Y, Kao G, Al-Mehdi AB, Bernhard EJ, Muschel RJ. 2001. Apoptosis: an early event in metastatic inefficiency. Cancer Research 61:333–338.

Yamasaki H. 1990. Gap junctional intercellular communication and carcinogenesis. Carcinogenesis 11:1051–1058.

Yao Y, Jin L, Fuchs A, Joseph A, Hastings HM, Goldberg ID, Rosen EM. 1996. Scatter factor protein levels in human breast cancers: clinicopathological and biological correlations. American Journal of Pathology 149:1707–1717.

Yoneda T, Williams PJ, Hiraga T, Niewolna M, Nishimura R. 2001. A bone-seeking clone exhibits different biological properties from the MDA-MB-231 parental human breast cancer cells and a brain-seeking clone *in vivo* and *in vitro*. The Journal of Bone and Mineral Research 16:1486–1495.

CHAPTER 10

THE ORGAN PREFERENCE OF METASTASIS FORMATION

Generally, colon cancer metastasizes into the liver, while liver cancer and rectal carcinoma disseminate predominantly into the lungs. Melanoma and lung cancer tend to colonize the liver, while neuroblastoma, breast, thyroid, prostate, and kidney carcinoma metastasize into bones. The presence of bone marrow micrometastases may be an independent prognostic factor. The brain is a frequent target for metastases from breast, kidney, gastrointestinal tract, and lung. Brain metastasis may also cause the first clinical manifestations of melanoma. Ovarian cancer invades the peritoneal cavity, without utilizing the blood or lymph system. For some cancers, the organ of origin is also the target organ. Basalioma, chondrosarcoma, and some forms of myelogenous leukemia typically grow locally invasive but do not form distal metastases. For most solid tumors, the presence of metastases in the draining lymph nodes is a critical prognostic factor for distal metastases and for survival.

The consistent patterns of organ preference associated with individual malignancies imply that topology of metastasis formation is encoded in the subsets of metastasis gene products (homing receptors and adhesion molecules) expressed on the tumor cell surface (Table 10.A). This pattern often matches the organ-specific expression of genes by tissue-resident macrophages in the target organ. Thus, cancer cells that metastasize to the liver express homing receptors akin to Kupffer cells, cancers with organ preference for bone resemble osteoclasts in their expression of homing receptors, cancers that disseminate to the lungs share gene-expression patterns with alveolar macrophages, and brain metastases overlap in gene expression with glia cells [Weber and Ashkar 2000].

The regulation of function of metastasis gene products occurs largely through structural modifications on the posttranslational and posttranscriptional levels. The resulting permutations make it possible to encode a large number of anatomical locations with a limited number of genes. As a case in point, cells expressing various variants of the homing receptor CD44 may bind particular ligands, including hyaluronate, Osteopontin, and Fibronectin, with overlapping but distinct functional consequences. Several metastasis gene products have one or more alternative splice sites and contain multiple sites for phosphorylation, glycosylation, calcium binding, and chondroitin sulfate binding [Patarca et al. 1993; Ashkar et al. 1993; Günthert 1993]. Distinct splice variants of CD44 are associated with diverse patterns of dissemination. Thus, the expression of CD44 variant exons 6 and 7 mediates the metastasis of pancreas carcinoma to the lungs, while CD44v10 mediates the homing of multiple myeloma cells to the bone marrow.

Some tumors spread predominantly hematogenously, while others disseminate predominantly through the lymphatic system. Lymphatic capillaries differ from blood capillaries by having a poorly developed basal lamina and being devoid of pericytes. Lymphatic endothelium is highly attenuated, and its cells are connected directly to the interstitial Collagen via anchoring filaments [Pepper and Skobe 2003]. Genes that encode constituents of adherens junctions, such as *desmoplakin* (*dsp*), *plakoglobin*, *plakophilin 2* (*pkp2*), *H-cadherin*, and *tight junction protein 2* (*zona occludens 2*), are expressed in lymphatic endothelial cells. JAM-2, a member of the

Table 10.A. Homing receptors as molecular basis for the topology of metastasis formation. Specific homing receptors expressed on the surface of certain tumor cells enable their migration to selective target organs. This may be due to an abundance of the cognate ligand in the targeted microenvironment

Tumor	Surface molecule	Target organ	Target molecule	Reference
Breast carcinoma	CD44 v4-v7		Osteopontin, hyaluronate	Günthert et al. 1991
Breast carcinoma	CD44 D3 (v6-v7)		Osteopontin, hyaluronate	Matsumura and Tarin 1992
Breast carcinoma	CXCR4	Lymph node, lung, liver, bone marrow	CXCL12	Mueller et al. 2001
Breast carcinoma	CCR7	Lymph node	CCL21	Mueller et al. 2001
Breast carcinoma	Surface Fibronectin	Lungs	Dipeptidyl Dipeptidase IV	Rouslahti 2000
Breast carcinoma	PECAM-1 (CD31)	Endothelium	PECAM-1	Buck 1995
Breast carcinoma	CAM 120/180			Buck 1995
Breast carcinoma	Integrin $\alpha_V\beta_5$		Bone Sialoprotein	
Prostate cancer	P-Selectin	High endothelial venules	Peripheral Node Addressin	Diacovo 1996
Prostate cancer	L-Selectin	Peyer's patches	MADCAM-1	Butcher and Picker 1996
Prostate cancer	Integrin $\alpha_4\beta_7$	Peyer's patches, appendix, nonpulmonary mucosa	MADCAM-1, VCAM-1	Butcher and Picker 1996; Buck 1995
Prostate cancer	Integrin $\alpha_2\beta_1$	Bone matrix	Collagen type I	
Prostate cancer	Integrin $\alpha_2\beta_3$	Bone	Fibronectin	
Prostate cancer	Integrin $\alpha_V\beta_3$, Integrin $\alpha_V\beta_5$	Bone	Osteonectin	Jacob et al. 1999; De et al. 2003
Prostate cancer	Galectin-3	Endothelial cells		Lehr and Pienta 1998
Pancreas carcinoma	CD44 v6,v7	Lung lymph nodes	Osteopontin, hyaluronate	Günthert et al. 1991
Colon carcinoma	CD44 D3 (v6-v7) CD44R (v8-v10) CD44R1		Osteopontin, hyaluronate hyaluronate	Matsumura and Tarin 1992; Finn et al. 1994; Tanabe et al. 1993
Small cell lung carcinoma	N-CAM		N-CAM FGF Receptor	Cunningham 1991 O'Brien 1995
Lymphoma	CD44	Lymph nodes	Hyaluronate	Zahalka et al. 1995
Lymphoma	L-Selectin	Lymph nodes	GlyCAM-1, MADCAM-1, Peripheral Node Addressin (CD34)	Bargatze et al. 1986
Lymphoma	LPAM-1 ($\alpha_4\beta_P$)	Intestinal lymph nodes		Roos 1991
Lymphoma	LFA-1	Spleen	ICAM-1,2,3	Zahalka et al. 1995
Multiple myeloma	CD44 v10	Bone marrow		Asosingh et al. 2001
T-cell tumor	LFA-1	Liver	ICAM-1,2,3	Roos 1991
Mycosis fungoides	CTLA	Skin	E-Selectin, secreted E-Selectin	Santamaria Babi et al. 1995
Melanoma	Integrin $\alpha_4\beta_1$	Central nervous system, heart, skin	VCAM-1, Fibronectin	Butcher and Picker 1996
Melanoma	Integrin $\alpha_E\beta_7$	Mucosal epithelia	E-Cadherin	Shaw and Brenner 1995
Melanoma		Central nervous system	HECA-452	
Melanoma	CCR10	Skin	CCL27	Mueller et al. 2001
Melanoma	CXCR3	Joints, lymph nodes	CXCL9 (Mig), CXCL10 (IP10)	
Neuroblastoma	N-CAM		N-CAM, FGF Receptor	Cunningham 1991; O'Brien 1995
Fibrosarcoma	CD44 v8-v10	Lung	Hyaluronate	Finn et al. 1994
Ewing sarcoma	N-CAM		N-CAM, FGF Receptor	Cunningham 1991; O'Brien 1995
Wilms tumor	N-CAM		N-CAM, FGF Receptor	Cunningham 1991; O'Brien 1995
Squamous cell carcinoma	Desmoglein I (DG I)	Epithelium		Buck 1995

Immunoglobulin superfamily, is expressed in tight junctions of lymphatic vessels and facilitates lymphocyte transmigration. The endothelial cells in the lymphatic system differ from blood vessel endothelial cells in the expression of specific cell-surface markers that serve as homing receptors in leukocyte trafficking [Al-Rawi et al. 2005]. These markers include PDPN, PROX-1, LYVE-1, VEGFR-3, ANG-2, and CCBP-2.

- The 162 amino acid, 38 kD transmembrane glycoprotein Podoplanin (PDPN, T1α2, GP36, Aggrus) localizes to podocytes. It is expressed in budding lymphatic progenitor cells and in the luminal plasma membrane of lymphatic vessels.
- PROX-1 [Oliver et al. 1993] is a homeobox protein involved in the growth and elongation of the lymphatic vessel sprouts. PROX-1 (Prospero-Related Homeobox 1) {1q32.2-q32.3} expression in embryonal development localizes to a subpopulation of endothelial cells in the veins, which are committed to lymphatic development.
- LYVE-1 (Lymphatic Vessel Endothelial Hyaluronan Receptor-1) {11} [Banerji et al. 1999] is a hyaluronate receptor on lymphatic endothelium. It is a member of the Link protein family, whose only other receptor for hyaluronate, CD44, is directly involved in leukocyte migration and tumor metastasis.
- The receptor VEGFR-3 (FLT-4) {5q25.3} is primarily expressed in lymphatic endothelial cells. Its ligands VEGF-C (VRP) and VEGF-D enhance the growth of lymphatic vessels.
- ANG-2 (Angiopoietin-2) {8p23} is expressed by lymphatic endothelial cells and is required for the proper development of the lymphatic system [Gale et al. 2002].
- CCBP-2 (Chemokine Binding Protein-2, D6, CMKBR9) {3p21.3} [Nibbs et al. 1997] is a chemokine receptor that is only expressed on a subset of lymphatics in the skin, intestine, and lymphoid tissues.

CCL21 (Secondary Lymphoid Chemokine, SLC, 6Ckine, Exodus-2) is constitutively expressed by lymph endothelial cells, suggesting an active role for these endothelia in tumor cell homing.

Macrophage Mannose Receptor I (MR-I) is expressed by lymphatic endothelial cells and mediates the adhesion of lymphocytes to lymphatics in lymph nodes [Irjala et al. 2001]. It supports lymphocyte binding to lymphatic vessels in a L-Selectin-dependent fashion, an interaction that may control lymphocyte exit from the lymph nodes. This mechanism is used by some tumors that express mannose-type oligosaccharides on their surface.

Molecular and cellular mechanisms governed by the target organs that determine sites for homing include Chemokines, Addressins, site-specific peptide motifs, and recruited bone marrow cells.

Chemotactic cytokines. Circulating tumor cells can respond to the diffusion of soluble factors from target organs. These target organs for metastasis may contribute to the patterns of dissemination by secreting chemotactic cytokines. The interaction between CCR7 and CCL21 is important for lymph node metastases, while the engagement of CXCR4 by CXCL12 contributes importantly to lung, liver, bone marrow, and brain metastases. The Chemokines CCL21 and CXCL12 may direct breast cancer cells bearing cognate receptors on their surface [Mueller et al. 2001]. Osteopontin is physiologically produced by osteoclasts in bone and in the lungs by alveolar macrophages. Both are common organs for CD44v expressing cancer cells to disseminate to [Weber 2001].

Addressins. Adhesion and stabilization of circulating tumor cells to endothelial cells in target blood vessels play an important role in the process of metastasis. Vascular endothelial cells recognize circulating cells and allow them to extravasate in a tissue-specific manner. The differential expression of adhesion receptors on tumor cells, and Addressins [Streeter et al. 1988] on endothelial cells communicate signals that determine the organ specificity of dissemination. Endothelial cells from lymph nodes express Peripheral Lymph Node Addressins, endothelial cells from nonlymphoid tissues express ICAM-1 and CD49, while endothelial cells from mucosal sites express MADCAM-1 (Mucosal Addressin Cell-Adhesion Molecule-1). P-Selectin is differentially expressed among endothelial cells in various locations [Kieda et al. 2002]. Cell surface receptors are involved in the adhesion of lymphoma cells to hepatic sinusoidal endothelial cell. An interaction of Integrin $\alpha_4\beta_1$ on lymphoma cells with liver endothelial VCAM-1 occurs during the early stages of the adhesion process and may be important in liver metastasis [Papadimitriou et al. 1999].

Peptide motifs. The vascular bed of an individual tissue can express molecules specific for that tissue.

Metastasis formation may therefore be a consequence, in part, of adhesive interactions between the tumor cells and organ-specific endothelial markers. According to evidence derived from peptide libraries, the binding motifs are often tripeptides or tetrapeptides [Ruoslahti 2000].

– The sequence SRL encodes a brain homing motif
– The RDV motif homes to the retina
– CGFE is a motif in lung homing peptides recognized by Membrane Dipeptidase.
– RGD, which is important in Integrin binding, homes to sites of neovascularization

Mobilization of bone marrow cells. Tumors can recruit bone marrow-derived cells to other regions in the body. There the recruited cells change the local environment to support developing metastases. This is accomplished through secreted molecules from the tumor cells, which act on VEGFR1 on the bone marrow cells, and which stimulate fibroblasts in the target organ to secrete Fibronectin. The mobilized hematopoietic precursor cells from the bone marrow express Integrin $\alpha_4\beta_1$, through which they adhere to Fibronectin. These cells create a pre-metastatic niche in the target organ [Kaplan et al. 2005].

Lungs. Organ specificity of lymphocyte homing is mediated by homing receptors on the white blood cells and Addressins on high endothelial venules. The same mechanism is used by certain disseminating tumors. Three proteins that are selectively expressed in the lung endothelia, LU-ECAM-1, Dipeptidyl Peptidase IV, and Membrane Dipeptidase, may be capable of facilitating adhesion by metastasizing tumor cells [Ruoslahti and Rajotte 2000]. LU-ECAM-1 (Lung Endothelial Cell Adhesion Molecule-1) is an Addressin that mediates the organ-specific binding by melanoma cells, but not by tumors of different origin. Lung endothelial cells also display constitutive expression of VCAM-1, whereas skin-derived endothelial cells do not.

Metadherin (Metastasis Adhesion Protein, LYRIC) is a 579 amino acid cell surface molecule of type II (the COOH-terminal end is extracellular), which causes homing specifically to the lung vasculature when it is expressed on tumor cells. Breast cancers express high levels of Metadherin and may initially disseminate to the lungs. Furthermore, the *metadherin* {19q13.2} expression levels in breast cancer patients are significantly correlated with a poor prognosis due to metastasis [Brown and Ruoslahti 2004]. Metadherin is a putative CEACAM-1 (CD66, Biliary Glycoprotein-1, BGP-1) associated protein in colon carcinoma.

The expression of CD44 containing the variant exons 6 and 7 in the membrane proximal extracellular region mediates metastasis formation by pancreas carcinoma to the lungs [Günthert et al. 1991]. This may reflect binding to the ligand Osteopontin, which is abundantly expressed by alveolar macrophages.

The Chemokine CXCL12 (SDF-1), which is highly expressed in the lungs binds to CXCR4 receptors on the surface of metastasizing breast cancer cells. This causes the cancer cells to migrate and invade [Mueller et al. 2001]. In colon cancer cells, CXCR4 is low at early stages of dissemination but is upregulated by the microenvironment of the lungs. Isolated metastatic cells are likely to require CXCR4 signals to initiate the outgrowth of micrometastases [Zeelenberg et al. 2003]. Lung metastases develop in up to 30% of patients with osteosarcoma. CXCL12 induces the directed migration and secretion of MMP-9 by osteosarcoma cells expressing CXCR4. The adhesion of the osteosarcoma cells to endothelial cells is promoted by CXCL12 [Perissinotto et al. 2005].

Liver. Cell surface glycans are involved in the adhesion of colon cancer cells to liver tissue. The adhesion is independent of E-Selectin, but dependent on calcium and is mediated by a surface glycan with the estimated structure NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1(±Fucα1-3)GlcNAcβ1-6(NeuAcα2-3Galβ1-3)GalNAc-pNP [Ota et al. 2000]. The expression of Mannose Receptors on hepatic sinusoidal endothelium facilitates the adhesion of melanoma cells through oligosaccharides on their surface and leads to increased liver metastasis. Inflammation can enhance this process through the production of IL-1, which mediates an increase in the expression of Mannose Receptors on the endothelium [Vidal-Vanaclocha et al. 1996; Mendoza et al. 1998].

Integrin-mediated adhesion between metastasizing tumor cells and hepatocytes has an important role in the formation of liver metastases. Integrin $\alpha_L\beta_2$ (CD11a/CD18, LFA-1) on metastasizing cells interacts with ICAM-1 (CD54) on liver cells. Transformed T-cells metastasize predominantly into the liver but spare the lungs. Their mutants that lack LFA-1 display reduced metastatic activity [Roos 1991].

The Chemokine CXCL12, which is highly expressed in the liver, binds to CXCR4 receptors on the surface of metastasizing breast cancer or colon cancer cells and aids their homing to the liver [Mueller et al. 2001; Zeelenberg et al. 2003].

Lymph nodes. The colonization of proximal lymph nodes (sentinel lymph nodes) is often the first step in the dissemination of cancers. Before there is evidence of metastasis, the regional lymph nodes that drain tumor areas may be enlarged, a condition termed tumor-reactive lymphadenopathy. Primary tumors may induce a reorganization of the vasculature and lymphatic channels in proximal lymph nodes before the arrival of metastatic cancer cells. The emerging vessels develop from high endothelial venules, in which the proliferation rate of endothelial cells is increased. The dilated lymph vessels contain relatively few lymphocytes [Ioachim 2002; Qian et al. 2006].

The Chemokine Receptors CXCR4 and CCR7 are important in lymph node metastasis. CXCR4 is upregulated in malignant melanoma and in breast cancer, and its ligand CXCL12 is highly expressed in lymph nodes. CCR7 is highly expressed by malignant melanoma and breast cancer cells. CCR7 and its ligands, the chemokines CCL19 and CCL21, are of crucial importance for the migration of lymphocytes and dendritic cells to the lymph nodes [Mueller et al. 2001]. Its expression is also associated with lymph node metastasis in gastric cancer [Mashino et al. 2002] and in non-small cell lung cancer [Takanami 2003].

The dissemination into draining lymph nodes is frequently an early step in metastasis formation and constitutes an adverse prognostic marker. The expression of CD44 forms containing variant exon 6 on the tumor surface is associated with invasiveness by certain lymphomata [Salles et al. 1993; Koopman et al. 1993].

Lymphoma cells expressing the lymph node homing receptor L-Selectin metastasize extensively and exclusively to peripheral lymph nodes, a homing phenotype displayed by mature T-cells leaving the thymus [Bargatze et al. 1986; Baumhueter et al. 1993].

While lymph node metastasis is common, the spleen is rarely a target for cancer metastases. Whereas the invasion of lymph nodes by lymphoma is dependent on CD44 and hyaluronate, but not on the Integrin $\alpha_L\beta_2$ (LFA-1, CD11a/CD18), the invasion of spleen by the same tumor is dependent on CD18 but not on CD44 [Zahalka et al. 1995].

Bone. Osteolytic bone metastasis by breast cancer is caused, in part, by the cooperative actions of a multigenic program, most of which encodes secreted and cell surface proteins. They include *il-11* and *ctgf*, which produce angiogenic and osteolytic factors. The prometastatic cytokine TGF-β, which may cause epithelial–mesenchymal transition, induces their expression [Kang et al. 2003].

Bone metastases by breast cancer and prostate cancer depend on the expression of CD44 splice variants and their ligand Osteopontin. Multiple myeloma cells preferentially home to bone marrow and adhere to bone marrow endothelial cells. This is mediated by CD44v10 [Asosingh et al. 2001].

Spinal tumors are mostly metastases from breast, prostate, or lung cancers. Their location may be extradural, intradural, or intramedullary. They cause symptoms due to spinal nerve compression and weakening of the vertebral structure, including back pain, incontinence, and decreased sensitivity in the buttocks.

Brain. The brain is a unique target for metastasis formation. It is confined by the skull and protected by the blood/brain barrier (BBB). The anatomic structure of the blood/brain barrier is defined by tight junctions among the endothelial cells, a thick basement membrane, and an underlying layer of astrocytes. It strictly regulates the access of soluble materials and cells to the brain. Tumor cells that have the capacity to colonize the brain need specific molecular determinants.

A paracrine form of Transferrin is important in brain metastasis. Cells metastatic to the brain respond to low levels of Transferrin and express high levels of Transferrin Receptors. This may facilitate their survival and growth in the brain micro-environment [Nicolson et al. 1990; Menter et al 1995].

Neurotrophins are important in brain invasive steps. Melanoma cells express the low affinity Neurotrophin Receptor $P75^{NTR}$, with their invasive properties being regulated by NGF (Nerve Growth Factor). They also express TRK-C, the receptor for the invasion promoting NT-3. In melanoma cells metastasizing to the brain, Neurotrophins promote invasion by enhancing the production of extracellular matrix-degrading enzymes, such as Heparanase [Denkins et al. 2004].

Interactions of Integrin $\alpha_3\beta_1$ and Laminin play important roles in the process of brain metastasis by non-small cell lung cancer [Yoshimasu et al. 2004].

Skin. T-cells in nearly all mucosal epithelial sites express Integrin $\alpha_E\beta_7$, which mediates lymphocyte binding to epithelial cell E-Cadherin. The Cutaneous Lymphocyte-Associated Antigen and its counterreceptor E-Selectin (ELAM-1) are involved in the selective targeting of memory T-cells reactive with skin-associated antigens to cutaneous inflammatory sites [Picker et al. 1991; Santamaria Babi et al. 1995]. The Cutaneous Lymphocyte-Associated Antigen is expressed by the malignant cells in chronic phase cutaneous T-cell lymphoma (mycosis fungoides, Sézary syndrome).

CCR10, the receptor for CCL 27, is important in skin metastases. It is expressed by melanoma cells.

Intestines. Lymphocytes expressing Integrin $\alpha_4\beta_7$ home to the intestine through the recognition of MADCAM-1 expressed on Peyer's patch high endothelial venules [De Keyser et al. 1996]. Because tumors rarely disseminate to this site the importance of this interaction in metastasis is uncertain.

REFERENCES

Al-Rawi MA, Mansel RE, Jiang WG. 2005. Molecular and cellular mechanisms of lymphangiogenesis. European Journal of Surgical Oncology 31:117–121.

Ashkar S, Teplow DB, Glimcher MJ, Saavedra RA. 1993. In vitro phosphorylation of mouse osteopontin expressed in *E. coli*. Biochemical and Biophysical Research Communications 191:126–133.

Asosingh K, Günthert U, De Raeve H, Van Riet I, Van Camp B, Vanderkerken K. 2001. A unique pathway in the homing of murine multiple myeloma cells: CD44v10 mediates binding to bone marrow endothelium. Cancer Research 61:2862–2865.

Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. 1999. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. Journal of Cell Biology 144:789–801.

Bargatze RF, Wu NW, Weissman IL, Butcher EC. 1986. High endothelial venule binding as a predictor of the dissemination of passaged murine lymphomas. The Journal of Experimental Medicine 166:1125–1131.

Baumhueter S, Singer MS, Henzel W, Hemmerich S, Renz M, Rosen SD, Lasky LA. 1993. Binding of L-selectin to the vascular sialomucin CD34. Science 262:436–438.

Brown DM, Ruoslahti E. 2004. Metadherin, a cell surface protein in breast tumors that mediates lung metastases. Cancer Cell 5:365–374.

Buck CA. 1995. Adhesion mechanisms controlling cell-cell and cell-matrix interactions during the metastatic process. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 172–205.

Butcher EC, Picker LJ. 1996. Lymphocyte homing and homeostasis. Science 272:60–66.

Cunningham BA. 1991. Cell adhesion molecules and the regulation of development. American Journal of Obstetrics and Gynecology 164:939–948.

De S, Chen J, Narizhneva NV, Heston W, Brainard J, Sage EH, Byzova TV. 2003. Molecular pathway for cancer metastasis to bone. The Journal of Biological Chemistry 278:39044–39050.

De Keyser F, Elewaut D, De Wever N, Bensbaho K, Cuvelier C. 1996. The gut associated addressins: lymphocyte homing in the gut. Bailliere's Clinical Rheumatology 10:25–39.

Denkins Y, Reiland J, Roy M, Sinnappah-Kang ND, Galjour J, Murry BP, Blust J, Aucoin R, Marchetti D. 2004. Brain metastases in melanoma: roles of neurotrophins. Neuro-oncology 6:154–165.

Diacovo TG, Puri KD, Warnock RA, Springer TA, von Andrian UH. 1996. Platelet-mediated lymphocyte delivery to high endothelial venules. Science 273:252–255.

Finn L, Dougherty G, Finley G, Meisler A, Becich M, Cooper DL. 1994. Alternative splicing of CD44 pre-mRNA in human colorectal tumors. Biochemical and Biophysical Research Communications 200:1015–1022.

Gale NW, Thurston G, Hackett SF, Renard R, Wang Q, McClain J, Martin C, Witte C, Witte MH, Jackson D, Suri C, Campochiaro PA, Wiegand SJ, Yancopoulos GD. 2002. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by angiopoietin-1. Developmental Cell 3:411–423.

Günthert U. 1993. CD44: A multitude of isoforms with diverse functions. Current Topics in Microbiology and Immunology 184:47–63.

Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. 1991. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 65:13–24.

Ioachim HLRH. 2002. Tumor-reactive lymphadenopathy. Philadelphia/Baltimore/New York/London/Buenos Aires/Hong Kong Sydney/Tokyo: Lippincott, Williams & Wilkins, pp. 254–258.

Irjala H, Johansson EL, Grenman R, Alanen K, Salmi M, Jalkanen S. 2001. Mannose receptor is a novel ligand for L-selectin and mediates lymphocyte binding to lymphatic endothelium. The Journal of Experimental Medicine 194:1033–1042.

Jacob K, Webber M, Benayahu D, Kleinman HK. 1999. Osteonectin promotes prostate cancer cell migration and invasion: a possible mechanism for metastasis to bone. Cancer Research 59:4453–4457.

Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. 2003. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 3:537–549.

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820–827.

Kieda C, Paprocka M, Krawczenko A, Zalecki P, Dupuis P, Monsigny M, Radzikowski C, Dus D. 2002. New human microvascular endothelial cell lines with specific adhesion molecules phenotypes. Endothelium 9:247–261.

Koopman G, Heider K-H, Horst E, Adolf GR, van den Berg F, Ponta H, Herrlich P, Pals ST. 1993. Activated human lymphocytes and aggressive non-Hodgkin's lymphomas express a homologue of the rat metastasis-associated variant of CD44. The Journal of Experimental Medicine 177:897–904.

Lehr JE, Pienta KJ. 1998. Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. Journal of the National Cancer Institute 90:118–123.

Mashino K, Sadanaga N, Yamaguchi H, Tanaka F, Ohta M, Shibuta K, Inoue H, Mori M. 2002. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Research 62:2937–2941.

Matsumura Y, Tarin D. 1992. Significance of CD44 gene products for cancer diagnosis and disease evaluation. The Lancet 340:1053–1058.

Mendoza L, Olaso E, Anasagasti MJ, Fuentes AM, Vidal-Vanaclocha F. 1998. Mannose receptor-mediated endothelial cell activation contributes to B16 melanoma cell adhesion and metastasis in liver. The Journal of Cell Physiology 174:322–330.

Menter DG, Herrmann JL, Nicolson GL. 1995. The role of trophic factors and autocrine/paracrine growth factors in brain metastasis. Clinical and Experimental Metastasis 13:67–88.

Mueller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56.

Nibbs RJB, Wylie SM, Yang J, Landau NR, Graham GJ. 1997. Cloning and characterization of a novel promiscuous human β-chemokine receptor D6. The Journal of Biological Chemistry 272:32078–32083.

Nicolson GL, Inoue T, Van Pelt CS, Cavanaugh PG. 1990. Differential expression of a Mr approximately 90,000 cell surface transferrin receptor-related glycoprotein on murine B16 metastatic melanoma sublines selected for enhanced brain or ovary colonization. Cancer Research 50:515–520.

O'Brien C. 1995. Neuronal adhesion molecules signal through FGF receptor. Science 267:1263–1264.

Oliver G, Sosa-Pineda B, Geisendorf S, Spana EP, Doe CQ, Gruss P. 1993. Prox 1, a prospero-related homeobox gene expressed during mouse development. Mechanisms of Development 44:3–16.

Ota M, Takamura N, Irimura T. 2000. Involment of cell surface glycans in adhesion of human colon carcinoma cells to liver tissue in a frozen section assay: role of endo-β-galactosidase-sensitive structures. Cancer Research 60:5261–5268.

Papadimitriou MN, Menter DG, Konstantopoulos K, Nicolson GL, McIntire LV. 1999. Integrin $\alpha_4\beta_1$/VCAM-1 pathway mediates primary adhesion of RAW117 lymphoma cells to hepatic sinusoidal endothelial cells under flow. Clinical and Experimental Metastasis 17:669–676.

Patarca R, Saavedra RA, Cantor H. 1993. Molecular and cellular basis of genetic resistance to bacterial infection: the role of the early T-lymphocyte activation-1/osteopontin gene. Critical Reviews in Immunology 13:225–246.

Pepper MS, Skobe M. 2003. Lymphatic endothelium: morphological, molecular and functional properties. The Journal of Cell Biology 163:209–213.

Perissinotto E, Cavalloni G, Leone F, Fonsato V, Mitola S, Grignani G, Surrenti N, Sangiolo D, Bussolino F, Piacibello W, Aglietta M. 2005. Involvement of chemokine receptor 4/stromal cell-derived factor 1 system during osteosarcoma tumor progression. Clinical Cancer Research 11:490–497.

Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 349:796–799.

Qian CN, Berghuis B, Tsarfaty G, Bruch MB, Kort EJ, Ditlev J, Tsarfaty H, Hudson E, Jackson DG, Petillo D, Chen J, Resau JH, The BT. 2006. Preparing the "soil": The primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells. Cancer Research 66:10365–10376.

Roos E. 1991. Adhesion molecules in lymophoma metastasis. Cancer Metastasis Revievs 10:33–48.

Ruoslahti E. 2000. Targeting tumor vasculature with homing peptides from phage display. Seminars in Cancer Biology 10:435–442.

Ruoslahti E, Rajotte D. 2000. An address system in the vasculature of normal tissues and tumors. Annual Reviews in Immunology 18:813–827.

Salles G, Zain M, Jiang W-M, Boussiotis VA, Shipp MA. 1993. Alternatively spliced CD44 transcripts in diffuse large-cell lymphomas: characterization and comparison with normal activated B cells and epithelial malignancies. Blood 82:3539–3547.

Santamaria Babi LF, Picker LJ, Perez Soler MT, Drzimalla K, Flohr, P, Blaser K, Hauser C. 1995. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. Journal of Experimental Medicine 181:1935–1940.

Shaw SK, Brenner MB. 1995. The β 7 integrins in mucosal homing and retention. Seminars in Immunology 7:335–342.

Streeter PR, Berg EL, Rouse BT, Bargatze RF, Butcher EC. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. Nature 331:41–46.

Takanami I. 2003. Overexpression of CCR7 mRNA in nonsmall cell lung cancer: correlation with lymph node metastasis. International Journal of Cancer 105:186–189.

Tanabe KK, Ellis LM, Saya H. 1993. Expression of CD44R1 adhesion molecule in colon carcinomas and metastases. The Lancet 341:725–726.

Vidal-Vanaclocha F, Alvarez A, Asumendi A, Urcelay B, Tonino P, Dinarello CA. 1996. Interleukin 1 (IL-1)-dependent melanoma hepatic metastasis in vivo; increased endothelial adherence by IL-1-induced mannose receptors and growth factor production in vitro. Journal of the National Cancer Institute 88:198–205.

Weber GF. 2001. The metastasis gene osteopontin: a candidate target for cancer therapy. Biochimica et Biophysica Acta 1552:61–85.

Weber GF, Ashkar S. 2000. Stress response genes: the genes that make cancer metastasize. The Journal of Molecular Medicine 78:404–408.

Yoshimasu T, Sakurai T, Oura S, Hirai I, Tanino H, Kokawa Y, Naito Y, Okamura Y, Ota I, Tani N, Matsuura N. 2004.

Increased expression of integrin α3β1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Science 95:142–148.

Zahalka MA, Okon E, Gosslar U, Holzmann B, Naor D. 1995. Lymph node (but not spleen) invasion by murine lymphoma is both CD44- and hyaluronate-dependent. The Journal of Immunology 154:5345–5355.

Zeelenberg IS, Ruuls-Van Stalle L, Roos E. 2003. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Research 63:3833–3839.

CHAPTER 11

ANGIOGENESIS

Blood vessels are required to supply oxygen and nutrients and to remove waste products from tissue. In the healthy adult organism, angiogenesis (the formation of new blood vessels) is a rare event that occurs in wound healing and for a few days each month in the female reproductive tract. In contrast to developmental angiogenesis, stress-induced angiogenesis is largely independent of anatomical boundaries, but rather occurs in a refined area of hypoxia or tissue damage. The genetic program directing this form of neovascularization is part of the stress response and is overlapping with, but distinct from the genetic program that directs blood vessel formation in morphogenesis during embryonic development. After wounding, platelets produce chemotactic factors, including TGF-β and PDGF. These factors activate fibroblasts to produce MMPs and growth factors, which degrade the extracellular matrix, stimulate the infiltration by macrophages, and promote blood vessel formation. The production of MMPs, UPA, and TPA facilitates the reepithelialization of the wound. Repair is also reflected in the secretion of the Fibroblast Growth Factors -1 and -2, Interleukin-8, and Osteopontin, as well as in the expression of the Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$, and possibly $\alpha_1\beta_1$ and $\alpha_2\beta_1$, on endothelial cells during angiogenesis, but not prominently on endothelial cells within mature blood vessels. The blood vessel formation in neoplasms is akin to wound healing [Dvorak 1984] or inflammation.

The onset of neovascularization, called the angiogenic switch, is a discrete step that can occur at any stage of tumor progression, depending on the type of tumor and its microenvironment. The signals for neoangiogenesis are initiated by local hypoxia. Tumors grow as chords around blood vessels [Goldman 1907; Thomlinson and Gray 1955] and their growth is limited by constraints on the diffusion of nutrients and catabolic products. Tumor cells that are located more than about 180 μm away from blood vessels necrose. This corresponds to the calculated distance that oxygen diffuses, as it passes from the capillaries to cells, before it is completely metabolized. Therefore, a prerequisite for exponential expansion is neovascularization [Folkman et al. 1971; Folkman 1972]. The fast growth rate and poor vascularization of solid tumors causes hypoxia, nutrient deprivation, and pH changes. These conditions activate cellular stress response pathways, including the unfolded protein response (UPR), which is induced when the physiological environment of the endoplasmic reticulum is altered and disrupts the proper folding and maturation of proteins in the secretory pathway. HIF-1 (Hypoxia Inducible Factor-1) is one of the master regulators that orchestrate the cellular responses to hypoxia. It is a heterodimeric transcription factor, composed of α and β subunits. HIF-1α is stable in hypoxic conditions but is rapidly degraded in normoxia. Upon stabilization or activation, HIF-1 translocates to the nucleus and induces the transcription of its downstream target genes. Most of these hypoxia-induced signal transduction pathways support tumor promotion and may activate genetic programs that promote an aggressive tumor phenotype. Low oxygen tension in tumors is associated with increased metastasis and poor survival [Hockel and Vaupel 2001; Hockel et al. 1999]. Tumor cells produce many of the same growth factors that activate the adjacent stroma in regenerating tissue and under inflammatory conditions.

Table 11.A. Molecular regulators of angiogenesis. The generation of new blood vessels in tumors is controlled by the balance of positive and negative regulators of angiogenesis. Hypoxia induces the production of positive regulators and thus induces neovascularization

Positive regulators			
VEGF (VPF)	45 kD	Renal cancers	VEGFR restricted to endothelium of blood vessels, four forms with various levels of heparin affinity, adipocytokine
Angiopoietins	70 kD	Colon cancer, glioma	Protect from apoptosis after binding to their receptors
Angiogenin	14 kD	Colon cancer	Chemotactic for endothelial cells, stimulates the secretion of prostacyclin
FGF-2 (basic FGF)	18 kD	Brain tumor, fibrosarcoma, ovarian cancer, breast cancer, bladder cancer	High affinity for heparin
FGF-1 (acidic FGF)	16 kD		
TGF-α	5.5 kD		
TGF-β	25 kD		Secreted by pericytes
PD-ECGF	45 kD	Breast cancer, endometrial cancer	Chemotactic for endothelial cells, Thymidine Phosphorylase
Placental Growth Factor	25 kD	Renal cell cancer	
Hepatocyte Growth Factor	92 kD	Glioblastoma	Adipocytokine
TNF-α	17 kD		Adipocytokine
HB-EGF			Adipocytokine, inducible by TNF-α
Leptin	16 kD	Breast cancer	Adipocytokine, signals through the Leptin Receptor
IL-6	21 kD	Breast cancer	Adipocytokine, stimulates estrogen biosynthesis
IL-8	40 kD	Gastric cancer	Chemokine that binds to CXCR2
G-CSF	17 kD	Glioblastoma, ovarian cancer	Regulates CXCR4 signaling
Osteopontin	57 kD	Breast cancer, colon cancer ovarian cancer, liver cancer	Induces endothelial cell migration
Proliferin	35 kD		
Endothelins		Ovarian cancer, prostate cancer	
Ephrins			Control endothelial cell migration and proliferation
Prostaglandins E_1 and E_2			
nicotinamide			
adenosine			
MMPs			Cleavage of Collagen
fibrinolytic proteinases			
Heparanase			Cleaves heparin sulfate in the extracellular matrix
Negative Regulators			
Angiostatin	38 kD		Internal Plasminogen fragment
Endostatin	20 kD		Internal Collagen XVIII fragment
Fibrinogen E fragment	50 kD		Fragment derived from Plasmin cleavage, inhibits endothelial cell migration and tubule formation by VEGF and FGF-2
Tumstatin			Collagen IV fragment
Thrombospondin			Inhibits endothelial cell proliferation
Platelet Factor 4		Kaposi sarcoma, melanoma, colon cancer, renal cell carcinoma	CXC Chemokine, inhibits endothelial cell, proliferation, and migration
Interferon-α2a, interferon-β		Renal carcinoma, colon carcinoma, osteogenic sarcoma, lymphoma, hairy-cell leukemia, hemangioma	Inhibition of endothelial cell, migration, downregulated expression of FGF
Interferon-γ			
Serpins			
Adiponectin			Adipocytokine
Prolactin fragment	16 kD		
Angiostatic steroids			Work in conjunction with heparin, dissolve basement membranes of growing capillaries
1α,25-dihydroxy-vitamin D_3			Metabolite has no effect on calcium, vitamin D_3 is not effective
Heparan sulfate oligo saccharide			-[GlcA-β 1,4-GlcNAc-α 1,4]$_n$-
TIMP-1			
TIMP-2			

Their blood vessels express markers that are not present in resting blood vessels of normal tissues, reflecting the stress-induced nature of this form of angiogenesis. (Table 11.A)

While existing lymphatic vessels are important for the spread of solid tumors, the role for lymphangiogenesis in carcinogenesis and the existence of lymphatic vessels in solid tumors or in their periphery is still subject to debate [Clarijs et al. 2001; Pepper 2000; Padera et al. 2002; Skobe et al. 2001; Stacker et al. 2001]. VEGF-C and VEGF-D can induce the growth of new lymphatic vessels. VEGF-C promotes enhanced spreading of tumor cells to the regional lymph nodes and the degree of tumor lymphangiogenesis may be correlated with the occurrence of lymph node metastases [Mattila et al. 2002].

- Glioblastomata, like other solid tumors, have extensive areas of hypoxia and necrosis. The resulting activation of HIF-1 in these cancers leads to the transcriptional induction of *vascular endothelial growth factors, vascular endothelial growth factor receptor, endothelin-1, angiopoietins, and angiopoietin receptors.*
- There is a direct correlation between the vascular density and the likelihood of metastasis in breast cancer patients, implying that the blood vessel supply can function as an independent prognostic variable in breast cancer [Weidner et al. 1991].
- During tumor angiogenesis, tumor endothelial markers are expressed at elevated levels. The respective transcripts identified are designated as *tem1* through *tem46* [St Croix et al. 2000; Carson-Walter et al. 2001].
- In lymphangiogenesis, VEGF-C ligates and activates VEGFR-3. Lymphatic spread is an important prognostic factor in patients with lung adenocarcinoma. VEGF-C and VEGFR-3 status may be indicative of survival rates for patients with early stage lung adenocarcinoma. Patients with expression of both tumor cell VEGF-C and endothelial cell VEGFR-3 exhibit the most unfavorable prognoses [Kojima et al. 2005].

11.1 INDUCTION OF NEOVASCULARIZATION

Endothelial cells must degrade their own basement membrane, develop sprouts from preexisting microvessels, invade the extracellular matrix, form tubes, and connect the tips of these tubes to create loops capable of handling blood flow. Outward budding during angiogenesis occurs due to a combination of mesenchymal influences (localized gradients of growth factors and quiescence of neighboring cells).

– Basement membrane degradation and synthesis are among the essential steps in angiogenesis [Folkman 1995].
– This is followed by emigration of the endothelial cells, often dependent on Integrin $\alpha_V\beta_3$, toward the source of the angiogenic factor, forming a sprout.
– An integral part in the conversion of sprouts into new blood vessels is the proliferation of the endothelial cells.
– Finally, the sprout develops a lumen. Then, vascular loops are formed and capillary tubes develop with the formation of tight junctions and deposition of new basement membrane.

The vascular basement membrane plays an active role in regulating the plasticity of blood vessel formation. Angiogenesis depends on vascular basement membrane components that support the growth and survival of the vascular endothelium. On the other hand, cryptic domains within basement membrane proteins are released during its turnover and breakdown and may act to inhibit angiogenesis [Sund et al. 2004].

The newly formed blood vessels are characterized by arteriovenous asymmetry with molecular markers discriminating the arteries from the veins. Endothelial cell markers include the arterial markers DeltaC, Delta-Like-4, Gridlock, VEGFR-2, Notch-1, Notch-5, TBX20, BMX, and Neuropilin-1, as well as the venous endothelial cell markers COUP-TFII, Neuropilin-2, TIE-2 (Tyrosine Kinase with Immunoglobulin and EGF Homology Domains-2), and VEGFR-3.

11.1.1 Secreted Factors and their Receptors

The coordination among multiple contributing cells in angiogenesis is accomplished through the actions of secreted factors and their cognate receptors. Secreted molecules that regulate the formation of new blood vessels include growth factors, adipocytokines, chemotactic cytokines, and guidance molecules. Among these, the VEGF/VEGFR system acts early on in endothelial cell differentiation and angiogenesis. Following this, the Ephrin/Ephrin Receptor system transduces positional guidance cues on cells of the vascular system to control the establishment of arteriovenous asymmetry in the developing vasculature. The Angiopoietin/TIE system acts at later stages of the angiogenic cascade controlling vascular maturation and quiescence.

Further, extracellular matrix-degrading enzymes are needed to remodel the tissue microenvironment.

VEGF. The cytokine Vascular Endothelial Growth Factor (VEGF, VPF) [Senger et al. 1983; Ferrara and Henzel 1989] acts as a mitogen selectively on endothelial cells and promotes neovascularization. It is not mitogenic to fibroblasts or epithelial cells (Figure 11.1.1.A). VEGF is a homodimeric glycoprotein that is structurally related to PDGF. The NH_2-terminal region contains eight cysteines that enable dimerization. The COOH-terminal portion, generated after cleavage by Plasmin, contains the residues 111–165, which are essential for the mitogenic activity. VEGF family members bear three loops produced by three intramolecular disulfide bonds. Cooperation between loop-1 and loop-3 is necessary for the specific binding and activation of the receptor VEGFR-2.

VEGF is upregulated during wound repair or hypoxia:

- Hypoxia activates SRC and a hypoxia response element in the *vegf* promoter.
- *vegf* is transcriptionally induced by HIF-1. This may be an early event in tumor angiogenesis.
- The *vegf* promoter has six GC boxes for binding of the transcription factor SP-1 and several sites for AP-1 and AP-2 binding [Sherbet and Lakshmi 1997]. P53 inhibits the transcription of *vegf*. This involves the binding of P53 to the transcription factor SP-1, which inactivates SP-1.
- The endoplasmic reticulum chaperone ORP-150 (Oxygen -Related Protein-150) plays a critical role in VEGF processing and secretion.

The secretion of newly synthesized VEGF is controlled by GRP170, which is a HSP70 family member localized in the endoplasmic reticulum, that is upregulated during both endoplasmic reticulum stress and hypoxia. Increased expression of GRP170 increases VEGF secretion, whereas decreased levels result in retention of VEGF by the endoplasmic reticulum.

vegf mRNA and protein are induced in fibroblastic and epithelial cells by TGF-β and EGF. In

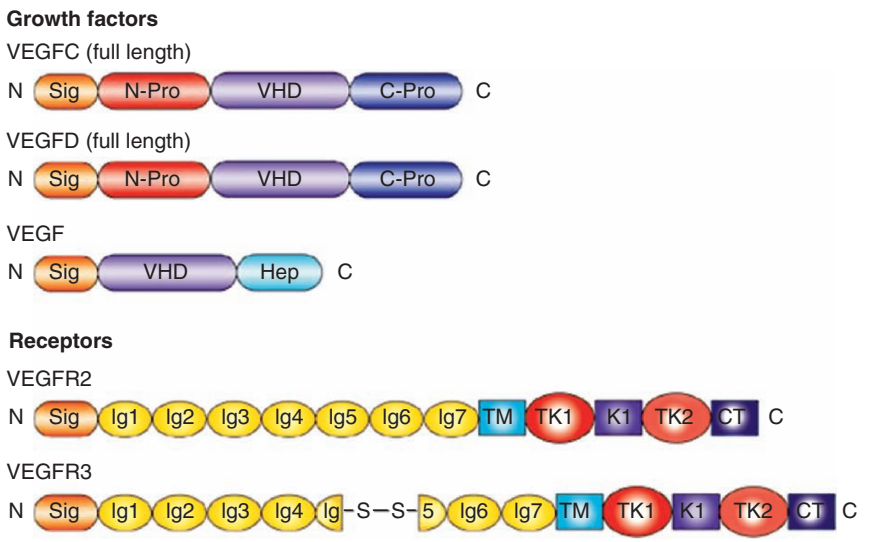


Figure 11.1.1.A. Structure of VEGF and VEGF Receptors. VEGFs (Vascular Endothelial Growth Factors) are secreted homodimeric polypeptides that contain a hydrophobic NH_2-terminal signal sequence for protein secretion (Sig). All VEGF family members contain a central region that is termed the VEGF homology domain (VHD) and contains a cysteine knot motif, a structural feature of each subunit that consists of three intra-subunit disulfide bonds forming the shape of a knot. Some forms of the angiogenic factor VEGF contain a COOH-terminal heparin binding domain (Hep). A subfamily of these growth factors contains VEGF-C and VEGF-D, defined by their ability to bind VEGFR3 and the presence of NH_2- and COOH-terminal propeptides (N-Pro and C-Pro). The receptors for VEGF-C and VEGF-D are VEGFR2 and VEGFR3, both of which can be expressed on lymphatic endothelial cells. These receptors are closely related in structure, consisting of an extracellular domain with seven Immunoglobulin-like domains (Ig), a transmembrane domain (TM), a split tyrosine kinase domain (TK) with a kinase insert sequence (KI), and a cytoplasmic tail (CT) at the COOH-terminus. The fifth Ig-like domain of VEGFR3 is proteolytically processed and the subunits are held together by a disulphide bond (-S-S-). Signaling by VEGFR3 is sufficient to induce lymphangiogenesis. VEGF binds VEGFR1 (not shown here) and VEGFR2, but not VEGFR3. [Reproduced from Stacker et al. 2002. With permission from Macmillan.]

fibroblasts, the expression of *vegf* may be induced after PKC activation. The expression of VEGF can be downregulated by the Retinoblastoma family member $P130^{RB-2}$ [Claudio et al. 2001].

The VEGF family of angiogenic growth factors includes the gene products VEGF-A {6p12}, -B {11q13}, -C {4q34}, and -D {Xp22.31}, as well as Placenta Growth Factor (PLGF) {14q24-q31}.

The *vegf-A* gene contains eight exons. VEGF-A forms a homodimer of approximately 45 kD. Alternative splicing generates at least nine variants. Four prominent forms that are generated by alternative splicing of *vegf-A* are designated by the number of amino acid residues in the mature protein as $VEGF_{121}$, $VEGF_{165}$, $VEGF_{180}$, and $VEGF_{206}$. $VEGF_{165}$ lacks the residues encoded by exon 6, while $VEGF_{121}$ lacks the residues encoded by exons 6 and 7.

vegf-B (*vrf*) spans approximately 5 kb and is composed of eight exons. The gene generates two alternatively spliced messages with open reading frames of 621 and 564 bp, respectively. Exons 6 and 7 are contiguous and the two forms of VEGF-B arise through the alternative splicing of exon 6. The resulting 186 amino acid and 167 amino acid polypeptides are distinct in their COOH-termini, resulting from a shift in the open reading frame. *vegf-B* is ubiquitously expressed as two transcripts of 2.0 and 5.5 kb.

VEGF-C (VRP) is a hemangiogenic and lymphangiogenic factor that is synthesized as a preproprotein. It has a mature form that consists of dimers of the VEGF homology domain (VHD), which contains receptor-binding sites that engage the VEGF Receptors-2 and -3 on blood vessels and lymphatic endothelia. VEGF-C induces angiogenesis through endothelial cell migration and proliferation. It can induce tumor lymphangiogenesis and direct metastasis to the lymphatic vessels and lymph nodes.

VEGF-D (c-FOS Induced Growth Factor, FIGF) stimulates angiogenesis and lymphangiogenesis. It shares 61% sequence identity with VEGF-C and is functionally similar to it. In normal tissues, *vegf-D* transcripts are present in lung, heart, skeletal muscle, skin, adrenal glands, and the gastrointestinal tract. *vegf-D* expression is under the control of c-FOS. VEGF-D engages the VEGFR-2 (KDR, FLK-1) and VEGFR-3 (FLT-4) receptor tyrosine kinases, both of which are essential for vascular development. In adults, VEGFR-3 expression is restricted mainly to lymphatic and fenestrated epithelium. VEGF-D can induce tumor lymphangiogenesis and direct metastasis to the lymphatic vessels and lymph nodes.

Angiogenic vessels express elevated levels of the receptors for VEGFs. VEGF engages the endothelium specific tyrosine kinase receptors VEGFR-1 (FLT-1) {13q12}, VEGFR-2 (KDR, FLK-1) {4q12}, and VEGFR-3 (FLT-4) {5q35.3}. The proto-oncogene *vegfr-1* (*flt-1*, *FMS like tyrosine kinase-1*) belongs to the *src* gene family and is related to the proto-oncogene *ros*. Like other members of this family, its gene product has protein tyrosine kinase activity that is important for its control of cell proliferation. VEGFR-2 is a high affinity receptor for VEGF-A and mediates most of its endothelial growth and survival signals. VEGFR-2 has a typical tyrosine kinase receptor structure with seven immunoglobulin-like domains in the extracellular region, as well as a catalytic domain. It utilizes a unique signaling system for DNA synthesis in vascular endothelial cells, which involves a PLC-$\gamma \rightarrow$RAF$\rightarrow$MAP Kinase pathway. The Integrin $\alpha_V\beta_3$ physically associates with VEGF Receptor-2 and, after ligation, increases VEGF signaling. VEGFR-3 expression is restricted to the lymphatic endothelium in the adult. Its signaling proceeds through the ERK and Phosphatidylinositol 3-Kinase$\rightarrow$PKB pathways.

Neoangiogenesis involves the mobilization of VEGFR-1 expressing myeloid precursor cells form the bone marrow and VEGFR-2 expressing circulating endothelial precursor cells. These cells are incorporated into the lining of tumor blood vessels. Expression of the *id* genes *id-1* and *id-3* may be required for VEGF signaling. In their absence, bone marrow-derived precursor cells are not recruited to the peripheral circulation [Lyden et al. 1999; Lyden et al. 2001]. VEGF induced neovascularization occurs, in part, through the induction of expression of the Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$, which allow the migration and attachment of endothelial cells on Collagen [Senger et al. 1997]. Furthermore, VEGF activates RAF-1 via the kinase CSK (Cytoplasmic Tyrosine Kinase, SRC Tyrosine Kinase), leading to phosphorylation of tyrosines 340 and 341 and MEK1-dependent protection from the extrinsic pathway to apoptosis [Alavi et al. 2003]. In general, RAF Kinases are linked to epithelial cell survival.

Neuropilin-1 (NRP1) {10p12} and -2 (NRP2) {2q34} are related transmembrane receptors that function as mediators of neuronal guidance and angiogenesis. NRPs bind members of the class 3 Semaphorin family, regulators of neuronal guidance, and members of the VEGF family of angiogenesis factors [Soker et al. 1998]. NRPs are expressed in endothelial cells and bind $VEGF_{165}$. NRP1 is a coreceptor for VEGFR-2 that strengthens the binding of $VEGF_{165}$ to VEGFR-2 and enhances $VEGF_{165}$-mediated chemotaxis. NRP1 expression is regulated in endothelial cells by TNF-α, by the transcription factors dHAND and ETS-1, and by vascular injury. NRP1 plays a critical role in embryonic vascular development. Overexpression of NRP1 results in the formation of excess capillaries and hemorrhaging. Deficiency of NRP1 leads to defects in yolk sac, embryo and neuronal vascularization, and in the development of large vessels in the heart.

- Many tumor types express abundant levels of VEGF, and the tumor cell population itself is usually the main source of this factor. In some cases, infiltrating host stromal cells may also make a significant contribution. VEGF expression is increased in breast carcinoma. Upregulation of VEGF and its receptors VEGFR-1 (FLT-1) and VEGFR-2 (FLK-1) occurs in endometrial carcinoma and germ cell tumors. In glioblastoma cells, the expression of the *vegf* gene is upregulated after engagement of the EGF Receptor via signal transduction through RAS and Phosphatidylinositol 3-Kinase.
- VEGF-C levels in primary tumors correlate with lymph node metastases in thyroid, prostate, gastric, colorectal, lung, and esophageal carcinomata.
- VEGF-D is upregulated in malignant melanoma and in inflammatory breast carcinoma. VEGF-D is expressed in melanoma cells and small cell lung cancer. VEGF-D serves as a prognostic marker in colorectal carcinoma.
- VEGFR-2 is expressed exclusively on endothelial cells. In glioblastoma, VEGFR-2 is essential for tumor growth [Millauer et al. 1994].
- VEGFR-3 expression is upregulated in various tumors, including breast cancer.
- Tumor cells express NRPs and bind $VEGF_{165}$. NRP1 upregulation is positively correlated with the progression of various tumors. Overexpression of NRP1 in tumor cells results in enlarged tumors and substantially enhanced tumor angiogenesis. On the other hand, truncated soluble NRP1 (sNRP1), generated through an alternate transcript, is an antagonist of tumor angiogenesis.

Angiopoietins. There are three known Angiopoietins, Angiopoietin-1 (ANG-1, ANGPT-1) [Davis et al. 1996], -2 (ANG-2, ANGPT-2), and -4 (ANG-4, ANGPT-4) that regulate endothelial cell survival. The *ang-1* gene {8q22} contains nine exons and spans 48.3 kb. Exons 1–5 encode the NH_2-terminus, the coiled-coil domain, and part of the hinge region, while exons 5–9 encode the remainder of the hinge region, the Fibrinogen-like domain, and the COOH-terminus. The 70 kD Angiopoietin-1 induces endothelial cells to recruit pericytes and smooth muscle cells to become incorporated in the vessel wall. Following activation of the receptor TIE-2 by Angiopoietin-1, pericyte, and smooth muscle recruitment are mediated by endothelial production of PDGF-BB. The combined actions of Angiopoietin and VEGF cause the loosening of the support cells and the ability of the newly exposed endothelial cells to multiply. The secreted, homodimeric glycoprotein Angiopoietin-2 is a naturally occurring antagonist of Angiopoietin-1 that prevents the activation and autophosphorylation of its receptor TIE-1. Angiopoietin-2 {8p23} plays a role during vascular remodeling. While Angiopoietin-2 is widely expressed in the embryo, it appears in the adult specifically in places where angiogenesis is critical, including the placenta, the ovaries (especially postovulation), and the uterus.

– If Angiopoietin-2 inhibits the TIE-2 kinase in the absence of VEGF, the endothelium of the blood vessel separates from the periendothelial cells, the endothelial cells lose contact with each other, and the blood vessels regress.
– If Angiopoietin-2 blocks the TIE-2 signal in the presence of a VEGF signal, angiogenesis occurs.

A splice variant, $ANG\text{-}2_{443}$ lacks 53 amino acids compared to the full length Angiopoietin-2 protein. The missing residues, amino acids 96–148, are encoded by exon B of the *ang-2* gene and include part of the NH_2-terminal coiled-coil domain. $ANG\text{-}2_{443}$ retains four of the six potential *N*-glycosylation sites present in the full length molecule [Kim I et al. 2000]. Angiopoietin-4 is an agonist of TIE-2 activation and of angiogenesis [Lee et al. 2004]. The *ang-4* gene {20p13} contains nine exons and spans 20 kb. *angiopoietin-4* is expressed as a

major 5 kb and a minor 4 kb transcript primarily in the lungs. A gene related to *angiopoietins* is *angptl-3* (*ang-5*). It encodes a 460 amino acid protein with the characteristic structure of angiopoietins, including a signal peptide, an extended helical domain, a short linker peptide, and a globular Fibrinogen homology domain (FHD). ANGPTL-3 contains the four conserved cysteine residues that form the intramolecular disulfide bonds within the Fibrinogen homology domain, but it does not have two other cysteines in this domain. ANGPTL-3 also does not contain the characteristic calcium binding motif of Angiopoietins.

Angiopoietins are ligands for the endothelium-specific dimeric tyrosine kinase receptor TIE-2 (Tyrosine kinase with Immunoglobulin and EGF Homology Domains-2, TEK, Endothelial Tyrosine Kinase). TIE-2 {9p21} is almost exclusively expressed on endothelial cells and early hematopoietic cells. This receptor possesses a unique extracellular domain that contains two Immunoglobulin like loops separated by three EGF-like repeats, which are connected to three Fibronectin type III-like repeats. Whereas Angiopoietin-1 binds and induces the tyrosine phosphorylation of TIE-2, it does not directly promote the growth of endothelial cells. TIE-2 signals through SHP-2→GRB-2→MAPK and through PI 3-K→PKB.

- Enhanced expression of the TIE family receptors, TIE-1 {1p34-p33} [Partanen et al. 1992] and TIE-2, occurs in the blood vessels of various cancers. They include gastric cancer, lung cancer, and astrocytoma.
- Blockade of the stabilizing action of Angiopoietin-1 may contribute to tumor vessel regression. Consistently Angiopoietin-1 acts antiapoptotically for endothelial cells and the expression of its antagonist Angiopoitein-2 is induced in the endothelium of coopted tumor vessels before their regression [Holash et al. 1999].

Angiogenin. Angiogenin (RNASE 5) {14q11} is a 123 amino acid polypeptide and a potent inducer of new blood vessel formation. It is the only member with angiogenic activity in the superfamily of pancreatic RNASEs. Angiogenin has a unique specificity of dinucleotide cleavage. Although this catalytic activity of the protein is rather weak, it is important for its blood vessel forming properties. Inflammation provokes the upregulation of *angiogenin* expression in the liver, resulting in increased Angiogenin protein in the blood.

Angiogenin binds with low affinity to the extracellular matrix. There it facilitates the adhesion of endothelial cells and fibroblasts. On confluent endothelial cells, Angiogenin binds to cell surface Actin. It dissociates with the Actin, and activates the cell associated Protease system. This results in endothelial cell invasion into the surrounding matrix. As the cells migrate into the basement membrane, their density is reduced, which triggers the expression of the 170 kD Angiogenin Receptor. Following the engagement of this high affinity proteoglycan receptors on the surface of endothelial cells, Angiogenin is endocytosed and transported to the nucleus, where it accumulates in the nucleolus. In this process, the cells are stimulated mitogenically and proliferate, filling the space opened up by the migrating cells. Once confluence is reached again, the Angiogenin Receptor expression is downmodulated, a process that regulates the new blood vessel network [Strydom 1998].

- Angiogenin is abundantly released from colon cancer cells and prostate adenocarcinoma cells.
- High levels of Angiogenin correlate with a poor prognosis in patients affected by acute myeloid leukemia, myelodysplastic syndromes, and myeloid leukemia [Musolino et al. 2004].
- The plasma levels of Angiogenin are significantly higher in patients with bladder carcinoma compared to healthy individuals. They are also elevated in patients with recurrent superficial bladder carcinoma compared to patients without recurrence. An elevated plasma level of Angiogenin may also serve as a predictor for the risk of bladder carcinoma [Zhao et al. 2005].

FGF. Fibroblast Growth Factors constitute about 20 structurally related growth factors, of which FGF-1 and FGF-2 act as endothelial growth factors.

FGF-1 (acidic Fibroblast Growth Factor, Heparin Binding Growth Factor-1) has angiogenic activity [Lobb et al. 1985]. The endothelial cell growth factors α-ECGF and β-ECGF are differentially processed forms of the *fgf-1* gene. β-ECGF represents a 20 amino acid NH_2-terminal extension of α-ECGF and a 14 amino acid NH_2-terminal extension of FGF-1 [Burgess et al. 1986].

The single copy *fgf-2* gene encodes multiple FGF-2 (basic FGF) forms of molecular weights ranging from 18 kD to 24 kD, with the low molecular weight forms

being NH_2-terminal truncations of the 24 kD protein. Mechanical stress induces the release of FGF-2 from the endothelium. FGF-2 can form complexes with heparan sulfate proteoglycans (HSPGs) [Shing et al. 1984]. Like other heparin-binding factors, it can link through a heparin bridge to CD44 forms containing the variant exon 3. In this state, FGF-2 may be presented to target cells [Bennett et al. 1995]. FGF-2 induces angiogenesis through several pathways.

– Both low and high molecular weight forms of FGF-2 induce endothelial cell proliferation.
– FGF-2 may induce the expression of Integrin $\alpha_V\beta_3$, chemotaxis, and matrix invasion by endothelial cells [Brooks et al. 1994a].
– FGF-2 may induce the expression and secretion from endothelial cells of proteinases that dissolve the basement membrane. They include Urokinase-Type Plasminogen Activator and its receptors [Mignatti et al. 1991], as well as other proteases.
– RAF family kinases are linked to endothelial cell survival. FGF-2 activates $P21^{PAK}$, which phosphorylates RAF-1 on serines 338 and 339. This results in mitochondrial translocation of RAF-1 and endothelial protection from the intrinsic pathway of apoptosis [Alavi et al. 2003].

LAM5 is a polysulfate derivative of the glucan Laminarin that counteracts angiogenesis by inhibiting FGF-2.

- Neovascularization may be triggered by molecules that are released by tumor cells or infiltrating inflammatory cells, which induce FGF-2 upregulation in the quiescent endothelium.
- Renal cancer patients with high levels of the angiogenic factor FGF-2 in their primary tumors have a poorer survival rate than patients with lower FGF-2 levels, suggesting that increased angiogenesis due to FGF-2 production may lead to increased metastatic potential and consequently decreased survival [Nanus et al. 1993].

PD-ECGF. Platelet-Derived Endothelial Cell Growth Factor (PD-ECGF, ECGF1, Gliostatin, TP) {22q13.32-qter} is a Thymidine Phosphorylase. This enzyme catalyzes the phosphorolysis of pyrimidine 2′-deoxynucleosides to 2′-deoxyribose-1-phosphate and their respective pyrimidine bases. PD-ECGF [Ishikawa et al. 1989] is often induced by stress, it protects cells from apoptosis and helps cell survival by stimulating nucleoside metabolism [Toi et al. 2005]. PD-ECGF also mediates the chemotaxis of endothelial cells and angiogenesis, possibly through its catalytic product 2-deoxy-D-ribose. 2-deoxy-D-ribose stimulates the formation of focal adhesions and the activation of Integrin $\alpha_5\beta_1$ in endothelial cells. In most healthy tissues, the expression of PD-ECGF is very low. Its transcription is activated by SP-1 family members. The protein is stored in platelets as a 45 kD single polypeptide chain and becomes active as a homodimer.

- Various tumors, such as breast cancer, produce elevated levels of PD-ECGF. The expression often correlates with the abundance of other enzymes of the nucleoside metabolism, such as Thymidylate Synthase.
- Hypoxia and proinflammatory cytokines, such as TNF-α, IL-1, and Interferon-δ, can upregulate *pd-ecgf* expression in malignant cells.
- Angiogenesis in uterine endometrial cancers is complex because the hormone dependency in growth also modifies the angiogenic potential. Progesterone primed with estrogen induces *pd-ecgf* in the endometrium, and PD-ECGF is highly expressed in early stage and well-differentiated endometrial cancers [Fujimoto et al. 2005].
- PD-ECGF and VEGF are coexpressed in various cancers. These angiogenic molecules have different specificities with respect to endothelial stimulation and may have a cooperative role in neovascularization.
- Education of stromal cells by tumor cells occurs in local microenvironments. PD-ECGF may be produced by the tumor stroma. Its release from infiltrating macrophages correlates with increased microvessel density and poor prognosis in astrocytic tumors [Yao et al. 2001].

HGF. HGF, VEGF, TNF-α, HB-EGF, Leptin, and IL-6 form a group of angiogenic Adipocytokines. Adipocytokines are polypeptides that are produced predominantly or exclusively by fat cells. They act by endocrine, paracrine, or autocrine mechanisms. Most have proangiogenic effects. The Adipocytokine Adiponectin suppresses angiogenesis.

The Hepatocyte Growth Factor (HGF, Scatter Factor) is physiologically secreted by adipocytes and fibroblasts. The blood levels of HGF correlate with the body mass index and are high in obese subjects. HGF is mitogenic for endothelial cells and stimulates capillary formation. It also induces the chemotaxis and secretion of MMPs from endothelial cells.

Its receptor is a tyrosine kinase encoded by the *c-met* proto-oncogene, which is expressed on endothelial cells.

- On head and neck cancer cells, HGF can induce the expression of *il-8* and *vegf* through its receptor c-MET via MEK and Phosphatidylinositol 3-Kinase-dependent pathways.
- HGF and its receptor tyrosine kinase MET are key determinants of brain tumor growth and angiogenesis [Abounader and Laterra 2005].

Chemotactic cytokines. Osteopontin (OPN, SPP1, ETA-1, 2AR) may be secreted by vascular smooth muscle cells, and in this setting contributes to neovascularization after injury. Osteopontin and its receptors are implicated in blood vessel formation in various ways.

– Osteopontin is among the genes differentially expressed during angiogenesis [Prols et al. 1998].
– A splice variant of CD44 contributes to endothelial cell proliferation, migration, and angiogenesis [Henke et al. 1996; Trochon et al. 1996]. CD44 variants containing the exons v3–v6 are receptors for Osteopontin.
– The coordinate expression of β_3 Integrins and Osteopontin by regenerating endothelial cells stimulates their migration [Liaw et al. 1995].
– The Osteopontin receptor Integrin $\alpha_V\beta_3$ is essential for the promotion of tumor neovascularization [Brooks et al. 1994b; Arap et al. 1998].
– VEGF-induced endothelial cell migration is mediated through cooperative mechanisms involving Integrin $\alpha_V\beta_3$, Osteopontin, and Thrombin [Senger et al. 1996].

Endothelial cells in angiogenic vessels express a repertoire of Integrins that differs from resting endothelial cells. This includes the expression of Integrin $\alpha_V\beta_3$, Integrin $\alpha_V\beta_5$, and the Fibronectin Receptor Integrin $\alpha_5\beta_1$. Signals through Integrin $\alpha_5\beta_1$ suppress PKA and support the survival of endothelial cells during angiogenesis. Oncofetal Fibronectin is an alternatively spliced form of Fibronectin that contains an extra Fibronectin type III domain. Oncofetal Fibronectin is selectively expressed in the extracellular matrix of angiogenic blood vessels, but not in mature blood vessels. The Integrin $\alpha_V\beta_3$ is of particular importance in angiogenesis [Brooks et al. 1994b; Arap et al. 1998], because it is expressed selectively on growing blood vessels. Angiogenesis induced by FGF-2 or by TNF-α is dependent on the Integrin $\alpha_V\beta_3$. This Integrin acts, in part, through protecting endothelial cells from apoptosis by activating NF-κB [Scatena et al. 1998].

Chemokines are a group of small, structurally related molecules that regulate cell mobility through their interactions with G-Protein coupled receptors. Chemokines also exert effects on endothelial cells involved in angiogenesis or angiostasis. The Chemokine of the CXC group Interleukin-8 (IL-8, Neutrophil-Activating Peptide-1, NAP1, SCYB8, Monocyte-Derived Neutrophil Chemotactic Factor, MDNCF) {4q12-q13} is typically secreted in response to inflammatory stimuli. It stimulates the formation of new blood vessels.

- The Integrin $\alpha_5\beta_1$ is involved in neoangiogenesis in renal cell carcinoma, melanoma, and pancreas carcinoma.
- Altered Integrin expression may contribute to tumor progression and angiogenesis. The pattern of expression of Integrin $\alpha_V\beta_3$ and Integrin $\alpha_V\beta_5$ changes in neoplastic compared to normal kidneys. These variations may play a role in the process of transformation [Rabb et al. 1996].
- Osteopontin secreted from neuroblastoma cells stimulates endothelial cell migration and neovascularization [Takahashi et al. 2002].
- Interleukin-8 is an inducer of angiogenesis that is frequently secreted by prostate carcinoma cells.

Molecules of neuronal guidance. Ephrins (EFN) and Netrins provide guidance for axons and for blood vessel growth. Ephrins are essential for remodeling of the capillary network. Based on their structures and sequence relationships, Ephrins are divided into the Ephrin-A (EFN-A) class, whose members are membrane anchored by glycosylphosphatidylinositol linkage, and the Ephrin-B (EFN-B) class, which are transmembrane proteins. Ephrin A1 is an immediate early response gene after cytokine stimulation of endothelial cells. It induces neovascularization by controlling the migration and proliferation of endothelial cells. Ephrin B2 is expressed in arteries, but not in veins. Conversely, Ephrin B4 is expressed in veins but not in arteries. Receptors in the EPH family typically have a single kinase domain, an extracellular region containing a cysteine-rich domain, and two Fibronectin type III repeats. The EPH family of receptors are divided into two groups based on the similarity of their extracellular domain sequences and their affinities for binding Ephrin-A or Ephrin-B ligands. The Ephrin Receptors B2 and

B4 are required for vascular remodeling during blood vessel maturation.

Endothelial cells express ROBO-1. The gene *robo-1* encodes a protein that is a member of the NCAM family of receptors. ROBO-1 (Roundabout-1) acts as an axon guidance receptor that belongs to a subfamily of Immunoglobulin domain proteins. SLIT ligands and their ROBO family receptors may contribute to guidance in angiogenesis. There is functional alteration between ROBO and Netrin. Activation of the SLIT receptor ROBO silences the attractive effect of Netrin-1 through direct binding of the cytoplasmic domain of ROBO to that of the Netrin receptor DCC.

Semaphorins and Neuropilins contribute to directing the formation of blood vessels. Neuropilin-1 ($VEGFR_{165}$, NP-1, BDCA4) is a tyrosine kinase receptor for $VEGF_{165}$ and for Semaphorin-3A. Neuropilin-1 enhances the binding of $VEGF_{165}$ to its cognate receptor VEGFR-2 and the ensuing $VEGF_{165}$-mediated chemotaxis.

- EPHA2 is upregulated in prostate tumors, colorectal tumors, lung tumors, and melanomata. The highest levels of EPHA2 correlate with tumor stage and tumor progression.
- EPHB4 is expressed in various carcinomata including those of the mammaries, lungs, colon, and ovaries. Correspondingly, EPHB4 may also be a prognostic marker for mammary tumors.
- Angiogenesis is an essential event in the pathogenesis of malignancies. Ephrin B2 is a marker of aggressive differentiated thyroid cancer [Kebebew et al. 2005].
- A wide variety of tumor cells produce SLIT-2.

Osteonectin. Extracellular matrix proteins associated with the metastatic phenotype of cancers frequently also contribute to tumor neovascularization. This is the case for SPARC (Secreted Protein Acidic and Rich in Cysteine, Osteonectin) [Rempel et al. 1999]. The COOH-terminal domain of the 32 kD phosphoprotein, which can be selectively expressed by the endothelium in response to certain types of injury, induces rounding in adherent endothelial cells. SPARC regulates the endothelial barrier function through F-Actin-dependent changes in cell shape, coincident with the appearance of intercellular gaps that provide a paracellular pathway for the extravasation of macromolecules. SPARC regulates extracellular matrix organization through its binding to ILK (Integrin-Linked Kinase) and modulation of ILK activity [Barker et al. 2005].

Endothelin. The family of Endothelins includes Endothelin-1 (EDN-1, ET-1) {6p24-p23}, Endothelin-2 {1p34}, and Endothelin-3 {20q13.2-q13.3}, which are 21 amino acid polypeptides [Inoue et al. 1989]. Endothelin-1 is expressed as a pre-propeptide that needs to be processed for activation. It is a vasoactive factor and a pain mediator implicated in the pathogenesis of the pain associated with ischemic states, inflammation, and cancer. Endothelin-1 also acts as a mitogenic and angiogenic factor selectively through the Endothelin Receptor A. Endothelin-1 is synthesized by keratinocytes in normal skin and is locally released after cutaneous injury. In the regulation of cell responses to hypoxia, the transcription factor HIF-1 activates the expression of *edn-1*. Endothelin-2 has the most potent vasoconstrictor activity among the Endothelins.

The Endothelin Receptor A (EDNRA, ETRA, ET_AR) binds Endothelins -1 and -2 with high affinity and Endothelin-3 with low affinity. In contrast, the Endothelin Receptor B binds all Endothelins with comparable affinity.

- Endothelin-1 is overexpressed by primary and metastatic ovarian carcinoma. It promotes ovarian carcinoma cell invasion by engaging Endothelin Receptor A and upregulating the secretion of multiple tumor proteinases [Bagnato et al. 1995; Rosano et al. 2001].
- Osteoblastic bone metastases are common in prostate cancer patients. Endothelin-1 is integrally involved in the progression of prostate cancer. Tumor-produced Endothelin-1 stimulates new bone formation in osteoblastic metastases via engagement of the Endothelin Receptor A [Yin JJ et al. 2003].

Endoglin. Endoglin (EDG, CD105) is a homodimer glycoprotein and a proliferation-associated cell membrane antigen on endothelial cells. EDG is a component of the TGF-β Receptor complex, which binds to TGF-β1 and TGF-β3. Endoglin is preferentially, but not exclusively, expressed in angiogenesis.

- Endoglin is expressed at high levels on tumor-associated angiogenic vascular endothelium.

Glycosaminoglycans. Sugars are essential modulators in the regulation of angiogenesis. Glycosaminoglycans

(GAGs) are negatively charged polysaccharides composed of repeating disaccharide units. They normally exist as proteoglycans composed of one or more polysaccharide chains attached to a core protein. The subclass of heparan sulfate proteoglycans (HSPGs) is involved in the modulation of the neovascularization that takes place in various physiologic and pathologic conditions. This occurs through

– The interaction of heparan sulfate proteoglycans with angiogenic growth factors or with negative regulators of angiogenesis. Endothelial cell surface heparan sulfate proteoglycans also act as coreceptors for a wide spectrum of angiogenic growth factors. Many of these growth factors, including VEGF, FGF, TGF-β, and HGF, bind heparan sulfate proteoglycans , which may aid in their localization on the cell membrane
– The necessity of heparan sulfate proteoglycans for the structural and functional integrity of the endothelium, because they act as matrix receptors for a variety of basement membrane proteins. Moreover, basal heparan sulfate proteoglycans are responsible for the charge selectivity of filtration in the endothelium
– The inhibition of smooth muscle cell proliferation and migration by heparan sulfate proteoglycans
– The major role played by luminal heparan sulfate proteoglycans in determining the anticoagulative properties of the vessel surface through binding to proteases of the intrinsic coagulation cascade, including Antithrombin III, Thrombin, and protease inhibitors

The cell surface molecule CSPG4 (NG2, Melanoma-Associated Chondroitin Sulfate Proteoglycan, MCSP) is a marker of angiogenic pericytes. It is a membrane spanning protein that carries chondroitin sulfate side chains. CSPG4 enhances the ability of PDGF-AA to signal through its receptor, PDGFR-α. CSPG4 also binds to FGF-2, and to type VI Collagen.

- CSPG4 is expressed highly and selectively on pericytes in the neovasculature of tumors and regenerating tissues.
- CSPG4 is expressed on more than 90% of melanoma tissues. It contributes to growth control, adhesion, cell–substratum interactions, and cell–cell contacts.

PECAM-1 is capable of mediating calcium-dependent heterophilic interactions. They are dependent on the binding of PECAM-1 to specific glycosaminoglycans on adjacent cells via a glycosaminoglycan consensus binding sequence in the second Immunoglobulin homology domain. PECAM-1 is involved in angiogenesis and endothelial cell migration [Cao et al. 2002; Wartenberg et al. 2001]. M-CAM is also expressed on tumor endothelia, where it may lead to proliferation and angiogenesis.

11.1.2 Matrix-Degrading Proteases

A prerequisite for the budding and formation of new capillaries is the degradation and remodeling of the surrounding extracellular matrix. Type I Collagen is the major constituent of the extracellular matrices, to which proliferating endothelial cells are exposed in injured tissues. Fibrillar Collagen type I cleavage at Collagenase specific sites is required for endothelial cell invasion and is a rate limiting event in neoangiogenesis [Seandel et al. 2001].

Matrix Metalloproteinases (MMPs) degrade the extracellular matrix, which allows the endothelial cells to invade the tumor stroma. Multiple MMPs are expressed in angiogenic tissue.

– MMP-2 is essential in tumor angiogenesis. Cleavage of Collagen type IV by MMP-2 exposes a cryptic Integrin $\alpha_V\beta_3$ binding site and allows the Collagen cleavage product to induce endothelial cell migration [Xu et al. 2001].
– MMP-9 increases the availability of VEGF.
– MMP-14 promotes tumor angiogenesis by degrading the Fibrin matrix that surrounds newly formed vessels. This allows further invasion of the endothelial cells.

In many solid tumors, MMPs are produced by the stromal cells, rather than by tumor cells. This expression pattern is regulated, in part, by tumor–stroma interactions that involve CD147. CD147 (Extracellular MMP Inducer, EMMPRIN, Basigin, BSG, Tumor Collagenase Stimulatory Factor, TCSF, M6 Leukocyte Activation Antigen) {19p13.3} is a member of the Immunoglobulin superfamily. It mediates

– MMP secretion and activation
– Hyaluronate secretion for anchorage independent growth

Following their induction by CD147, the MMPs release CD147 from the cell membrane through proteolytic cleavage, generating a soluble form. Furthermore, CD147 and MMPs synergize to induce angiogenesis via stimulation of VEGF production [Tang et al. 2005].

- CD147 is a carrier of an onco-developmental carbohydrate marker expressed in teratocarcinoma stem cells.
- CD147 is expressed by bladder cancer cells. The urinary concentrations of CD147 are elevated in patients with bladder cancer, more so in invasive tumors than in benign tumors [Guirguis et al. 1990].
- CD147 is highly expressed in oral squamous cell carcinoma, facilitates tumor cell motility, and mediates Tenascin-C matrix deposition. This contributes to regulating oral squamous cell carcinoma invasion [Bordador et al. 2000].

Components of the fibrinolytic system, including Plasminogen, UPA, UPAR, PAI, and Tissue Plasminogen Activator may be important in the degradation of the extracellular matrix during angiogenesis.

– Components of the fibrinolytic system activate MMPs.
– Cellular binding to Vitronectin may be mediated by UPAR and is blocked by PAI-1. Diminished PAI-1 expression can result in enhanced adhesion of endothelial cells to the matrix protein Vitronectin, thus adversely affecting cell motility and neovascularization [Mignatti 1993].
– The expression of the Thrombin Receptor PAR-1 plays a central role in blood vessel recruitment. PAR-1-induced angiogenesis requires VEGF. Activation of PAR-1 markedly augments the expression and splicing of *vegf* mRNAs and of functional VEGF forms. The signal transduction from PAR-1 activation to *vegf* expression proceeds through PKC and PI 3-Kinase [Yin YJ et al. 2003].

Heparan sulfate is an important component of the extracellular matrix and vascular basal lamina. Heparan sulfate in the extracellular matrix of the blood vessel wall inhibits the proliferation of vascular smooth muscle cells. The potent proangoigenic and prometastatic properties of Heparanase (endo-β-D-Glucuronidase, Heparan Sulfate-Degrading Endoglycosidase, HPSE) {4q21.3} [Hulett et al. 1999; Vlodavsky et al. 1999] are tightly regulated by its cellular localization and secretion [Goldschmidt et al. 2002]. Activated endothelial cells degrade the extracellular matrix by secretion of Heparanase, which is expressed on vascular sprouts but not on mature blood vessels. The secretion of Heparanase may release some of the angiogenic growth factors that are sequestered in the extracellular matrix [Parish et al. 2001]. Heparanase-mediated degradation of heparan sulfate in the extracellular matrix releases endothelial cell growth factors, most prominently FGF-2, that are stored in the matrix by high affinity binding to heparan sulfate.

11.1.3 Angiogenic Transcription Factors

Various products of *hox* genes are transcription factors responsible for tissue remodeling. The expression of *hoxB7* upregulates the expression of proangiogenic cytokines, including VEGF, FGF-2, IL-8, and Angiopoietin-2.

Several ETS transcription factors are expressed in vascular endothelial cells. Angiogenic growth factors, including VEGF, FGF-2, and TNF-α, induce the transcription factor ETS-1. This induction in endothelial cells conveys an angiogenic phenotype, including the secretion of UPA, MMP-1, MMP-3, and MMP-9, and the expression of Integrin β_2 [Sato 1998].

11.2 INHIBITION OF NEOVASCULARIZATION

Several angiogenesis inhibitors are stored in the extracellular matrix or in blood proteins and need to be released to become active. This typically occurs through targeted proteolysis. These angiogenesis inhibitors often interact with adhesion proteins, suggesting that the shared ability of the various inhibitors to form such complexes is an integral part of their antiangiogenic effects. Complexes formed in this manner may engage Integrin receptors on the surface of endothelial cells.

There are various modes of action for inhibitors of angiogenesis. Individual angiogenesis inhibitors may use several of these mechanisms in concert to prevent blood vessel formation.

– Some inhibitors engage surface receptors on endothelial cells and directly prevent them from migrating or proliferating.
– Inhibitors of angiogenesis often bind to heparin and neutralize it.
– Some inhibitors of angiogenesis block the activities of FGF or other angiogenic gene products.

Inhibitors of angiogenesis may be produced by primary tumors and suppress the growth of distant metastases. In these cases, the surgical removal of the tumors leads to accelerated growth of the dissemi-

nated tumor colonies. Frequently, inhibitors of metastasis formation also inhibit angiogenesis. This is the case for the Serpin family of protease inhibitors and for Tissue Inhibitors of Metalloproteinases (TIMPs).

11.2.1 Secreted Factors and their Receptors

Secreted factors that negatively regulate the formation of new blood vessels include proteolytic cleavage fragments of extracellular matrix proteins and cytokines.

Endostatin. Endostatin [O'Reilly et al. 1997] is a 20 kD cleavage product of the COOH-terminal domain of type XVIII Collagen {21q22.3}. It is a potent inhibitor of angiogenesis (Figure 11.2.1A). The Endostatin domain (NC1) can be cleaved from Collagen XVIII by Cathepsin-L, Elastin, or Matrilysin downstream of a trimerization region, in an area containing a protease sensitive hinge. Endostatin binds to cells through high affinity and low affinity interactions. While the high affinity receptor has not been identified, the cell surface proteoglycans Glypican-1 and Glypican-4 constitute the low affinity receptors [Karumanchi et al. 2001]. A specific octasulfated hexasaccharide in the heparan sulfate glycosaminoglycans of Glypican is critical for Endostatin binding. Endostatin inhibits endothelial cell migration and proliferation, and it may induce apoptosis.

– Endostatin inhibits endothelial cell invasion by forming stable complexes with pro-MMP-2 and inhibiting the catalytic activities of MMP-1 and MMP-2 [Kim YM et al. 2000]. This is similar to inhibitors of Metalloproteinases, such as TIMP-2, which may inhibit angiogenesis by suppressing MMP activity.
– Endostatin blocks the binding of the VEGF forms $VEGF_{165}$ and $VEGF_{121}$ to the receptor VEGFR-2, and the subsequent tyrosine phosphorylation of this receptor, even though it does not bind to VEGF itself.
– Endothelial cells exposed to Endostatin are unable to migrate in response to FGF-2 and are impaired in their ability to deposit Fibronectin in their extracellular matrices. Endostatin associates with Integrin $\alpha_5\beta_1$ and Caveolin-1. This activates SRC via a Tyrosyl Phosphatase-dependent pathway in endothelial cells [Wickstroem et al. 2002].
– The inhibition of endothelial cell migration and entry into S phase by Endostatin is due to modulation of the WNT pathway. Endostatin modulates the WNT pathway by regulating β-Catenin stability via a GSK3-independent mechanism [Hanai et al. 2002].
– The exposure of endothelial cells, but not cells of distinct differentiation, to Endostatin leads to a marked reduction of the antiapoptotic proteins BCL-2 and $BCL\text{-}X_L$, whereas the levels of BAX are unaffected [Dhanabal et al. 1999].

• A single nucleotide polymorphism D104N (G4349A) in Endostatin is associated with elevated

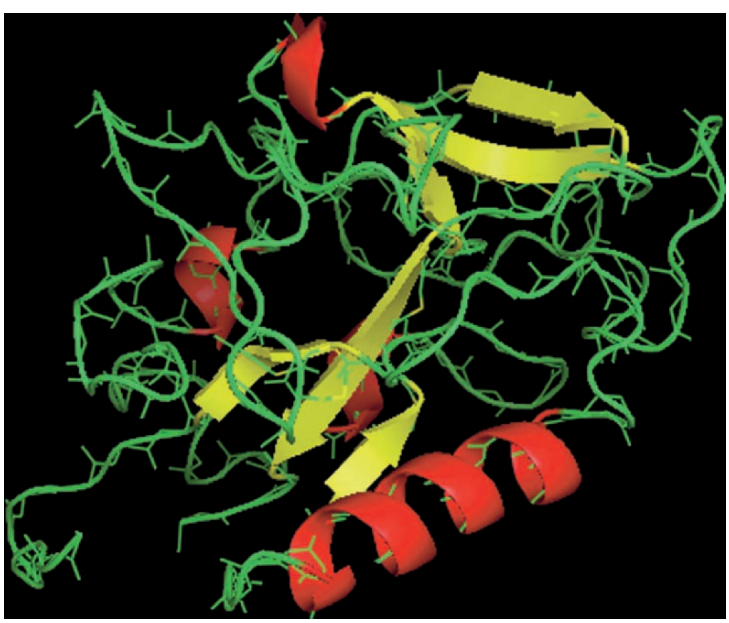


Figure 11.2.1.A. Structure of Endostatin. The three-dimensional structure of Endostatin was modeled using the program loopp and visualized with PyMOL.

risk of prostate cancer. This may reflect an impairment of the protein function by N104 [Iughetti et al. 2001]. However, this polymorphism does not increase the risk for leukemia [Liu et al. 2003].

- Circulating auto-antibodies to self antigens overexpressed by the tumor cells are common in cancer patients. As specific proteins are expressed during neoangiogenesis, a similar phenomenon may occur in the tumor vessels. Collagen XVIII, from which Endostatin is cleaved, is highly expressed in the perivascular basement membrane of tumor-associated blood vessels. A natural immune reaction to Endostatin can occur in breast cancer patients and in glioblastoma patients. This implies a possible role of this humoral reaction in the neoplastic process [Ratel et al. 2000; Bachelot et al. 2006].

Angiostatin. Angiostatin [O'Reilly et al. 1994] is a 38 kD internal fragment of Plasminogen {6q26}, which contains four Kringle domains. Kringle domains are autonomous structural domains, occurring in multiple copies in blood clotting and fibrinolytic proteins, that may play a role in binding mediators and in regulating proteolytic activity. Kringle domains are characterized by a triple loop and three disulfide bridge organization, whose conformation is defined by a number of hydrogen bonds, and small areas folded into an antiparallel β-sheet. The Kringle structure is typically associated with inhibitors of angiogenesis.

Angiostatin is produced by auto-proteolytic cleavage of Plasminogen, involving extracellular disulfide bond reduction by free sulfhydryl donors or Phosphoglycerate Kinase. UPA or Cathepsin D may generate Angiostatin. It is processed by the Matrix Metalloproteinases MMP-3, MMP-7, and possibly by MMP-2, -9, and -12.

A shared mechanism of action for antiangiogenic factors derived from extracellular matrix proteins and plasma proteins is their formation of complexes with adhesion proteins in the plasma to create an active antiangiogenic substance [Yi et al. 2003]. Angiostatin binds Fibrin and Vitronectin. The Integrins $\alpha_V\beta_3$, $\alpha_9\beta_1$, and to a lesser extent $\alpha_4\beta_1$, on endothelial cells specifically bind to Angiostatin, with Integrin $\alpha_V\beta_3$ being a predominant receptor [Tarui et al. 2001]. Angiostatin, but not Plasminogen, binds to ATP Synthase on the cell surface and thus downregulates endothelial cell proliferation [Moser et al. 1999].

- Angiostatin, which specifically inhibits endothelial proliferation, induces dormancy of metastases defined by a balance of apoptosis and proliferation.
- As secreted proteases from prostate carcinoma cells, UPA and Cathepsin D may be responsible for the generation of Angiostatin [Tsukuba et al. 2000].
- Angiostatin inhibits hemangioendothelioma tumor growth [Lannutti et al. 1997].

Tumstatin. Tumstatin is a 232 amino acid peptide in the α chain of Collagen IV that potently inhibits angiogenesis [Maeshima et al. 2000]. MMP-9 is essential for Tumstatin function, because it mediates the cleavage of Collagen IV in the basement membrane that leads to its release. Tumstatin exerts its antiangiogenic effect by specifically inducing apoptosis in proliferating endothelial cells. This requires the its interaction with β_3 Integrin. Tumstatin ligates Integrin $\alpha_V\beta_3$ and inhibits DNA synthesis in endothelial cells. Tumstatin also causes an inhibition of protein translation, mediated through the negative regulation of mTOR (mammalian Target of Rapamycin) signaling. In the absence of Tumstatin, the physiological angiogenesis during development and tissue repair proceeds normally, indicating that the molecule is important in remodeling but not in organogenesis.

Endorepellin. Endoreprellin is a vascular basement membrane derived protein fragment with antiangiogenic activity. It constitutes a 81 kD COOH-terminal fragment cleaved from the ubiquitous basement membrane protein Perlecan [Mongiat et al. 2003]. Endorepellin inhibits endothelial cell migration, Collagen-induced tube formation, and blood vessel growth. However, Endorepellin also binds Endostatin and potentially counteracts the antiangiogenic effect of this molecule.

Anastellin. Anastellin is an antiangiogenic protein fragment derived from Fibronectin. Anastellin forms polymers with Fibronectin and Fibrinogen [Ruoslahti 2002].

Platelet Factor-4. The CXC Chemokine Platelet Factor-4 inhibits endothelial cell proliferation [Sharpe et al. 1990]. The inhibitory activity is associated with the COOH-terminal, heparin binding region of the molecule, is based on the neutralization of heparin, and is abrogated by excess heparin.

This indicates that sulfated polysaccharides modulate the angiostatic activity of Platelet Factor-4 [Maione et al. 1990]. PF-4 binds to FGF-2, and this interaction is stabilized by heparin sulfate. PF-4 released at sites of angiogenesis may bind to proangiogenic growth factors attached to endothelial cell surface heparin sulfate to disrupt or prevent them from interacting with their signaling receptors [Chadderton and Stringer 2003].

Interferons. Interferon-α (IFN-α, Leukocytic Interferon) {9p22} and Interferon-β (IFN-β, Fibroblast Interferon) {9p21} belong to the type I Interferons. All type I Interferons bind to a specific cell surface receptor complex composed of IFNAR1 and IFNAR2 (Interferon-α -β and -ω Receptors 1 and 2). These receptors convey IFN signaling through the JAK→STAT pathway. Interferon-α is produced predominantly by B-lymphocytes. The Interferons-α and -β have antiangiogenic properties.
- Interferon-α and -β inhibit endothelial cell migration [Brouty-Boye and Zetter 1980]
- Interferons inhibit the secretion of such angiogenic factors as FGF-2 from tumor cells
- Interferon inhibits the transcription and translation of *fgf-1* by cancer cells
- IFN-β is able to decrease MMP-2 (72 kD Gelatinase) production by suppressing its gene expression. This compromises the formation of new blood vessels

- Interferon can induce the regression of angiosarcomata and angioblastomata [Marler et al. 2002; Kaban et al. 2002]. These tumors all express high levels of FGF-2 as their major angiogenic mediator.

TIMPS, Serpins, and Cystatins. TIMP-1 and TIMP-2 inhibit MMPs and may thus exert antiangiogenic effects [Takigawa et al. 1990]. TIMPs also have effects on angiogenesis that are separate from their MMP inhibitory activities. A mechanism involves TIMP-2 binding to $\alpha_3\beta_1$ Integrins. This interaction causes a transfer of the Tyrosine Phosphatase SHP-1 from these Integrins to growth factor-stimulated receptor tyrosine kinases, which results in a decrease in their kinase activity [Seo et al. 2003].

The Serpin family member Pigment Epithelium-Derived Factor (PEDF) is a potent antiangiogenic mediator. The Serpin Maspin also effectively inhibits angiogenesis by blocking endothelial cell migration in response to VEGF or FGF-2 [Zhang et al. 2000]. Antithrombin (AT-III, Serpin C1), a member of the Serpin family, functions as an inhibitor of Thrombin and other enzymes. Cleavage of the COOH-terminal loop of Antithrombin induces a conformational change in the molecule. The cleaved conformation of Antithrombin has potent antiangiogenic and antitumor activity. The latent form of intact Antithrombin, which is similar in conformation to the cleaved molecule, also inhibits angiogenesis and tumor growth [O'Reilly et al. 1999]. Antithrombin forms complexes with Thrombin and Vitronectin.

The cleaved form of high molecular weight Kininogen has antiangiogenic activity. Histidine–Proline-Rich Glycoprotein is a plasma component related to Kininogen. It acts strongly antiangiogenic, which is mediated through its histidine- and proline-rich domain.

Integrin ligands. Integrin interactions play prominent roles in regulating angiogenesis. The Integrins $\alpha_V\beta_3$, $\alpha_V\beta_5$, and $\alpha_5\beta_1$, which are selectively expressed in angiogenic vessels are likely targets of antiangiogenic protein complexes. The Integrin $\alpha_V\beta_3$ is engaged by Angiostatin, Endostatin, Antithrombin, and Anastellin. Both Fibronectin and Vitronectin, frequent binding partners for antiangiogenic factors, also are ligands for the Integrin $\alpha_V\beta_3$. Endostatin forms complexes with Fibulin and Nidogen and binds the Integrin $\alpha_V\beta_3$.

Thrombospondin-1 (TSP-1) inhibits endothelial cell growth, migration, and tube formation. It does so, in part, by suppressing the function of FGF-2. Thrombospondin also contains the sequence motif RFK, which binds and activates TGF-β. The interaction between Thrombospondin and TGF-β inhibits tumor growth. The antiangiogenic activity of Thrombospondin is mediated by its ligation of CD36 through the motif VTCG. The molecule also contains a RGD sequence and binds to Integrin $\alpha_V\beta_3$ [Lawler 2002]. In addition, antiangiogenic activity of Thrombospondin maps to the pro-Collagen homology domain and the type 1 repeats. At the boundary between the first and second type 1 repeats, Thrombospondin-1 inhibits endothelial cell proliferation. This effect is suppressed by binding of heparin to Thrombospondin.

P53 can suppress angiogenesis by regulating the expression of *thrombospondin-1*. ID proteins are

helix-loop-helix transcription factors that modulate tumor angiogenesis. Thrombospondin-1, a potent inhibitor of endothelial migration and angiogenesis, is a target of transcriptional repression by ID-1.

The active site of Laminin promotes cell adhesion and has an inhibitory effect on angiogenesis. The β1 chain of Laminin contains the sequence YIGSR (amino acids 929–933) that may mediate this effect [Sherbet and Lakshmi 1997].

- The expression of *thrombospondin* is inversely related to *p53* expression and to angiogenesis in bladder cancer [Grossfeld et al. 1997].

Adiponectin. Adiponectin is a 30 kD protein synthesized exclusively by adipocytes. It is the most abundant gene product in adipose tissue and constitutes about 0.01% of all plasma proteins. It plays roles in inhibiting inflammatory processes and modulating endothelial functions. In contrast to other adipocytokines, Adiponectin acts as an angiogenesis suppressor. It induces endothelial cell apoptosis through the activation of Caspases [Brakenhielm et al. 2004].

- Low serum Adiponectin levels are significantly associated with an increased risk for breast cancer. Further, tumors arising in women with low serum Adiponectin are more likely to be afflicted by an aggressive phenotype. The association between obesity and breast cancer risk might be partly due to Adiponectin [Miyoshi et al. 2003]. This connection seems to affect particularly postmenopausal women [Mantzoros et al. 2004].

Antiangiogenic steroids. In conjunction with heparan sulfate, cortisone, and hydrocortisone act as angiogenesis inhibitors. This is independent of their corticosteroid activity and is governed by distinct structural configurations of the pregnane nucleus [Crum et al. 1985]. In contrast, progesterone has no antiangiogenic activity. The sulfation of heparin is functionally important for its adjuvant effects. In the presence of steroid/heparin combinations, the basement membranes of growing capillaries undergo rapid dissolution.

11.2.2 Antiangiogenic Signaling

Hypoxia and growth factors are critical modulators of angiogenesis. PTEN regulates hypoxia-induced signal transduction. Although PTEN fails to completely inhibit DNA synthesis, its PKB inhibiting activity blocks the expression of endogenous *cox-1*, *pgk1*, and *pfk*, which are hypoxia inducible genes implicated in angiogenesis. PTEN expression completely suppresses the stabilization of HIF-1α by hypoxia. HIF-1 transactivates the promoters of various angiogenic genes like *vegf*, *pai1*, and *upa*.

Microvascular endothelial apoptosis is a homeostatic factor that may be rate limiting to tumor growth. The expression levels of *acid sphingomyelinase* (*asmase*) and *bax* are important in determining the sensitivity of the microvasculature to programmed cell death.

- The loss of PTEN may contribute to tumor expansion through the deregulation of PKB activity and HIF-1-dependent gene expression.
- The expression of PTEN blocks the induction of PKB-1 phosphorylation and kinase activity by hypoxia or IGF-1 in glioblastoma cells.

REFERENCES

Abounader R, Laterra J. 2005. Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. Neuro-oncology 7:436–451.

Alavi A, Hood JD, Frausto R, Stupack DG, Cheresh DA. 2003. Role of Raf in vascular protection from distinct apoptotic stimuli. Science 301:94–96.

Arap W, Pasqualini R, Ruoslahti E. 1998. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 279:377–380.

Bachelot T, Ratel D, Menetrier-Caux C, Wion D, Blay JY, Berger F. 2006. Autoantibodies to endostatin in patients with breast cancer: correlation to endostatin levels and clinical outcome. British Journal of Cancer 94:1066–1070.

Bagnato A, Tecce R, Moretti C, Di Castro V, Spergel D, Catt KJ. 1995. Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells. Clinical Cancer Research 1:1059–1066.

Barker TH, Baneyx G, Cardo-Vila M, Workman GA, Weaver M, Menon PM, Dedhar S, Rempel SA, Arap W, Pasqualini R, Vogel V, Sage EH. 2005. SPARC regulates extracellular matrix organization through its modulation of integrin-linked kinase activity. The Journal of Biological Chemistry 280:36483–36493.

Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, Stamenkovic I, Plowman G, Aruffo A. 1995. CD44 isoforms containing exon v3 are responsible for the presentation of heparin-binding growth factor. Journal of Cell Biology 128:687–698.

Bordador LC, Li X, Toole B, Chen B, Regezi J, Zardi L, Hu Y, Ramos DM. 2000. Expression of emprin by oral squamous cell carcinoma. International Journal of Cancer 85:347–352.

Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, Funahashi T, Cao Y. 2004. Adiponectin-induced

antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proceedings of the National Academy of Sciences USA 101:2476–2481.

Brooks PC, Clark RA, Cheresh DA. 1994a. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 264:569–571.

Brooks PC, Montgomery AMP, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA. 1994b. Integrin $\alpha_V\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 79:1157–1164.

Brouty-Boye D, Zetter BR. 1980. Inhibition of cell motility by interferon. Science 208:516–558.

Burgess WH, Mehlman T, Marshak DR, Fraser BA, Maciag T. 1986. Structural evidence that endothelial cell growth factor β is the precursor of both endothelial cell growth factor α and acidic fibroblast growth factor. Proceedings of the National Academy of Sciences USA 83:7216–7220.

Cao G, O'Brien CD, Zhou Z, Sanders SM, Greenbaum JN, Makrigiannakis A, DeLisser HM. 2002. Involvement of human PECAM-1 in angiogenesis and in vitro endothelial cell migration. American Journal of Physiology Cell Physiology 282:C1181–C1190.

Carson-Walter EB, Watkins DN, Nanda A, Vogelstein B, Kinzler KW, St Croix B. 2001. Cell surface tumor endothelial markers are conserved in mice and humans. Cancer Research 61:6649–6655.

Chadderton NS, Stringer SE. 2003. Interaction of platelet factor 4 with fibroblast growth factor 2 is stabilised by heparan sulphate. The International Journal of Biochemistry and Cell Biology 35:1052–1055.

Clarijs R, Ruiter DJ, de Waal RM. 2001. Lymphangiogenesis in malignant tumours: does it occur? Journal of Pathology 193:143–146.

Claudio PP, Stiegler P, Howard CM, Bellan C, Minimo C, Tosi GM, Rak J, Kovatich A, De Fazio P, Micheli P, Caputi M, Leoncini L, Kerbel R, Giordano GG, Giordano A. 2001. RB2/p130 gene-enhanced expression down-regulates vascular endothelial growth factor expression and inhibits angiogenesis *in vivo*. Cancer Research 61:462–468.

Crum R, Szabo S, Folkman J. 1985. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science 230:1375–1378.

Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD. 1996. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87:1161–1169.

Dhanabal M, Ramchandran R, Waterman MJ, Lu H, Knebelmann B, Segal M, Sukhatme VP. 1999. Endostatin induces endothelial cell apoptosis. The Journal of Biological Chemistry 274:11721–11726.

Dvorak HF. 1984. Tumors: wounds that do not heal. The New England Journal of Medicine. 315:1650–1659.

Ferrara N, Henzel WJ. 1989. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochemical and Biophysical Research Communications 161:851–858.

Folkman J. 1972. Anti-angiogenesis: New concept for therapy of solid tumors. Annales of Surgery 175:409–416.

Folkman J. 1995. Tumor angiogenesis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, 206–232.

Folkman J, Mersler E, Abernathy C, Williams G. 1971. Isolation of a tumour factor responsible for angiogenesis. Journal of Experimental Medicine 133:275–288.

Fujimoto J, Toyoki H, Jahan I, Alam SM, Sakaguchi H, Sato E, Tamaya T. 2005. Sex steroid-dependent angiogenesis in uterine endometrial cancers. The Journal of Steroid Biochemistry and Molecular Biology 93:161–165.

Goldman E. 1907. The growth of malignant disease in man and the lower animals with special reference to the vascular system. The Lancet II:1236–1240.

Goldschmidt O, Zcharia E, Abramovitch R, Metzger S, Aingorn H, Friedmann Y, Schirrmacher V, Mitrani E, Vlodavsky I. 2002. Cell surface expression and secretion of heparanase markedly promote tumor angiogenesis and metastasis. Proceedings of the National Academy of Sciences USA 99:10031–10036.

Grossfeld GD, Ginsberg DA, Stein JP, Bochner BH, Esrig D, Groshen S, Dunn M, Nichols PW, Taylor CR, Skinner DG, Cote RJ. 1997. Thrombospondin-1 expression in bladder cancer: association with p53 alterations, tumor angiogenesis, and tumor progression. Journal of the National Cancer Institute 89:219–227.

Guirguis R, Javadpour N, Sharareh S, Biswas C, el-Amin W, Mansur I, Kim JS. 1990. A new method for evaluation of urinary autocrine motility factor and tumor cell collagenase stimulating factor as markers for urinary tract cancers. The Journal of Occupational Medicine 32:846–853.

Hanai J, Gloy J, Karumanchi SA, Kale S, Tang J, Hu G, Chan B, Ramchandran R, Jha V, Sukhatme VP, Sokol S. 2002. Endostatin is a potential inhibitor of Wnt signalling. The Journal of Cell Biology 158:529–539.

Henke CA, Roongta U, Mickelson DJ, Knutson JR, McCarthy JB. 1996. CD44-related chondroitin sulfate proteoglycan, a cell surface receptor implicated with tumor cell invasion, mediates endothelial cell migration on fibrinogen and invasion into a fibrin matrix. Journal of Clinical Investigation 97:2541–2552.

Hockel M, Schlenger K, Hockel S, Vaupel P. 1999. Hypoxic cervical cancers with low apoptotic index are highly aggressive. Cancer Research 59:4525–4528.

Hockel M, Vaupel P. 2001. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. Journal of the National Cancer Institute 93:266–276.

Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ. 1999. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284:1994–1998.

Hulett MD, Freeman C, Hamdorf BJ, Baker RT, Harris MJ, Parish CR. 1999. Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. Nature Medicine 5:803–809.

Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proceedings of the National Academy of Sciences USA 86:2863–2867.

Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W, Heldin CH. 1989. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338:557–562.

Iughetti P, Suzuki O, Godoi PH, Alves VA, Sertie AL, Zorick T, Soares F, Camargo A, Moreira ES, di Loreto C, Moreira-Filho CA, Simpson A, Oliva G, Passos-Bueno MR. 2001. A polymorphism in endostatin, an angiogenesis inhibitor, predisposes for the development of prostatic adenocarcinoma. Cancer Research 61:7375–7378.

Kaban LB, Troulis MJ, Ebb D, August M, Hornicek FJ, Dodson TB. 2002. Antiangiogenic therapy with interferon α for giant cell lesions of the jaws. Journal of Oral and Maxillofacillary Surgery 60:1103–1111.

Karumanchi SA, Jha V, Ramchandran R, Karihaloo A, Tsiokas L, Chan B, Dhanabal M, Hanai JI, Venkataraman G, Shriver Z, Keiser N, Kalluri R, Zeng H, Mukhopadhyay D, Chen RL, Lander AD, Hagihara K, Yamaguchi Y, Sasisekharan R, Cantley L, Sukhatme VP. 2001. Cell surface glypicans are low-affinity endostatin receptors. Molecular Cell 7:811–822.

Kebebew E, Peng M, Reiff E, Duh QY, Clark OH, McMillan A. 2005. Diagnostic and prognostic value of angiogenesis-modulating genes in malignant thyroid neoplasms. Surgery 138:1102–1109.

Kim I, Kim J-H, Ryu YS, Jung SH, Nah JJ, Koh GY. 2000. Characterization and expression of a novel alternatively spliced human angiopoietin-2. The Journal of Biological Chemistry 275:18550–18556.

Kim YM, Jang JW, Lee OH, Yeon J, Choi EY, Kim KW, Lee ST, Kwon YG. 2000. Endostatin inhibits endothelial and tumor cellular invasion by blocking the activation and catalytic activity of matrix metalloproteinase. Cancer Research 60:5410–5413.

Kojima H, Shijubo N, Yamada G, Ichimiya S, Abe S, Satoh M, Sato N. 2005. Clinical significance of vascular endothelial growth factor-C and vascular endothelial growth factor receptor 3 in patients with T1 lung adenocarcinoma. Cancer 104:1668–1677.

Lannutti BJ, Gately ST, Quevedo ME, Soff GA, Paller AS. 1997. Human angiostatin inhibits murine hemangioendothelioma tumor growth in vivo. Cancer Research 57:5277–5280.

Lawler J. 2002. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. Journal of Cellular and Molecular Medicine 6:1–12.

Lee HJ, Cho CH, Hwang SJ, Choi HH, Kim KT, Ahn SY, Kim JH, Oh JL, Lee GM, Koh GY. 2004. Biological characterization of angiopoietin-3 and angiopoietin-4. FASEB Journal 18:1200–1208.

Liaw L, Skinner MP, Raines EW, Ross R, Cheresh DA, Schwartz SM, Giachelli CM. 1995. The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. Role of $\alpha_v\beta_3$ in smooth muscle cell migration to osteopontin in vitro. Journal of Clinical Investigations 95:713–724.

Liu TC, Peng CT, Lin SF, Chang CS, Chen TP, Chang JG. 2003. Association of endostatin D104N with leukemia. The Kaohsiung Journal of Medical Sciences 19:1–5.

Lobb RR, Alderman EM, Fett JW. 1985. Induction of angiogenesis by bovine brain derived class 1 heparin-binding growth factor. Biochemistry 24:4969–4973.

Lyden D, Young AZ, Zagzag D, Yan W, Gerald W, O'Reilly R, Bader BL, Hynes RO, Zhuang Y, Manova K, Benezra R. 1999. Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. Nature 401:670–677.

Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S. 2001. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nature Medicine 7:1194–1201.

Maeshima Y, Colorado PC, Torre A, Holthaus KA, Grunkemeyer JA, Ericksen MB, Hopfer H, Xiao Y, Stillman IE, Kalluri R. 2000. Distinct antitumor properties of a type IV collagen domain derived from basement membrane. The Journal of Biological Chemistry 275:21340–21348.

Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ. 1990. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 247:77–79.

Mantzoros C, Petridou E, Dessypris N, Chavelas C, Dalamaga M, Alexe DM, Papadiamantis Y, Markopoulos C, Spanos E, Chrousos G, Trichopoulos D. 2004. Adiponectin and breast cancer risk. The Journal of Clinical Endocrinology and Metabolism 89:1102–1107.

Marler JJ, Rubin JB, Trede NS, Connors S, Grier H, Upton J, Mulliken JB, Folkman J. 2002. Successful antiangiogenic therapy of giant cell angioblastoma with interferon α 2b: report of 2 cases. Pediatrics 109:E37.

Mattila MM, Ruohola JK, Karpanen T, Jackson DG, Härkönen PL. 2002. VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumours. Interantional Journal of Cancer 98:946–951.

Mignatti P. 1993. The fibrinolytic system and angiogenesis. Fibrinolysis 7:20–21.

Mignatti P, Mazzieri R, Rifkin DB. 1991. Expression of the urokinase receptor in vascular endothelial cells is stimulated by basic fibroblast growth factor. The Journal of Cell Biology 113:1193–1201.

Millauer B, Shawver LK, Plate KH, Risau W, Ullrich A. 1994. Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1 mutant. Nature 367:576–579.

Miyoshi Y, Funahashi T, Kihara S, Taguchi T, Tamaki Y, Matsuzawa Y, Noguchi S. 2003. Association of serum adiponectin levels with breast cancer risk. Clinical Cancer Research 9:5699–5704.

Mongiat M, Sweeney SM, San Antonio JD, Fu J, Iozzo RV. 2003. Endorepellin, a novel inhibitor of angiogenesis derived from the C terminus of perlecan. The Journal of Biological Chemistry 278:4238–4249.

Moser TL, Stack MS, Asplin I, Enghild JJ, Hojrup P, Everitt L, Hubchak S, Schnaper HW, Pizzo SV. 1999. Angiostatin binds ATP synthase on the surface of human endothelial cells. Proceedings of the National Academy of Sciences USA 96:2811–2816.

Musolino C, Alonci A, Bellomo G, Loteta B, Quartarone E, Gangemi D, Massara E, Calabro L. 2004. Levels of soluble angiogenin in chronic myeloid malignancies: clinical implications. European Journal of Haematology 72:416–419.

Nanus DM, Schmitz-Drager BJ, Motzer RJ, Lee AC, Vlamis V, Cordon-Cardo C, Albino AP, Reuter VE. 1993. Expression of basic fibroblast growth factor in primary human renal tumors: correlation with poor survival. Journal of the National Cancer Institute 85:1597–1599.

O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. 1997. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88:277–285.

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. 1994. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 79:315–328.

O'Reilly MS, Pirie-Shepherd S, Lane WS, Folkman J. 1999. Antiangiogenic activity of the cleaved conformation of the serpin antithrombin. Science 285:1926–1928.

Padera TP, Kadambi A, di Tomaso E, Carreira CM, Brown EB, Boucher Y, Choi NC, Mathisen D, Wain J, Mark EJ, Munn LL, Jain RK. 2002. Lymphatic metastasis in the absence of intratumor lymphatics. Science 296:1883–1886.

Partanen J, Armstrong E, Makela TP, Korhonen J, Sandberg M, Renkonen R, Knuutila S, Huebner K, Alitalo K. 1992. A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. Molecular and Cellular Biology 12:1698–1707.

Parish CR, Freeman C, Hulett MD. 2001. Heparanase: a key enzyme involved in cell invasion. Biochimica et Biophysica Acta 1471:M99–M108.

Pepper MS. 2000. Lymphangiogenesis and tumour metastasis: more questions than answers. Lymphology 33:144–147.

Prols F, Loser B, Marx M. 1998. Differential expression of osteopontin, PC4, and CEC5, a novel mRNA species, during in vitro angiogenesis. Experimental Cell Research 239:1–10.

Rabb H, Barroso-Vicens E, Adams R, Pow-Sang J, Ramirez G. 1996. α_V/β_3 and α_V/β_5 integrin distribution in neoplastic kidney. American Journal of Nephrology 16:402–408.

Ratel D, Nasser V, Dupre I, Benabid AL, Berger F. 2000. Antibodies to endostatin in a multifocal glioblastoma patient. The Lancet 356:1656–1657.

Rempel SA, Ge S, Gutierrez JA. 1999. SPARC: A potential diagnostic marker of invasive meningiomas. Clinical Cancer Research 5:237–241.

Rosano L, Varmi M, Salani D, Di Castro V, Spinella F, Natali PG, Bagnato A. 2001. Endothelin-1 induces tumor proteinase activation and invasiveness of ovarian carcinoma cells. Cancer Research 61:8340–8836.

Ruoslahti E. 2002. Specialization of tumor vasculature. Nature Reviews Cancer 2:83–90.

Sato Y. 1998. Transcription factor ETS-1 as a molecular target for angiogenesis inhibition. Human Cell 11:207–214.

Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM. 1998. NF-κB mediates $\alpha_v\beta_3$ integrin-induced endothelial cell survival. The Journal of Cell Biology 141: 1083–1093.

Seandel M, Noack-Kunnmann K, Zhu D, Aimes RT, Quigley JP. 2001. Growth factor-induced angiogenesis in vivo requires specific cleavage of fibrillar type I collagen. Blood 97:2323–2332.

Senger DR, Claffy KP, Benes JE, Peruzzi CA, Sergiou AP, Detmar M. 1997. Angiogenesis promoted by vascular endothelial growth factor: Regulation through $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins. Proceedings of the National Academy of Sciences USA 94:13612–13617.

Senger DR, Galli SJ, Dvorak AM, Peruzzi CA, Harvey VS, Dvorak HF. 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983–985.

Senger DR, Ledbetter SR, Claffey KP, Papadopoulos-Sergiou A, Peruzzi CA, Detmar M. 1996. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the αvβ3 integrin, osteopontin, and thrombin. American Journal of Pathology 149:293–305.

Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei BY, Stetler-Stevenson WG. 2003. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 114:171–180.

Sharpe RJ, Byers HR, Scott CF, Bauer SI, Maione TE. 1990. Growth inhibition of murine melanoma and human colon carcinomas by recombinant human PF-4. Journal of the National Cancer Institute 82:848–853.

Sherbet GV, Lakshmi MS. 1997. The Genetics of cancer. Genes associated with cancer invasion, metastasis and cell proliferation. San Diego/London/Boston/New York/Sydney/Tokyo/Toronto: Academic Press.

Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. 1984. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science 223:1296–1298.

Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo K, Claffey K, Detmar M. 2001. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nature Medicine 7:192–198.

Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. 1998. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 92:735–745.

Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG. 2001. VEGF-D promotes the metastatic spread of tumour cells via the lymphatics. Nature Medicine 7:186–191.

St Croix B. Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW. 2000. Genes expressed in human tumor endothelium. Science 289:1197–1202.

Strydom DJ. 1998. The angiogenins. Cellular and Molecular Life Sciences 54:811–824.

Sund M, Xie L, Kalluri R. 2004. The contribution of vascular basement membranes and extracellular matrix to the mechanics of tumor angiogenesis. Acta Pathologica, Microbiologica, et Immunologica Scandinavica 112:450–462.

Takahashi F, Akutagawa S, Fukumoto H, Tsukiyama S, Ohe Y, Takahashi K, Fukuchi Y, Saijo N, Nishio K. 2002. Osteopontin induces angiogenesis of murine neuroblastoma cells in mice. International Journal of Cancer 98:707–712.

Takigawa M, Nishida Y, Suzuki F, Kishi J, Yamashita K, Hayakawa T. 1990. Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). Biochemical and Biophysical Research Communications 171:1264–1271.

Tang Y, Nakada MT, Kesavan P, McCabe F, Millar H, Rafferty P, Bugelski P, Yan L. 2005. Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases. Cancer Research 65:3193–3199.

Tarui T, Miles LA, Takada Y. 2001. Specific interaction of angiostatin with integrin $\alpha_v\beta_3$ in endothelial cells. The Journal of Biological Chemistry 276:39562–39568.

Thomlinson R, Gray L. 1955. The histological structure of some human lung cancers and the possible implications for radiotherapy. British Journal of Cancer 9:539–549.

Toi M, Atiqur Rahman M, Bando H, Chow LW. 2005. Thymidine phosphorylase (platelet-derived endothelial-cell growth factor) in cancer biology and treatment. Lancet Oncology 6:158–166.

Trochon V, Mabilat C, Bertrand P, Legrand Y, Smadja-Joffe F, Soria C, Delpech B, Lu H. 1996. Evidence of involvement of CD44 in endothelial cell proliferation, migration and angiogenesis in vitro. International Journal of Cancer 66:664–668.

Tsukuba T, Okamoto K, Yasuda Y, Morikawa W, Nakanishi H, Yamamoto K. 2000. New functional aspects of cathepsin D and cathepsin E. Molecules and Cells 10:601–611.

Vlodavsky I, Friedmann Y, Elkin M, Aingorn H, Atzmon R, Ishai-Michaeli R, Bitan M, Pappo O, Peretz T, Michal I, Spector L, Pecker I. 1999. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nature Medicine 5:793–802.

Wartenberg M, Donmez F, Ling FC, Acker H, Hescheler J, Sauer H. 2001. Tumor-induced angiogenesis studied in confrontation cultures of multicellular tumor spheroids and embryoid bodies grown from pluripotent embryonic stem cells. FASEB Journal 15:995–1005

Weidner N, Semple JP, Welch WR, Folkman J. 1991. Tumor angiogenesis correlates with metastasis in invasive breast carcinoma. New England Journal of Medicine 324:1–8.

Wickstroem SA, Alitalo K, Keski-Oja J. 2002. Endostatin associates with integrin $\alpha_5\beta_1$ and caveolin-1, and activates Src via a tyrosyl phosphatase-dependent pathway in human endothelial cells. Cancer Research 62:5580–5589.

Xu J, Rodriguez D, Petitclerc E, Kim JJ, Hangai M, Moon YS, Davis GE, Brooks PC. 2001. Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. The Journal of Cell Biology 154: 1069–1079.

Yao Y, Kubota T, Sato K, Kitai R. 2001. Macrophage infiltration-associated thymidine phosphorylase expression correlates with increased microvessel density and poor prognosis in astrocytic tumors. Clinical Cancer Research 7:4021–4026.

Yi M, Sakai T, Fässler R, Ruoslahti E. 2003. Antiangiogenic proteins require plasma fibronectin or vitronectin for in vivo activity. Proceedings of the National Academy of Sciences USA 100:11435–11438.

Yin JJ, Mohammad KS, Kakonen SM, Harris S, Wu-Wong JR, Wessale JL, Padley RJ, Garrett IR, Chirgwin JM, Guise TA. 2003. A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. Proceedings of the National Academy of Sciences USA 100:10954–10959.

Yin YJ, Salah Z, Maoz M, Ram SC, Ochayon S, Neufeld G, Katzav S, Bar-Shavit R. 2003. Oncogenic transformation induces tumor angiogenesis: a role for PAR1 activation. FASEB Journal 17:163–174.

Zhang M, Volpert O, Shi YH, Bouck M. 2000. Maspin is an angiogenesis inhibitor. Nature Medicine 6:196–199.

Zhao H, Grossman HB, Delclos GL, Hwang LY, Troisi CL, Chamberlain RM, Chenoweth MA, Zhang H, Spitz MR, Wu X. 2005. Increased plasma levels of angiogenin and the risk of bladder carcinoma: from initiation ot recurrence. Cancer 104:30–35.

CHAPTER 12

INTERACTIONS WITH THE IMMUNE SYSTEM

Interactions between a tumor and its host involve the immune system on various levels and may have diverse consequences. They include the protection of the host by immune surveillance, tumor-induced immune deviation, and a predisposition to cancer due to chronic inflammation. Furthermore, tumors may recruit cells of the immune system to facilitate their progression.

12.1 IMMUNE SURVEILLANCE

The immune system monitors the integrity of cells within the organism [Burnet 1970; Burnet 1971]. It neutralizes or destroys infectious particles as well as infected cells. It may also eliminate cells that are altered in structure or function without being infected, such as transformed cells. Components of the immune system are adaptive and innate, cellular and humoral (Figure 12.1.A). They contribute to immune surveillance in various ways. Immune responses are mediated by leukocytes that derive from precursors in the bone marrow. Pluripotent hematopoietic stem cells give rise to the lymphocyte lineages (responsible for adaptive immunity) and the myeloid lineages (participate mostly in innate immunity).

– Granulocytes comprise neutrophils, eosinophils, and basophils. They circulate in the blood until they are recruited to act as effector cells at sites of infection and inflammation. They kill pathogens by phagocytosis, followed by free radical generation, pH changes, and the secretion of proteases.
– Macrophages, phagocytose, pathogens, and cell debris. They complete their differentiation in specific tissues and become monocytes. Macrophages present foreign antigens on their cell surface to stimulate lymphocyte activation.
– Mast cells secrete various mediators of inflammation and thus orchestrate host-defense mechanisms. They complete their differentiation in specific tissues.
– Dendritic cells [Steinman and Cohn 1973] enter specific tissues as immature phagocytes. They specialize in antigen processing and presentation.
– NK cells (Natural Killer cells) constitute a major component of the innate immune system. They are large granular lymphocytes that do not express T-Cell Antigen Receptors (TCRs), or the pan-T-cell marker CD3, or surface Immunoglobulins (B-Cell Antigen Receptors). They kill target cells independently of the expression of HLA molecules. NK cells were discovered and named because of their innate ability to recognize tumor cells as abnormal and eliminate them.
– B-lymphocytes mature in the bone marrow. They produce antibodies to specific antigens.
– T-lymphocytes mature in the thymus. They recognize antigens presented in HLA (MHC) molecules. Mature lymphocytes recirculate continually through the peripheral or secondary lymphoid organs. Adaptive immune responses are typically triggered when recirculating T-lymphocytes recognize their cognate antigens on the surface of dendritic cells.

The B-lymphocytes and T-lymphocytes are effector cells of the adaptive immune response. Their reactivity is determined by clonal selection during maturation. The immune responses directed by them are typically stronger upon repeated encounter with their cognate antigens.

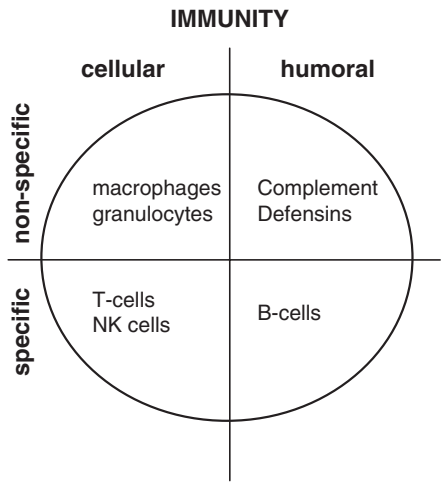


Figure 12.1.A. Components of the immune system. The T-lymphocytes, NK cells, and B-lymphocytes respond to particular antigens and comprise the specific components of the immune system. The responses of macrophages, granulocytes, Complement, and Defensins to pathogens are not restricted to particular antigens. They comprise the nonspecific arm of the immune system. Immune responses mediated by soluble agents, such as members of the Complement system, Defensins, or the Antibodies secreted by B-lymphocytes, are termed humoral. Other responses are cellular.

The role of immune surveillance in cancer protection may be limited (Table 12.1.A). Most of the cancers that develop during states of immune deficiency are rare cancers associated with viral infection [Frish et al. 2001]. Many of these cancers have strong association with Epstein-Barr virus (EBV), such as skin cancers after solid organ transplantation [Otley and Pittelkow 2000] or lymphoid neoplasia after hematopoietic stem cell transplantation [Aguilar et al. 1999]. Lymphoid malignancies may be caused by HTLV-1 in donor blood [Gonzalez-Perez et al. 2003; Hoshida and Aozasa 2004]. EBV infection is strongly associated with leiomyosarcomata and lymphoid tumors in children with AIDS [McClain et al. 1995; McClain et al. 2000]. Immune deficiency caused by HIV infection predisposes to Kaposi sarcoma, a tumor induced by the herpes virus HHV-8 [Chang et al. 1994]. HPV can cause cervical cancer. Immunocompromised women have an increased risk for squamous intraepithelial lesions, a small fraction of which persists and progresses to cancer [Petry et al. 1994]. Transplant recipients are heavily immune suppressed. They are highly susceptible to tumors caused by viral infections. Those include a 400-fold increased risk for Kaposi sarcoma, a 100-fold increased risk for squamous cell vulvar and anal carcinomata (caused by HPV), a 40-fold increased risk for EBV-induced lymphoproliferative diseases, and a 30-fold increase in hepatocellular carcinoma.

The absence of a thymus is not associated with an increased risk of cancer. This suggests a limited role for T-lymphocyte surveillance against tumors. However, neoplasms of the lymphoreticular system frequently occur in patients with genetically determined immune deficiencies. In some of these cases, a viral origin of the neoplasms has been established.

– Common variable immunodeficiency (CVID) is caused by mutations in the *tnfrsf13B* gene {17p11.2} [Salzer et al. 2005]. A central feature in the pathogenesis of this condition is an arrest of B-lymphocyte differentiation. Affected individuals have a normal number of IgA bearing B-cell precursors, but a profound deficit in IgA producing plasma cells. The patients are at high risk for B-cell lymphomata. Infection with the B-lymphotropic virus HHV-8 is linked to lymphoproliferative disorders in people with immune deficiencies. HHV-8 infection may be an important factor in the pathogenesis of the lymphoproliferative disorders associated with common variable immune deficiency [Wheat et al. 2005].
– Granulomatous/lymphocytic interstitial lung disease (GLILD) patients are at high risk for B-cell lymphomata. The lymphoproliferative disorders associated with this disease may be caused by infections with HHV-8.
– Chediak–Higashi syndrome (CHS) is caused by mutations in the lysosomal trafficking regulator gene *lyst* (*chs1*) {1q42.1-q42.2}. As a consequence, peptide loading onto HLA class II molecules and antigen presentation are strongly delayed. Clinically, Chediak-Higashi syndrome is characterized by reduced pigmentation of the hair and eyes (partial albinism), photophobia, nystagmus, neutropenia, abnormal susceptibility to infection, and a unique form of malignant lymphoma [Efrati and Jonas 1958]. The lymphoproliferative syndrome becomes manifest in more than 85% of patients. Its accelerated phase is characterized by generalized lymphohistiocytic infiltrates, fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia, and bleeding [Spritz 1999].
– Wiskott–Aldrich syndrome (WAS) is a rare X-linked disorder caused by mutations in the *wasp* gene {6q21-q22}. WASP (WAVE, SCAR1) is a key regulator of Actin polymerization in hematopoietic

Table 12.1.A. Immune surveillance in cancer. The role of the immune system in host protection from cancer has been subject to much debate. Most cancers occurring in immunocompromised individuals are of infectious origin. Beside those cases, the lack of a correlation between various immunocompromised states and cancer susceptibility implies only limited benefit from immune surveillance

Evidence that suggests a reduction in tumor incidence through the elimination of predisposed cells:
– Solid tumors often contain infiltrates of lymphocytes (tumor infiltrating lymphocytes or TILs) and macrophages, suggesting the activation of an inflammatory host response is induced by the tumor, possibly secondary to tissue invasion and destruction by malignant neoplasms.
– On the basis of histologic evidence, the prognosis of cancer may bear a direct relationship to the expression of immune surveillance [Underwood 1974].
– Tumor associated antigens exist that have immunostimulatory effects.
Evidence that tumors may not induce an immune response:
– Tumor cells are part of the host and display mostly self antigens. Therefore, the immune system should not recognize them.
– Various immunocompromised mouse strains do not have elevated incidence of spontaneous tumors [Stutman 1975].
– The risk of developing the most common types of epithelial cancers is not elevated in immunocompromised states and may even be reduced [Stewart et al. 1995; Gallagher et al. 2001]. Lymphoma incidence is not correlated to affliction with diseases or to intake of medications that result in altered immunity [Hoover 1992].
– In general, immunosuppressed individuals are not typically susceptible to a higher incidence of cancers. When this is the case, it is frequently due to transformation induced by infectious agents.

cells. It plays important roles in lymphoid development and in the maturation of myeloid monocytic cells. Disease causing mutations inhibit or dysregulate normal WASP function. Classic Wiskott-Aldrich Syndrome occurs when WASP is absent, X-linked thrombocytopenia occurs when mutated WASP is expressed, and X-linked neutropenia arises when missense mutations are located in the CDC42 binding site. Patients are prone to develop EBV-related lymphoproliferative disorders [Okano et al. 1984].

– Ataxia teleangiectasia (AT) is caused by a loss-of-function mutation in the signaling protein kinase ATM {11q22.3} that causes chromosome breakage and immune deficiency. The defect in repair of double-strand breaks is inherited autosomally recessively. Patients with ataxia teleangiectasia suffer from rare entities of pediatric non-Hodgkin lymphoma or lymphocytic leukemia. EBV contributions to these lymphoproliferative states are likely [Saemundsen et al. 1981].
– X-linked agammaglobulinemia (XLA) is characterized by the inability to produce mature B-lymphocytes due to a failure of *immunoglobulin heavy chain* rearrangement. The defect in this disorder resides in *btk* (*Bruton tyrosine kinase*, *bpk*, *atk*) {Xq21.3-q22}, which encodes a key regulator in B-lymphocyte development. The patients are highly prone to bacterial infections, but not to viral infections. Lymphomata may arise in antibody deficiency, and they are predominantly of B-cell origin.
– X-linked lymphoproliferative (XLP, Duncan disease) disorder is a rare, often fatal, primary immunodeficiency that has profound and damaging effects on the immune system of the affected individuals. It is characterized by a dysregulated immune response, most commonly to EBV infection. The disease is caused by mutations in *SH2d1A* (*sap-slam*, *sap, SH2 domain protein 1A*) {Xq25}. SH2D1A is an adapter molecule that acts as an inhibitor of SLAM (Signaling Lymphocyte Activation Molecule) receptors by blocking the recruitment of the Tyrosine Phosphatase SHP2 to the phosphorylated cytoplasmic domain of SLAM.

Beyond protecting from oncogenic viruses, immune surveillance also plays a role in defending the host against cancers that do not have infectious origins. In immunosuppressed transplant recipients, the incidence of nonmelanoma skin cancer, thyroid cancer, head and neck cancer, cancers of the urinary tract (kidney, ureter, bladder), and colorectal cancer is substantially elevated compared to the general population [Kinlen 1992; Vajdic et al. 2006]. Conversely, the prognosis for carriers of ovarian carcinoma or melanoma is more favorable when these tumors are infiltrated with lymphocytes. Critical roles in the protection from cancer are played by Interferon-γ [Dighe et al. 1994] (produced by γδ T-cells) and by the ability to kill (through the actions of NK cells [Smyth et al. 2001], Perforin [van den Broek et al. 1996], and TRAIL [Takeda et al. 2002; Cretney et al. 2002]). Interferon-γ exerts its effects through

– Promoting the generation of tumor-specific $CD4^+$ T_H1 T-cells

– Promoting the generation of cytolytic T-cells (CTL)
– Activation of cytocidal activity in macrophages
– Direct effects on the tumor cells.

Additional contributions to antitumor immunity come from T-lymphocytes [Girardi et al. 2001; Gao et al. 2003] and B-lymphocytes.

12.1.1 Tumor-Associated Antigens

The recognition of tumor cells as abnormal is a prerequisite for immune stimulation. This may occur based on the expression of altered gene products. For tumor-derived molecules to be recognized by the adaptive immune system as tumor-associated antigens it is required that they reach the cell surface. Cells that display those antigens in a suitable context are eliminated by cytolytic T-lymphocytes (CTLs) or NK cells. Additional roles are played by B-lymphocytes and cytokines. In advanced tumor growth, hypoxia, and tissue damage induce stress responses, which also activate the immune system.

In adaptive immune responses, antigens are recognized by two distinct sets of lymphocyte receptor molecules: the Immunoglobulins that serve as antigen receptors on B-lymphocytes and the antigen-specific receptors (TCRs) of T-lymphocytes. The antigens that are recognized by B-lymphocytes may be epitopes within complex macromolecules, which may be cell bound or soluble. The T-lymphocytes are activated only by antigens that are displayed on cell surfaces in the HLA (MHC) complexes of other cells. Their TCRs recognize features of both the peptide antigen and the HLA molecule to which it is bound. Virtually all cells express HLA class I molecules on their surface, which display intracellular antigens and may include the antigens of intracellular pathogens. During antigen processing by such antigen presenting cells as dendritic cells or macrophages, proteins from ingested pathogens are degraded into peptides, which are then carried to the cell surface bound to HLA class II molecules.

- In some tumors, the recognition by infiltrating $CD8^+$ cytolytic T-lymphocytes is diminished through the downregulation of HLA class I expression by the tumor cells. This is often due to transcriptional repression. The loss of HLA-I expression is frequently associated with increasing metastatic potential. In more than half of advanced breast cancers, surface HLA-I is absent, and such loss correlates with poor prognosis.
- Certain high-grade tumors are characterized by the inability of HLA-I molecules to reach the cell surface. This may be due to a lack of β_2-Microglobulin synthesis, which is an essential binding partner. Other tumors have defects in TAP (Transporter -Associated with Antigen Presentation) molecules.

Tumor-associated antigens are surface structures that are recognized by effector cells [van der Bruggen et al. 1991]. On the majority of cancer cells, strong tumor-associated antigens are not expressed. Because most tumor-derived gene products are part of self rather than being tumor specific, the failure of immune protection in these cases is due to the intrinsic inability of tumors to activate an effective immune response. The majority of the purported immune escape mechanisms are not specifically acquired by selected tumor cells, but are common mechanisms, shared between solid tumors and normal healthy tissues. Immune responses to them are poor because tumor antigens do not efficiently localize to lymph follicles in lymphoid tissues, and are not efficiently presented to cytolytic T-lymphocytes in an immunogenic context [Speiser and Ohashi 1998]. However, there are mechanisms that can generate new antigenic domains (Table 12.1.1.A), which can act immunostimulatory.

– Tumor rejection antigens may be encoded by genomes of oncogenic viruses, including *v-src*, *v-myc*, and *HPV e6*. Antigens produced by the genes of the EBV are associated with Burkitt lymphoma and are xenogenic.
– Mutations of cancer related genes can result in the expression of altered proteins that differ from normal ones by a single amino acid residue [Monach et al. 1995]. One such protein is *ras*. The point mutations that result in amino acid substitutions in positions 12, 13, or 61 can generate new epitopes. Other alterations result in missense mutations, as they often arise in *p53*.
– Translocations of proto-oncogenes frequently occur in the leukemias. The resulting transcripts and their fusion proteins are specific for the transformed cells. At the joining region between the translocated genes, a unique antigenic domain may be generated.
– The immune system can recognize products from alternative transcripts, including those from cryptic start sites, alternative reading frames, and pseudogenes [Moreau-Aubry et al. 2000]. T-cell epitopes may be translated from introns. The RNA

Table 12.1.1.A. Immunostimulatory antigens on cancer. Atypical gene products are unique antigens for individual tumors. They are the products of damaged genes. Cancer testis antigens are expressed in melanoma and certain other tumors, but in normal tissues only in the testes. Silencing in other cell types is due to gene methylation. Differentiation antigens are the physiologic gene products of specific cell lineages, associated with certain stages of differentiation. Some malignancies are of viral origin. They may express viral antigens

Category	HLA-I	HLA-II	Antigen	Cancer
Atypical gene products	HLA-A2		Mutated *cdk4*	Melanoma
			Mutated *p53*	Colorectal cancer, lung cancer, head and neck cancer
	HLA-A24		Mutated *β-catenin*	Melanoma
		HLA-DR4	Mutated *cdc27*	
			Mutated *ras*	Pancreas cancer, colon cancer, lung cancer
			bcr-abl	Chronic myeloid leukemia
			cia0205	Bladder cancer
			immunoglobulin idiotype	B-cell non-Hodgkin lymphoma, multiple myeloma
	HLA-B44		Mutated *mum1*	Melanoma
	HLA-B35		Mutated *caspase-8*	Squamous cell carcinoma
		HLA-DR1	Mutated *tpi*	
		HLA-DR1	Fusion *ldlr*	
Cancer testis antigens	HLA-A1		*mage-1*	Melanoma
	HLA-Cw16			
			mage-2	Melanoma
	HLA-A1	HLA-DR11	*mage-3*	Melanoma
	HLA-A2	HLA-DR13		
	HLA-B44			
			mage-12	Melanoma
	HLA-Cw16		*bage*	
	HLA-A29		*gage-1*	
	HLA-Cw6			
			gage-2	
	HLA-B7		*rage*	
	HLA-A2		*lage-1* (*camel*)	
	HLA-A2		*ny-eso-1* (*cag3*)	
	HLA-A31			
Differentiation antigens	HLA-A1	HLA-DR4	*tyrosinase*	Melanoma
	HLA-A2	HLA-DR15		
	HLA-A24			
	HLA-B44			
	HLA-31		*trp-1*	Melanoma
	HLA-A2		*trp-2*	Melanoma
	HLA-A31			
	HLA-A33			
	HLA-A68			
	HLA-A2	HLA-DR4	*gp100* (*pmel17*)	Melanoma
	HLA-A3			
	HLA-A2		*melanA* (*mart-1*)	Melanoma
	HLA-B45			
			mc1r	
			α-fetoprotein	Liver cancer
			cea	Colon cancer, breast cancer, pancreas cancer
			pap	Prostate cancer
			psma	Prostate cancer
Viral antigens			*EBV lmp2a*	Hodgkin lymphoma, nasopharyngeal cancer
			HPV e6	Cervical cancer, penile cancer
			HPV e7	Cervical cancer, penile cancer
			HBV	Liver cancer
			HCV	Liver cancer
			HHV-8	Kaposi sarcoma
			HTLV-1	T-cell leukemia

message of *trp-1* has alternative, overlapping open reading frames, one of which generates a T-cell epitope [Wang R-F et al. 1996]. A cryptic promoter within one of the introns of *N-acetylglycosaminyl transferase* generates an aberrant transcript that can form a T-cell epitope.
– In some cases, posttranslational modifications of proteins differ between transformed and non-transformed cells, which may expose previously cryptic epitopes. This occurs with the mucins MUC-1 and CA-125. MUC-1 is hypoglycosylated in some cancers and displays an epitope of the tandem repeat that is normally cryptic. The gangliosides (neuraminic acid containing glycosphingolipids) GM2 and GD2 are present at very high densities on melanomata as compared to normal melanocytes. They can be antigenic.
– Some genes are silent or expressed at very low levels in normal tissue. They may be selectively present on cells of a given differentiation or during specific time periods early during development. Full T-lymphocyte tolerance to these antigens may not always occur because they are not expressed in the thymus. The aberrant expression of normal differentiation antigens in tumor cells is frequent. Among them are *mage* [van der Bruggen et al. 1991], *bage*, *gage*, *rage*, *erbB2*, and splice variants of *cd44*. Likewise, *telomerase* is not typically expressed in differentiated cells. Oncofetal proteins, such as CEA or AFP, may be produced by tumors, but not in the healthy adult organism.
– Malignancies of T- and B-lymphocytes express unique antigenic determinants in their TCR or surface Immunoglobulin. The idiotype of a given malignant clone provides a tumor specific marker. The majority of these tumor-associated antigens are nonmutated self proteins.

Most tumor-associated antigens belong to four functional groups.
– Differentiation antigens are expressed in a developmental stage-dependent manner. They may contribute to the development of specific organs.
– Some cancers of internal organs express oncofetal antigens, such as CEA (Carcinoembryonic Antigen) or AFP (α-Fetoprotein), which exert their physiologic functions in early development.
– The cancer testis antigens are expressed on melanomata as well as breast, prostate, colorectal, and lung cancers, but not in normal tissues with the exception of the testes. A related entity are the brain/testis cancer antigens, which may be sources of paraneoplastic neuropathies.
– Mutated or aberrantly expressed proteins can alter homeostasis and generate novel epitopes.

Due to the high immunologic activity of the skin, melanomata frequently express strong antigenic determinants.
– The melanoma/melanocyte differentiation antigens are antigens expressed by melanomata and normal melanocytes at some stage of differentiation. They include GP100 (MEL17, PMEL, ME20, SILV), MART-1 (Melan-A), Tyrosinase, TRP-1 (Tyrosinase-Related Protein-1, GP75), TRP-2, and SOX-10. Physiologically, these genes contribute to the pigmentation of the skin. Their protein products can be recognized by antibodies and by T-lymphocytes.
– Cancer testis antigens expressed on melanomata include MAGE (Figure 12.1.1.A), BAGE, GAGE, PRAME, and NY-ESO. Because testis may be an immune privileged site and germ cells lack the expression of HLA-I (MHC-I) on their surface, these antigens are relatively tumor specific. They contain several HLA class I epitopes and are potentially very immunogenic. There are four gene clusters (A, B, C, and D) of the *mage* family. Some *mage* genes are silent and may represent pseudogenes. Some members of the *mage* gene family are ubiquitously expressed on normal cells, while others occur on various tumor cells, but are silent in normal adult tissues except in the male germline. A conserved MAGE domain of about 200 amino acids is shared by all MAGE family members. The COOH-terminal domain of MAGE-D3 is identical to Trophinin. MAGE-D1 may bind to the P75 Neurotrophin Receptor [Chomez et al. 2001].
– Mutated or aberrantly expressed proteins include MUM-1, β-Catenin, CDK4, GP100-in4, P15, and *N*-Acetylglucosaminyl Transferase V (MGAT5, GNT-V). MUM-1 (Melanoma Ubiquitous Mutated-1) may be subject to a point mutation in melanoma, which allows the presented peptide to be recognized by T-lymphocytes [Wang 1999]. Mutations in β-Catenin are common in various tumors. A mutation occurring in melanoma, generates a new T-lymphocyte epitope that stimulates cytolytic T-lymphocyte activity [Wang 1999]. GP100 is a tumor regression antigen. Some tumor antigens are HLA-II restricted and activate $CD4^+$ cells. One such antigen is derived from a mutated

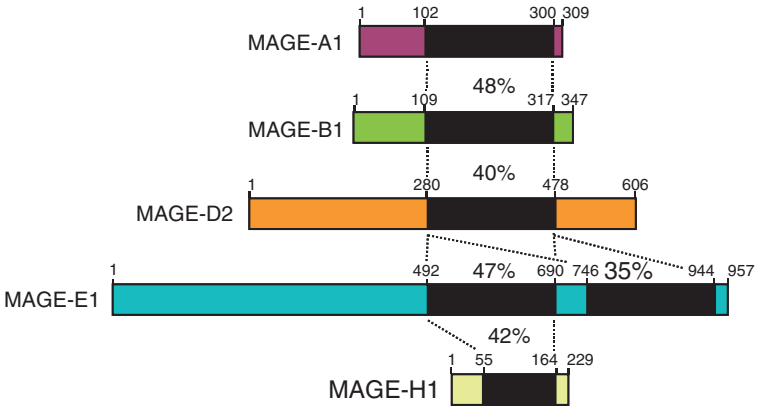


Figure 12.1.1.A. Structure of MAGE proteins. The filled blocks indicate the MAGE-conserved domains, with the percentage of identical amino acids indicated between pairs of MAGE proteins. Unique domains are shown in distinct colors.

form of CDC27, which gives rise to a HLA-DR4 restricted melanoma antigen. Similarly, a mutated form of Triosephosphate Isomerase in melanoma is a HLA-DR1 (a form of HLA-II)-restricted antigen. [Wang et al. 1999].

– Up to 20% of melanoma cells may retain the expression of specific antigens, yet fail to adequately present immunogenic epitopes due to defects in the antigen presenting machinery. They comprise alterations in the expression of HLA alleles, β_2-Microglobulin, or TAP (Transporter of Antigen Processing) [Wang Z et al. 1996].

Tumor-associated antigens are expressed on some gynecologic cancers. Some may also be expressed on other tumors of epithelial origin.

– ERBB2 (HER-2/NEU) is a shared tumor antigen recognized by T-lymphocytes in breast and ovarian cancers [Ioannides et al. 1993]. The recognition and lysis of ovarian cancer cells by cytolytic T-lymphocytes correlates with their expression levels of ERBB2. Two epitope peptides are commonly recognized. The epitope peptide GP2 (amino acids 654–662) is shared by various epithelial tumors, including breast, ovarian, pancreas, and non-small cell lung cancers.
– The MUC-1 protein is a highly *O*-glycosylated transmembrane molecule that is expressed at the luminal surface of most glandular epithelial cells and is upregulated in carcinomata. Activation of the ERBB2→RAS→RAF→Phosphatidylinositol 3-Kinase pathway increases the expression of the *muc-1* gene in normal and transformed mammary epithelial cells. In normal resting, pregnant, and lactating breasts, Mucin *O*-glycans are largely extended (core 2 type) structures. In contrast, Mucin *O*-glycans in breast carcinomata and pancreas adenocarcinomata are often truncated (core 1 type). An underlying mechanism for this increase in core 1 structures is a change in the expression of Glycosyl Transferases, particularly an increase in the expression of the Sialyl Transferase ST3Gal-1. The loss of core 2-based glycans is a consistent feature of MUC-1 Mucin, when it is expressed by mammary tumors. This causes the unmasking of the SM3 epitope, consisting of multiple 20 amino acid sequence repeats, in more than 90% of breast carcinomata. In cancer cells, the reduction in *O*-glycosylation of MUC-1 leads to the exposure of cancer-associated peptide epitopes within the tandem repeat region of MUC-1.

Many mutated cancer gene products are mostly intracellular and therefore depend on their presentation on the cell surface, typically by HLA molecules, for the induction of antigenicity. Heat shock proteins play a role in this process. The generation of anti-P53 antibodies in breast cancer depends on the formation of P53/HSP70 complexes [Davidoff et al. 1992]. These antigens are presented by HLA-I. In about 10% of all cancers, complete loss of HLA-I occurs, which curtails the ability to display antigens on the cell surface. In some types of malignancies, the loss of 1 allele of HLA-I is frequent.

The diverse antigens expressed by tumor cells can have varied effects on the immune system. Two opposing effects include immunodominance and epitope spreading.

– Although tumors may express a multitude of antigens on their surface, the immune system often responds to very few of them. This phenomenon has been dubbed immunodominance [Wortzel et al. 1983]. Individual tumor cells that do not

express the immunodominant epitopes are less susceptible to immune attack.
– On the other hand, T-cell recruitment and selection can lead to changes in the specificity of the anti-self response during the course of disease. These changes are due to alterations in self-antigen presentation that lead to the display of previously cryptic self-determinants, a phenomenon called epitope spreading [Lehmann et al. 1993].

12.1.2 Adaptive Cellular Immunity

$CD8^+$ T-lymphocytes are cytolytic effector cells. $CD4^+$ T-lymphocytes are helper cells that secrete cytokines to amplify cellular or humoral immune responses. Whereas $CD4^+$ cells protect predominantly from extracellular pathogens, $CD8^+$ T-lymphocytes eliminate cells that are infected by viruses or intracellular pathogens as well as cells that display signs of malfunction.

Cytolytic pathways. Tumor infiltrating lymphocytes are typically $CD8^+$ cells. The reactivity of $CD8^+$ cytolytic T-lymphocytes is restricted by shared class I HLA (MHC class I) determinants. They recognize peptides of 8–10 amino acids in length bound to the antigen presenting groove of HLA-I. They kill their target cells through the engagement of death receptors and through the secretion of Perforin and Granzyme B.
– In the CD95L pathway, lymphocytes exhibit the death ligand CD95L on the cell surface and trigger apoptosis through the receptor CD95 on the target cells. In renal cell carcinoma, the tumor infiltrating lymphocytes express higher levels of CD95L compared to peripheral blood lymphocytes. This reflects a state of activation and lytic potency of the lymphocytes surrounding the tumor [Elasser-Beile et al. 2003]. The antiapoptotic protein FLIP (FLICE-Like Inhibitory Protein) is overexpressed in melanomata and protects them from CD95-mediated apoptosis induced by cytolytic T-lymphocytes.
– In the granule exocytosis pathway, T-lymphocytes secrete Perforin, which induces membrane permeability, and Granzymes, which are enzymes from cytolytic granules. In the presence of calcium, Perforins polymerize and initiate the formation of pores in the target cell membrane that allow Granzymes to pass into the cell and activate Caspases, leading to programmed cell death. Granzyme B activates the cytosolic protein BID, which induces changes in the mitochondrial membrane potential and thereby the release of products that induce apoptosis. Perforin-dependent cytotoxicity is a crucial mechanism of resistance to carcinogenesis [van den Broek et al. 1996].

Cell–cell contact is a prerequisite for target killing by cytolytic T-lymphocytes. Disaccharides on tumor cells interact with platelets through P-Selectin. The platelets may protect the tumor cells from cytolytic T-lymphocytes lysis [Fuster et al. 2003], possibly by epitope masking. Shielding of antigenic cancer cells from cytolytic T-lymphocytes may also occur at immune-privileged sites, such as the brain and the testes, which are exempt of some monitoring by the immune system.

Helper cell pathways. Although endogenous antigens are rarely taken up into the class II HLA (MHC class II), some tumor-associated antigens need to be presented to $CD4^+$ helper T-cells in physical association with HLA-II to induce an immune response. Those antigens must be endocytosed before presentation to the $CD4^+$ T-lymphocytes. The help from the stimulated $CD4^+$ cells is then needed for antibody production, which is executed by B-lymphocytes, as well as for the effectiveness of a cytolytic response, which is executed by cytolytic T-lymphocytes and NK cells.

Regression in melanoma is often associated with lymphoid infiltrates, and the infiltrates have a preponderance of $CD4^+$ T-lymphocytes in areas of regression. These $CD4^+$ cells kill melanoma cells predominantly by TRAIL-induced apoptosis. CD95L and TNF-α seem to have little involvement in this process in melanoma [Thomas and Hersey 1998; Kayagaki et al. 1999].

Immunoediting. For effective anticancer immune responses the participation of both $CD4^+$ cells (helper T-lymphocytes) and $CD8^+$ cells (cytolytic T-lymphocytes) is important. The selective pressure exerted by T-lymphocytes against tumor cells expressing specific cancer antigens may cause the alteration of gene expression in those tumor cells. Tumors can evade immunity in part through genetic aberrancies in antigen processing or antigen presentation. The genetic instability of cancers facilitates these alterations. This mechanism promotes the emergence of primary tumors with reduced

immunogenicity that are capable of escaping immune recognition, a process that is referred to as immunoediting [Dunn et al. 2002]. It has three phases, comprising the elimination of antigenic tumor cells, an equilibrium due to immune-mediated latency, and the escape from recognition by the immune system following genetic or epigenetic changes that reduce the antigenicity of the tumor cells [Dunn et al. 2004].

T-lymphocyte priming. The priming of tumor antigen-specific T-lymphocytes is critical for the initiation of successful antitumor immunity. Efficient immune responses only occur if antigens reach the secondary lymphoid organs and are expressed there for sufficient periods of time and at sufficient levels [Zinkernagel 2000]. Antigen presenting cells are central to the priming of T-lymphocytes, and dendritic cells are the most potent stimulatory antigen presenting cells. Dendritic cells take up antigens and deliver them to lymphoid organs. Infiltrating dendritic cells are present in melanoma, nasopharyngeal carcinoma, lung carcinoma, breast carcinoma, stomach carcinoma, and prostate carcinoma, but only sparsely in renal cell carcinoma. The ability of these tumor-infiltrating dendritic cells to induce efficient antitumor responses is compromised, in part, by the absence of PAMP (Pathogen-Associated Molecular Pattern)-related activation signals, which are typical of bacteria. The migration of tumor-infiltrating dendritic cells to the draining lymph nodes may involve the ligation of CCR7 on the dendritic cell surface by CCL21 (6Ckine, SCL, SCYA21), which is produced by the lymphatic endothelium. The upregulation of CCR7 on the dendritic cell surface or the production of CCL21 by lymph endothelial cells may be impaired in the tumor microenvironment [Vicari et al. 2002].

Regulatory T-cells. Regulatory T-lymphocytes (suppressor T-lymphocytes) are able to inhibit responses to self tissue, thus maintaining self-tolerance and avoiding autoimmune disease. They include NKT subsets (cells expressing invariant T-Cell Antigen Receptors and Natural Killer cell receptors), $CD4^+CD25^+$ cells, and $CD8\alpha\alpha^+$ $\gamma\delta$ T-lymphocytes. Regulatory cells may also inhibit responses to tumors. Their recruitment through the interaction between CCL22, secreted by the cancer cells, and CCR4, expressed on the surface of the regulatory cells, constitutes a mechanism of immune evasion. NKT cells prevent tumor regression by producing IL-13 and inhibiting cytolytic T-lymphocytes [Terabe et al. 2000]. The $CD4^+CD25^+$ T-lymphocyte population suppresses the activation and proliferation of other T-lymphocytes in an antigen-independent manner. Its absence may be sufficient for the induction of tumor rejection [Shimizu et al. 1999; Onizuka et al. 1999].

12.1.3 Immunity Mediated by Natural Killer Cells

Natural Killer cells (classical NK cells and natural cytotoxic cells) are components of the innate immune system. They participate in early responses to infected or transformed cells by producing cytokines and executing direct cytotoxicity. NK cells are bone marrow-derived lymphocytes, which kill targets in a manner that complements cytolytic T-lymphocytes. They recognize a lack of normal HLA-I expression on cells ("missing self" [Karre et al. 1986]) and hence kill independently of HLA restriction. Tumor cells that have downregulated their HLA class I expression can be killed by these cells [Whiteside and Herberman 1995]. NK cells exert their cytotoxicity almost exclusively through the Granzyme/Perforin system. Lymphokine activated killer cells (LAK cells) are Interleukin-2 or Interferon-γ stimulated NK cells, they therefore kill target cells with increased effectiveness. NK activity is regulated by groups of inhibitory and activating surface receptors [Long 2002]. Because potential target cells signal their identity as part of self through the expression of HLA-I, NK cells have HLA class I specific inhibitory receptors on their surface, which block NK cytotoxicity when ligated. This occurs through the recruitment of the phosphatase SHP-1 to phosphorylated ITIM (immunoreceptor tyrosine-based inhibition) motifs in their cytoplasmic tail. NK cells become activated when inhibition is removed and stimulatory receptors are engaged, which results in target cell killing. The genes for many of the regulatory receptors cluster on chromosome 19q13.4.

– KIR (Killer Immunoglobulin-Like Receptor) belong to the Immunoglobulin superfamily. Several members of the KIR receptor family are inhibitory and signal through ITIMs. The KIR family also contains the stimulatory KIR-2DS (CD158H) and KIR-3DS (CD158E2). KIR-2DS is an activating receptor on NK cells that forms a complex with the

cell surface adapter proteins DAP10 (DNAX Activation Protein 10) or DAP12.
– NKG-2 receptors belong to the C-type Lectin superfamily. The NKG-2 (KC Lectin Receptor, KLR) family includes CD94 (KLRD1), LY49L (KLRA1), NKR (KLRB1), NKG-2A (KLRC1), NKG-2C (KLRC2), NKG-2E (KLRC3), NKG-2D (KLRC4), and KLRF1. The virtual absence of a cytoplasmic tail in CD94 requires that it functions in conjunction with other cell surface molecules. Several forms of NKG2 can pair with the CD94 common chain, but only CD94/NKG-2A is inhibitory. CD94 containing receptors are heterodimeric type II transmembrane proteins with C-type lectin domains that bind to the nonclassical HLA-E. The NKG family also entails stimulatory receptors. NKG-2D is an activating receptor on NK cells that pairs with the cell surface adapter proteins DAP10 (DNAX Activation Protein 10) or DAP12. NKG-2D may be engaged by MIC-A or MIC-B on target cells. This receptor/ligand interaction activates NK-mediated killing.
– Receptors of the ILT (Immunoglobulin-Like Transcript, Leukocyte Immunoglobulin-Like Receptor, LIR, Macrophage Inhibitory Receptor, MIR) family bind to most HLA class I types. They are receptors of the Immunoglobulin superfamily.
– LAIR-1 (Leukocyte-Associated Inhibitory Receptor-1) and LAIR-2 are inhibitory receptors.
– The receptors NKp30, NKp44, and NKp46 carry ITAMs (immunoreceptor tyrosine-based activation motifs) that signal through SRC and SYK [Pende et al. 1999]. They may interact with non-HLA ligands and are stimulatory.

After NK cells have initiated an innate immune response by recognizing and attacking target cells, they secrete IFN-γ to attract macrophages. This mounts a second phase of the host defense reaction.

- NK cell activity can inhibit metastasis formation through the elimination of intravascular tumor cells [Herberman 1995]. NK cell activity may be depressed in some patients with cancer. It is a poor prognostic marker for patients with head and neck cancer or with recurrent melanoma.
- MIC-A is broadly expressed on tumor cells of epithelial origin. Diverse tumors may release soluble MIC-A after cleavage by Metalloproteinases. This leads to elevated serum levels of soluble MIC-A, and is followed by the internalization and degradation of NKG-2D receptors on NK cells [Groh et al. 2002; Salih et al. 2002] and may protect the tumors from NK-mediated cytotoxicity.
- Leukemic cells have downregulated HLA class I expression and may have allelic loss. NK cells participate in antileukemia immune responses, which is reflected in an inverse relationship between the number or activity of NK cells and prognosis in acute leukemia. NK cells also contribute to the graft-versus-leukemia effect in allogeneic bone marrow transplantation [Fauriat et al. 2003].
- Immunodeficiency syndromes that are associated with impaired NK cell activity have increased frequencies of lymphoid hematologic malignancies.

12.1.4 Adaptive Humoral Immunity and Cytokines

Antibodies are the secreted forms of the B-lymphocyte antigen receptors. They generally recognize only small regions (epitopes, antigenic determinants) on the surface of large molecules, such as polysaccharides or proteins. The coating of infectious particles or cells with antibodies results in their neutralization. Antibodies can kill tumor cells indirectly by two distinct mechanisms,
– In Complement-dependent cytotoxicity, the formation of complexes between antigen and humoral antibody activates the Complement system and activates platelets. This results in the release of histamine and vasoactive amines, as well as the release of proteolytic enzymes,
– In antibody-dependent cellular cytotoxicity (ADCC), antibodies binding to cell surface antigens activate phagocytic cells and killer cells to destroy the cell expressing the target antigen.

The induction of an antibody response is enhanced by helper T-lymphocytes ($CD4^+$ cells) through the secretion of T_H2 cytokines, which stimulate humoral immune responses.

- Immune complexes circulating in the blood stream may be a reflection of tumor growth. Circulating immune complexes sometimes reach high levels in individuals with malignant melanoma. They include complexes containing tumor antigen/antibody as well as antibody/anti-antibody components. These complexes accumulate in the kidneys. The coexistence of anti-antibodies, immune complex disease, and anergy in melanoma patients may indicate a deranged immune regulation consequent to chronic antigenic stimulation by the tumor [Jerry et al. 1976].

- Two highly homologous genes encode SCCA-1 (Squamous Cell Carcinoma Antigen-1) {18q21.3} and SCCA-2 {18q21.3}. They belong to the family of Serpins (Serine Protease Inhibitors). SCCA antigens are expressed by squamous cell carcinomata of the uterine cervix and by hepatocellular carcinomata. The antigens circulate in immune complexes with IgM [Kato and Torigoe 1977; Beneduce et al. 2005].
- Most healthy cells are protected from Complement-mediated cytotoxicity through the expression of the membrane-bound Complement regulatory proteins (anticomplement proteins, mCRPs) CD46, CD55, and CD59. These molecules are also overexpressed on a variety of cancer cells.

In specific settings, cytokines may exert antitumor effects [Mendelsohn and Gabrilove 1995], including:
– Restoration of normal hematopoiesis
– Augmentation of the host defense
– Stimulation and production of functionally primed antitumor effector cells
– Clonal extinction of malignant disease by induction of differentiation

Dendritic cells are the major antigen presenting cells to undifferentiated T_H0 $CD4^+$ T-cells in lymph nodes. Upon activation, these naïve $CD4^+$ T-lymphocytes can differentiate either into T helper 1 (T_H1) cells or into T helper 2 (T_H2) cells, which differ in the types of cytokines they produce. Polarized T_H1 cells secrete Interleukin-2 (IL-2), IL-12, and Interferon-γ (IFN-γ), whereas polarized T_H2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13. While the T_H1 cytokines induce a predominantly cellular immune response with cytolytic T-lymphocytes and NK cells as effectors, the T_H2 cytokines induce a predominantly humoral immune response with B-lymphocytes as effectors. The decision on the direction of the differentiation is made during the clonal expansion that ensues after the initial antigenic stimulation. Antitumor immune responses are most effective if they contain cellular and humoral components. Therefore, the induction of a broad spectrum of cytokines is beneficial.

An endogenous tumor surveillance system is dependent on Interferon-γ [Dighe et al. 1994; Kaplan et al. 1998; Shankaran et al. 2001]. Interferon-γ has direct effects on tumor cell immunogenicity, because it upregulates the expression of HLA-I and thus plays an important role in promoting tumor cell recognition and elimination by $CD8^+$ T-lymphocytes. In addition, Interferon-γ is required for the migration of the T-cells to tumors. Lymphocytes and Interferon-γ synergize to protect against the development of carcinogen-induced sarcomata and spontaneous epithelial carcinomata, but they also select for tumor cells with reduced immunogenicity [Shankaran et al. 2001]. After activation, NK cells secrete Interferon-γ to attract macrophages and enhance the innate immune response. γδ T-lymphocytes are a population of T-cells with unique TCRs that reside predominantly in the skin. They are also an important source of Interferon-γ in antitumor immunity [Gao et al. 2003].

The Interferons α and β, sometimes referred to as type I Interferons, are generally induced by viral infections. They inhibit T_H2 cytokine synthesis by hematopoietic cells and $CD4^+$ T-lymphocytes and they enhance T_H1 cytokine synthesis with ensuing activation of the adaptive immunity. This immune response can protect from virallyn-induced tumors. The expression of HPV *e6* and *e7* genes in differentiating keratinocytes directly alters the expression of genes that influence the host resistance to infection. One function of E7 is to prevent the induction of Interferon-α inducible genes.

Colony Stimulating Factors (CSFs) are proteins necessary for the survival, proliferation, and differentiation of hematopoietic progenitor cells. GM-CSF (CSF2) is produced by macrophages, monocytes, and activated T-lymphocytes. It has the ability to enhance immune responses by inducing a broad spectrum of cytokines, which results in a combined T_H1 (cellular) and T_H2 (humoral) response. GM-CSF also contributes to the maturation and recruitment of dendritic cells, which can potently present tumor antigens. GM-CSF is one of the most potent cytokines to induce antitumor immunity [Grabstein et al. 1986; Dranoff et al. 1993].

GM-CSF is located on a chromosomal region {5q31.1} that are sometimes deleted in leukemias of the myeloid lineage [Le Beau et al. 1986]. This may reflect the stalled maturation of macrophages in the absence of this cytokine, resulting in increased susceptibility to transformation.

Interleukin-2 (IL-2, T-Cell Growth Factor) is an immunostimulatory Lymphokine that is produced

by activated T-lymphocytes. It acts as a growth factor for T-cells and B-cells. It induces cellular immune responses and plays a role in T-lymphocyte differentiation. T-lymphocytes activated by IL-2 may then target and kill the cancer cells.

- Some tumors secrete factors that downregulate T-lymphocyte help (suppressor factors). In the absence of substantial T-lymphocyte help, antibody production is profoundly compromised. This may limit antitumor immunity.
- The 28 kD protein CML28 (RRP46p) is a component of the exosome, a multiprotein complex involved in the 3′ processing of RNA. CML28 is highly expressed in leukemia and in epithelial tumors, but not in normal tissue. It elicits a high titer of a specific IgG antibody response [Yang et al. 2002].

12.2 TUMOR-INDUCED IMMUNE DEVIATION

Some tumors produce cytokines that alter the normal functions of the immune system and cause immune deviation. Similarly, cancer cells may express surface molecules that exert immune modulatory functions or lack molecules that exert immune stimulatory functions. In many cases, this leads to increased susceptibility to infections.

12.2.1 Immunomodulatory Cytokines

Immunomodulatory cytokines secreted by cancer cells include TGF-β, Interleukin-10 (IL-10), and prostaglandins.

A variety of cancer types secrete TGF-β, including malignant gliomata, breast cancers, prostate cancers, and leukemias. TGF-β is frequently potently immunosuppressive.

– The proliferation of thymocytes, T-lymphocytes, B-lymphocytes, NK cells, monocytes, and macrophages is inhibited by TGF-β.
– TGF-β induces apoptosis in B-lymphocytes and T-lymphocytes.
– TGF-β downregulates cytolytic T-cell activation by shifting the balance of the cytokine profile towards type II (T_H2) and inhibiting type I (T_H1) cytokines. This includes the suppression of IL-12 expression and the downregulation of IL-2 Receptors on T-lymphocytes. The shift of the cytokine balance toward a humoral immune response (type II, T_H2) is not effective for anticancer immune responses, which depend on cellular participation.
– TGF-β inhibits antigen presentation on HLA class II molecules. The inhibition of antigen presentation prevents T-lymphocytes from recognizing the cancer cells as foreign.
– The TGF-β-induced downregulation of adhesion molecules prevents T-lymphocytes from reaching the tumor site.
– For the fraction of T-lymphocytes that do manage to get to the tumor site and recognize the tumor, a lack of costimulation can cause anergy.

Many tumor types secrete IL-10, which exerts immunosuppressive effects on subsets of T-lymphocytes. Through its inhibition of the cytokine production by macrophages, IL-10 indirectly reduces the cytokine synthesis by helper T-lymphocytes. This downregulates the functions of T_Hl cells, compromising the activation of cytolytic T-lymphocytes. The secretion of IL-10 in the vicinity of a tumor can render the tumor insensitive to cytolytic T-lymphocytes-mediated lysis. However, IL-10 is a B-cell differentiation factor and may possess other roles in the development of antitumor immune responses and actually benefit host defenses under certain circumstances.

Prostaglandins inhibit lymphocyte mitogenesis, cytolysis, and antibody production. They also affect cell differentiation as well as target cell interactions. Monocytes and macrophages are the primary physiologic sources of prostaglandin production. If induced accordingly, these cells can inhibit immune responses by releasing prostaglandin E_2. Prostaglandins are also produced by tumor cells at elevated levels when they interact with effector lymphocytes. Prostaglandins play important roles in the interactions between the immune system and tumor cells:

– A variety of prostaglandins are produced by cells that are active in the expression and regulation of immune response activity.
– The production of prostaglandins is linked to the production of effector lymphocytes and tumor targets.
– Prostaglandins produced during these interactions influence the expression of lymphocyte and macrophage cytoxicity against tumor targets.

Prostaglandin synthesis is regulated by *cox* (*cyclooxygenase*, *prostaglandin endoperoxide synthase*, *pdgs*) gene expression. Two separate gene products are expressed, COX-1 {9q32-q33.3} is

constitutively expressed and COX-2 {1q25.2-q25.3} is mitogen inducible. They catalyze the rate limiting step in prostaglandin synthesis. COX-1 and COX-2 are expressed in large amounts by various cancers. Specifically, COX-2 is present at high levels in intestinal tumors.

12.2.2 Inhibition of the Immune System

Anergy. T-lymphocyte activation requires two signals. The primary signal is conveyed through the TCR and mediates the specificity of the immune response. The secondary signal is transduced through the engagement of costimulatory receptors on the interacting cells. The multiple contacts between antigen presenting cells and T-lymphocytes that are needed for effective activation are referred to as immunologic synapse (supramolecular activation cluster, smac). In the absence of a secondary signal, TCR engagement causes the T-lymphocytes to fail developing full effector function and they are rendered anergic [Schwartz 1990; Harding et al. 1992], which is a state of unresponsiveness. Anergizing TCR signals result in increased intracellular concentrations of the second messenger cyclic AMP, which upregulates $P27^{KIP1}$, sequestering Cyclin/CDK complexes, and preventing the progression of T-lymphocytes through the G_1 restriction point of the cell cycle.

Tumor-specific T-lymphocytes are rendered anergic upon encountering tumor antigen on the HLA molecules of the cancer cells in the absence of costimulatory signals. Endogenous tumor-associated antigens may be displayed by transformed cells in the HLA class I but costimulatory receptors are not typically present on the surface of tumor cells. Thus anergy is induced in T-lymphocytes that recognize these antigens. The induction of antigen specific T-cell unresponsiveness can occur early in the course of T-lymphocyte interactions with a tumor and significantly precede the development of a more generalized state of immunosuppression.

B7 molecules are the most important costimulatory receptors on antigen presenting cells. The costimulatory molecule B7-2 is constitutively expressed on dendritic cells, and is upregulated more rapidly than B7-1 (CD80), indicating that B7-2 (CD86) participates in initiating an immune response, whereas B7-1 may be involved in sustaining or regulating the activation process. Both B7-1 and B7-2 bind to the costimulatory T-lymphocyte receptor CD28 with low affinity and to the negatively coregulatory T-lymphocyte receptor CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4, CD152) with high affinity. The differences between the binding of B7-1 and B7-2 to either CD28 or CTLA-4 are small, although B7-1 binds more weakly to CD28 and has a faster off-rate from CTLA-4 than B7-2. Alternatively, the B7 molecules may engage the coreceptor ICOS on activated T-lymphocytes.

- CD28 is constitutively expressed as a homodimer on the surface of T-lymphocytes, with its levels increasing slightly upon activation. The CD28 cytoplasmic domain contains a YMNM motif, which binds PI 3-K and GRB-2. In the presence of an antigenic signal, the engagement of CD28 by its ligands B7-1 or B7-2 on antigen presenting cells transduces activating signals to the T-cell. This promotes antigen-specific T-cell proliferation, enhances the production of IL-2 and other cytokines, and induces the differentiation of effector T-lymphocytes. CD28 ligation also induces *bcl-*X_L expression, which promotes T-cell survival.
- CTLA-4 (CD152) is homologous to CD28 and is not present on naïve T-cells, but it is rapidly upregulated upon activation. Signaling via B7-1 or B7-2 on antigen presenting cells through the homodimeric CTLA-4 on activated T-lymphocytes may deliver a signal that inhibits T-cell proliferation, IL-2 production, and IL-2 Receptor expression.
- The CD28 family member ICOS (Inducible Co-Stimulator, Activation-Inducible Lymphocyte Immunomediatory Molecule, AILIM) is expressed on the T-lymphocyte surface as a homodimer. It can costimulate T-cell growth, and induce IL-10 and IL-4 production. An increase in IL-10 production is usually associated with a reduced cellular and increased humoral immune response, and with anergy of antigen-specific T-cells. ICOS contains a YMFM motif, which binds PI 3-K, but not GRB-2.
- Homologs for B7 include B7-H1, B7-H2, and B7-H3. Most normal tissues, except for cells of the macrophage lineage, do not express B7-H1. In contrast, B7-H1 is abundant in carcinomata of lung, ovary, and colon, as well as in melanomata. The cancer cell-associated B7-H1 increases T-cell apoptosis [Dong et al. 2002], which could be an immune escape mechanism.

Costimulation engages a variety of receptors and occurs through the formation of receptor complexes surrounding the TCR/HLA complex (the formation of the immunologic synapse). This includes the

interactions of the Integrin LFA-1 (Leukocyte Function-Associated Antigen-1, CD11a/CD18, Integrin $\alpha_L\beta_2$) on T-lymphocytes with ICAM-1 on antigen presenting cells.

- Tumor attack by cytolytic T-lymphocytes and macrophages is mediated by the interaction of LFA-1 on the lymphocytes with ICAM-1 on the tumor surface. Thus, a low expression of ICAM-1 on tumor cells can contribute to their escape from host immune surveillance.
- In adenoid cystic carcinoma of the head and neck, patients with high expression levels of ICAM-1 have a significantly better disease-free survival rate than patients with low level expression. The reduced expression of ICAM-1 may promote immune evasion and metastasis, resulting in a poor prognosis [Shirai et al. 2003].

Adenocarcinomata often abundantly express and secrete underglycosylated MUC-1 mucin. The lack of sugars exposes tandem repeat peptide sequences, which are normally cryptic. High levels of MUC-1 are correlated with a poor prognosis and immunosuppression in these adenocarcinoma patients, because the cancer-associated MUC-1 can downregulate T-lymphocyte proliferative responses [Agrawal et al. 1998]. This occurs most likely through the engagement of suppressive T-lymphocyte surface receptors by MUC-1. This mechanism may underlie the phenomenon that lymphocytes present in the vicinity of tumor cells are often anergic. Affected tumors include breast cancer and colon cancer.

Activation-induced cell death. Certain stimuli can induce a transient expansion of T-lymphocytes followed by programmed cell death [Webb et al. 1990; Jones et al. 1990]. Thus, the stimulation of primed T-cells frequently leads to a burst of cytokine production, but is then followed by extensive apoptosis of the reactive cells.

Tumor cells induce T-lymphocyte apoptosis in a receptor-dependent fashion. Histologically disparate tumors aberrantly express elevated levels of various ligands of the TNF superfamily, including CD95L, TRAIL, and CD70. The expression of CD95L (FAS Ligand) on the surface of cancer cells may kill infiltrating cytolytic T-lymphocytes [O'Connell et al. 1996; Hahne et al. 1996; Strand et al. 1996]. Tumor-specific $CD4^+$ T-cells play a critical role in the augmentation of immune effector mechanisms. However, in the context of an extensive tumor burden, chronic stimulation of such $CD4^+$ T-cells often leads to the upregulation of both CD95 and CD95L [Tinhofer et al. 1998]. The coexpression of these molecules can potentially result in activation-induced cell death followed by the subsequent loss of effector activity.

Soluble factors may contribute to tumor-induced T-lymphocyte apoptosis. Many tumors exhibit enhanced synthesis of select gangliosides, some of which are shed into the tumor microenvironment. Renal cell carcinomata secrete gangliosides that sensitize T-lymphocytes to undergo activation-induced cell death [Finke et al. 2001].

The transmembrane growth inhibitory molecule RCAS-1 (Receptor-Binding Cancer Antigen Expressed on SiSo Cells, EB9, Estrogen Receptor Binding Site-Associated Antigen 9, EBAG9) is expressed at a high frequency on uterine and ovarian tumor cells, where it is correlated with poor prognosis. It also occurs on nongynecological cancers, including esophageal squamous cell carcinomata, gastric cancers, colon cancers, lung cancers, and pancreatic adenocarcinomata. RCAS-1 on tumor cells may act as a defense by tumor cells against cells of the immune system. It induces T-lymphocyte and NK cell apoptosis. In cervical cancer of the uterus, RCAS1 expression in lymph node metastases is higher than that in primary lesions, which may reflect a mechanism of immune evasion [Nakashima et al. 1999; Sonoda et al. 2005].

Renal cell carcinomata can induce several immune defects. Their infiltrating lymphocytes display poor proliferative and lytic capacities, leading to a global functional anergy. There is a potential role of inhibitory NK Receptors, KIRs (Killer Cell Immunoglobulin-Like Receptors), in the alteration of tumor-infiltrating lymphocyte (TIL) cytolytic function. About 5–40% of the lymphocytes that infiltrate renal cell carcinoma express KIRs that inhibit the anti-tumor $CD8^+$ T-cell functions and may contribute to local self-tolerance. TCR activation by cognate tumor cells induces apoptosis in KIR^+ Cytolytic T-Lymphocytes, but not in KIR^- Cytolytic T-Lymphocytes. The coengagement of T-Cell Antigen Receptors and KIRs by tumor cells decreases the tumor-mediated T-lymphocyte apoptosis, suggesting that tumor cells selectively favor the local persistence of nonfunctional KIR^+ Cytolytic

T-Lymphocytes by promoting their survival [Gati et al. 2003].

Ignorance. The rejection of cancer cells by cytolytic T-lymphocytes depends critically on whether immune responses are primed in the secondary lymphoid organs, such as the draining lymph nodes. This priming may occur through tumor cells entering the draining lymph nodes or through cross-presentation of tumor antigens by dendritic cells or bone marrow cells. In this regard, the tumor stroma provides a barrier from the induction of an immune response to low levels of antigen presentation. Frequently, T-lymphocytes from patients do not display reactivity to autologous cancer cells. However, cytolytic responses and Interferon-γ secretion can be generated by stimulation with dendritic cells that have processed autologous tumor cells. Therefore, the peripheral T-lymphocyte repertoire may contain cells that have the potential to recognize autologous tumor [Dhodapkar et al. 2002], but do not attack it. The insufficient presentation of tumor derived antigens to T-lymphocytes may lead to this phenomenon, referred to as ignorance. In this setting, T-lymphocytes that potentially recognize tumor-derived antigens are neither anergized nor deleted.

Other mechanisms. T-lymphocytes are sensitive to tryptophan availability. The activity of the tryptophan catabolizing enzyme Indoleamine 2,3-Dioxygenase (IDO) inhibits T-lymphocyte proliferation. Tumor cells frequently express IDO, thus depleting the local tryptophan pool and limiting T-lymphocyte-mediated antitumor immunity [Uyttenhove et al. 2003]. Furthermore, the tumor draining lymph nodes contain a subset of plasmacytoid dendritic cells that constitutively express immunosuppressive levels of IDO. Despite comprising only 0.5% of all lymph node cells, these dendritic cells potently suppress T-lymphocyte responses to antigens presented by the dendritic cells themselves and by nonsuppressive antigen presenting cells. This may create a local microenvironment that is potently suppressive of host antitumor T-lymphocyte responses [Munn et al. 2004].

Tumor infiltrating lymphocytes (TILs) may be dysfunctional. The molecular basis for this involves the loss of the ζ chain of the TCR [Mizoguchi et al. 1992]. TCRζ contributes to signal transduction by undergoing an ordered series of tyrosine phosphorylations in response to receptor engagement. In the absence of the ζ chain, the T-lymphocytes are prone to activation-induced cell death. In head and neck cancer patients with metastases, those who expresses normal levels of the TCRζ chain have a better prognosis than those who display ζ chain loss [Reichert et al. 2002; Kuss et al. 2003].

Splenic red pulp macrophages purge red blood cells at the end of their life spans. CD47 (Integrin-Associated Protein, IAP) functions as a marker of self on erythrocytes. It prevents the elimination of the intact red blood cells by splenic red pulp macrophages through binding to the phagocyte transmembrane glycoprotein SIRPα (SHPS-1) and inducing an inhibitory signal. Thus, the differentiation between self and foreign by splenic macrophages does not have to rely on activating receptors alone [Oldenborg et al. 2000].

Ovarian cancer cells express high levels of CD47 [Campbell et al. 1992; Poels et al. 1986]. Using an alternative mechanism, malignancies of myeloid origin downregulate SIRPα [Seiffert et al. 1999]. This may convey selective advantages to these malignancies by suppressing a host defense mechanism.

12.2.3 Susceptibility to Infections

One consequence of tumor-induced immune deviation may be the increased susceptibility to infections. About 80% of patients with acute leukemia, 75% of patients with lymphoma, and 50% of patients with multiple myeloma develop infections during the course of their disease, and infection is the proximate cause of death in a substantial fraction of these patients.

– Neutropenia is the most common predisposing factor for infections in cancer patients [Bodey 1986]. It may be caused by hematologic neoplasms or iatrogenically through anticancer therapy. The resulting infections are characterized by a profound lack of inflammatory host responses and may therefore lead to rapid dissemination. Most serious infections, including bacteremia, develop during severe and prolonged neutropenia, and occur in virtually every patient whose neutrophil count is less than 100/mL for 3 weeks or more. The most common sites of infection include the lungs, oropharynx, blood, urinary tract, skin, and soft tissues. Infections are generally caused by opportunistic organisms that normally colonize the afflicted organs.

– Inadequate neutrophil function may be associated with acute and chronic leukemia and with Hodgkin disease. Defects include the inability to migrate to sites of inflammation, impaired phagocytosis, and reduced killing of ingested bacteria. Abnormalities in neutrophil maturation and bactericidal activity can occur in patients with acquired immunodeficiency syndrome (AIDS)-associated tumors. The frequency of infection in acute leukemia is higher among patients whose neutrophils have reduced bactericidal capacity than among patients whose neutrophils function normally.
– Defects in the mononuclear phagocytic system arise in patients with monocytic leukemia. These patients are especially susceptible to infection with intracellular organisms. Chronic myelomonocytic leukemia (CMML) results from the uncontrolled proliferation of abnormal monocytes. It may lead to clinical symptoms that include splenomegaly, hepatomegaly, leukemia cutis, and serous effusions.
– Defects in the T-lymphocyte system result in increased susceptibility to infection. Cell-mediated immunity plays a primary role in protecting against intracellular pathogens. Patients with Hodgkin disease or with chronic or acute lymphocytic leukemia have impaired cell-mediated immunity.
– Hypo-gammaglobulinemia is present in 30–40% of patients with chronic lymphocytic leukemia. Infection occurs in nearly 90% of these patients compared with only 15% in patients with normal γ-Globulin levels. Patients with multiple myeloma and chronic lymphocytic leukemia are especially susceptible to infections caused by encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae* because specific opsonizing antibodies that play a major role in the defense against such pathogens are greatly reduced.

12.3 CANCER PREDISPOSITION THROUGH CHRONIC INFLAMMATION

Infections are likely responsible for 15% of malignancies world wide [Kuper et al. 2000]. Chronic states of inflammation may increase the risk of carcinogenesis by leading to tissue damage, which can include the induction of DNA mutations via elevated levels of reactive oxygen intermediates. The accumulation of these mutations increases the risk of transformation. The tissue reorganization caused by the inflammation may also interfere with the tumor-suppressive effects of the extracellular matrix.

Inflammatory responses to infectious agents are associated with EBV infections that cause lymphomata and head and neck cancers, cervical cancers caused by exposure to high risk HPV, liver cancer secondary to HBV infection, HHV-8 induced Kaposi sarcomata in AIDS patients, chronic gastritis leading to gastric cancer, possible influences by *Escherichia coli* on colon carcinogenesis, and infection with *Schistosoma hematobium* resulting in bladder cancer. Furthermore, the cancer risk is elevated in Barrett esophagus, chronic pancreatitis, primary sclerosing cholangitis, and ulcerative cholitis.

EBV infection is prevalent in over 90% of adults. Following the primary infection, an individual remains a lifelong carrier of the virus [Lopes et al. 2003]. The main reservoir of EBV are memory B-lymphocytes. In some cases, the latent infection with EBV may lead to cancers. Cell transformation by EBV occurs through targeting by EBNA-3C (EBV Nuclear Antigen-3C) of a molecule that normally regulates cell cycle progression. This ultimately initiates uncontrolled growth. The EBV induced tumors are characterized by the presence of multiple extrachromosomal copies of the circular viral genome, and by the expression of some EBV encoded latent genes, which contribute to the transformed phenotype.
– Undifferentiated nasopharyngeal carcinoma shows the most consistent world wide association with EBV. In areas of China and Southeast Asia, undifferentiated nasopharyngeal carcinoma is particularly common, reaching a peak incidence of around 20–30 cases per 100,000 inhabitants.
– Butkitt lymphoma has a high incidence in areas of equatorial Africa and Papua New Guinea. In these regions, *Plasmodium falciparum* malaria infection is holoendemic. Malaria plays a role as a cofactor in causing immunosuppression and promoting the proliferation of EBV transformed cells. Consistent genetic features are translocations involving the long arm of chromosome 8 {8q24} in the region of the *myc* proto-oncogene and either chromosome 14 in the region of the *immunoglobulin heavy chain* gene or, less frequently, chromosomes 2 or 22 in the region of the *immunoglobulin light chain* genes. These

translocations result in the deregulated expression of *myc*.
- In up to 50% of all Hodgkin lymphomata, EBV is present and may play a pathogenetic role. This is influenced by factors including the country of residence, histologic subtypes, sex, ethnicity, and age.
- Lymphoproliferative disease may occur in various states of immune deficiency, including X-linked lymphoproliferative syndrome, Wiscott-Aldrich syndrome, and AIDS. They are most often of B-lymphocyte origin and represent a family of lesions ranging from atypical polyclonal B-cell proliferations to aggressive monomorphic non-Hodgkin lymphomata. Most cases are positive for EBV and show a latency III pattern of gene expression.
- Children with AIDS and recipients of organ transplants have an unusually high incidence of smooth muscle tumors, comprising leiomyomata and leiomyosarcomata. EBV can infect smooth muscle cells in these immunocompromised hosts, and it may contribute to the pathogenesis of the leiomyomata and leiomyosarcomata. In contrast, EBV may not play a role in smooth muscle tumors of otherwise healthy children [McClain et al. 1995; Rogatsch et al. 2000; Brichard et al. 2001].

In EBV-mediated cancers, those viral latent proteins to which immunodominant cytolytic T-lymphocyte responses can be directed, are downregulated in overtly immunocompetent individuals. Thus, these cancer cells evade immune surveillance.

HPV is one of the most common sexually transmitted infectious agents. Women who have many sexual partners have a higher chance of developing an HPV infection in the cervix, and consecutively have increased odds to develop cervical cancer. The use of tobacco has also been associated with elevated susceptibility to HPV infection. Of more than 100 strains of HPV fewer than 20 are considered to convey a high risk of cancer development. The HPV high risk strains -16 and -18, but also -45 and -58 are major pathogenetic factors for the development of cervical cancer. The oncogenic HPV types can be present as episomes or may integrate into the host chromosomes. The integration of HPV DNA into the genome occurs early in cervical cancer development and is probably an important event in malignant transformation. While most infections with HPV remain without symptoms and a small fraction progresses to cervical cancer, some patients develop genital warts (condylomata acuminata, venereal warts, papillomata), which are benign growths. The warts are mostly caused by HPV-6 and -11.

Infection with hepatitis B virus may cause liver cancer. The route of transmission is through blood and sexual contact. In the initial period of infection, this virus infiltrates the body, causing acute hepatitis. About 5–10% of all patients with acute hepatitis progress to chronic disease, which may result in liver cirrhosis or liver cancer. There is a high rate of HBV infection world wide, with an incidence over 50 million new cases a year and a prevalence of over 350 million HBV surface antigen positive chronic carriers. The geographic variability in the rates of chronic HBV infection is substantial. In many high prevalence regions in Africa and Asia, most infected individuals acquire the infection perinatally or during early childhood, many of them develop immune tolerance to the virus, and have lifelong chronic infection. About 90% of infants infected during the first year of life and 30–50% of children infected between 1 and 4 years also develop chronic infection. By contrast, in low prevalence regions, most infections are acquired in young adulthood and lead to symptomatic acute hepatitis, followed by clearing of the virus. About 3–5% of these afflicted individuals will develop chronic hepatitis B and be predisposed to liver cancer.

The DNA virus Human Herpesvirus 8 (HHV-8, Kaposi-Associated Herpesvirus, KSHV) causes Kaposi sarcoma in hosts with a compromised immune system. This includes infections with HIV-1 and immunosuppression after organ transplantation. The HHV-8 latent proteins have the ability to induce cell growth or to block apoptosis, leading to uncontrolled expansion of the afflicted cells. The expression of the gene for vGPCR (viral G-Protein Coupled Receptor, ORF74) transforms endothelial cells through inducing high PKB activity as well as through inducing the production of large amounts of VEGF and its receptor VEGFR-2.

Gastric cancer can evolve from a transition of normal mucosa to gastritis, which eventually leads to adenocarcinoma. *Helicobacter pylori* is commonly present in the gastrointestinal tract. Its ability to induce superficial gastritis increases the risk of stomach neoplasia. Strains carrying the *cagA* gene are particularly virulent. The CagA protein recruits

the phosphatase SHP-2 to the plasma membrane and binds to it. SHP-2 alters cell migration and adhesion and may play a role in early carcinogenesis. Host factors also play a predisposing role. Polymorphisms of immune response genes are associated with gastric cancer. Individuals with polymorphisms in the promoter region of type I (T_H1) cytokines that are associated with high expression have an increased risk of developing gastric cancer consecutive to infection with *Helicobacter pylori*. The elevated cytokine levels increase the probability of hypochlorhydria, gastric atrophy, and consecutive distal gastric adenocarcinoma. The underlying polymorphisms include:

– *interleukin-1β* -31 C/C, -511 T/T
– *interleukin-1β receptor* penta-allelic 86 bp tandem repeat in intron 2
– *tnf-α* -308 A/A
– *interleukin-10* -592 ATA/ATA, -819 ATA/ATA, -1082 ATA/ATA (low expression polymorphism of a T_H2 cytokine).

Host possession of the HLA allele DQA1*0102 increases the risk of atrophic gastritis and intestinal-type gastric adenocarcinoma. Activating mutations of *cdh1*, the gene encoding E-Cadherin, are associated with familial diffuse-type gastric cancer.

The loss of certain antigens from tumor cells may cause a susceptibility to pathogens that promote tumor progression. Expression of the Sd^a (Cad, Sid, named after Sidney Smith) transplantation antigen is genetically polymorphic with autosomal dominant inheritance. Sd^a describes the group carbohydrate structure GalNAcβ1-4[NeuAcα2-3]Galβ1-4GlcNAc-R that is expressed on glycolipids and glycoproteins. The Sd^a antigen is present in the gastrointestinal mucosa where it serves as a marker for colonic cell differentiation [Montiel et al. 2003]. It is also present on erythrocytes, as well as on the surface of activated cytolytic T-lymphocytes, where it is largely associated with the extracellular domain of CD45, but it is not expressed on resting T-cells. Furthermore, the Sd^a structure is present in the kidney medulla and on the Tamm-Horsfall urinary glycoprotein. Members of the UDP-*N*-Acetyl-α-D-Galactosamine:Polypeptide *N*-Acetylgalactosaminyl Transferase (GalNAcT) family catalyze the initiation step of Mucin type *O*-linked protein glycosylation, in which *N*-acetylgalactosamine (GalNAc) is transferred to serine and threonine amino acid residues. The activity of the β1,4*N*-Acetylgalactosaminyl Transferase (Sd^a-GalNAcT, β4GalNT1), which transfers GalNAc to NeuAcα2-3Galβ1-4Glc(NAc)-R, correlates with the expression of the Sd^a immunoepitope. While *Sd^a-galNact* mRNA is expressed in normal stomach and small intestine, it is absent from a fraction of gastric and colonic cancers due reduced transcription [Malagolini et al. 1989; Dohi et al. 1996]. This causes a dramatic decrease in the expression of the Sd^a determinant in these cancers. Sd^a on the surface of gastrointestinal epithelial cells may protect them from the adhesion of *Escherichia coli*, suggesting a potential role for these bacteria in tumor promotion.

Ulcerative colitis and Crohn disease are chronic inflammatory diseases of the rectum and colon. They are associated with increased risk for the development of colorectal cancer. The chronic inflammation caused by ulcerative colitis stimulates epithelial cyst formation and progression to tumors [Farrell and Peppercorn 2002]. Chronic inflammatory processes induce oxidative stress and lipid peroxidation, hereby generating DNA reactive aldehydes such as trans-4-hydroxy-2-nonenal (HNE). The abundance of 4-hydroxy-2-nonenal leads to an increase in etheno-DNA adducts in the colonic mucosa. The DNA damage may predispose to transformation [Nair et al. 2006]. Fatty Acid Synthase is an enzyme that catalyzes the synthesis of long chain fatty acids. The enzyme expression is minimal in healthy adult tissues, but it is very high in many cancers. Fatty acid synthase is overexpressed in ulcerative colitis [Consolazio et al. 2006].

Schistosoma haematobium may increase the risk of bladder cancer because the incidence of this malignancy is high in endemic areas [Gelfand et al. 1967; Tawfik 1988]. Most cancers that are likely attributable to *Schistosoma haematobium* infection are squamous cell carcinomata, while transitional cell carcinomata are less frequent.

12.4 TUMOR PROGRESSION THROUGH THE RECRUITMENT OF IMMUNE SYSTEM CELLS

Mast cell secretions are integral components of wound healing and tissue repair. They secrete various mediators of inflammation and thus orchestrate host defense mechanisms.

– In addition to their role in inflammation, mast cells provide mitogens for fibroblasts, endothelial cells, and nerve cells to enhance tissue

remodeling. Through this function, they are potential epigenetic contributors to the growth of cancer cells.
– Through their roles in tissue remodeling, mast cells may facilitate tumor dissemination and angiogenesis.

The infiltration of squamous cell carcinomata by mast cells and the activation of MMP-9 coincide with the activation of angiogenesis in premalignant lesions. Mast cells infiltrate hyperplasias, dysplasias, and invasive fronts of carcinomata, but not the core of solid tumors. There, they degranulate in close apposition to capillaries and epithelial basement membranes, releasing the mast cell specific serine proteases MCP-4 (Chymase) and MCP-6 (Tryptase). Neoplastic progression in this setting involves the exploitation of an inflammatory response to tissue abnormality [Coussens et al. 1999].

Tumor infiltrating macrophages participate in a host driven stress response. Inflammatory cytokines secreted by macrophages can exert trophic effects on the surrounding tumor cells [Mantovani et al. 1992]. They may secrete soluble mediators that are also used by the tumor cells to disseminate. Tumor-associated Osteopontin is often localized to infiltrating macrophages [Brown et al. 1994]. Furthermore, tumor infiltrating macrophages have high amounts of Cathepsin D in advanced endometrial adenocarcinomata, transitional cell carcinomata of the bladder, and gastric carcinomata [Sherbet and Lakshmi 1997]. Macrophages are recruited through the local expression of chemoattractants such as CSF-1 (Colony Stimulating Factor-1) and MCP-1 (Macrophage Chemoattractant Protein-1). They activate NF-κB and the expression of trophic cytokines by the macrophages. The overexpression of both of these chemoattractants is correlated with a poor prognosis in a variety of tumors.

REFERENCES

Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. 1998. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nature Medicine 4:43–49.

Aguilar LK, Rooney CM, Heslop HE. 1999. Lymphoproliferative disorders involving Epstein-Barr virus after hemopoietic stem cell transplantation. Current Opinions in Oncology 11:96–101.

Beneduce L, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnu L, Calabrese F, Gatta A, Pontisso P, Fassina G. 2005. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. Cancer 103:2558–2565.

Bodey GP. 1986. Infections in cancer patients: a continuing association. American Journal of Medicine 81(Suppl 1A):11–26.

Brichard B, Smets F, Sokal E, Clapuyt P, Vermylen C, Cornu G, Rahier J, Otte JB. 2001. Unusual evolution of an Epstein-Barr virus-associated leiomyosarcoma occurring after liver transplantation. Pediatric Transplantation 5:365–369.

Brown LF, Papadopoulos-Sergiou A, Berse B, Manseau EJ, Tognazzi K, Perruzzi CA, Dvorak HF, Senger DR. 1994. Osteopontin expression and distribution in human carcinomas. American Journal of Pathology 145:610–623.

Burnet FM. 1970. The concept of immunological surveillance. Progress in Experimental Tumor Research 13:1–27.

Burnet FM. 1971. Immunological surveillance in neoplasia. Transplantation Reviews 7:3–25.

Campbell IG, Freemont PS, Foulkes W, Trowsdale J. 1992. An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. Cancer Research 52:5416–5420.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994. Identification of herpes-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869.

Chomez P, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. 2001. An overview of the *MAGE* gene family with the identification of all human members of the family. Cancer Research 61:5544–5551.

Consolazio A, Al Ograve PL, Rivera M, Iacopini F, Paoluzi OA, Crispino P, Pica R, Paoluzi P. 2006. Overexpression of Fatty Acid synthase in ulcerative colitis. American Journal of Clinical Pathology 126:1–6.

Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D. 1999. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes and Development 13:1382–1397.

Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ, Smyth MJ. 2002. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. The Journal of Immunology 168:1356–1361.

Davidoff AM, Iglehart D, Marks JR. 1992. Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancer. Proceedings of the National Academy of Sciences USA 89:3439–3442.

Dhodapkar MV, Krasovsky J, Olson K. 2002. T cells from the tumor microenvironment of patients with progressive myeloma can generate strong, tumor-specific cytolytic responses to autologous, tumor-loaded dendritic cells. Proceedings of the National Academy of Sciences USA 99:13009–13013.

Dighe AS, Richards E, Old LJ, Schreiber RD. 1994. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN γ receptors. Immunity 1:447–456.

Dohi T, Yuyama Y, Natori Y, Smith PL, Lowe JB, Oshima M. 1996. Detection of *N*-acetylgalactosaminyltransferase mRNA which determines expression of Sda blood group carbohydrate structure in human gastrointestinal mucosa and cancer. International Journal of Cancer 67:626–631.

Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. 2002. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nature Medicine 8:793–800.

Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC. 1993.

Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proceedings of the National Academy of Sciences USA 90:3539–3543.

Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. 2002. Cancer immunoediting: from immunosurveillance to tumor escape. Nature Immunology 3:991–998.

Dunn GP, Old LJ, Schreiber RD. 2004. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21:137–148.

Efrati P, Jonas W. 1958. Chediak's anomaly of leukocytes in malignant lymphoma associated with leukemic manifestations: case report with necropsy. Blood 13:1063–1073.

Elasser-Beile U, Gierschner D, Welchner T, Wetterauer U. 2003. Different expression of Fas and Fas ligand in tumor infiltrating and peripheral lymphocytes of patients with renal cell carcinomas. Anticancer Research 23:433–437.

Farrell RJ, Peppercorn MA. 2002. Ulcerative colitis. The Lancet 359:331–340.

Fauriat C, Marcenaro E, Sivori S, Rey J, Gastaut JA, Moretta A, Olive D, Costello RT. 2003. Natural killer cell-triggering receptors in patients with acute leukaemia. Leukemia and Lymphoma 44:1683–1689.

Finke JH, Rayman P, George R, Tannenbaum CS, Kolenko V, Uzzo R, Novick AC, Bukowski RM. 2001. Tumor-induced sensitivity to apoptosis in T cells from patients with renal cell carcinoma: role of nuclear factor-κB suppression. Clinical Cancer Research 7:940S–946S.

Frish M, Biggar RJ, Engels EA, Goedert JJ. 2001. Association of cancer with AIDS-related immunosuppression in adults. Journal of the American Medical Association 285:1736–1745.

Fuster MM, Brown JR, Wang L, Esko JD. 2003. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. Cancer Research 63:2775–2781.

Gallagher B, Wang Z, Schymura MJ, Kahn A, Fordyce EJ. 2001. Cancer incidence in New York State acquired immunodeficiency syndrome patients. American Journal of Epidemiology 154:544–556.

Gao Y, Yang W, Pan M, Scully E, Girardi M, Augenlicht LH, Craft J, Yin Z. 2003. γδ T cells provide an early source of interferon γ in tumor immunity. The Journal of Experimental Medicine 198:433–442.

Gati A, Guerra N, Gaudin C, Da Rocha S, Escudier B, Lecluse Y, Bettaieb A, Chouaib S, Caignard A. 2003. CD158 receptor controls cytotoxic T-lymphocyte susceptibility to tumor-mediated activation-induced cell death by interfering with Fas signaling. Cancer Research 63:7475–7482.

Gelfand M, Weinberg RW, Castle WM. 1967. Relation between carcinoma of the bladder and infestation with *Schistosoma haematobium*. The Lancet 1:1249–1251.

Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, Hobby P, Sutton B, Tigelaar RE, Hayday AC. 2001. Regulation of cutaneous malignancy by γδ T cells. Science 294:605–609.

Gonzalez-Perez MP, Munoz-Juarez L, Cardenas FC, Zarranz Imirizaldu JJ, Carranceja JC, Garcia-Saiz A. 2003. Human T-cell leukemia virus type I infection in various recipients of transplants from the same donor. Transplantation 75:1006–1011.

Grabstein KH, Urdal DL, Tushinski RJ, Mochizuki DY, Price VL, Cantrell MA, Gillis S, Conlon PJ. 1986. Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. Science 232:506–508.

Groh V, Wu J, Yee C, Spies T. 2002. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 419:734–738.

Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Schneider P, Bornand T, Fontana A, Lienard D, Cerottini J, Tschopp J. 1996. Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape. Science 274:1363–1366.

Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. 1992. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature 356:607–609.

Herberman RB. 1995. Cellular immunity. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 511–533.

Hoover RN. 1992. Lymphoma risks in populations with altered immunity–a search for mechanism. Cancer Research 52:5477S–5478S.

Hoshida Y, Aozasa K. 2004. Malignancies in organ transplant recipients. Pathology International 54:649–658.

Ioannides CG, Fisk B, Pollack MS, Frazier ML, Wharton JT, Freedman RS. 1993. Cytotoxic T-cell clones isolated from ovarian tumour infiltrating lymphocytes recognizes common determinants on nonovarian tumour clones. Scandinavian Journal of Immunology 37:413–424.

Jerry LM, Rowden G, Cano PO, Phillips TM, Deutsch GF, Capek A, Hartmann D, Lewis MG. 1976. Immune complexes in human melanoma: a consequence of deranged immune regulation. Scandinavian Journal of Immunology 5:845–859.

Jones LA, Chin LT, Longo DL, Kruisbeek AM. 1990. Peripheral clonal elimination of functional T cells. Science 250:1726–1229.

Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD. 1998. Demonstration of an interferon γ-dependent tumor surveillance system in immunocompetent mice. Proceedings of the National Academy of Sciences USA 95:7556–7561.

Karre K, Ljunggren HG, Piontek G, Kiessling R. 1986. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. Nature 319:675–678.

Kato H, Torigoe T. 1977. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer 40:1621–1628.

Kayagaki N, Yamaguchi N, Nakayama M, Kawasaki A, Akiba H, Okumura K, Yagita H. 1999. Involvement of TNF-related apoptosis-inducing ligand in human $CD4^+$ T cell-mediated cytotoxicity. The Journal of Immunology 162:2639–2647.

Kinlen LJ. 1992. Immunosuppression and cancer. In: Vainio H, Magee PN, McGregor DB, McMichael AJ (eds.). Mechanisms of carcinogenesis in risk identification. Lyon: International Agency for Research on Cancer, pp. 237–253.

Kuper H, Adami HO, Trichopoulos D. 2000. Infections as a major preventable cause of human cancer. Journal of Internal Medicine 248:171–183.

Kuss I, Donnenberg AD, Gooding W, Whiteside TL. 2003. Effector $CD8^+$ $CD45RO^-$ $CD27^-$T cells have signalling defects in patients with squamous cell carcinoma of the head and neck. British Journal of Cancer 88:223–230.

Le Beau MM, Westbrook CA, Diaz MO, Larson RA, Rowley JD, Gasson JC, Golde DW, Sherr CJ. 1986. Evidence for the

involvement of GM-CSF and FMS in the deletion (5q) in myeloid disorders. Science 231:984–987.

Lehmann PV, Sercarz EE, Forsthuber T, Dayan CM, Gammon G. 1993. Determinant spreading and the dynamics of the autoimmune T-cell repertoire. Immunology Today 14:203–208.

Long EO. 2002. Tumor cell recognition by natural killer cells. Cancer Biology 12:57–61.

Lopes V, Young LS, Murray PG. 2003. Epstein-Barr virus-associated cancers: aetiology and treatment. Herpes 10:78–82.

Malagolini N, Dall'Olio F, Di Stefano G, Minni F, Marrano D, Serafini-Cessi F. 1989. Expression of UDP-GalNAc:NeuAc α 2,3Gal β-R β 1,4(GalNAc to Gal) N-acetylgalactosaminyltransferase involved in the synthesis of Sda antigen in human large intestine and colorectal carcinomas. Cancer Research 49:6466–6470.

Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. 1992. The origin and function of tumor-associated macrophages. Immunology Today 13:265–270.

McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Hutchison RE, Murphy SB. 2000. Molecular and virologic characteristics of lymphoid malignancies in children with AIDS. Journal of Acquired Immune Deficiency Syndromes 23:152–159.

McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Parmley RT, DiCarlo FJ, Chadwick EG, Murphy SB. 1995. Association of Epstein-Barr virus with leiomyosarcomas in children with AIDS. New England Journal of Medicine 332:12–18.

Mendelsohn J, Gabrilove J. 1995. Growth factors in malignancy. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 432–459.

Mizoguchi H, O'Shea JJ, Longo DL, Loeffler CM, McVicar DW, Ochoa AC. 1992. Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. Science 258:1795–1798.

Monach PA, Meredith SC, Siegel CT, Schreiber H. 1995. A unique tumor antigen produced by a single amino acid substitution. Immunity 2:45–59.

Montiel MD, Krzewinski-Recchi MA, Delannoy P, Harduin-Lepers A. 2003. Molecular cloning, gene organization and expression of the human UDP-GalNAc:Neu5Acα2–3Galβ-R β1,4-*N*-acetylgalactosaminyltransferase responsible for the biosynthesis of the blood group Sd^a/Cad antigen: evidence for an unusual extended cytoplasmic domain. Biochemical Journal 373:369–379.

Moreau-Aubry A, Le Guiner S, Labarriere N, Gesnel MC, Jotereau F, Breathnach R. 2000. A processed pseudogene codes for a new antigen recognized by a $CD8^+$ T cell clone on melanoma. The Journal of Experimental Medicine 191:1617–1624.

Munn DH, Sharma MD, Hou D, Baban B, Lee JR, Antonia SJ, Messina JL, Chandler P, Koni PA, Mellor AL. 2004. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. Journal of Clinical Investigation 114:280–290.

Nair J, Gansauge F, Beger H, Dolara P, Winde G, Bartsch H. 2006. Increased etheno-DNA adducts in affected tissues of patients suffering from Crohn's disease, ulcerative colitis, and chronic pancreatitis. Antioxididants and Redox Signaling 8:1003–1010.

Nakashima M, Sonoda K, Watanabe T. 1999. Inhibition of cell growth and induction of apoptotic cell death by the human tumor-associated antigen RCAS1. Nature Medicine 5:938–942.

O'Connell J, O'Sullivan GC, Collins JK, Shanahan F. 1996. The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. The Journal of Experimental Medicine 184:1075–1082.

Okano M, Mizuno F, Osato T, Takahashi Y, Sakiyama Y, Matsumoto S. 1984. Wiskott-Aldrich syndrome and Epstein-Barr virus-induced lymphoproliferation. The Lancet 2:933–934.

Oldenborg P-A, Zheleznyak A, Fang Y-F, Lagenaur CF, Gresham HD, Lindberg FP. 2000. Role of CD47 as a marker of self on red blood cells. Science 288:2051–2054.

Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. 1999. Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. Cancer Research 59:3128–3133.

Otley CC, Pittelkow MR. 2000. Skin cancer in liver transplant recipients. Liver Transplantation 6:253–262.

Pende D, Parolini S, Pessino A, Sivori S, Augugliaro R, Morelli L, Marcenaro E, Accame L, Malaspina A, Biassoni R, Bottino C, Moretta L, Moretta A. 1999. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. The Journal of Experimental Medicine 190:1505–1516.

Petry KU, Scheffel D, Bode U, Gabrysiak T, Kochel H, Kupsch E, Glaubitz M, Niesert S, Kuhnle H, Schedel I. 1994. Cellular immunodeficiency enhances the progression of human papillomavirus-associated cervical lesions. International Journal for Cancer 57:836–840.

Poels LG, Peters D, van Megen Y, Vooijs GP, Verheyen RN, Willemen A, van Niekerk CC, Jap PH, Mungyer G, Kenemans P. 1986. Monoclonal antibody against human ovarian tumor-associated antigens. Journal of the National Cancer Institute 76:781–791.

Reichert TE, Strauss L, Wagner EM, Gooding W, Whiteside TL. 2002. Signaling abnormalities, apoptosis, and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. Clinical Cancer Research 8:3137–3145.

Rogatsch H, Bonatti H, Menet A, Larcher C, Feichtinger H, Dirnhofer S. 2000. Epstein-Barr virus-associated multicentric leiomyosarcoma in an adult patient after heart transplantation: case report and review of the literature. American Journal of Surgical Pathology 24:614–621.

Saemundsen AK, Berkel AI, Henle W, Henle G, Anvret M, Sanal O, Ersoy F, Caglar M, Klein G. 1981. Epstein-Barr-virus-carrying lymphoma in a patient with ataxia-telangiectasia. British Medical Journal (Clinical Research Edition) 282:425–427.

Salih HR, Rammensee H-G, Steinle A. 2002. Down-regulation of MICA on human tumors by proteolytic shedding. The Journal of Immunology 169:4098–4102.

Salzer U, Chapel HM, Webster ADB, Pan-Hammarstrom Q, Schmitt-Graeff A, Schlesier M, Peter HH, Rockstroh JK, Schneider P, Schaffer AA, Hammarstrom L, Grimbacher B. 2005. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nature Genetics 37:820–828.

Schwartz RH. 1990. A cell culture model for T lymphocyte clonal anergy. Science 248:1349–1356.

Seiffert M, Cant C, Chen Z, Rappold I, Brugger W, Kanz L, Brown EJ, Ullrich A, Buhring HJ. 1999. Human signal-regulatory protein is expressed on normal, but not on subsets

of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 94:3633–3643.

Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. 2001. IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 410:1107–1111.

Sherbet GV, Lakshmi MS. 1997. The Genetics of cancer: genes associated with cancer invasion, metastasis and cell proliferation. San Diego/London/Boston/New York/Sydney/Tokyo/Toronto: Academic Press.

Shimizu J, Yamazaki S, Sakaguchi S. 1999. Induction of tumor immunity by removing $CD25^+$ $CD4^+$ T cells: a common basis between tumor immunity and autoimmunity. The Journal of Immunology 163:5211–5218.

Shirai A, Furukawa M, Yoshizaki T. 2003. Expression of intercellular adhesion molecule (ICAM)-1 in adenoid cystic carcinoma of the head and neck. Laryngoscope 113:1955–1960.

Smyth MJ, Crowe NY, Godfrey DI. 2001. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. International Immunology 13:459–463.

Sonoda K, Miyamoto S, Hirakawa T, Yagi H, Yotsumoto F, Nakashima M, Watanabe T, Nakano H. 2005. Association between RCAS1 expression and microenvironmental immune cell death in uterine cervical cancer. Gynecologic Oncology 97:772–779.

Speiser DE, Ohashi PS. 1998. Activation of cytotoxic T cells by solid tumours? Cellular and Molecular Life Sciences 54:263–271.

Spritz RA. 1999. Multi-organellar disorders of pigmentation: tied up in traffic. Clinical Genetics 55:309–317.

Steinman RM, Cohn ZA. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. The Journal of Experimental Medicine 137:1142–1162.

Stewart T, Tsai SC, Grayson H, Henderson R, Opelz G. 1995. Incidence of *de novo* breast cancer in women chronically immunosuppressed after organ transplantation. The Lancet 346:796–798.

Strand S, Hofmann WJ, Hug H, Muller M, Otto G, Strand D, Mariani SM, Stremmel W, Krammer PH, Galle PR. 1996. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells–a mechanism of immune evasion? Nature Medicine 2:1361–1366.

Stutman O. 1975. Immunodepression and malignancy. Advances in Cancer Research 22:261–422.

Takeda K, Smyth MJ, Cretney E, Hayakawa Y, Kayagaki N, Yagita H, Okumura K. 2002. Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. The Journal of Experimental Medicine 195:161–169.

Tawfik H. 1988. Carcinoma of the urinary bladder associated with schistosomiasis in Egypt: the possible causal relationship. In: Miller R, Watanabe S, Fraumeni JJF, Sugimura T, Takamaya S, Sugano H. (eds). Unusual occurrences as clues to cancer etiology. Tokyo: Japan Scientific Societies Press, pp. 197–209.

Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, Carbone DP, Paul WE, Berzofsky JA. 2000. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL4R-STAT6 pathway. Nature Immunology 1:515–520.

Thomas WD, Hersey P. 1998. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. The Journal of Immunology 161:2195–2200.

Tinhofer I, Marschitz I, Kos M, Henn T, Egle A, Villunger A, Greil R. 1998. Differential sensitivity of $CD4^+$ and $CD8^+$ T lymphocytes to the killing efficacy of Fas (Apo-1/CD95) ligand$^+$ tumor cells in B chronic lymphocytic leukemia. Blood 91:4273–4281.

Underwood JC. 1974. Lymphoreticular-cell infiltration in human tumors: prognostic and histological implications. A review. British Journal of Cancer 30:538–548.

Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, Boon T, Van den Eynde BJ. 2003. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nature Medicine 9:1269–1274.

Vajdic CM, McDonald SP, McCredie MRE, van Leeuwen MT, Stewart JH, Law M, Chapman JR, Webster AC, Kaldor JM, Grulich AE. 2006. Cancer incidence before and after kidney transplantation. The Journal of the American Medical Association 296:2823–2831.

van den Broek ME, Kagi D, Ossendorp F, Toes R, Vamvakas S, Lutz WK, Melief CJ, Zinkernagel RM, Hengartner H. 1996. Decreased tumor surveillance in perforin-deficient mice. The Journal of Experimental Medicine 184:1781–1790.

Van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. 1991. A gene encoding an antigen recognized by cytolytic T lymphocytes on human melanoma. Science 254:1643–1647.

Vicari AP, Caux C, Trinchieri G. 2002. Tumor escape from immune surveillance through dendritic cell inactivation. Cancer Biology 12:33–42.

Wang R-F. 1999. Human tumor antigens: implications for cancer vaccine development. The Journal of Molecular Medicine 77:640–655.

Wang R-F, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA. 1996. Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. The Journal of Experimental Medicine 183:1131–1140.

Wang R-F, Wang X, Atwood AC, Topalian SL, Rosenberg SA. 1999. Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen. Science 284:1351–1354.

Wang Z, Margulies L, Hicklin DJ, Ferrone S. 1996. Molecular and functional phenotypes of melanoma cells with abnormalities in HLA class I antigen expression. Tissue Antigens 47:382–390.

Webb S, Morris C, Sprent J. 1990. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell 63:1249–1256.

Wheat WH, Cool CD, Morimoto Y, Rai PR, Kirkpatrick CH, Lindenbaum BA, Bates CA, Ellison MC, Serls AE, Brown KK, Routes JM. 2005. Possible role of human herpesvirus 8 in the lymphoproliferative disorders in common variable immunodeficiency. The Journal of Experimental Medicine 202:479–484.

Whiteside TL, Herberman RB. 1995. The role of natural killer cells in immune surveillance of cancer. Current Opinions in Immunology 7:704–710.

Wortzel RD, Urban JL, Phillipps C, Fitch FW, Schreiber H. 1983. Independent immunodominant and immunorecessive

tumor-specific antigens on a malignant tumor: antigenic dissection with cytolytic T cell clones. The Journal of Immunology 130:2461–2466.

Yang XF, Wu CJ, Chen L, Alyea EP, Canning C, Kantoff P, Soiffer RJ, Dranoff G, Ritz J. 2002. CML28 is a broadly immunogenic antigen, which is overexpressed in tumor cells. Cancer Research 62:5517–5522.

Zinkernagel R. 2000. Localization dose and time of antigens determine immune reactivity. Seminars in Immunology 12:163–171.

CHAPTER 13

ENDOCRINE DYSREGULATION

Clinical symptoms of malignancy typically occur late in disease and are frequently characterized by pain resulting from tissue destruction. The generic symptoms of cachexia or fatigue are also common. In specific cases, however, cancer may present with more specific disease manifestations. The proliferation of cells with particular differentiation may lead to an overabundance of factors secreted by them. If such factors have hormone-like properties they may cause symptoms. Conversely, tissue destruction by cancer growth may damage hormone-producing cells causing endocrine deficiency or imbalance. Clinically, tumor-induced endocrine dysregulations present as paraneoplastic syndromes (Table 13.A). Approximately 15% of hospitalized cancer patients have a paraneoplastic syndrome. The risk that a patient with cancer will develop a paraneoplastic syndrome is 50–75%.

The range of soluble mediators that can be dysregulated in neoplastic diseases is wide. Tumors may secrete substances like

– Carcinoembryonic Antigen (CEA) in cancers of the gastrointestinal tract, the pancreas, the breasts, or the lungs
– α-Fetoprotein (AFP) in hepatoma or embryonal carcinoma of the testes
– Regan Enzyme (Placental-Type Alkaline Phosphatase) in gonadal and urologic cancers
– Chorionic Gonadotropin by hydatidiform mole (gestational trophoblastic disease), choriocarcinoma, or testicular cancer
– Acid Phosphatase in prostate carcinoma
– Vitamin B_{12}-Binding Protein by acute and chronic myelogenous leukemias
– Lysozyme in monocytic leukemia

Endothelial cells may produce growth factors, including FGF, PDGF, IGF-I, G-CSF, and EGF, which can stimulate tumor cells in a paracrine fashion [Folkman 1995].

13.1 HORMONE-DEPENDENT MALIGNANCIES

Various tumors, including breast cancer, prostate cancer, and liver cancer, depend on the influence of growth stimulating hormones.

Estrogen is the primary growth factor for breast epithelial cells. Most breast cancers depend on estrogen signaling for growth promotion, although in a fraction of cancers estrogen signaling is mimicked by ligand-independent activation of the Estrogen Receptor. In Estrogen Receptor expressing breast cancer cells, the expression and secretion of autocrine growth factors, including TGF-α and IGF-2, are stimulated by estrogens and suppressed by antiestrogens.

Steroid hormones have important roles in prostate biology. Androgens are crucial for the normal development of the prostate gland, and in maintaining its functional state in the adult. Androgens and estrogens both play some part in the development of benign prostatic hyperplasia, which is common in old age. The prolonged presence of androgens, which act as growth factors on prostate cells, is an important component in the development of prostate cancer. Androgen ablation slows the progression of prostate cancer because of testosterone reduction. However, eventually

Table 13.A. Paraneoplastic syndromes. Cancers may cause systemic clinical symptoms through diverse mechanisms. The proliferation of cells with particular differentiation may lead to an overabundance of factors secreted by them. If such factors have hormone-like properties they may cause disease. Conversely, tissue destruction by cancer growth may damage hormone-producing cells causing endocrine deficiency or imbalance

Syndrome	Molecular defect	Cancers
Cushing syndrome	ACTH	Lung cancer (small cell or oat cell), thymoma, pancreas cancer, thyroid cancer, neurologic tumors
Thyroid dysregulation		
Hyperthyroidism	TSH	Choriocarcinoma, testicular carcinoma
Hyperparathyriodism	PTH	Kidney cancer, squamous lung cancer, pancreas cancer, ovarian cancer
Altered glucose metabolism		
Doege-Potter hypoglycemia		Fibrosarcoma
Nadler-Wolfer hypoglycemia		Liver carcinoma
Anderson hypoglycemia		Adrenal tumors
Rosenfeld hypoglycemia		Pseudomyxoma
Neuro- and myelopathies		
Central cerebral		
Progressive multifocal leukoencephalopathy		Lymphoproliferative disorders, myeloproliferative disorders
Limbic encephalitis	anti-MA2 antibodies	Seminoma, bronchus cancer
Central cerebellar		
Degeneration of the cerebellar cortex		Bronchus cancer, ovarian cancer, mammary cancer
Subacute cerebellar degeneration		Bronchus cancer, ovarian cancer, mammary cancer, lymphoma
Cerebellar opsoclonus-myoclonus (children)	Antibodies to CNS antigens	Neuroblastoma
Cerebellar opsoclonus-myoclonus (adult)	anti-NOVA Antibodies breast cancer (RI syndrome)	Small cell lung cancer
CV2 syndrome	Antibodies to cytoplasmic oligodendrocyte protein	Small cell lung cancer, thymoma
YO syndrome	Anti-YO antibodies	Ovarian cancer, mammary cancer
TR syndrome	Antibodies to Purkinje cells	Hodgkin lymphoma
Stiff man syndrome	Antibody to amphiphysin	Mammary cancer, lung cancer, thymoma
Amyotrophic lateral sclerosis		Bronchus cancer, mammary cancer
Peripheral		
Sensory neuropathy	anti-HU Antibodies	Bronchus cancer, neuroblastoma
Sensorimotoric neuropathy		Plasmocytoma, Hodgkin lymphoma, bronchus cancer
Autonomic neuropathy	Antibodies to α3 subunit of the Acetylcholine Receptor	
Myasthenia gravis	Antibodies to nicotinic Acetylcholine receptor Antistriational antibodies	Thymoma
Lambert-Eaton myasthenia	Antibodies to P-type (α1A) Calcium Channel	Small cell lung cancer
Isaac neuromyotonia	Antibodies to potassium channel	Thymoma
Myopathies		
Subacute necrotizing myelopathy		Bronchus cancer, ovarian cancer
Dermatomyositis		Ovarian cancer, nasopharyngeal cancer
Rippling muscle syndrome		thymoma
Visual loss syndromes		
Cancer associated retinopathy	Anti-Recoverin antibodies	Renal cell cancer, breast cancer, small cell lung cancer

(continued)

Table 13.A. (continued)

Syndrome	Molecular defect	Cancers
Acquired night blindness	Antibodies to bipolar retinal neurons	Melanoma
Bilateral diffuse uveal melanotic proliferation		Ovarian cancer, gastrointestinal cancer, non-small cell lung adenocarcinoma
Optic neuropathy		Non-small cell lung cancer, multiple myeloma
Blood disorders		
Aplastic anemia		Thymoma
Erythrocytosis	Erythropoietin	Renal cancer, liver cancer, uterine cancer, cerebellar hemangioma
Multiple thromboses		Pancreatic cancer, bronchus cancer, stomach cancer
Disseminated intravascular coagulation		Mucinous adenocarcinomas
Hemolytic anemia		Leukemias, Hodgkin lymphoma
Hypertension	Renin	Wilms tumor, renal cancer
Raynaud vasospasm	Cryoproteinemia	Multiple myeloma
Trousseau migratory		Lung cancer, pancreatic cancer
Venous thrombophlebitis		
Skin alterations		
Dermal depositions		
Amyloidosis	Amyloid	Multiple myeloma
Reactive erythemata		
Urticaria		Lung cancer
Erythema gyratum repens		Lung cancer, mammary cancer, stomach cancer, esophageal cancer
Necrolytic erythema migrans erythema annulare centrifugum	Glucagon	Glucagonoma
Neutrophilic dermatoses		
Pyoderma gangrenosum		AML, multiple myeloma
Sweet syndrome		AML, genitourinary cancers, mammary cancer, gastrointestinal neoplasias
Inflammation		
Dermatitis herpetiformis		
Exfoliative dermatitis		Cutaneous T-cell lymphoma
Dermatomyositis		Mammary cancer, stomach cancer
Polyarteriitis nodosa		Hairy cell leukemia
Leukocytoclastic vasculitis		Hairy cell leukemia
Papulosquamous disorders		
Acanthosis nigricans maligna		Stomach cancer
Pityriasis rotunda		Hepatocellular cancer, gastric cancer
Florid cutaneous papillomatosis		Gastric cancer
Others		
Porphyria cutanea tarda		Hepatoma
Nodular fat necrosis	Lipases	Pancreas carcinoma
Acrokeratosis		Tongue carcinoma, tonsil carcinoma
Pemphigus vulgaris		Lymphoma
Electrolyte imbalance		
Hypercalcemia	PTHrP	Breast cancer, prostate cancer
	prostaglandin E	Breast cancer, hepatocellular carcinoma, renal tumors, fibrosarcoma
Hypocalcemia	Calcitonin	Lung cancer, renal cancer, breast cancer
Hypokalemia	Renin	Renal cancers, Wilms tumor, lung cancer
Hyponatriemia	ADH	Bronchus cancer, pancreas cancer, thymoma, lymphosarcoma
Hyperuricemia		Leukemia, lymphoma

(continued)

Table 13.A. (continued)

Syndrome	Molecular defect	Cancers
Others		
Carcinoid paraneoplasia	serotonin	Bronchus cancer, intestinal carcinoid
Retinopathy		Small cell lung cancer
Digital clubbing		Lung cancer
Gynecomastia	HCG	Oat cell carcinoma of lung, liver tumors
Diarrhea	Somatostatin	Pancreatic islet cell tumor
Zollinger-Ellison syndrome	Astrin	Pancreatic islet cell tumor
Conn syndrome	Aldosterone	Adrenal carcinoma
Adrenogenital syndrome	Androgen	Adrenal carcinoma

prostate cancer progresses, as a result of acquiring an androgen insensitive state.

The exposure to androgenic steroids can lead to liver tumors [Johnson et al. 1972]. Depending on the type of androgen, they may manifest as hepatic adenomata or as hepatocellular carcinomata. Patients with Fanconi anemia are more susceptible to this mode of carcinogenesis than healthy individuals [Shapiro et al. 1977; Velazquez and Alter 2004].

Aspects of the metastatic process are sensitive to the cyclic hormonal fluctuations of the menstrual cycle. At various phases of the menstrual cycle (proestrus, estrus, metestrus, diestrus), breast cancers display variable expression of genes associated with metastasis [Saad et al. 1998]. The occurrence of ovarian metastases by melanoma and gastrointestinal cancers depends on the estrous phase at the time of entry of the cancer cells into the circulation [Webb et al. 1975; Vantyghem et al. 2003]. The estrous influence is exerted in several ways:

– Menstrual regulation affects the adrenergic system, which impacts cellular immune competence [Shakhar et al. 2000]. NK cell activity fluctuates with the estrous and circadian stages, IL-2 levels are highest in proestrus and estrus. These stages within the fertility cycle are also associated with the lowest metastatic potential [Hrushesky et al. 1988]. The modulation by the estrous cycle of the adrenergic suppression of NK cell activity may determine the resistance of the host to metastasis [Ben-Eliyahu et al. 1996; Ben-Eliyahu et al. 2000],
– While tumor blood vessel density and blood volume do not vary throughout the menstrual cycle, tumor capillary permeability is highest in diestrus, the cycle stage associated with the highest cancer growth rate and the highest frequency of postresection cancer metastasis [Wood et al. 2005]. However, there is no difference in serum concentrations of VEGF during the distinct phases of the menstrual cycle in healthy women [McIlhenny et al. 2002], suggesting that these effects are limited to specific organs.

The expression of TGF forms can be a predictor of tumor fate. The growth of colon cancer cells in the liver is facilitated by the expression of growth factor receptors on the cancer cells and growth factors, including TGF-α, in the liver. Although the autocrine factor TGF-β_1 is a potent growth inhibitor of normal epithelial cells, including colonocytes, it is implicated as an enhancer of colon cancer metastasis. Increased expression of TGF-β_1 by colon cancer is correlated with tumor progression, because it supports the epithelial–mesenchymal transition.

13.2 ALTERATIONS IN THE ACTH AXIS

The cortisol levels in the body are regulated on multiple levels through the hypothalamic-pituitary-adrenal axis. The hypothalamus produces Corticotropin Releasing Hormone (CRH), which causes the pituitary to secrete Adrenocorticotropic Hormone (ACTH). When the adrenals receive the ACTH, they respond by releasing cortisol into the bloodstream. Excess amounts of cortisol lead to Cushing syndrome (hypercortisolism). It is characterized by a red full face (moon face), central obesity with protruding abdomen and thin extremities, weakness, headache, thirst and increased urination, purple striations on the skin of the abdomen and thighs, mental changes, and impotence or cessation of menses. The secretion of ACTH or β-Melanocyte Stimulating Hormone (MSH) by certain tumor cells causes ectopic ACTH syndrome (ectopic Cushing syndrome). ACTH is derived from pro-Opiomelanocortin (POMC) by proteolytic cleavage. In contrast to pituitary-dependent Cushing syndrome, the cancer-induced disease is characterized by

substantially elevated plasma and urine ACTH and cortisol, which do not respond to suppression by dexamethasone or induction by CRH. Ectopic ACTH syndrome tends to present with less impressive classic features, but more dramatic high blood pressure and low potassium. It is characterized by mineral–corticosteroid excess, with hypokalemic alkalosis in 95–100% of all cases, in contrast to less than 10% in other forms of Cushing syndrome. The hypokalemia is caused by ACTH-induced substrate saturation and consecutive inhibition of 11-β-HSD-2 (11-β-Hydroxysteroid Dehydrogenase Type 2). The inhibition depresses the conversion of cortisol to cortisone, so that cortisol acts as a mineral–corticosteroid. This underlies the mineral–corticosteroid excess state that characterizes the ectopic ACTH syndrome.

Ectopic ACTH syndromes are associated with overt malignancies, such as small cell lung cancer, and with occult neoplasms, most commonly bronchial carcinoid tumors. Bronchial carcinoid tumor, one of the most frequent sources for ectopic ACTH secretion, often displays numerous features of the corticotroph phenotype. This differentiation is accompanied by the induction of *c-fos* and *kiaa1775*, and the repression of *tm4sf5* [Pascual-Le Tallec et al. 2002]. Some nonendocrine tumors, particularly oat cell carcinoma of the lung, secrete polypeptide hormones that can induce ectopic ACTH syndromes. The *pro-opiomelanocortin* gene is occasionally expressed in nonpituitary tumors, leading to Cushing syndrome.

Common symptoms of Addison disease (chronic adrenal insufficiency, hypocortisolism) include chronic fatigue that gradually worsens, muscle weakness, loss of appetite, weight loss, nausea and vomiting, diarrhea, low blood pressure (orthostatic hypotension), areas of hyperpigmentation (darkened skin, melasma suprarenale), irritability, depression, craving for salt and salty foods.

- Hematogenous metastasis to the adrenal glands is common, exceeded in frequency only by hematogenous metastasis to the lung, liver, and bone. About 10–25% of patients who die from malignant illness have adrenal metastasis. In rare cases, this may lead to adrenal insufficiency. Most of the adrenal tissue must be destroyed before corticosteroid production, under both basal and stress conditions, is impaired.
- Addison disease can result from primary lymphoma of the adrenals. This distinct entity has some unique clinical features, including an enlargement of the adrenal gland with maintenance of the adreniform shape and elevated serum Lactate Dehydrogenase [Pimentel et al. 1997].
- Other causes of primary adrenal insufficiency in cancer patients include autoimmune adrenalitis, adrenal hemorrhage, and granulomatous diseases.
- Secondary adrenal insufficiency because of metastasis to the pituitary or hypothalamus, or because of sustained elevated glucocorticosteroids may occur. The resulting hypothalamic–pituitary suppression causes ACTH deficiency.

13.3 THYROID DYSREGULATION

The hormones, Thyroxine (T_4) and Triiodothyronine (T_3), are tyrosine-based secreted messengers produced by the thyroid gland. Iodine is an important component in their synthesis from the precursor Thyroglobulin. The major form to circulate in the blood is Thyroxine, which is converted to the active Triiodothyronine within cells by Deiodinases. The thyroid hormones increase the basal metabolic rate, affect protein synthesis, and increase the sensitivity to catecholamines including adrenaline. These hormones also regulate the metabolism of protein, fat, and carbohydrates by affecting the use of energetic compounds. Thyroid disorders and abnormalities in thyroid function are commonly associated with cancer, and they may be iatrogenically induced as adverse effects of cancer therapy.

Hyperthyroidism (thyrotoxicosis) is the clinical syndrome caused by an excess of circulating free Thyroxine (T_4) or free Triiodothyronine (T_3). Symptoms are weight loss (often accompanied by a ravenous appetite), hyperactivity, irritability, depression, polyuria, and sweating. It is a possible development in patients with thyroid metastasis from lymphoma or pancreatic cancer [Shimaoka et al. 1976; Eriksson et al. 1977]. In these cases, the etiology of the thyrotoxicosis is probably similar to that in subacute thyroiditis, with follicular destruction resulting in an unregulated release of thyroid hormones and their precursor glycoprotein Thyroglobulin.

Hypothyroidism in cancer is often a consequence of damage to or removal of the thyroid in therapeutic intervention. The incidence of metastasis to the thyroid gland varies from 1% to 25%. The primary tumor sites include the kidney (30%), lung (15%), breast (15%), esophagus (9%), and uterus (7%).

However, hypothyroidism secondary to metastatic infiltration and replacement of the thyroid by cancer cells is extremely rare [Nakhjavani et al. 1997]. The condition manifests in slowed speech, and a hoarse breaking voice, impaired memory, temperature sensitivity, slow heart rate, sluggish reflexes, dry puffy skin, thinning of the outer edge of the eyebrows, weight gain, slowed metabolism, and constipation.

Euthyroid sick syndrome (SES, sick euthyroid syndrome) may arise in up to 70% of moderately to seriously ill cancer patients. It is characterized by low serum T_3 (Triiodothyronine) levels that are due to a decrease in the extrathyroidal conversion of T_4 (Thyroxine) to T_3. Consequently, the serum concentrations of free T_4 are usually normal or high, while the concentrations of free T_3 are below normal. The patients are clinically euthyroid, and serum TSH level as well as TRH stimulation are normal. With disease progression, the low-T_3 syndrome may evolve into the low-T_3/low-T_4 syndrome, in which the low level of total T_4 is caused by decreased binding of T_4 to serum proteins, decreased serum TBG (Thyroxine-Binding Globulin, T_4-Binding Globulin) levels, decreased pre-Albumin or Albumin levels, or increased T_4 clearance.

Calcitonin is a 32 amino acid polypeptide hormone that is produced primarily by the C-cells (parafollicular cells) of the thyroid. The hormone participates in calcium and phosphorus metabolism. Medullary carcinoma of the thyroid develops from the C-cells. Therefore, thyroid medullary carcinoma may be associated with hypercalcitoninemia. In this setting, the serum concentrations of calcium and phosphorus are normal. Serum 1,25-dihydroxy vitamin D levels are increased in spite of reduced serum 25-hydroxy vitamin D, reflecting an enhanced activity of the renal 1 α-Hydroxylase [Emmertsen et al. 1982]. The condition is associated with increased trabecular bone remodeling.

The parathyroid glands are four small structures located on the posterior surface of the thyroid gland. They release Parathyroid Hormone (PTH), which is a small protein that contributes to calcium and phosphorus homeostasis, as well as bone physiology. It regulates the blood calcium level within a very narrow range for proper function of the nervous and muscular systems. When the blood calcium levels drop, the parathyroid glands are activated to secrete PTH, which then stimulates osteoclasts to break down bone and release calcium into the blood.

Hyperparathyroidism with elevated levels of PTH causes hypercalcemia and increases the concentrations of 1α,25-dihydroxy vitamin D_3 through induction of the enzyme 1α-Hydroxylase. Tumors, such as ovarian cancer and breast cancer, often induce hypercalcemia through the secretion of PTHrP (PTH-Related Peptide) rather than PTH.

Hyperparathyroidism–jaw tumor syndrome (familial cystic parathyroid adenomatosis, primary familial hyperparathyroidism with ossifying jaw fibromata) is a familial multitumor syndrome, resulting from inactivating mutations in the *hrpt2* (*c10rf28*) {1q25-q31} tumor suppressor gene, which encodes Parafibromin [Jackson 1958; Carpten et al. 2002]. Wilms tumors, renal hamartomata, and cystic kidney disease can occur in conjunction with this syndrome.

13.4 ALTERED GLUCOSE METABOLISM

A large fraction of the cellular chemical energy is derived from glucose, which can be metabolized through aerobic and anaerobic pathways. The glucose blood levels need to be tightly regulated because too little glucose leads to starvation while excess levels are toxic. When glucose enters the blood stream after intestinal absorption, it stimulates the pancreatic β-cells to secrete Insulin. Insulin then facilitates the uptake of glucose into its target cells, resulting in a downmodulation of the blood glucose to nontoxic levels. At times of low glucose intake, the α-cells of the pancreas release the enzyme Glucagon, which induces the breakdown of stored glycogen into glucose in the liver and results in rising blood glucose concentrations.

Insulinomata of the pancreas (β-cell tumors) often lead to clinical signs of hypoglycemia due to elevated Insulin secretion, even before metastases occur. In this condition, plasma pro-Insulin and C-Peptide are elevated and the Insulin/glucose ratio is high. The hypoglycemia causes symptoms of neuroglycopenia and catecholamine response (increases in norepinephrine and epinephrine concentrations in response to stresses that challenge the blood glucose homeostasis).

Mesenchymal tumors, hepatic carcinomata, and adrenal carcinomata are frequently associated with hypoglycemia. This state may be induced by a tumor-secreted pro-form of IGF-2 (Insulin-Like Growth Factor 2). IGF-1 and -2 suppress Growth Hormone secretion in a feedback regulation, which perpetuates hypoglycemia.

Hypoglycemia occurs in fewer than 5% of fibrous tumors. The Doege–Potter syndrome is constituted by recurrent solitary fibrous tumors with associated hypoglycemia. These tumors are rare, tend to be large with a high mitotic rate, and have a 12–13% rate of malignancy. The typical cause of the hypoglycemia is the production of IGFs by these tumors [Zafar et al. 2003]. Augmented glucose utilization may also be an important pathogenetic factor in tumor-associated hypoglycemia with suppressed Insulin and C-Peptide levels [Herrmann et al. 2000].

13.5 NEURO- AND MYELOPATHIES

Neuronal symptoms may be caused by direct infiltration of the neuromuscular system by a tumor.

- Lung cancer can invade the sympathetic nerve causing paralysis and Horner syndrome (ptosis, myosis, enophthalmus).
- Pancoast syndrome (superior sulcus tumor) results from local extension of a lung tumor growing in the apex with involvement of the eighth cervical through second thoracic nerves. This causes shoulder pain that characteristically radiates into the ulnar distribution of the arm.
- Brain metastases are common in melanoma and breast cancer. In contrast, brain metastases from uterine cervical cancer are rare, estimated at 0.5–1.2%. They may generate symptoms of increased intracranial pressure and cerebellar dysfunction [Omari-Alaoui et al. 2003].

Various tumors express antigens, to which antibodies are produced that cross-react with molecules on healthy neural tissue. Such cross-reactivities cause autoimmune syndromes. Antibodies that recognize tumor and host antigens are typically polyclonal and are present in the serum and in the cerebrospinal fluid. Among membrane proteins, ion channels are frequently the antigens. The relevant antibodies typically affect peripheral nerves and muscles, resulting in focal damage due to membrane dysfunction. In contrast, intracellular antigens tend to prompt the generation of antibodies that cross-react with the central nervous system.

Cerebellar manifestations of auto-antibodies to Purkinje cells:

- Autoimmune paraneoplastic cerebellar degeneration (PCD, YO syndrome) affects cerebellar Purkinje cells and can cause ataxia [Anderson et al. 1988]. Some breast and ovarian cancer patients have paraneoplastic cerebellar degeneration [Greenlee and Brashear 1983]. Patients with lung cancer or Hodgkin disease may also be afflicted. In most of these individuals, there is a specific immune response to the shared tumor and Purkinje cell antigen CDR2 (Cerebellar Degeneration-Related 2, CDR-62, Autoantigen-2, PCD17, Paraneoplastic Cerebellar Degeneration-Associated Antigen-17, YO) [Furneaux et al. 1990]. Consistently, the syndrome is characterized by the presence of antibodies to CDR2 (anti-YO, PCA1, Purkinje cell Antibody 1). The 62 kD protein CDR2 is a member of a family of the leucine zipper DNA binding proteins, which acts as a transcriptional repressor by binding to the senescence gene product MRG15. MRG15 derepresses the E2F responsive *B-myb* promoter, and CDR2 inhibits this derepression, as a result repressing the promoter. Anti-YO antibodies inhibit the repression of the *B-myb* promoter activity by CDR2. This mechanism may contribute to neuronal death [Sakai et al. 2004]. The cross-presentation of CDR2, derived from apoptotic tumor cells, by dendritic cells also activates a potent specific cytolytic T-lymphocyte response. This is reflected in expanded populations of HLA-I restricted, CDR2 specific cytolytic T-lymphocytes in the blood of patients. The peripheral activation of CDR2-specific CTLs is likely to contribute to the subsequent development of the autoimmune neuronal degeneration. The other antigen commonly recognized by anti-YO antibodies is CDR-34 (CDR1, Autoantigen-1). It consists almost entirely of tandem repeats of the six amino acid consensus sequence (L/F)LEDVE, which gives rise to a number of leucine zipper elements [Dropcho et al. 1987]. This suggests that anti-YO sera specifically recognize leucine zipper structures [Fathallah-Shaykh et al. 1991].
- The TR syndrome (anti-TR antibodies to Purkinje cell cytoplasm and dendrites) is associated with some cases of Hodgkin lymphoma. It affects predominantly the cerebellum, leading to ataxia [Graus et al. 1997; Bernal et al. 2003].
- Antibodies to CARP (Carbonic Anhydrase-Related Protein, Carbonic Anhydrase 8, CA8) affect the Purkinje cell cytoplasm and dendrites. They cause ataxia and can occur secondary to the onset of melanomata that express CARP [Bataller et al. 2004].
- In smokers with small cell lung cancer, an autoantibody (PCA2) to a 280 kD antigen in Purkinje

cell cytoplasm commonly arises. It is also reactive with neurons in the internal granular layer and dentate nucleus, and with neuronal elements in gut and kidneys. It may result in varied clinical presentations including cerebellar ataxia, limbic encephalitis, Lambert–Eaton myasthenic syndrome, or autonomic neuropathy [Vernino and Lennon 2000].
– The metabotropic Glutamate Receptor-1 (mGLUR1) transmits signals through its association with G-Proteins. This type of receptor is located at the peri-synaptic site of the Purkinje cell dendritic spines, which form excitatory synapses with parallel fibers or climbing fibers. The activation of these receptors is necessary for the induction of cerebellar long-term depression, a likely mechanism of cerebellar motor learning. In Hodgkin disease, cerebellar ataxia can develop during remission, due to the occurrence of antibodies that bind to mGLUR1 [Sillevis Smitt et al. 2000].

Manifestations of antibodies to cytoplasmic proteins:
– The MA2 syndrome (antibodies to antigens in neuronal cytoplasm and nuclei) causes limbic encephalitis (hallucinations, lethargy, seizures, memory loss) and brain stem encephalitis (dysarthria, ataxia). It can be associated with narcolepsy and cataplexy [Landolfi and Nadkarni 2003]. Paraneoplastic limbic or brain stem encephalitis occurs more frequently with testicular cancer than with most other cancers. The 40 kD MA2 antigen (PNMA2, Paraneoplastic Antigen MA2) is selectively expressed by normal brain tissue and by the testicular tumors of the afflicted patients [Voltz et al. 1999]. It is therefore a brain/testis cancer antigen.
– The 37 kD MA1 is a brain/testis cancer antigen that can become a target in paraneoplastic syndromes [Dalmau et al. 1999]. Cancer patients harboring antibodies to MA1 display brain stem and cerebellar dysfunction, dysphagia, and motor weakness. There is a loss of Purkinje cells, Bergmann gliosis, and deep cerebellar white matter inflammation. The sites of extensive neuronal degeneration and gliosis have infiltrates that are mainly composed of $CD8^+$ T-lymphocytes.
– The CV2 syndrome is caused by antibodies to the 66 kD cytoplasmic oligodendrocyte antigen CV2 (Collapsin Response Mediator Protein 5, CRMP5, Dihydropyrimidinase-Like 5, DPYSL5) [Honnorat et al. 1996; Yu et al. 2001]. It is characterized by limbic encephalitis, polyneuropathy, and intestinal pseudo-obstruction. Anti-CV2 antibodies are frequently associated with a paraneoplastic sensorimotor axonal neuropathy and small cell lung cancer. In other paraneoplastic manifestations, the anti-CRMP5 IgG antibodies can cause optic neuritis, uveitis, and coexisting retinitis. They are associated with subacute vision loss and scotomas (field defects). Vitreous inflammatory cells, primarily cytotoxic T-lymphocytes, are present [Cross et al. 2003].

Antibodies to RNA-binding proteins:
– The anti-HU syndrome [Denny-Brown 1948] is sometimes associated with small cell lung cancer. In this syndrome, neuronal loss, gliosis, and an inflammatory cell reaction are variably distributed in the central nervous system, as well as in neurons, in sensory ganglia, and in the myenteric plexi. Symptoms include limbic encephalitis, myelitis with patchy weakness, fasciculations, and subacute sensory neuronopathy (ganglionopathy) [Moll et al. 1990; Dalmau et al. 1990]. The condition is caused by antibodies to the nuclear RNA-binding protein HU. This implies that the disorder results from an immune reaction primarily directed against the cancer and cross-reacting with an antigen in the nervous system [Graus et al. 1985]. The mechanisms leading to nerve involvement are unclear, as HU antigens are not expressed in the peripheral nerves. The reason for involvement might be an autoimmune reaction simultaneously directed against several antigens.
– The NOVA syndrome (RI syndrome, ANNA-2 syndrome, opsoclonus-myoclonus ataxia syndrome) occurs in breast and gynecologic cancers [Budde-Steffen et al. 1988], in about 2% of children with neuroblastoma [Weizman and Leong 2004], and may be a consequence of renal carcinoma [De Luca et al. 2002]. It is associated with antibodies (Anti-Neuronal Nuclear Antibody 2, ANNA-2) to the RNA-binding protein RI that cross-react with NOVA (Neuro-Oncologic Ventral Antigens) [Buckanovich et al. 1996]. This leads to opsoclonus-myoclonus and ataxia (paraneoplastic opsoclonus-myoclonus ataxia, POMA). The opsoclonus is characterized by irregular, continual, and conjugated chaotic saccades of the eyes. It is increased with eye closure and fixation and persists during sleep. The disease is characterized by a rapid onset of symptoms also including

vertigo, nausea, vomiting, and encephalopathy. The *nova* gene is expressed exclusively in a subset of central nervous system neurons, primarily in the brain stem and ventral spinal cord neurons, at all developmental stages. The NOVA-1 and 2 constitute a family of regulators of RNA metabolism in neurons. The proteins harbor 3 KH-type RNA-binding motifs. The third KH domain (KH3) of NOVA-2 binds to stem loop RNA. NOVA regulates alternative splicing in neurons.

Neuromuscular manifestations by auto-antibodies to ion channels and receptors:

- Lambert–Eaton myasthenic syndrome is a paraneoplastic neuromuscular disorder, in which an immune response directed to a small cell lung cancer or thymoma cross-reacts with antigens in the neuromuscular junction. It is caused by antibodies to the β_2 subunit of voltage-gated calcium channels (CACNB2, CAVB2, MYSB). This leads to peripheral neuropathies, subacute cerebellar degeneration, cortical degeneration, and polymyositis.
- Neuromyotonia is a spontaneous muscular activity that results from repetitive motor unit action potentials of peripheral origin. Isaac neuromyotonia occurs in thymoma. It is caused by antibodies to potassium channels, which reduce the channel functions.
- The stiff person syndrome (stiff man syndrome, progressive encephalomyelitis with rigidity, PER) is characterized by progressive rigidity of the body musculature with superimposed painful spasms. The condition occurs in breast cancer, small cell lung cancer, and mediastinal cancer.
  - 60% of patients harbor auto-antibodies directed to Glutamic Acid Decarboxylase (GAD2, GAD65), the enzyme that synthesizes γ-amino butyrate (GABA) [Folli et al. 1993].
  - A fraction of patients, all women affected by breast cancer, have auto-antibodies directed to the synaptic protein Amphiphysin, which functions in Clathryn-coated vesicle endocytosis [De Camilli et al. 1993]. The auto-antibodies from patients with the stiff person syndrome bind to an Amphiphysin epitope located in the COOH-terminal region, which contains a SH3 domain.
  - In mediastinal cancer, high titer auto-antibodies directed to Gephyrin may lead to stiff person syndrome. Gephyrin is a cytosolic protein selectively concentrated at the postsynaptic membrane of inhibitory synapses, where it is associated with GABA and Glycine Receptors. This implies a link between autoimmunity directed against components of the inhibitory synapses and neurologic conditions characterized by chronic rigidity and spasms [Butler et al. 2000].
- All three targets for auto-antibodies that cause the stiff person syndrome are involved in the regulation of synaptic transmission. Both GAD and Amphiphysin represent nonintrinsic membrane proteins that are concentrated in nerve terminals, where a pool containing these proteins is associated with the cytoplasmic surface of synaptic vesicles. Gephyrin is essential for both the postsynaptic localization of inhibitory neurotransmitter receptors in the central nervous system and the biosynthesis of the Molybdenum Co-Factor (an essential cofactor for Sulfite Oxidase, Xanthine Dehydrogenase, and Aldehyde Oxidase) in various peripheral organs.
- Myasthenia gravis may be caused by antibodies to the nicotinic Acetylcholine Receptor or by antistriational antibodies. It may be a consequence of thymoma. The synthesis of auto-antibodies to the Acetylcholine Receptor results from an antigen-driven immune reaction that starts inside the thymus, is maintained there, but spreads to extrathymic sites and initiates the early phase of myasthenia gravis.
- Autonomic neuropathy is caused by antibodies to the α3 subunit of the Acetylcholine Receptor.

Antibodies that affect peripheral nerves and muscles:

- Neoplasms may secrete antibodies that affect peripheral nerves and muscles. Frequently, these are IgM that recognize carbohydrates or membrane lipids (GM1 gangliosides on motoric nerves; GD1b gangliosides on sensory nerves; sulfatides, MAG, or GALOP on Myelin) [Noguchi et al. 2003].
- Tumor secreted IgG or IgA M-Proteins can generate POEMS syndrome (polyneuropathy organomegaly endocrinopathy M-Protein and skin changes, Crow–Fukase syndrome), polyneuropathy, or cryoglobulinemia resulting in vasculopathy. Tumor-derived antibodies are typically monoclonal and present in the serum but not in the cerebrospinal fluid.
- Guillain–Barré syndrome (GBS, acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculoneuritis, acute idiopathic polyneuritis, French Polio, Landry ascending

paralysis) is an acquired, immune-mediated inflammatory disorder of the peripheral nervous system [Landry 1859; Guillain et al. 1916]. The pathologic hallmark of the disease is a loss of myelin in peripheral nerves due to an acute and progressive inflammation. The syndrome may manifest in acute inflammatory demyelinating poly-radiculoneuropathy (AIDP), in the Miller–Fisher variant (ataxia, loss of tendon reflexes, difficulty moving eye muscles, without weakness or sensory loss), or in acute motor and sensory axonal neuropathy (AMSAN). While in classical Guillain–Barré syndrome, onconeural antibodies are mostly absent, anti-GQ1b antibodies are typically associated with the Miller-Fisher variant [Chiba et al. 1992]. The cancer most often associated with Guillain–Barré syndrome is Hodgkin lymphoma. The relationship to other malignancies is less clear.

Peripheral neuropathy is common in many vasculitic syndromes and may be the only manifestation of the underlying disease. Paraneoplastic vasculitic neuropathy may present as a painful sensorimotor neuropathy or as multiple mononeuropathies, which mostly affect older men. The underlying tumors include small cell lung cancer, and less frequently cancers of the colon, kidney, bile duct, stomach, prostate, and tongue epidermoid [Oh 1997; Antoine et al. 1999].

Motor neuron diseases can occur as paraneoplastic syndromes. Lymphoma and breast cancer may be linked to pure lower motor neuron syndromes, possibly resulting in quadriparesis. No autoimmune mechanism is known [Schold et al. 1979; Ferracci et al. 1999]. A lower motor neuron syndrome may also arise in patients with renal cell carcinoma. In these individuals, nephrectomy leads to improvement of the weakness [Evans et al. 1990; Forman et al. 1999].

13.6 MYOPATHY

Myopathies are neuromuscular defects, in which the muscle fibers dysfunction, resulting in their weakness. Tumors may damage muscle structure and function in multiple ways, causing paraneoplastic myopathies. This may be reflected in atrophy, necrosis, or inflammation.

Muscle atrophy (muscle wasting, loss of muscles) can result from damage to the nerves that supply the muscles or from damage to the muscles themselves. The most frequent myopathy in cancer is type 2 fiber atrophy (affecting the fast contracting muscles) in the context of cachexia, which occurs in about half of all cancer patients. Proteolysis through ATP-dependent and Ubiquitin-dependent pathways is prominent in this form of muscle wasting.

Necrotizing myopathy is often characterized by subacutely progressive, symmetric, proximal weakness that results in severe disability. It occurs in lung [Levin et al. 1998], gastrointestinal [Lorimer and Eidus 1994], or breast cancer, and it may be a consequence of leukemia [Crowley et al. 1997]. Neutropenia, secondary to the tumor or to chemotherapy, can lead to infections with *Aeromonas veronii* or *Clostridium septicum*, overwhelming sepsis, myonecrosis, vascular occlusion, and necrotizing enterocolitis [Hiew et al. 1993; Masuya et al 2003].

Dermatomyositis is an autoimmune inflammatory myopathy with cutaneous vasculitis. The myopathy (loss of type I and type II fibers) produces symmetric weakness of the limb-girdle muscles and anterior neck flexors with or without muscle tenderness. This weakness progresses over weeks to months, with variable involvement of the pharynx, upper esophagus, or respiratory muscles. Dermatomyositis may be caused by breast, lung, ovarian, or nasopharyngeal cancers. The presence of T-lymphocytes in the involved sites suggests that cell-mediated immunity plays a role in the pathogenesis of dermatomyositis. Tumor antigens that stimulate the immune system may induce cross-reactivity with self antigens in the muscle and skin, leading to autoimmunity. The condition may also be due to an autoimmune response induced by chronic infection with hepatitis C virus associated with hepatocellular carcinoma. Autoantibodies associated with dermatomyositis include anti-Histidyl-tRNA s-Synthetase (anti-JO1), anti-Signal Recognition Particle, and anti-MI2. The risk for dermatomyositis is increased in patients with the *tnf-*α allele 308A and in maternal-fetal microchimerism.

Polymyositis is an inflammatory myopathy that affects multiple skeletal muscles. The most common symptom is weakness, usually affecting the muscles that are closest to the trunk of the body. Muscles may ache and be tender to touch. The condition is associated with lung cancer and non-Hodgkin lymphoma.

13.7 BLOOD DISORDERS

Red cells. Various malignant tumors cause disorders in the cellularity of the blood. Erythrocytosis, although rare, may be a consequence of renal, liver, uterine, or cerebellar tumors. Erythropoietin is physiologically produced by renal cells. Its elevation in malignancies leads to polycythemia. Conversely, pancytopenia may have several reasons.

– Bone marrow hematopoietic cells can be replaced by primary tumor in hematologic malignancies or by metastatic spread to the marrow from neoplasms of other organs. Hodgkin and non-Hodgkin lymphoma, malignant melanoma, neuroblastoma, as well as carcinoma of the breast, prostate, lung, adrenal, thyroid, and kidney commonly manifest marrow involvement. The result is pancytopenia.
– Aplastic anemia can arise in the presence of thymomata. It is caused by antibodies to erythroblast nuclei. Aplastic anemia and pure red cell aplasia are types of immune-mediated cytopenias that can be associated with large granular lymphocyte leukemia.
– Red blood cell life span can be significantly shortened in patients with advanced cancer, without typical clinical or laboratory evidence of hemolysis. Erythrophagocytosis or hypersplenism may account for this decrease in red cell survival.
– Immune hemolytic anemias are associated with neoplasms of the lymphocytic and reticuloendothelial systems. Anemia is usually moderate to severe and associated with jaundice, splenomegaly, and increased urine and fecal urobilinogen excretion. Red cell survival is decreased and the reticulocyte count is increased, unless erythropoiesis is profoundly impaired.

White cells. Peripheral blood leukocyte counts are usually normal in patients with cancer. Elevated circulating leukocyte counts, termed leukemoid reactions [Hill and Duncan 1941], may occur most commonly in patients with metastatic tumors of the breast, lung, and stomach. They can consist either of mature leukocytes or of early forms, such as myeloblasts and promyelocytes, in the peripheral blood.

Multiple myeloma is a malignant proliferation of plasma cells that can cause monoclonal gammopathies.

– M-Proteins, composed of either heavy chain and light chain or only light chain of antibodies typically accumulate in the serum and urine (Bence-Jones proteinuria) and may cause kidney damage. Some M-Proteins have known target antigens, such as Decorin (causing myopathy), GALOP antigen (causing gait ataxia, late onset polyneuropathy), Myelin-Associated Glycoprotein (causing sensory and motor neuropathy). In addition, M-Proteins may cause scleromyxedema (skin papules, myopathy, and Raynaud syndrome).
– Waldenström macroglobulinemia (lymphoplasmacytic lymphoma) has elevated levels of monoclonal pentameric IgM in the blood. The IgM molecules are secreted by transformed plasma cells in this form of B-lymphocyte non-Hodgkin lymphoma. They cause generalized lymph node swelling and hepatomegaly. Symptoms include weakness, severe fatigue, bleeding from the nose or gums, weight loss, and bruises or other skin lesions. Severely high levels of IgM can lead to hyperviscosity syndrome, in which there occur visual symptoms (blurring or loss of vision) and neurologic symptoms (headache, dizziness, vertigo). Renal failure rarely occurs. Normal hemoglobin and low levels of serum β_2-Microglobulin are favorable prognostic factors. The disease may result in anti-Decorin myopathy [al-Lozi et al. 1997].

Platelets. Platelets contribute essentially to blood coagulation. Primary haemostasis is initiated when platelets adhere to Collagen fibers via their receptor Glycoprotein Ia/IIa and via cross-links formed by von Willebrand Factor (vWF, Factor VIII). The platelets are then activated to release the contents of their granules, thus activating other platelets and white blood cells. The platelets also undergo a change in their shape, which exposes a surface phospholipid that activates coagulation factors. Secondary hemostasis is then executed by the coagulation factors.

Platelet abnormalities are a common complication of myeloproliferative disorders. Mechanisms for increased platelet activation in malignancy include:

– Tumor-induced Thrombin generation
– ADP release by the tumor cells
– Elevated levels of von Willebrand Factor

In addition, TNF (Tumor Necrosis Factor) is able to dramatically shift the predisposition of vascular endothelial cells toward procoagulant properties. This is mediated by an increase in the expression of

leukocyte adhesion molecules, Platelet-Activating Factor, and Tissue Factor. Further contributing to hypercoagulability may be vascular stasis due to vessel obstruction by the tumor, patient immobility, hepatic dysfunction, or advanced age. Multiple thromboses are typically due to pancreatic, gastrointestinal tract, ovary, prostate, or lung cancers.

Megakaryocyte hyperplasia and thrombocytosis are frequently associated with cancers of the lungs, ovaries, breasts, and stomach, and with Hodgkin disease. Deregulated blood coagulation may result and become manifest in multiple thromboses or in disseminated intravascular coagulation (DIC). Disseminated intravascular coagulation occurs in virtually all patients with acute promyelocytic leukemia and is often caused by mucinous adenocarcinomata. The thrombocyte-dependent activation of the Complement Factor cascade may be caused by the release of Cancer Procoagulant or of Tissue Factor.

- Cancer Procoagulant is a calcium-dependent cysteine protease that is expressed in malignant and fetal tissue, but not in differentiated tissue. It activates Factor X independently of the Tissue Factor/Factor VIIa complex [Falanga and Gordon. 1985]. Cancer Procoagulant is produced in acute promyelocytic leukemia, malignant melanoma, and cancers of the colon, breast, lung, and kidney.
- Tissue Factor activates Factor X after forming a complex with Factor VIIa. It is a transmembrane protein expressed by some normal parenchymal and connective tissue cells and many transformed cell types, including sarcoma, melanoma, neuroblastoma, lymphoma, pancreatic cancer, and acute promyelocytic leukemia [Williams 1977].

Thrombembolic disease (Trousseau syndrome, Nygaard–Brown syndrome), associated with some cancers, is classically characterized by migratory superficial phlebitis involving the upper or lower extremities (phlegmasia alba dolens) and is often combined with disseminated intravascular coagulation. The thromboses are preventable by anticoagulation therapy with heparin but not with warfarin [Callander and Rapaport 1993]. The disease is classically associated with pancreatic cancer, but other tumors, particularly adenocarcinomata, can also cause it.

Nonbacterial thrombotic endocarditis (NBTE) consists of sterile vegetations, composed of platelets and Fibrin, on the heart valves. It is highly associated with malignant tumors, mostly adenocarcinomata and lung cancer. Emboli released from the heart valves may affect the spleen, the kidneys, the extremities, the central nervous system, and the coronary arteries.

Acquired Glanzmann thrombasthenia is an uncommon event in association with leukemia. It can present as severe hemorrhagic syndrome without disseminated intravascular coagulation. In Glanzmann throbasthenia based on ALL, anti-Glycoprotein IIb/IIIa complex antibodies may be produced [Andre et al. 2005].

Thrombocytopenia may be due to splenic sequestration in patients who have splenomegaly. In this setting, increased numbers of megakaryocytes are present, unless the marrow is extensively infiltrated by the underlying cancer.

Vasculitis. Vasculitis is the inflammation of the blood vessel walls. It can occur as a paraneoplastic syndrome in malignancies. The most common forms of vasculitis in this context are cutaneous leukocytoclastic vasculitis, giant cell arteritis, polyarteritis nodosa, and Wegener granulomatosis. Adult Henoch-Schoenlein purpura is an IgA-associated vasculitis that may be caused by malignant neoplasms.

Cutaneous lymphocytic vasculopathy is a relatively common paraneoplastic skin manifestation in patients with lymphoproliferative diseases. It is characterized histologically by lymphocytic vasculitis with perivascular infiltration by nontransformed T-lymphocytes. The recurrent vasculitic changes involve exclusively the skin and are characterized by a maculo-papular rash, most frequently in the upper and lower extremities. Pruritus of varying intensity occurs in about 80% of the patients.

Paraneoplastic vasculitic neuropathy is characterized by damage to nerves due to inflammation of their associated blood vessels. The cancers most commonly associated with it are small cell lung carcinomata and lymphomata. Asymmetric or multifocal painful sensorimotor axonal neuropathy is the most common form of manifestation. Patients with vasculitic neuropathy may present with either mono-neuritis multiplex or asymmetric sensorimotor neuropathy. Symmetrical neuropathy is rare. It can arise as subacute relapsing, progressive, or relapsing progressive disease. The erythrocyte

sedimentation rate and cerebrospinal fluid protein content are high.

13.8 SKIN ALTERATIONS

Some cancers tend to form skin metastases. They include leukemia cutis, cutaneous T-cell lymphoma, and Paget disease of the breast. Cancer may also manifest in the skin in the form of nonspecific metabolic effects, through infections related to immunosuppression, or as results of compromise or dysfunction of other affected organs (such as jaundice due to liver failure). Furthermore, paraneoplastic syndromes can affect the skin. The cutaneous manifestations may develop before the detection of the underlying malignancy.

Papulosquamous lesions. Acanthosis nigricans and sudden eruption of multiple seborrheic keratoses (sign of Leser-Trélat) are cutaneous markers of stomach cancer [Yeh et al. 2000], and of other cancers in the gastrointestinal tract and the pancreas. Acanthosis nigricans manifests as a hyperpigmented, velvety thickening of the skin that usually occurs in the intertriginous zones. It is associated with hyperkeratosis and papillomatosis of the epidermis. The sign of Leser-Trélat in conjunction with eruptive acrochordons (small benign tumors that form primarily in areas where the skin forms creases) may be a paraneoplastic manifestation of melanoma, with TGF-α playing a pathogenetic role [Ellis et al. 1987]. A high EGF content is present in the hyperkeratinized portions of the seborrheic keratoses.

Bazex syndrome (follicular atrophoderma and basal cell carcinomata, Bazex–Dupre–Christol Syndrome, BDCS, acrokeratosis paraneoplastica) [Bazex et al. 1965; Bazex et al. 1966] is a manifestation of hyperkeratosis in the extremities associated with cancer in the upper respiratory and digestive tracts, and with tumors that have formed cervical or mediastinal lymph node metastases. Clinical manifestations include red, violaceous psoriasiform plaques with poorly defined margins and a symmetric, bilateral, and acral distribution, affecting mainly the hands, feet, ears, nose, and, to a lesser extent, elbows and knees. The skin may be thickened mainly in the fingers and the helices of the ears. In about two thirds of cases with Bazex syndrome, the paraneoplastic manifestations precede other clinical symptoms caused by the underlying tumor.

Pityriasis rotunda is characterized by circular scaly, hyperpigmented patches on the trunk, buttocks, and thighs. The lesions are at least 1–3 cm in diameter. They are hyperpigmented in dark-skinned individuals and are hypopigmented in light-skinned individuals. About 5% of cases are paraneoplastic. They may be due to hepatocellular carcinoma, gastric carcinoma, prostate carcinoma, or some forms of leukemia.

Acquired ichtyosis is very rare. It manifests with small white to brown polygonal scales that lift up at the free edge and are widely distributed on the trunk and extensor surfaces of the extremities. The palms and soles are usually spared. About 70% of the cases of paraneoplastic acquired ichtyosis involve Hodgkin disease. Kaposi sarcoma, cutaneous T-cell lymphoma, non-Hodgkin lymphoma, leukemias, breast cancer, lung cancer, or bladder cancer may also cause this condition.

Florid cutaneous papillomatosis is characterized by the eruption of pruritic papules, which are indistinguishable from common viral warts, in patients with cancer. The lesions first appear on the dorsal aspects of the hands and wrists and then spread to the trunk and occasionally to the face.

Erythematous conditions. Reactive erythemata (necrolytic migratory erythema, erythema gyratum repens) arise in tumor patients. Their lesions may be flat or slightly raised and consist of erythematous concentric rings with scales, which cause a woodgrain appearance. They are localized to the trunk and proximal extremities, sparing the feet, hands, and face. In erythema gyratum repens, the rings spread outward in a serpiginous pattern at a rate of 1 cm per day. Patients universally report severe pruritus. The oral manifestations of the spectrum of erythema range from tender superficial erythematous and hyperkeratotic plaques to painful deep hemorrhagic bullae and erosions. Other mucosal surfaces may be involved. In the reactive erythemata, the characteristic cutaneous target lesions and satellite cell necrosis of the epithelium may be sequelae of a cytotoxic immunologic attack on keratinocytes. Erythema gyratum repens is an annular erythema associated with lung, breast, stomach and esophageal cancer. Necrolytic migratory erythema arises in patients with a Glucagon-producing tumor in the pancreas.

Neutrophilic dermatoses are associated with myelodysplasia, leukemia, or carcinoma. They are often clinically indistinguishable from each other, but can be separated histologically by the presence of papillary dermal edema (Sweet syndrome), ulceration and necrosis (pyoderma gangrenosum), or vasculitis (pustular vasculitis). These distinctions may, however, reflect variations in the temporal course of the disease and in the degree of inflammation rather than different disease entities.

– Sweet syndrome (Gomm–Button disease, according to the names of the first two patients) [Sweet 1964] is an acute febrile neutrophilic dermatosis with pyrexia, neutrophilia, and erythematous painful cutaneous plaques primarily located on the upper extremities, head and neck. It arises in myeloid leukemia.
– Pyoderma gangrenosum is a neutrophilic dermatosis that occurs in acute myelogenous leukemia or multiple myeloma. Clinically, paraneoplastic pyoderma gangrenosum is often of the superficial bullous variant and is associated with a poor prognosis.
– Pustular vasculitis is often caused by a leukocytoclastic mechanism. It may afflict selectively the hands.

Multicentric reticulohistiocytosis is a rare disorder characterized by red to brown papular or nodular skin lesions in association with a progressive development of severe destructive arthritis. The lesions tend to involve the upper half of the body, preferentially including the face, hands, ears, and forearms. About 50% of patients have oral mucosal involvement. Arthritis of the hands, knees, and shoulders is typical and mimics rheumatoid disease with arthritis mutilans. Arthritic manifestations typically wax and wane, but they can rapidly progress to joint destruction. Approximately one thirds of cases are associated with malignancy. Underlying tumors include cancers of the breasts, lungs, muscles, gastrointestinal and genitourinary tracts, and hematologic system.

Bullous manifestations. Paraneoplastic pemphigus is an autoimmune mucocutaneous disease frequently associated with hematologic malignancies (close to 85% of cases). The most common malignancy among them is non-Hodgkin lymphoma (40%). Others comprise chronic lymphocytic leukemia (20%), Castleman disease (20%), and thymoma (5%). In about 15% of cases, the disease is combined with other malignancies, such as carcinomata, sarcomata, melanoma, and squamous cell carcinoma of the skin [Kaplan et al. 2004; Tilakaratne and Dissanayake 2005]. Most patients develop very severe oral and conjunctival ulceration. Genital ulceration is also a common symptom. Pemphigus is characterized by

– Mucocutaneous blistering and ulcerations
– Acantholytic changes of the epithelium and epidermis with interface dermatitis
– Deposition of IgG and C3 in intercellular areas or along the basement membrane
– Presence of serum antibodies
– Various Desmoplakins and Desmogleins in the serum [Anhalt et al. 1990]

Proteins of the Plakin and Desmoglein families play an important role in the pathogenesis of paraneoplastic pemphigus. Because Plakins have cytoplasmic location, they are not directly accessible to auto-antibodies in intact cells. This makes it likely that auto-antibodies directed to the Desmogleins-1 and -3 initiate the damage of the cell membranes, thus exposing the Plakins.

Others. Amyloid describes various forms of protein aggregations. Amyloidosis in malignancy possibly stems from immune deregulation. It may be caused by multiple myeloma and is a systemic disease with several potential skin manifestations, including purpura, plaques, papules, nodules, scleroderma-like infiltration, alopecia, nail changes, and bullous eruptions. Secondary amyloidosis is often associated with Hodgkin lymphoma or renal cell carcinoma.

Acquired hypertrichosis lanuginosa, which is an extremely rare disorder of hair growth, may be a specific marker of occult internal malignancy, especially lung and colon cancer. In women, it can be a paraneoplastic symptom of breast cancer. Abnormalities in virilizing hormones, or other hormone driven growths of lanugo hair may be the cause for the condition.

The syndrome of tender subcutaneous nodules, fever, eosinophilia, and polyarthritis of the small joints is produced by pancreatic adenocarcinoma. The subcutaneous nodules may undergo necrosis with oily discharge and are caused by circulating Lipases and other pancreatic enzymes.

13.9 ELECTROLYTE IMBALANCE

Electrolyte imbalance can be a result of catabolism in cancer. Tumor lysis syndrome is caused by the rapid death of predominantly malignant cells. This releases intracellular components into the systemic circulation faster than they can be eliminated, leading to hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. Symptoms include nausea, lethargy, vomiting, clouding of the urine, joint discomfort, and renal colic. They can ultimately lead to acute oliguric renal failure. Tumor lysis syndrome is most often associated with high grade myeloproliferative and lymphoproliferative disorders. It may also result from cell death upon initiation of therapy.

Uric acid is the end product of the purine metabolism. It is generated in the liver by oxidation of hypoxanthine and xanthine. Uric acid has a pK_a of 5.4 and is only moderately soluble at the renal luminal pH around 5.0. Hyperuricemia occurs most often in hematologic malignancies and is associated with hyperuricosuria and increased creatinine and urea in the serum. Acute uric acid nephropathy can result form crystal deposition in the distal tubules and collecting ducts. Possible complications are gout arthrtis, renal failure, and mental impairment.

Potassium is the major intracellular cation. Hyperkalemia (elevated blood potassium) results form the rapid release of intracellular potassium during extensive cell lysis. Due to the critical role of potassium in muscle function and neural transmission, neuromuscular abnormalities may occur. Hyperkalemia can lead to cardiac arrhythmia, and possibly to cardiac arrest.

The plasticity of the bone structure is maintained by a balance between osteoblastic and osteoclastic activities. This balance is regulated by various growth factors and hormones, notably TCF-β, FGF-1 and -2, IGF-1 and -2, Bone Morphogenic Protein 2, and PDGF (Platelet-Derived Growth Factor).

Complications of bone metastases include pain, fractures, and spinal cord compression. Increased osteoclastic or osteoblastic activity can also lead to electrolyte imbalance. Osteoclastic bone resorption is activated through a tumor-induced effect of osteoblasts on osteoclasts. The engagement of RANK (Receptor Activator of NF-κB, TNFRSF11A, Trancer) on osteoclast progenitors by RANK Ligand (RANKL, TNFSF11, Osteoclastic Differentiation Factor, ODF, Trance) on osteoblasts stimulates their maturation. RANKL is a 316 amino acid type II transmembrane protein of the TNF Ligand family. An important fate determination in the regulation of bone resorption includes the distinction between induction and activation of osteoclastogenesis, induced by binding of RANKL on osteoblasts to RANK on osteoclast progenitors, and the suppression of osteoclastogenesis, mediated by binding of RANKL to soluble Osteoprotegerin, which interferes with the engagement of RANK. The decoy receptor Osteoprotegerin (OPG, TNFRSF11B, OCIF) [Simonet et al. 1997] is a member of the TNF Receptor family. It is a negative regulator of osteoclast function. In cancer, Osteoprotegerin decreases skeletal tumor burden [Morony et al. 2001].

Tumor-produced PTHrP (Parathyroid Hormone-related Peptide) [Martin et al. 1989], a 141 amino acid polypeptide with NH_2-terminal homology to PTH, binds to the PTH Receptor on osteoblasts, resulting in the increasing production of RANKL and decreasing production of Osteoprotegerin. Beside facilitating bone resorption, circulating PTHrP can enhance hypercalcemia also by increasing renal calcium reabsorption and hypophosphatemia by renal phosphate wasting. PTHrP is expressed at low levels by normal keratinocytes. The pathophysiologic production of PTHrP in breast cancer cells is inducible by TGF-β and estrogen, and these two stimuli synergize. The *pthrp* promoter region contains three distinct elements, designated P1, P2, and P3. P1 and P3 are TATA box-like, while P2 is a GC-rich domain. Negative regulation of *pthrp* gene transcription may be exerted by 1,25-dihydroxy vitamin D. In contrast to PTH, PTHrP activates a feedback mechanism by decreasing the levels of 1,25-dihydroxy vitamin D. PTHrP is subject to RNA splicing, which may generate three forms of the message. PTHrP is also processed endoproteolytically,

- $PTHrP_{1–34}$ mediates the growth regulating and hypercalcemic effects. $PTHrP_{1–34}$ may stimulate Interleukin-6 production in osteoblasts. Interleukin-6 induces the expression of RANKL and serves as a mediator of bone resorption by promoting osteoclast formation
- $PTHrP_{35–94}$ promotes placental calcium transfer
- Peptides contained in $PTHrP_{109–141}$ inhibit osteoclast function

PTHrP peptides may localize in the nucleus, where they can inhibit apoptosis. In contrast, the PTH

Receptor is a 7-transmembrane spanning G-Protein coupled receptor expressed in the cell membranes of osteoblasts as well as breast cancer cells.

The incidence of hypercalcemia in cancer patients is approximately 1% [Vassilopoulou-Sellin et al. 1993]. It occurs as a consequence of bone resorption, possibly secondary to hyperparathyroidism. Symptoms include bone pain and pathologic fractures, pain in the flanks and frequent urination (with risk of nephrocalcinosis), muscle weakness and twitches, irritability.

– Osteolytic metastases induce fracture, hypercalcemia, and increased renal excretion of cAMP and phosphate. They may occur in neuroblastoma, breast carcinoma, prostate carcinoma, lung carcinoma, renal carcinoma, thyroid carcinoma, and lymphoma.
– Some cancers secrete PTH, and may thus cause bone resorption and elevated urinary cAMP excretion.
– Osteoclast activity may also be enhanced by tumor-derived M-CSF, IL-1, IL-11, TNF, and TGF.
– Prostaglandins are potent inducers of bone resorption. Some tumors, such as squamous lung cancers, produce prostaglandin E, causing paracrine osteolysis and hypercalcemia [Seyberth 1978].
– The tumor itself may exert lytic effects on the bone through the release of proteases.

Markers of bone resorption are NH_2-terminal and COOH-terminal telopeptides of Collagen breakdown products, and the pyridinium cross-links, pyridinoline and deoxypyridinoline, of Collagen. Urinary excretion of calcium and hydroxyproline (derived from Collagen degradation) may be elevated.

Tumor-induced osteomalacia [McCrance 1947] is a form of renal phosphate wasting that leads to severe hypophosphatemia, hyperphosphaturia, and a defect in vitamin D metabolism that causes low blood concentrations of 1,25-dihydroxy vitamin D (Figure 13.9.A, B). A frequent cause for tumor-induced osteomalacia is impaired renal reabsorption. This is

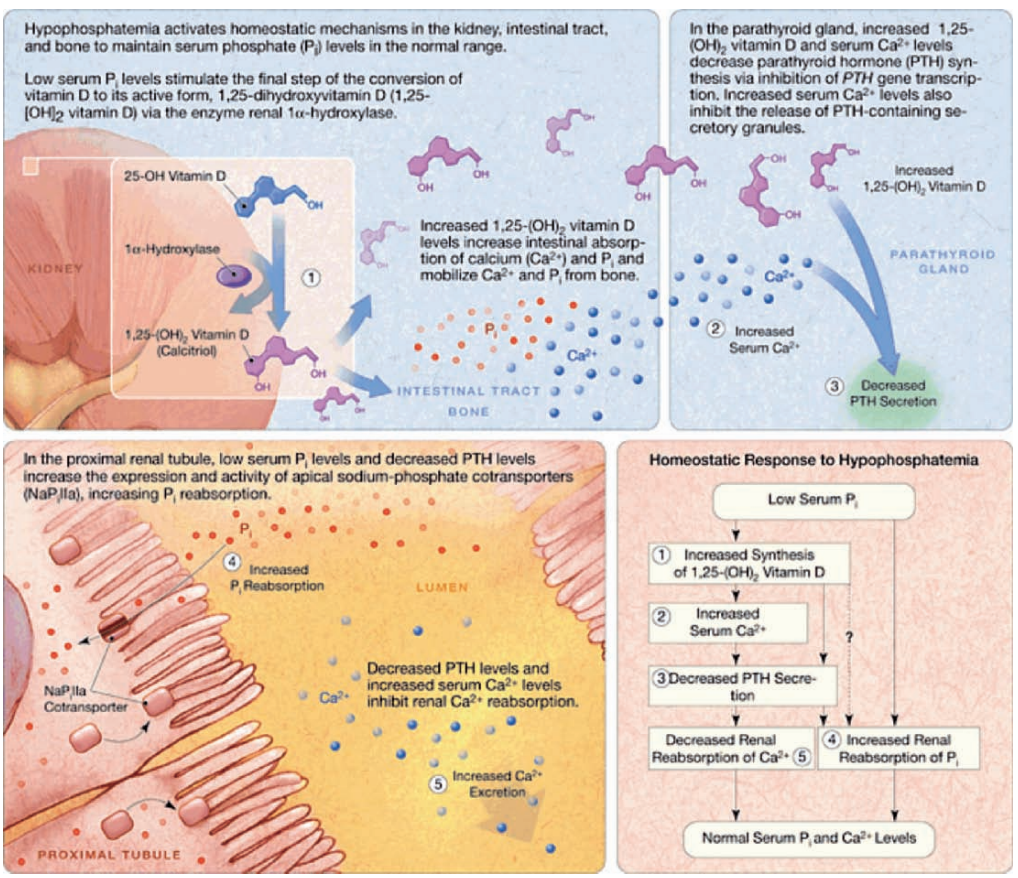


Figure 13.9.A. Phosphate homeostasis in osteomalacia. [Reproduced from Jan de Beur 2005. With permission.]

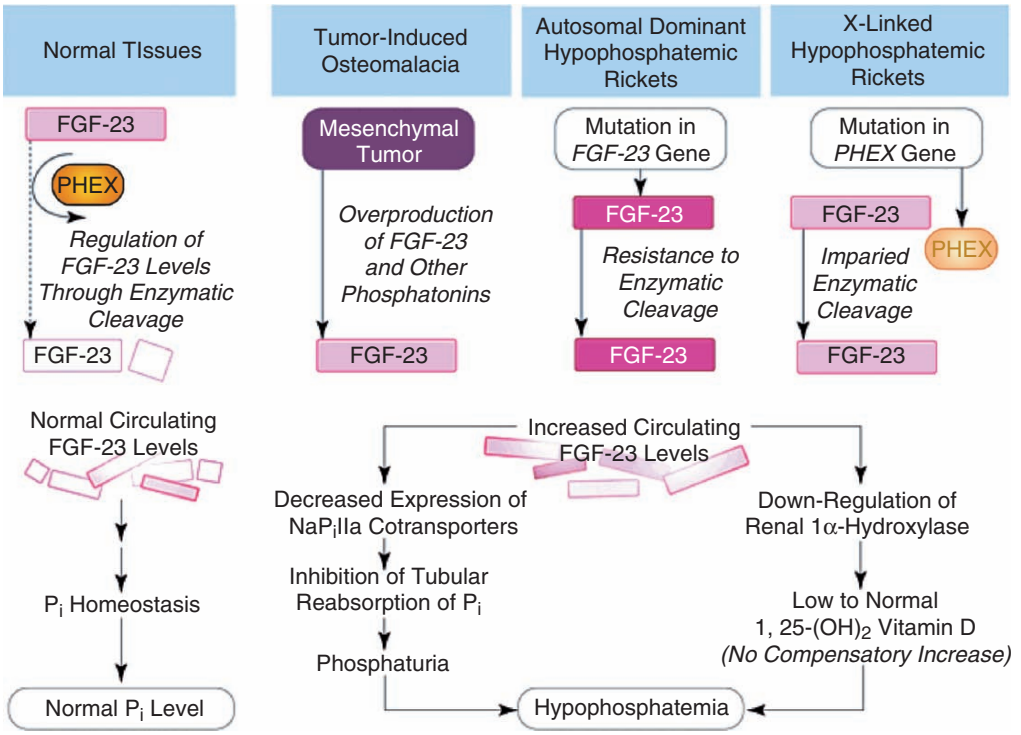


Figure 13.9.B. Osteomalacia. Mechanisms of FGF-23 Excess in Renal Phosphate-Wasting Syndromes. In tumor-induced osteomalacia, FGF-23 (Fibroblast Growth Factor 23) and other Phosphatonins ectopically produced by a mesenchymal tumor lead to excess circulating FGF-23 levels. In autosomal dominant hypophosphatemic rickets, FGF-23 excess results from mutations in the FGF-23 gene, which render the protein resistant to cleavage and inactivation. In X-linked hypophosphatemia, mutations in the PHEX endopeptidase (presumably located on osteoblasts or osteocytes) may either directly or indirectly result in FGF-23 excess by interfering with the processing and inactivation of FGF-23. [Reproduced from Jan de Beur 2005. With permission.]

compounded by a vitamin D synthesis defect that blocks the compensatory rise in Calcitriol. The bones suffer from a mineralization defect with increased mineralization lag time and excessive osteoid (unmineralized bone matrix). Symptoms include progressive fatigue, weakness, muscle and bone pain, and fractures. The occult nature of osteomalacia often delays its diagnosis.

Osteomalacia may be a manifestation of tumors either through accelerated bone formation with insufficient mineralization or through the production of a phosphaturic substance. A likely initiating factor for tumor-induced osteomalacia is FGF-23, which is produced at very low levels in normal tissues but may be highly expressed by certain tumors.

– Elevated levels of FGF-23 cause decreased expression of type IIa Sodium Dependent Phosphate Cotransporters (NaP_iIIa Cotransporters). At the proximal renal tubule, this leads to inhibition of phosphate reabsorption.
– FGF-23 downregulates renal 1α-Hydroxylase. This causes low levels of 1,25-dihydroxy vitamin D.

The abundance of circulating Immunoglobulin in multiple myeloma causes global proximal dysfunction of the kidneys, which may lead to hypophosphatemia. There are mesenchymal tumors associated with osteomalacia that are characteristically slow growing polymorphous neoplasms belonging to four groups [Jan de Beur 2005]:

– Phosphaturic mesenchymal tumor, mixed connective tissue type (PMTMC), including hemangiopericytomata
– Osteoblastoma-like tumors
– Ossifying fibrous-like tumors
– Nonossifying fibrous-like tumors

Prostate carcinoma typically induces osteoblastic lesions. This may be due, in part, to the secretion of osteoblast growth factors, including IGF-1 and -2 FGFs, Transforming Growth Factor-β (TGF-β), and Bone Morphogenic Proteins (BMPs). The NH_2-terminal fragment of the Urokinase Plasminogen Activator (UPA), released by prostate carcinoma

cells, engages an osteoblast receptor and acts as a growth factor. Prostate carcinomata also express the osteoblastogenic molecule Endothelin-1. The ligation of Endothelin-A Receptors on osteoblasts induces their proliferation. Endothelin-1 is increased in the circulation of patients with osteoblastic metastases. Markers of bone formation are Bone Alkaline Phosphatase, Osteocalcin, and pro-Collagen extension peptides. In patients with prostate cancer skeletal metastasis there is increased renal tubular reabsorption of inorganic phosphate despite higher urinary cAMP excretion. This suggests that renal tubular reabsorption is capable of adaptation to the need for minerals in enhanced bone formation [Buchs et al. 1998]. Osteoblastic lesions are less prone to electrolyte imbalance than osteoclastic lesions.

Low sodium levels can occur in small cell lung cancer. The syndrome of inappropriate Antidiuretic Hormone (SIADH) is characterized by hyponatremia, low serum osmolality and inappropriately high urine osmolality, heart failure, cirrhosis, adrenal insufficiency, and hypothyroidism. In cancer patients, SIADH may be caused by Vasopressin secreted by their tumors (up to 15% of small cell lung cancers), abnormal secretory stimuli (intrathoracic infection, positive pressure ventilation), or cytotoxicity affecting paraventricular and supraoptic neurons.

Hypernatremia secondary to central diabetes insipidus occurs frequently as a complication of destruction by the tumor of the anterior pituitary or the related hypothalamic nuclei.

13.10 VISUAL LOSS SYNDROMES

Cancer-induced autoimmune mechanisms may produce antibodies that cross-react with antigens in the eyes and compromise vision.

– Cancer associated retinopathy is reflected in night blindness, photopsia, and sudden visual loss. The onset is unilateral, often before detection of the underlying tumor, and the course is fluctuating but rapidly progressive. The retinal vessels at the fundus are narrow. Cancer-associated retinopathy is caused by an antibody to Recoverin. It can occur in small cell lung cancer, in renal cell cancer, or in breast cancer.
– Acquired night blindness causes impaired vision in darkness or in shimmering lights. The fundus is characterized by vitiligo and narrow retinal vessels. The disease causing auto-antibody reacts with bipolar retinal neurons. Acquired night blindness may be an autoimmune consequence of melanoma.
– In paraneoplastic optic neuropathy, visual loss can be sudden or progressive, and it can be associated with arcuate scotomata (defect in the field of vision arising near the blind spot and arching toward the nasal field) or centrocecal scotomata (horizontal oval defect in the field of vision embracing both the point of fixation and the blind spot). At the fundus, papillitis and disk pallor exist. The disease is caused by IgG M-Protein to retinal ganglion cells. Underlying cancers may be non-small cell lung cancer or multiple myeloma.

Bilateral diffuse uveal melanotic proliferation leads to a rapid loss of vision. It is characterized by pigment epithelial patches, uveal tumors, retinal detachment, and cataract. The condition may be caused by non-small cell lung adenocarcinoma, ovarian carcinoma, or gastrointestinal cancers.

Cone dystrophy presents with blindness after light exposure (sensitivity to bright lights) and achromatopsia (monochromatism, color blindness, maskun). The retinal arteries are narrow. The condition may be caused by renal cancer.

13.11 OTHERS

Weight loss and anorexia occur in about one half of all cancer patients. Cancer cachexia is a complex syndrome presenting anorexia, weight loss, wasting of muscle and adipose tissues, hyperlipidemia, and other metabolic abnormalities. Biochemically, cachexia is characterized by a dramatic loss of triglycerides from adipose tissue and proteins from skeletal muscle. A sulfated glycoprotein acting as a proteolysis inducing factor is produced by tumors. It brings about protein catabolism in muscle cells by activating the Ubiquitin-proteasome pathway, possibly through the intermediate molecule 15-hydroxyeicosatetraenoic acid (15-HETE), which increases the production of proteasome subunits. In synergy with this mechanism, cytokines, such as TNF-α together with IFN-γ, activate the transcription factor NF-κB. This leads to decreased expression of MYO-D, a transcription factor that may be important for replenishing wasted muscle [Guttridge et al. 2000] (Figure 13.11.A).

– Monocytes and macrophages can cause the systemic suppression of Lipoprotein Lipase activity and the development of hyper-triglyceridemia, a state frequently seen in cachexia. The mediator

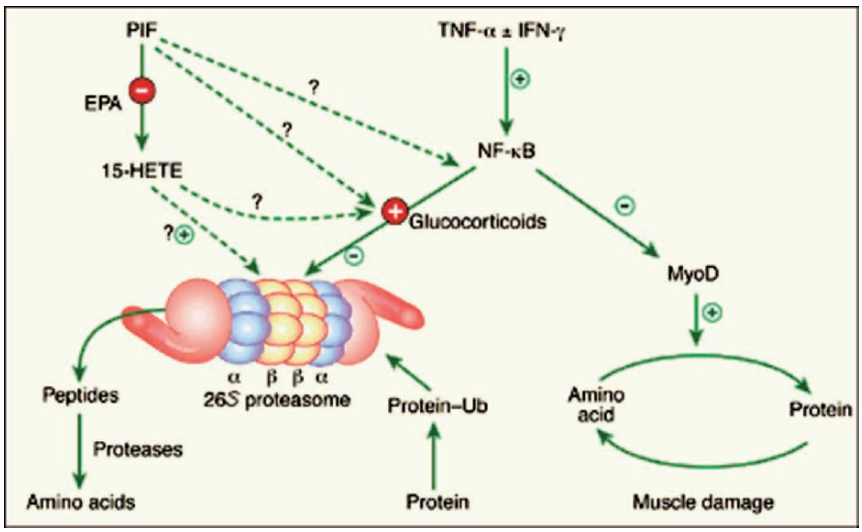


Figure 13.11.A. Cancer cachexia. Muscle breakdown is caused by malfunction of signaling pathways that regulate protein homeostasis in skeletal muscle. Cytokines such as TNF-α together with IFN-γ activate the transcription factor NF-κB. This leads to decreased expression of MYO-D, a transcription factor that may be important for replenishing wasted muscle. Activated NF-κB also acts as a repressor of proteasome subunit expression and hence suppresses protein degradation, an activity that is antagonized by glucocorticosteroids. Tumor factors such as PIF increase the production of proteasome subunits through the intermediary 15-HETE. Eicosapentaenoic acid (EPA) inhibits 15-HETE production in response to PIF and prevents muscle wasting in cancer patients. [Reproduced from Tisdale 2000. With permission.]

responsible for this is TNF-α (Cachectin) [Beutler et al. 1985], secreted by the cancer cells.
– Alimentary tract dysfunction may be a consequence of abnormalities in the perception of taste and smell in cancer patients.
– Tumors of the oropharynx, esophagus, stomach, pancreas, liver, and peritoneum may compromise oral intake by mechanical interference with anatomical structures. Intestinal obstruction is a common complication of cancer.
– In a rare event, anorexia nervosa can be caused by a tumor of the fourth ventricle, due to a direct inhibitory effect on the appetite center [Udvarhelyi et al. 1966].
– The anorexia/cachexia syndrome may reflect metabolic malabsorption. It may occur secondary to pancreatic insufficiency due to pancreas carcinoma or secondary to the infiltration of the intestine or mesentery by lymphoma.

Malnutrition in cancer patients is a predictor for susceptibility to infections [Hughes et al. 1974]. Therefore, weight loss is correlated with reduced life expectancy.

Carcinoid tumors are slowly growing neoplasms of enterochromaffine cells. The carcinoid syndrome can also be caused by tumors of other tissues derived from the embryonic foregut, such as bronchus, stomach, pancreas, and thyroid. Furthermore, teratomata can result in carcinoid symptoms.

The carcinoid syndrome is associated with paroxysms of cutaneous vasodilation, purple teleangiectasia, gastrointestinal hypermobility with cramping and diarrhea, cardiac vulvular lesions caused by fibrous deposits on the endocardium, and bronchial constriction. The typical cutaneous flush is erythematous, involving head and neck. The symptoms are caused, in part, by the secretion of 5-hydroxy tryptamine (serotonin) or 5-hydroxy tryptophan from the tumor. The metabolic product 5-hydroxy indoleacetate is excreted into the urine. Other mediators, including the vasodilator and intestinal motility inducer substance P, indoles, the vasodilator peptide Bradykinin, the anaphylactic mediator histamine, and ACTH may also be produced.

Zollinger–Ellison syndrome [Zollinger and Ellison 1955] is characterized by the presence of a Gastrin-producing tumor, severe and recalcitrant upper gastrointestinal ulcerative disease, excessive gastric acid secretion, and diarrhea in about 40% of the cases. The causative neoplasm is a gastrinoma, which may be located in the pancreas (non-β islet

cell tumor), the duodenal wall, the hilum of the spleen, or the stomach. Gastrinomata occur as single or as multiple tumors. About 50–65% of single gastrinomata are malignant tumors that most commonly spread to the liver and lymph nodes near the pancreas and small bowel. Gastrin secreted by these tumors stimulates the production of gastric acid in the stomach, which leads to intractable ulcers in the upper gastrointestinal tract and diarrhea. Some of these tumors secrete multiple hormones, including ACTH, Glucagons, Insulin, Pancreatic Polypeptide, and Vasoactive Intestinal Peptide. The Zollinger–Ellison syndrome is also a typical manifestation of multiple endocrine neoplasia (MEN).

Metastasis to the hypothalamic region or the pituitary gland is uncommon and clinical manifestations of endocrine dysfunction due to metastatic disease in this region are rare [Sioutos et al. 1996]. However, benign tumors, such as pituitary tumors and craniopharyngiomata frequently affect this anatomic region and cause endocrine dysfunction [Sklar 1994]. The resulting symptoms comprise fatigue and weakness. Hypothalamic and pituitary malfunction may include abnormal serum levels of Growth Hormone and IGF-1 as well as gonadal failure. Signs of overt hypopituitarism include hypoglycemia, hypotension, and hypothermia.

Abnormal elevations of the body temperature result from either hyperthermia or pyrexia (fever). In fever, the thermoregulatory mechanisms are intact, but the hypothalamic set point is elevated above normal by exogenous or endogenous pyrogens. Tumor fever (paraneoplastic fever) occurs in lymphomata, leukemias, renal cancer, and hepatomata. Tumor-associated fevers may be cyclic, occur at a specific time of the day, or be intermittent, alternating with afebrile periods lasting days or weeks.

– Tumor fevers are associated with solid tumors that have metastasized to the central nervous system or the spinal cord. They may affect thermoregulation.
– Fever may be caused by hormones or cytokines released from the tumors. IL-1, possibly also TNF and IL-6, play roles in this process.
– Fever may be a consequence of tumor necrosis. The cell debris causes an inflammatory response that includes an increase in body temperature.

PDGF is produced by some tumor cells, such as mammary carcinoma, that lack PDGF Receptors. This may cause the stimulation of adjacent nonmalignant fibroblasts, resulting in fibrosis [Mendelsohn and Gabrilove 1995].

REFERENCES

al-Lozi MT, Pestronk A, Choksi R. 1997. A skeletal muscle-specific form of decorin is a target antigen for a serum IgM M-protein in a patient with a proximal myopathy. Neurology 49:1650–1654.

Anderson NE, Rosenblum MK, Graus F, Wiley RG, Posner JB. 1988. Autoantibodies in paraneoplastic syndromes associated with small-cell lung cancer. Neurology 38:1391–1398.

Andre JM, Galambrun C, Trzeciak MC, Dargaud Y, Kaplan-Gouet C, Jallu V, Bertrand Y. 2005. Acquired Glanzmann's thrombasthenia associated with acute lymphoblastic leukemia. Journal of Pediatric Hematology and Oncology 27:554–557.

Anhalt GJ, Kim SC, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Ratrie H 3rd, Mutasim D, Ariss-Abdo L, Labib RS. 1990. Paraneoplastic pemphigus: an autoimmune mucocutaneous disease. The New England Journal of Medicine 323:1729–1735.

Antoine JC, Mosnier JF, Absi L, Convers P, Honnorat J, Michel D. 1999. Carcinoma associated paraneoplastic peripheral neuropathies in patients with and without anti-onconeural antibodies. Journal of Neurology, Neurosurgery, and Psychiatry 67:7–14.

Bataller L, Sabater L, Saiz A, Serra C, Claramonte B, Graus F. 2004. Carbonic anhydrase-related protein VIII: autoantigen in paraneoplastic cerebellar degeneration. Annals of Neurology 56:575–579.

Bazex A, Dupré A, Christol B. 1966. Atrophodermic folliculaire, proliferations baso-cellulaires et hypotrichose. Annales de Dermatologie et de Syphiligraphie 93:241–254.

Bazex A, Salvador R, Dupré A, Christol B. 1965. Syndrome paranéoplasique à type d'hyperkératose des extremités. Guérison après le traitement de l'épithelioma laryngé. Bulletin de la Societe Francaise de Dermatologie et de Syphiligraphie. 72:182.

Ben-Eliyahu S, Page GG, Shakhar G, Taylor AN. 1996. Increased susceptibility to metastasis during pro-oestrus/oestrus in rats: possible role of oestradiol and natural killer cells. British Journal of Cancer 74:1900–1907.

Ben-Eliyahu S, Shakhar G, Shakhar K, Melamed R. 2000. Timing within the oestrous cycle modulates adrenergic suppression of NK activity and resistance to metastasis: possible clinical implications. British Journal of Cancer 83:1747–1754.

Bernal F, Shams'ili S, Rojas I, Sanchez-Valle R, Saiz A, Dalmau J, Honnorat J, Sillevis Smitt P, Graus F. 2003. Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. Neurology 60:230–234.

Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, Ulevitch R, Cerami A. 1985. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature 316:552–554.

Buchs N, Bonjour JP, Rizzoli R. 1998. Renal tubular reabsorption of phosphate is positively related to the extent of bone

metastatic load in patients with prostate cancer. The Journal of Clinical Endocrinology and Metabolism 83:1535–1541.

Buckanovich RJ, Yang YY, Darnell RB. 1996. The onconeural antigen Nova-1 is a neuron-specific RNA-binding protein, the activity of which is inhibited by paraneoplastic antibodies. The Journal of Neuroscience 16:1114–1122.

Budde-Steffen C, Anderson NE, Rosenblaum MK, Grause F, Ford D, Synek BJ, Wray SH, Posner JB. 1988. An antineuronal autoantibody in paraneoplastic opsoclonus. Annals of Neurology 23:528–531.

Butler MH, Hayashi A, Ohkoshi N, Villmann C, Becker C-M, Feng G, De Camilli P, Solimena M. 2000. Autoimmunity to gephyrin in stiff-man syndrome. Neuron 26:307–312.

Callander N, Rapaport SI. 1993. Trousseau's syndrome. Western Journal of Medicine 158:364–371.

Carpten JD, Robbins CM, Villablanca A, Forsberg L, Presciuttini S, Bailey-Wilson J, Simonds WF, Gillanders EM, Kennedy AM, Chen JD, Agarwal SK, Sood R, Jones MP, Moses TY, Haven C, Petillo D, Leotlela PD, Harding B, Cameron D, Pannett AA, Hoog A, Heath H 3rd, James-Newton LA, Robinson B, Zarbo RJ, Cavaco BM, Wassif W, Perrier ND, Rosen IB, Kristoffersson U, Turnpenny PD, Farnebo LO, Besser GM, Jackson CE, Morreau H, Trent JM, Thakker RV, Marx SJ, Teh BT, Larsson C, Hobbs MR. 2002. HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. Nature Genetics 32:676–680.

Chiba A, Kusunoki S, Shimizu T, Kanazawa I. 1992. Serum IgG antibody to ganglioside GQ1b is a possible marker of Miller Fisher syndrome. Annals of Neurology 31:677–679.

Cross SA, Salomao DR, Parisi JE, Kryzer TJ, Bradley EA, Mines JA, Lam BL, Lennon VA. 2003. Paraneoplastic autoimmune optic neuritis with retinitis defined by CRMP-5-IgG. Annals of Neurology 54:38–50.

Crowley RS, Lembke A, Horoupian DS. 1997. Isolated meningeal vasculopathy associated with *Clostridium septicum* infection. Neurology 48:265–267.

Dalmau J, Furneaux HM, Gralla RJ, Kris MG, Posner JB. 1990. Detection of the anti-Hu antibody in the serum of patients with small cell lung cancer–a quantitative western blot analysis. Annals of Neurology 27:544–552.

Dalmau J, Gultekin SH, Voltz R, Hoard R, DesChamps T, Balmaceda C, Batchelor T, Gerstner E, Eichen J, Frennier J, Posner JB, Rosenfeld MR. 1999. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. Brain 122:27–39.

De Camilli P, Thomas A, Cofiell R, Folli F, Lichte B, Piccolo G, Meinck H-M, Austoni M, Fassetta G, Bottazzo G, Bates D, Cartlidge N, Solimena M, Kilimann MW. 1993. The synaptic vesicle-associated protein amphiphysin is the 128-kD autoantigen of stiff-man syndrome with breast cancer. The Journal of Experimental Medicine 178:2219–2223.

De Luca S, Terrone C, Crivellaro S, De Zan A, Polo P, Vigliani MC, Tizzani A. 2002. Opsoclonus-myoclonus syndrome as a paraneoplastic manifestation of renal cell carcinoma. a case report and review of the literature. Urology International 68:206–208.

Denny-Brown D. 1948. Primary sensory neuropathy with muscular changes associated with carcinoma. Journal of Neurologic and Neurosurgical Psychiatry 11:73–87.

Dropcho EJ, Chen YT, Posner JB, Old LJ. 1987. Cloning of a brain protein identified by autoantibodies from a patient with paraneoplastic cerebellar degeneration. Proceedings of the National Academy of Sciences USA 84:4552–4556.

Ellis DL, Kafka SP, Chow JC, Nanney LB, Inman WH, McCadden ME, King LE Jr. 1987. Melanoma, growth factors, acanthosis nigricans, the sign of Leser-Trelat, and multiple acrochordons: a possible role for alpha-transforming growth factor in cutaneous paraneoplastic syndromes. New England Journal of Medicine 317:1582–1587.

Emmertsen K, Melsen F, Mosekilde L, Lund B, Lund B, Sorensen OH, Nielsen HE, Solling H, Hansen HH. 1982. Altered vitamin D metabolism and bone remodelling in patients with medullary thyroid carcinoma and hypercalcitoninemia. Metabolic Bone Disease and Related Research 4:17–23.

Eriksson M, Ajmani SK, Mallette LE. 1977. Hyperthyroidism from thyroid metastasis of pancreatic adenocarcinoma. Journal of the American Medical Association 238:1276–1278.

Evans BK, Fagan C, Arnold T, Dropcho EJ, Oh SJ. 1990. Paraneoplastic motor neuron disease and renal cell carcinoma: improvement after nephrectomy. Neurology 40:960–962.

Falanga A, Gordon SG. 1985. Isolation and characterization of cancer procoagulant: a cysteine proteinase from malignant tissue. Biochemistry 24:5558–5567.

Fathallah-Shaykh H, Wolf S, Wong E, Posner JB, Furneaux HM. 1991. Cloning of a leucine-zipper protein recognized by the sera of patients with antibody-associated paraneoplastic cerebellar degeneration. Proceedings of the National Academy of Sciences USA 88:3451–3454.

Ferracci F, Fassetta G, Butler MH, Floyd S, Solimena M, De Camilli P. 1999. A novel antineuronal antibody in a motor neuron syndrome associated with breast cancer. Neurology 53:852–855.

Folkman J. 1995. Tumor angiogenesis. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 206–232.

Folli F, Solimena M, Cofiell R, Austoni M, Tallini G, Fassetta G, Bates D, Cartlidge N, Bottazzo GF, Piccolo G, De Camilli P. 1993. Autoantibodies to a 128-kD synaptic protein in three women with the stiff-man syndrome and breast cancer. The New England Journal of Medicine 328:546–551.

Forman D, Rae-Grant AD, Matchett SC, Cowen JS. 1999. A reversible cause of hypercapnic respiratory failure: lower motor neuronopathy associated with renal cell carcinoma. Chest 115:899–901.

Furneaux HM, Rosenblum MK, Dalmau J, Wong E, Woodruff P, Graus F, Posner JB. 1990. Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. The New England Journal of Medicine 322:1844–1851.

Graus F, Cordon-Cardo C, Posner JB. 1985. Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. Neurology 35:538–543.

Graus F, Dalmau J, Valldeoriola F, Ferrer I, Rene R, Marin C, Vecht CJ, Arbizu T, Targa C, Moll JW. 1997. Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. Journal of Neuroimmunology 74:55–61.

Greenlee JE, Brashear HR. 1983. Antibodies to cerebellar Purkinje cells in patients with paraneoplastic cerebellar degeneration and ovarian carcinoma. Annals of Neurology 14:609–613.

Guillain G, Barré JA, Strohl A. 1916. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des réflexes tendineux. Bulletins et Mémoires de la Société des Médecins des Hôpitaux de Paris 40:1462–1470.

Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin AS Jr. 2000. NF-κB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 289:2363–2366.

Herrmann BL, Saller B, Kiess W, Morgenroth K, Drochner K, Schroder T, Mann K. 2000. Primary malignant fibrous histiocytoma of the lung: IGF-II producing tumor induces fasting hypoglycemia. Experimental and Clinical Endocrinology and Diabetes 108:515–518.

Hiew CY, Silberstein M, Hennessy OF. 1993. Fatal *Clostridium septicum* myonecrosis. Australasian Radiology 37:399–400.

Hill JM, Duncan CN. 1941. Leukemoid reactions. American Journal of Medical Science 201:847.

Honnorat J, Antoine JC, Derrington E, Aguera M, Belin MF. 1996. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. Journal of Neurology, Neurosurgery, and Psychiatry 61:270–278.

Hrushesky WJ, Gruber SA, Sothern RB, Hoffman RA, Lakatua D, Carlson A, Cerra F, Simmons RL. 1988. Natural killer cell activity: age, estrous- and circadian-stage dependence and inverse correlation with metastatic potential. The Journal of the National Cancer Institute 80:1232–1237.

Hughes WT, Price RA, Sisko F, Havron WS, Kafatos AG, Schonland M, Smythe PM. 1974. Protein-calorie malnutrition: a host determinant for *Pneumocystis carinii* infection. American Journal of Diseases of Children 128:44–52.

Jackson CE. 1958. Hereditary hyperparathyroidism associated with recurrent pancreatitis. Annals of Internal Medicine 49:829–836.

Jan de Beur SM. 2005. Tumor-induced osteomalacia. The Journal of the American Medical Association 294:1260–1267.

Johnson FL, Lerner KG, Siegel M, Feagler JR, Majerus PW, Hartmann JR, Thomas ED. 1972. Association of androgenic-anabolic steroid therapy with development of hepatocellular carcinoma. The Lancet 2:1273–1276.

Kaplan I, Hodak E, Ackerman L, Mimouni D, Anhalt GJ, Calderon S. 2004. Neoplasms associated with paraneoplastic pemphigus: a review of literature with emphasis on non-haematologic malignancy and oral manifestations. Oral Oncology 40:553–562.

Landolfi JC, Nadkarni M. 2003. Paraneoplastic limbic encephalitis and possible narcolepsy in a patient with testicular cancer: case study. Neuro-oncology 5:214–216.

Landry JBO. 1859. Note sur la paralysie ascendante aiguê. Gazette Hebdomadaire de Médecine et de Chirurgie 6:472–474.

Levin MI, Mozaffar T, Al-Lozi MT, Pestronk A. 1998. Paraneoplastic necrotizing myopathy: clinical and pathological features. Neurology 50:764–767.

Lorimer JW, Eidus LB. 1994. Invasive *Clostridium septicum* infection in association with colorectal carcinoma. Canadian Journal of Surgery 37:245–249.

Martin TJ, Allan EH, Caple IW, Care AD, Danks JA, Diefenbach-Jagger H, Ebeling PR, Gillespie MT, Hammonds G, Heath JA, Hudson PJ, Kemp BE, Kubota M, Kukreja SC, Moseley JM, Ng KW, Raisz LG, Rodda CP, Simmons HA, Suva LJ, Wettenhall REH, Wood WI. 1989. Parathyroid hormone-related protein: isolation, molecular cloning, and mechanism of action. Recent Progress in Hormone Research 45:467–502.

Masuya A, Tamura K, Shishime M, Suzumiya J, Takata T. 2003. A successful treatment of progressive necrotizing myositis caused by *Aeromonas veronii* biotype sobria. Kansenshogaku Zasshi (Japanese) 77:991–995.

McCrance RA. 1947. Osteomalacia with looser's nodes (Milkman's syndrome) due to a raised resistance to vitamin D acquired about the age of 15 years. The Quarterly Journal of Medicine 16:33–46.

McIlhenny C, George WD, Doughty JC. 2002. A comparison of serum and plasma levels of vascular endothelial growth factor during the menstrual cycle in healthy female volunteers. British Journal of Cancer 86:1786–1789.

Mendelsohn J, Gabrilove J. 1995. Growth factors in malignancy. In: Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds). The molecular basis of cancer. Philadelphia: W.B. Saunders, pp. 432–459.

Moll JW, Henzen-Logmans SC, Vecht CJ. 1990. Anti-neuronal antibodies in paraneoplastic neurological disorders with small-cell lung carcinoma. Clinical Neurology and Neurosurgery 92:223–228.

Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. 2001. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastases. Cancer Research 61:4432–4436.

Nakhjavani MK, Gharib H, Goellner JR, van Heerden JA. 1997. Metastasis to the thyroid gland: a report of 43 cases. Cancer 79:574–578.

Noguchi M, Mori K, Yamazaki S, Suda K, Sato N, Oshimi K. 2003. Multifocal motor neuropathy caused by a B-cell lymphoma producing a monoclonal IgM autoantibody against peripheral nerve myelin glycolipids GM1 and GD1b. British Journal of Haematology 123:600–605.

Oh SJ. 1997. Paraneoplastic vasculitis of the peripheral nervous system. Neurologic Clinics 15:849–863.

Omari-Alaoui HE, Gaye PM, Kebdani T, El Ghazi E, Benjaafar N, Mansouri A, Errihani H, Kettani F, El Ouahabi A, El Gueddari BK. 2003. Cerebellous metastases in patients with uterine cervical cancer. Two cases reports and review of the literature. Cancer Radiotherapy 7:317–320.

Pascual-Le Tallec L, Dulmet E, Bertagna X, de Keyzer Y. 2002. Identification of genes associated with the corticotroph phenotype in bronchial carcinoid tumors. Journal of Clinical Endocrinology and Metabolism 87:5015–5022.

Pimentel M, Johnston JB, Allan DR, Greenberg H, Bernstein CN. 1997. Primary adrenal lymphoma associated with adrenal insufficiency: a distinct clinical entity. Leukemia and Lymphoma 24:363–367.

Saad Z, Bramwell VH, Wilson SM, O'Malley FP, Jeacock J, Chambers AF. 1998. Expression of genes that contribute to proliferative and metastatic ability in breast cancer resected during various menstrual phases. The Lancet 351:1170–1173.

Sakai K, Kitagawa Y, Saiki S, Saiki M, Hirose G. 2004. Effect of a paraneoplastic cerebellar degeneration-associated neural protein on B-myb promoter activity. Neurobiology of Disease 15:529–533.

Schold SC, Cho ES, Somasundaram M, Posner JB. 1979. Subacute motor neuronopathy: a remote effect of lymphoma. Annals of Neurology 5:271–287.

Seyberth HW. 1978. Prostaglandin-mediated hypercalcemia: a paraneoplastic syndrome. Klinische Wochenschrift 56:373–387.

Shakhar K, Shakhar G, Rosenne E, Ben-Eliyahu S. 2000. Timing within the menstrual cycle, sex, and the use of oral contraceptives determine adrenergic suppression of NK cell activity. British Journal of Cancer 83:1630–1636.

Shapiro P, Ikeda RM, Ruebner BH, Connors MH, Halsted CC, Abildgaard CF. 1977. Multiple hepatic tumors and peliosis hepatis in Fanconi's anemia treated with androgens. American Journal of Diseases of Children 131:1104–1106.

Shimaoka K, VanHerle AJ, Dindogru A. 1976. Thyrotoxicosis secondary to involvement of the thyroid with malignant lymphoma. Journal of Clinical Endocrinology and Metabolism 43:64–68.

Sillevis Smitt P, Kinoshita A, De Leeuw B, Moll W, Coesmans M, Jaarsma D, Henzen-Logmans S, Vecht C, De Zeeuw C, Sekiyama N, Nakanishi S, Shigemoto R. 2000. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. The New England Journal of Medicine 342:21–27.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. 1997. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319.

Sioutos P, Yen V, Arbit E. 1996. Pituitary gland metastases. Annals of Surgery and Oncology 3:94–99.

Sklar CA. 1994. Craniopharyngioma: endocrine abnormalities at presentation. Pediatric Neurosurgery 21:18–20.

Sweet RD. 1964. An acute febrile neutrophilic dermatosis. British Journal of Dermatology 76:349–356.

Tilakaratne W, Dissanayake M. 2005. Paraneoplastic pemphigus: a case report and review of literature. Oral Diseases 11:326–329.

Tisdale MJ. 2000. Biomedicine. Protein loss in cancer cachexia. Science 289:2363–2366.

Tisdale 2000. Science 289:2294.

Udvarhelyi GB, Adamkiewicz JJ Jr, Cooke RE. 1966. "Anorexia nervosa" caused by a fourth ventricle tumor. Neurology 16:565–568.

Vantyghem SA, Postenka CO, Chambers AF. 2003. Estrous cycle influences organ-specific metastasis of B16F10 melanoma cells. Cancer Research 63:4763–4765.

Vassilopoulou-Sellin R, Newman BM, Taylor SH, Guinee VF. 1993. Incidence of hypercalcemia in patients with malignancy referred to a comprehensive cancer center. Cancer 71:1309–1312.

Velazquez I, Alter BP. 2004. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. American Journal of Hematology 77:257–267.

Vernino S, Lennon VA. 2000. New Purkinje cell antibody (PCA-2): marker of lung cancer-related neurological autoimmunity. Annals of Neurology 47:297–305.

Voltz R, Gultekin SH, Rosenfeld MR, Gerstner E, Eichen J, Posner JB, Dalmau J. 1999. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. The New England Journal of Medicine 340:1788–1795.

Webb MJ, Decker DG, Mussey E. 1975. Cancer metastatic to the ovary: factors influencing survival. Obstetrics and Gynecology 45:391–396.

Weizman DA, Leong WL. 2004. Anti-Ri antibody opsoclonus-myoclonus syndrome and breast cancer: a case report and a review of the literature. Journal of Surgical Oncology 87:143–145.

Williams WJ. 1977. Thrombocytosis. In: Williams WJ, Beutler E, Erslev AJ, Rundles RW (eds). Hematology. New York: McGraw-Hill.

Wood PA, Bove K, You S, Chambers A, Hrushesky WJ. 2005. Cancer growth and spread are saltatory and phase-locked to the reproductive cycle through mediators of angiogenesis. Molecular Cancer Therapy 4:1065–1075.

Yeh JS, Munn SE, Plunkett TA, Harper PG, Hopster DJ, du Vivier AW. 2000. Coexistence of acanthosis nigricans and the sign of Leser-Trelat in a patient with gastric adenocarcinoma: a case report and literature review. Journal of the American Academy of Dermatology 42:357–362.

Yu Z, Kryzer TJ, Griesmann GE, Kim K, Benarroch EE, Lennon VA. 2001. CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Annals of Neurology 49:146–154.

Zafar H, Takimoto CH, Weiss G. 2003. Doege-Potter syndrome: hypoglycemia associated with malignant solitary fibrous tumor. Medical Oncology 20:403–408.

Zollinger RM, Ellison EH. 1955. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Annals of Surgery 142:709–723.

CHAPTER 14

DORMANCY AND MINIMAL RESIDUAL DISEASE

Relapse, even after many years of disease-free survival, is a common and potentially life threatening complication of tumors. Two major causes for this are the presence of dormant tumor cells in the body and the existence of minimal residual disease after therapy.

Dormancy is a state, in which tumor cells are present, but do not proliferate for extended periods of time. In early stage tumors and consecutive to clinically successful treatment of carcinomata, there is presence of tumor cells in the blood and bone marrow of the patients. Many of them do not develop into metastatic lesions, rather a large proportion of solitary cells disseminates throughout the body but remains dormant [Naumov et al. 2001]. Tumors or their metastases may sustain dormancy for years to decades, and metastases can arise long after the removal of a primary tumor [Meltzer 1990; Karrison et al. 1999]. Dormancy is likely to be a fairly common occurrence. Nearly one third of women between the ages of 40 and 50 have small in situ breast tumors, whereas only about 1% of women in this age group encounter breast cancer [Nielsen et al. 1987]. This suggests that the vast majority of these tumors stay dormant.

Although the molecular mechanisms of dormancy have not been defined, it is almost certain that they reflect tumor–host interactions.

– A lack of angiogenesis is a possible cause for the dormancy of metastases. In these cases, the rates of cell division and cell death are in a steady state. Micrometastases can be held dormant by blocks in angiogenesis that result in a balance of tumor cell proliferation and apoptosis [Holmgren et al. 1995]. If a nonangiogenic metastasis emerges in vascularized tissue it may form an in situ dormant microcylinder of tumor cells around capillary vessels (cooption of the blood vessels).
– Dormancy may be the result of immune mechanisms preventing the growth of microscopic metastases already present. Immunodepressive events often precede resurgent growth [Stewart 1996].
– Dormancy of primary or secondary tumors may be accounted for by endocrine factors. Certain hormones can regulate the proliferation of tumor cells. The absence of cues from growth promoting hormones or the presence of growth inhibiting hormonal signals can determine whether tumor cells remain dormant.
– Endoplasmic reticulum stress may have an important role in promoting dormancy. The relative activation levels of two MAP Kinase family members, the ERKs and $P38^{SAPK}$, can affect the fate of tumor cells. ERK is usually activated by proliferative signaling molecules and is associated with cell growth. Conversely, $P38^{SAPK}$ activation is usually downstream of death receptors, pro-Caspases, and DNA damage signals. Higher ratios of activated ERK to $P38^{SAPK}$ correlate with aggressive growth, whereas higher levels of $P38^{SAPK}$ activity help keep tumor cells in a growth-arrested, dormant state. The high $P38^{SAPK}$ activation in the growth arrested cells may be correlated with an induction of the unfolded protein response, whereas the cells with high ERK activation and aggressive growth do not show any indication of this activation. A characteristic of the unfolded protein response that could contribute to dormancy is the G_1 arrest that occurs in response

to the inhibition of Cyclin D_1 translation [Aguirre-Ghiso et al. 2003].

Minimal residual disease (MRD) is the remainder of tumor cells after primary therapy, which leads to clinically complete remission. The remaining cells often have long generation times or may be in G_0. Minimal residual disease is an indicator of incomplete therapeutic success and it poses a risk for relapse. In some cases, minimal residual disease causes an inflammatory response.

The recurrence of a cancer can be associated with the acquisition of resistance to chemotherapy. Typically, tumors that recur after an initial response to chemotherapy are resistant to multiple drugs (multidrug resistant). This may reflect the expansion of cancer resident stem cells, which are naturally resistant to chemotherapy through their quiescence, their capacity for DNA repair, and their high expression levels of efflux transporters of the ABC family.

REFERENCES

Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. 2003. ERK^{MAPK} activity as a determinant of tumor growth and dormancy; regulation by $p38^{SAPK}$. Cancer Research 63:1684–1695.

Holmgren L, O'Reilly MS, Folkman J. 1995. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nature Medicine 1:149–153.

Karrison TG, Ferguson DJ, Meier P. 1999. Dormancy of mammary carcinoma after mastectomy. Journal of the National Cancer Institute 91:80–85.

Meltzer A. 1990. Dormancy and breast cancer. Journal of Surgical Oncology 42:181–188.

Naumov GN, MacDonald IC, Chambers AF, Groom AC. 2001. Solitary cancer cells as a possible source of tumour dormancy? Seminars in Cancer Biology 11:271–276.

Nielsen M, Thomsen JL, Primdahl S, Dyreborg U, Andersen JA. 1987. Breast cancer and atypia among young and middle-aged women: a study of 110 medicolegal autopsies. British Journal of Cancer 56:814–819.

Stewart TH. 1996. Immune mechanisms and tumor dormancy. Medicina (Buenos Aires) 56(Suppl 1):74–82.

SECTION V

MOLECULAR MECHANISMS OF INDIVIDUAL MALIGNANCIES

Benign tumors are rarely life threatening, grow within a well-defined capsule that limits their size, and are usually well differentiated. Malignant tumors may be poorly differentiated; they invade surrounding tissues and various distant areas of the body. Carcinomata are malignant epithelial tumors. Carcinomata in situ (CIS) are their corresponding premalignant conditions, in which cytological signs of malignancy are present, but there is no histologic evidence of invasion through the basement membrane. Sarcomata are malignant mesenchymal tumors. They affect the connective tissue, including muscle, fat, bone, cartilage, or blood vessels. Blastomata are dedifferentiated tumors that cannot be characterized histogenetically. They are described as anaplastic.

Most tumors originate from somatic mutations. They are sporadic and not associated with a family history of cancer. Less frequently, predisposing mutations can be inherited. Hereditary cancer syndromes are characterized by an early age of onset, the presence of multiple tumors, or bilateral affliction, familial clustering, and specific patterns of inheritance.

Tumors are commonly characterized by staging and grading. Tumor staging describes the expansion of a tumor, while tumor grading describes its differentiation (Table 15.A).

Table 15.A. Tumor staging and grading. Staging and grading of tumors provide a uniform framework for their description. The criteria for stages and grades of tumors define the spread of the transformed cells throughout the body and the differentiation of these cells.

The TNM classification for cancer staging (extent of dissemination)	
Primary tumor (T)	T0 no tumor, T1–4 increasing size of primary tumor
Regional lymph nodes (N)	N0 local LN clean, N1–3 local LN afflicted, N4 LN afflicted beyond regional
Distant metastasis (M)	M0 no distal metastases, M1 evidence of metastasis

The Gleason system for grading cancer (level of differentiation)

According to the National Cancer Institute, one way of grading prostate cancer is the Gleason system. The lower the number, the lower the grade, and the slower the cancer is growing

Grades 2–4: the cancer cells look similar to normal cells, and the cancer is likely to be less aggressive
Grades 5–7: the cancer cells appear dysplastic, and are more likely to be aggressive and grow faster
Grades 8–10: the cancer cells are more likely to be very aggressive in growth

CHAPTER 15

EPITHELIAL TUMORS

The majority of human tumors arise from epithelial tissues (carcinomata). Most carcinomata are derived from either epithelial cells with secretory specialization (adenocarcinomata) or epithelial sheets that form protective surfaces (squamous cell carcinomata). Squamous cells are characterized histologically by keratinization and the presence of intercellular bridges. Beneath the epithelial cell layers lies a basement membrane (basal lamina) that separates them from the underlying layer of connective tissue cells (stroma). The disruption of the basement membrane is an early sign of carcinoma invasiveness.

15.1 LUNG CANCER

Until the 1930s, the incidence of lung carcinoma was low. It increased through the remainder of the 20th century. Nearly all lung cancers are associated with exposure to environmental carcinogens, predominantly tobacco smoke, and also arsenic and radiation. Specifically, the etiology of small cell lung cancer (SCLC) is strongly tied to cigarette smoking. Early symptoms of lung cancer include cough, hemoptysis, shortness of breath, and chest wall pain.

Basic forms of lung cancer include:

– Adenocarcinoma, the predominant histologic patterns of lung adenocarcinoma include acinar, solid, papillary, and bronchioloalveolar. Lung adenocarcinoma cells express Cytokeratins 7 and 20 (Figure 15.1.A).
– Small cell carcinoma (oat cell carcinoma), small cell lung cancer is characterized by small hyperchromatic cells with minimal cytoplasm, absent nucleoli, and variable degrees of necrosis. It constitutes about 20% of all primary lung cancers. Small cell lung cancer may be a neuroendocrine tumor (Figure 15.1.B).
– Large cell carcinoma.
– Squamous cell carcinoma.

Transforming genetic defects. Lung tumors are typically characterized by chromosomal structural instability, including deletions, duplications, rearrangements, and unbalanced translocations. Genome-wide allelic imbalances, leading to loss of heterozygosity are induced by carcinogens. Allelic losses of chromosomes that contain repair genes may be associated with an increase in chromosomal instability [Herzog et al. 2002]. Carcinogens that form bulky DNA adducts force chromosomal instability, presumably due to their propensity for causing DNA damage that leads to erroneous repair and recombination events.

– In lung adenocarcinogenesis, frequent allelic loss occurs on chromosome 9p21, which contains the *cdkn2A* locus, on chromosomes 13q, 1p36, 14q, and 3p.
– Deletions of chromosome 3p are common in small cell lung cancer [Whang-Peng et al. 1982].
– Loss of heterozygosity arises more frequently in squamous cell carcinoma than in adenocarcinoma. It includes loss of DNA sequences from the short arm of chromosome 17. This is often accompanied by loss of DNA sequences from chromosome 11, specifically 11pter–p15.5 and 11p13–q13.

Excessive activation of the EGFR {7p12.3–p12.1}, often caused by mutations, occurs in 70% of non-small cell lung carcinomata, especially in squamous cell

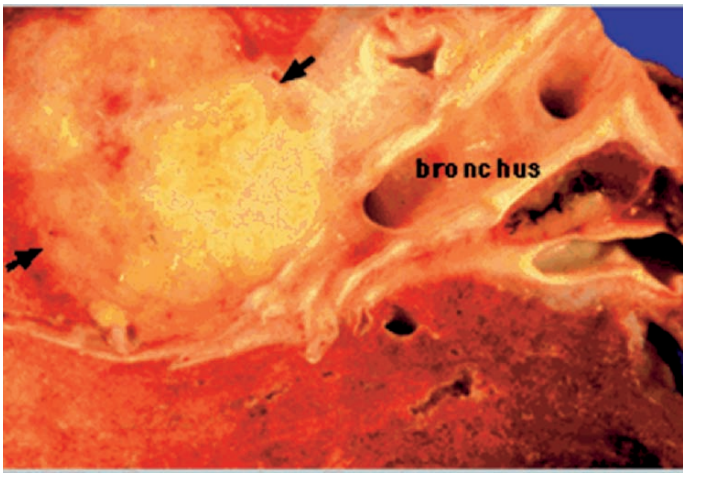


Figure 15.1.A. Lung adenocarcinoma. The neoplasm (*black arrows*) has a glistening yellowish surface reflecting the large amount of Mucin within it. The carcinoma is adjacent to a large bronchus and may have arisen from it. This tumor often has peripheral location, which associates with pleuritic chest pain and effusion. If it involves the pleura it causes puckering and scarring. The neoplasm can be unassociated with a large bronchus. Symptoms may include weight loss and dyspnea. Metastases locate to lymph nodes, brain, liver, and adrenals. The overall 5-year survival rate is 10%. Adenocarcinoma accounts for 25–40% of all lung cancers. [Reproduced from hrrp://pathweb.uchc.edu. With permission.]

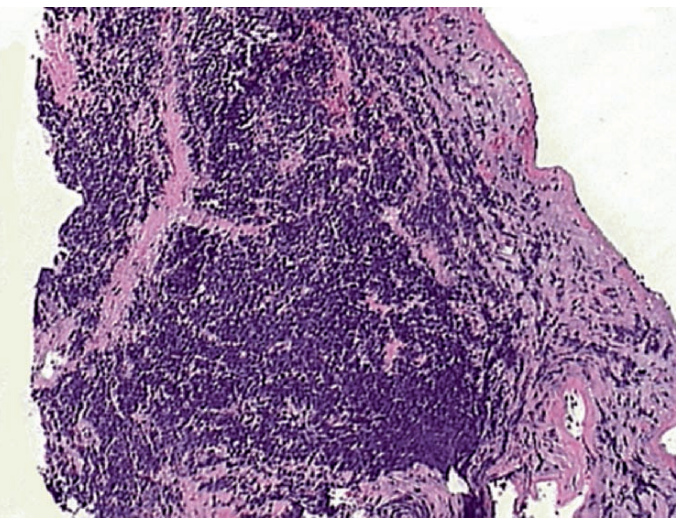


Figure 15.1.B. Small cell lung carcinoma. This is a very low power view of a small cell carcinoma. Note the dense cellularity, which shows profound hyperchromasia (deep blue staining). The neoplastic cells are arranged in a sheet with occasional intervening fibrous tissue (*arrows*). At this power these could be lymphocytes or a variety of small cell malignancies. These neoplasms tend to be perihilar and surround large bronchi. This leads to cough and hemoptysis as well as dyspnea. Small cell lung cancer is a highly aggressive neoplasm that frequently presents with evidence of metastatic disease. It is most likely associated with ectopic hormone production. The 2-year survival rate is 25%. [Reproduced from http://pathweb.uchc.edu. With permission.]

carcinomata and adenocarcinomata. The mutant EGFR typically activates PKB and STAT signal transduction, which promotes cell survival. Macrophage Migration Inhibitory Factor (MIF) and Cyclophilin A, downstream targets in these signaling pathways, are frequently overexpressed in non-small cell lung cancer.

In primary adenocarcinoma of the lung, *kras2* mutations are detected in close to 40% and are significantly more common in smokers compared with nonsmokers. The vast majority of *kras2* mutations has one of four changes in codon 12, the most common being G12C [Ahrendt et al. 2001].

The RB gene product is affected in close to 100% of small cell lung cancers. The absence of RB is sufficient to cause the hyperplasia of pulmonary neuroendocrine cells. Seven isoforms of 14-3-3 proteins are known. Deletion of 14-3-3ε in small cell lung cancers is associated with a defective G_2 checkpoint response. Down-regulation of *rb2* (*p130*) gene expression may occur in small cell lung cancer, whereas point mutations arise in small cell lung cancer and in non-small cell lung cancer.

p53 may be mutated in up to 50% of lung cancer cases. This is often caused by tobacco carcinogens, such as the high frequency of G→T transversions that may be induced by benzo[a]pyrene. In contrast, in lung cancers caused by radon exposure, *p53* mutations comprise C→A transversions, G→A transitions, and the hot spot mutation AGG→ATG in codon 249.

Proline Oxidase can mediate apoptosis in lung carcinoma cells. Its expression is induced by P53. In the absence of functional P53, the lack of *proline oxidase* expression may confer resistance to programmed cell death and predispose to cancer.

Polo-Like Kinases (PLK) are key regulators for mitotic progression. PLK-1 negatively regulates P53 function. PLK-1 is overexpressed in non-small cell lung cancer and may be a prognostic factor [Wolf et al. 1997].

Metastasis and angiogenesis. Lung cancers often metastasize to the mediastinal lymph nodes, the liver, and the brain (Figure 15.1.C). Lung cancer metastases can cause a variety of clinical symptoms:
– Superior vena cava syndrome from enlarged right paratracheal nodes
– Left recurrent laryngeal nerve paresis from subaortic nodal metastases
– Dysphagia from extrinsic esophageal compression by enlarged subcranial nodes
– Pancoast tumor with bone destruction of the first rib and the first chest vertebra, plexus neuralgia, Horner syndrome (ptosis, myosis, enophthalmus), and swelling of the arm

Lung cancer metastasis is frequently associated with the expression of CD44 variants. This is more common in squamous cell carcinomata than in adenocarcinomata. While the expression of CD44v6 is associated with an increased risk for local lymph node metastasis in non-small cell lung cancers, the presence of CD44v4–5 is typically reflective of squamoid differentiation. CD44v8–10 is the dominant splicing isoform in non-small cell lung cancer.

L-myc is amplified in a fraction of small cell lung cancers. *L-myc* restriction-length fragment polymorphism is a marker for the metastatic potential of lung cancer [Kawashima et al. 1988]. The up-regulation of the mRNA levels of the neuroendocrine markers *neurotensin* (*nts*), *neuroendocrine-specific*

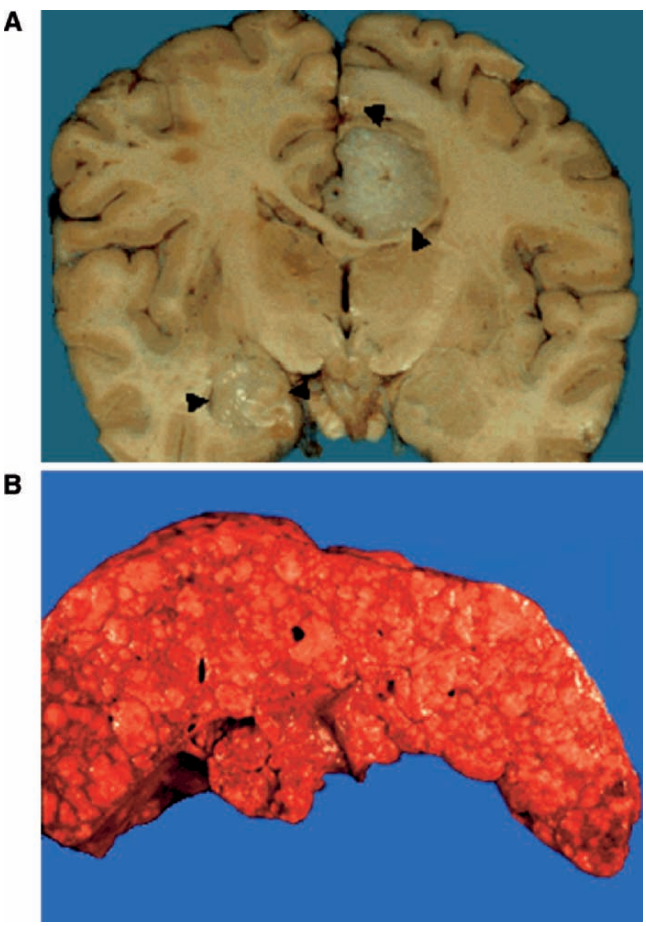


Figure 15.1.C. Metastatic lung carcinoma. (A) The lung primary cancer shows multiple brain metastatic lesions, the largest of which is in the cingulate gyrus and produces a mass effect sending the cingulate gyrus under the falx toward the contralateral side and compressing the lateral ventricle on the same side. Lung carcinomata, particularly small cell carcinomata, often metastasize to the brain. Tumor emboli may get trapped in small blood vessels (usually arterioles) and grow through the vascular wall into the parenchyma or more rarely the meninges. (B) The liver is studded with irregular tumor masses derived from small cell lung cancer. This is a classic case of secondary malignancy in the liver, which can be markedly enlarged. The patient may be jaundiced with elevated Alkaline Phosphatase and other marker enzymes. Metastases often tend to be multiple, nodular, and clearly demarcated from the rest of the liver tissue. Very often, the growth of the tumor outstrips blood supply with the development of central necrosis. [Reproduced from http://pathweb.uchc.edu. With permission.]

protein (*nsp*), *neural cell adhesion molecule 1* (*ncam1*), and *γ-aminobutyric acid B-type receptor* (*gpr51*) is associated with increased metastatic potential in large cell lung carcinoma. In contrast, *semaphorin 3B* (*sema3B*) is dramatically down-regulated [de Lange et al. 2003]. In lung cancer, *proteoglycan 1 secretory granule*, *tfII-D I*, *DNAJ-like heat shock protein 40*, *phosphoenolpyruvate carboxykinase 2*, and *soluble VEGF receptor* are overexpressed in noninvasive compared to invasive cells, whereas *calcyclin*, *axl*, *tumor-associated antigen L6*, *metallothionein I-B*, and *rtp* are overexpressed in highly invasive cells.

The Semaphorins are a family of secreted transmembrane, and membrane-associated proteins that cause the repulsion of nerve growth cone guidance and can induce retraction in nonneural cells. The genes for two related secreted Semaphorins, SEMA3F and SEMA3B, are located on 3p21.3. Because Semaphorins and VEGF-bind antagonistically to Neuropilins, the loss of *semaphorin* genes is likely to facilitate angiogenesis. The levels of SEMA3F correlate with stage and histologic subtypes, with more aggressive tumors showing increased VEGF and decreased SEMA3F [Roche and Drabkin 2001].

Paraneoplastic manifestations. Small cell lung cancers are frequently the source of neuromuscular autoimmune paraneoplastic syndromes. In the order of frequency, the IgG markers for neurological autoimmunity related to small cell carcinoma include ANNA-1 (HU), CRMP-5 (Collapsin Response Mediator Protein-5), Amphiphysin, SCA-2 (Purkinje Cell Cytoplasmic Antibody-2), ANNA-2 (RI), ANNA-3 [Chan et al. 2001].

The syndrome of inappropriate Antidiuretic Hormone (SIADH) can occur in small cell lung cancer. It is characterized by hyponatremia, low serum osmolality, and excessively high urine osmolality, heart failure, cirrhosis, adrenal insufficiency, and hypothyroidism. SIADH may be caused by Vasopressin, secreted by the tumor cells.

Small cell lung cancers and bronchial carcinoids often display corticotroph markers. They may lead to ectopic ACTH syndromes. Bombesin is secreted as an autocrine factor by small cell carcinoma of the lung, a tumor likely to be of Kultchitsky cell origin. It results in clonal proliferation. The syndromes of inappropriate secretion of Antidiuretic Hormone and Cushing's syndrome, occurring with small cell lung cancer, are due to the ectopic production of ADH and ACTH, respectively. Basic helix–loop–helix (bHLH) transcription factors of the MASH family are regulators of development in the neural crest. MASH-1 (ASCL-1, ASH-1) is highly expressed in small cell lung cance and may be a marker of neuroendocrine features.

Nonbacterial thrombotic endocarditis (NBTE) consists of sterile vegetations, composed of platelets and Fibrin, on the heart valves. It is highly associated with malignant tumors, mostly adenocarcinomata and lung cancer. Emboli released from the heart valves may affect the spleen, the kidneys, the extremities, the central nervous system, and the coronary arteries.

Genetic predisposition. Between 3% and 6% of lung cancer, patients have a family history of the disease [Tomizawa et al. 1998; Bromen et al. 2000]. Genetic predisposition constitutes a risk factor for lung cancer [Tokuhata and Lilienfeld 1963] with a susceptibility locus mapping to chromosome 6p [Bailey-Wilson et al. 2004].

Several genetic factors determine the susceptibility to lung cancer. Phase I metabolizing enzymes, including the Cytochrome P450 group, oxidize a wide range of substrates, potentially resulting in highly active procarcinogens. Phase II enzymes, including the Glutathione Transferases, play central roles in the detoxification of electrophilic intermediates. Their genes display multiple polymorphisms.

- Polymorphisms of *cyp1A1*, *cyp2E1*, and *cyp2D6* determine the predisposition to lung cancer. Major classes of carcinogens present in tobacco and tobacco smoke are converted into DNA-reactive metabolites by Cytochrome P450-related enzymes. CYP1A1 and GSTM1 play a major role in the metabolic activation and detoxification of polycyclic aromatic hydrocarbons, respectively, and CYP2E1 plays a major role in the metabolic activation of nitrosamines [Le Marchand et al. 1998; Bartsch et al. 2000].
- Microsomal Epoxide Hydroxylase (MEH, EPHX) is a phase I enzyme with roles in the metabolism of environmental carcinogens [Zhou et al. 2001], including those derived from tobacco. Polymorphisms in exons 3 and 4 of the gene are associated with variations in the resulting enzyme activity. There is a significant association between the MEH enzyme activity and the risk for lung cancer [Benhamou et al. 1998].

– Polymorphisms in *gstM1* and *gstP1*, but not *gstT1*, are associated with the risk for small cell lung cance and squamous cell carcinomata. A reduced activity of GSTM1 represents a factor of increased susceptibility. A polymorphism I105V in exon 5 of GSTP1 results in significantly lower enzyme activity among individuals with the 105 valine allele [Malats et al. 2000; Watson et al. 1998].

Noncarcinoma lung cancer. Bronchial adenomata are benign tumors that are typically centrally located and slowly growing. They may lead to bronchial obstruction, atelectasias, and recurrent pneumonias. These growths can become malignant.

Alveolar soft tissue sarcomata typically occur on the basis of specific chromosomal translocations. They often involve the MITF/TFE subfamily of transcription factors, and some share the underlying genetic defect with childhood renal cancers. TFE3 is a member of the basic helix-loop-helix (bHLH) family of transcription factors with the ability to bind to μE3 elements in the *immunoglobin heavy chain* intronic enhancer. The translocation t(X;1)(p11.2;q21.2) results in the fusion of *prcc* (*papillary renal cell carcinoma*) at 1q21.2 to the *tfe3* gene at Xp11.2. Through this fusion, reciprocal translocation products are formed. PRCC is ubiquitously expressed in normal adult and fetal tissues and encodes a protein of 491 amino acids with a relatively high content of prolines. The fusion of the NH_2-terminal region of the PRCC protein, which includes the proline-rich domain, to the entire TFE3 protein is accompanied by the complete loss of normal *tfe3* transcripts. The resulting gene fusion generates excessively active transcriptional activators, which are sufficient to cause transformation. The mitotic checkpoint protein MAD-2B interacts with PRCC. The PRCC–TFE3 fusion protein retains the MAD2B-binding domain, but their functional interaction is impaired [Weterman et al. 2001b]. Renal carcinomata associated with the t(X;17)(p11.2;q25), fusing *aspl* witrh *tfe3*, share this defect with a subset of alveolar soft tissue sarcomata, with the distinction that the translocation is balanced in renal cancers, while it is der(17)t(X;17)(p11.2, q25) in alveolar soft tissue sarcoma [Argani et al. 2001].

Benign tumors of the mesenchymal tissue in the lungs are most frequently chondromata. They are encapsulated cartilaginous neoplasms with a lobular growing pattern. The transformed chondrocytes resemble normal cells and produce the cartilaginous matrix consisting of amorphous basophilic material.

Bronchial carcinoid tumors are malignant tumors that represent 1–2% of all lung cancers. They arise from the neurosecretory cells of the bronchial mucosa and secrete serotonin.

– Typical carcinoid tumors are low-grade growths, with good prognosis. They are commonly located centrally within the major bronchi. Typical carcinoid tumors are capable of local invasion, including invasion of local lymph nodes, but they rarely form distant metastases.
– Atypical carcinoids are aggressive, with 5-year survival rates of 25–70%. Atypical carcinoids tend to arise from the peripheral and central bronchi with equal frequency.

Carcinoid tumors cause lobar obstruction, hemoptysis, dyspnea, cough, and lobar pneumonia. Both types of carcinoid tumors of the lung can occur without gender preference in patients of any age.

Cylindromata (adenoid cystic carcinomata, adenocycst, AdCCs) are aggressive tumors that metastasize into the pleura.

15.2 SKIN CANCER

The major carcinogenic agent for most skin cancers is solar UV light. It is absorbed by DNA with the formation of UV-specific dipyrimidine photoproducts. It causes DNA damage via two distinct mutational mechanisms.

– Cyclobutane pyrimidine dimers and 6-4 photoproducts are precursors to the C→T and CC→TT transitions that are the classic UV signature mutations.
– UV exposure also generates reactive oxygen intermediates, which causes replicative errors and base substitutions [Jhappan et al. 2003].

These lesions can be repaired by nucleotide excision repair or replicated by low fidelity class-Y Polymerases. Insufficient repair, followed by errors in reduplication produces the characteristic mutations in dipyrimidine sequences that may represent initiation events in carcinogenesis. Chronic exposure to Ultraviolet B results in the disruption of the epithelial structure and the expansion of premalignant clones, which undergo further genomic changes leading to full malignancy.

Pigmentary traits, including red hair, fair skin, and propensity to freckle (RHC phenotype) are genetic risk factors for skin cancers when combined with high UV light exposure. The Melanocortin-1 Receptor (MC1R) is a key determinant of the pigmentation process. Its coding sequence is highly polymorphic. The MC1R alleles R151C, R160W, and D294H are associated with increased risk for all forms of skin cancer. They also correlate with penetrance and age of onset in familial melanoma. There is a significant allele heterozygote carrier effect on skin phototype and skin cancer risk, which indicates that the variant alleles are not strictly recessive [Sturm 2002].

HPV is a double-stranded DNA virus in the family Papovaviridae. Premalignant lesions of the anogenital epithelium, such as Bowenoid papulosis, Buschke-Loewenstein tumors, and condylomata acuminata, are closely linked etiologically to infection with certain types of HPV (HPV 6, 11, 16, 18, and less commonly, 31, 33, 35, and 45) [Gross et al. 1985; Rüdlinger et al. 1989]. HPV is also associated with oncogenicity in epidermodysplasia verruciformis (HPV 5, 8, 9, 10, 12, 14, 15, 17, 19–29) and periungual squamous cell carcinomata (HPV16) [Moy et al. 1989]. The presence of arginine at codon 72 in P53 is a genetic risk factor in HPV-related carcinogenesis. Homozygosity for arginine at this polymorphic site is associated with the development of skin malignancies in epidermodysplasia veruciformis [De Oliveira et al. 2004].

15.2.1 Melanoma

While melanoma only accounts for about 6% of all invasive skin cancer cases, it is responsible for 75% of all skin cancer deaths. Over the last 25 years, the incidence of melanoma has increased at a rate of 3% per year.

Although originating in part of the skin or the retina, melanomata are strictly speaking not carcinomata (epithelial cancers), because melanocytes are derived from the neural crest. The majority of malignant melanomata fall into four groups:

– Malignant melanoma of the superficial-spreading type
– Malignant melanoma of the lentigo-maligna type
– Malignant melanoma of the nodular type
– Malignant melanoma of the eye

Primary malignant melanomas of the superficial-spreading type and of the lentigo-maligna type develop through a characteristic biphasic growth pattern. The initial radial growth phase of these melanomata is only rarely associated with the development of metastases, while the ensuing vertical growth phase is commonly associated with subsequent metastatic disease [Clark et al. 1975].

Transforming signaling pathways. Melanomata have activating mutations in signaling pathways that mediate proliferation in melanocyte development. Metastatic melanoma cells are usually highly aneuploid.

raf somatic mutations arise in about 65% of melanomata. Almost all of them contain the transversion of thymidine 1,796 to adenine [Davies et al. 2002], encoding the alteration V599E. This mutation may mimic the phosphorylation of the activation segment by insertion of an acidic residue close to a site of regulated phosphorylation at serine 598. It thus initiates a proliferative signal through B-RAF and ERK and represents a gain of function in a pathway that is physiologically engaged by α-Melanocyte Stimulating Hormone.

Epigenetic inactivation of the RAS effector and potential tumor suppressor *ras association domain family protein 1* (*rassf1*) {3p21.3} also occurs in malignant melanoma. Hypermethylation in two susceptible domains of the CpG island promoter region of a major alternative transcript of this gene, *rassf1A*, plays a key role in pathogenesis. This hypermethylation, upstream and within exon 1α, is associated with about half of all melanomata [Spugnardi et al. 2003].

APC is important for the maintenance of chromosome stability. Defects in APC-interacting molecules in melanoma may account for aneuploidy of the tumors. Melanoma is among the cancers with β-Catenin abnormalities, albeit at low frequency. Although detectable mutations are rare, nuclear localization of the protein occurs in about 30% of the cases. Thus, additional mechanisms for β-Catenin activation occur in these tumors. They include the WNT pathway components APC, ICAT, and LEF-1. β-Catenin can induce *myc* and *cyclin* D_1, as well as the lineage-specific transcription factor genes *brn-2*, *mitf-M*, and *dct*. MITF plays a critical role in melanocyte survival. BRN-2 is involved in melanoma proliferation.

APC suppresses RB phosphorylation and reduces the levels of Cyclin D_1. This inhibits G_1/S progression.

Inactivation of the RB-interacting zinc finger protein RIZ1 (PR-Domain Containing Protein 2, PRDM2) commonly occurs in melanoma.

The prevention of apoptosis plays a role in melanocyte transformation. Untransformed melanocytes respond to the c-KIT ligand Mast Cell Growth Factor (MGF) with tyrosine phosphorylation of c-KIT and its downstream targets, including ERK and MITF. *bcl*-2 is a MITF-dependent KIT transcriptional target in melanocytes. A lack of MITF would trigger profound apoptosis due to ensuing lack of BCL-2 expression. In melanoma cells, the expression of *mitf* and *bcl-2* is tightly linked [McGill et al. 2002].

IAPs (Inhibitors of Apoptosis) are a family of antiapoptotic signaling intermediates. They are characterized by the presence of at least one baculovirus IAP repeat (BIR) motif that is essential for their activity. IAPs function as sensors and inhibitors of death signals that emanate from a variety of pathways. ML-IAP (Livin, KIAP, BIRC7) [Vucic et al. 2000] is expressed at high levels in melanoma cells, but not in primary melanocytes. Melanoma cells that express ML-IAP are significantly more resistant to apoptosis than those that do not express ML-IAP.

A soluble form of CD95 (sCD95) can act as a decoy receptor and protect malignancies from cell death. High serum levels of sCD95 are associated with poor prognosis in melanoma patients.

Uveal melanoma is the most common primary intraocular malignancy. Its incidence is higher in lightly pigmented individuals than in dark-skinned persons. A predisposition is associated with xeroderma pigmentosum. Another predisposing condition is oculodermal melanocytosis (nevus of Ota), a developmental condition in which the ocular surface and the uveal tract are hyperpigmented. The orbit and meninges can also be involved. Uveal melanoma initially grows flatly within the choroid, later it elevates Bruch's membrane and then ruptures it. At that stage, the tumor assumes a characteristic shape, resembling a mushroom, as it grows toward the vitreous humor. This causes elevation and detachment of the retina (solid retinal detachment). Uveal melanoma may metastasize to distant sites including liver, lungs, bones, kidneys, and brain.

Susceptibility to this tumor is associated with mutations in chromosomes 3q and 3p [Tschentscher et al. 2001]. The expression of IGF1R (IGF-1 Receptor) contributes to cell growth in uveal melanoma. It is also related to the incidence of death due to metastatic disease. Physiologically, IGF1R is produced mainly in the liver, the preferential site for uveal melanoma metastases [All-Ericsson et al. 2002].

Metastasis. Melanoma forms frequent metastases in brain, spinal cord, lungs, liver, lymph nodes, and skin. Often, the first clinical signs of the cancer are generated by the metastases. Melanoma is a malignancy with one of the highest frequencies of brain metastases. At autopsy, 70–80% of melanomata have metastatic deposits in the central nervous system. If the brain is colonized, there is often a rapid decline in the quality of life and ensuing death. Astrocytes may provide paracrine signals that attract melanoma cells. In addition, the melanoma cells activate distinctive genetic programs that contribute to dissemination.

Specific homing receptors are involved in melanoma metastasis. The expression of Mannose Receptors on hepatic sinusoidal endothelium facilitates the adhesion of melanoma cells through oligosaccharides on their surface and leads to increased liver metastasis. Inflammation can enhance this process through the production of IL-1, which mediates an increase in the expression of Mannose Receptors on the endothelium.

Neurotrophins are a family of small, highly basic homodimers that are produced in the brain at relatively high levels. Neurotrophins are critical in brain metastatic steps, including invasion and survival. Their effects are mediated through two classes of receptors, both of which are present on the surface of metastatic melanoma. The Neurotrophin Receptors are abundant at the invasion front of melanoma brain metastases, while Neurotrophins are increased in the surrounding tissue. Melanoma cells express the low affinity Neurotrophin Receptor $P75^{NTR}$, with their invasive properties being regulated by the ligand NGF (Nerve Growth Factor). They also express TRK-C, the receptor for the invasion-promoting Neurotrophin-3 (NT-3, NTF-3). Neurotrophin signaling stimulates the activation of the nonreceptor tyrosine kinase YES, but not SRC, in brain metastatic melanoma cells. In melanoma cells metastasizing to the brain, Neurotrophins promote invasion by enhancing the production of extracellular matrix-degrading enzymes, such as Heparanase [Denkins et al. 2004].

CD44 forms that bear heparin sulfate chains can bind to HGF (Scatter Factor, SCF) and facilitate its presentation to c-MET. The ligation of c-MET, in turn, mediates the up-regulation of CD44v6 on melanoma cells. This is accomplished, in part, through transcriptional regulation of the immediate early gene *egr-1*. EGR-1 then binds to the *cd44* promoter, facilitating its activation.

Changes in the expression of cell surface adhesion molecules are associated with melanoma progression from in situ to invasive to metastatic tumors. They include the up-regulation of the Integrins $\alpha_V\beta_3$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_5\beta_1$, as well as the down-regulation of $\alpha_6\beta_1$.

The proteins RHO-C, Fibronectin, and Thymosin β4 are needed for melanoma metastasis. The small GTP hydrolyzing protein RHO-C contributes to adhesion site turnover and to the function of the contractile Actomyosin. Thymosin β4 is involved in lamellopodia extensions.

Melanoma metastasis to the central nervous system requires the penetration through the blood/brain barrier. The Endo-β-D-Glucuronidase Heparanase is a determinant for the successful degradation of heparan sulfate proteoglycans in the basement membrane of this barrier. The enzyme recognizes specific motifs within heparan sulfate chains, which are then cleaved. Heparanase also releases heparan sulfate-bound angiogenic factors at the target site, thus facilitating the outgrowth of metastases. The secretion of Heparanase is up-regulated by Neurotrophin signaling. Beside Heparanase, metastatic melanoma cells express high levels of type IV Collagenases, Cathepsins, and Plasminogen Activator.

In melanoma metastasis, transcription factors that regulate invasiveness are affected. Progression of melanoma is associated with a loss of expression of the transcription factor AP-2. In metastatic melanoma cells, this loss results in the overexpression of M-CAM and MMP-2, and the lack of expression of c-KIT [Nyormoi and Bar-Eli 2003]. Loss of AP-2 expression in metastatic melanoma cells results in the overproduction of the Thrombin Receptor PAR-1.

Additionally, the transition of melanoma cells from radial growth phase to vertical growth phase (a marker of progression) is associated with the overexpression of two transcription factors, CREB and ATF-1, both of which may act as survival factors during dissemination.

The down-regulation of metastasis suppressor genes is a factor in melanoma dissemination. *kiss1* is a melanoma metastasis suppressor gene located on chromosome 1q32–q41. Its expression may be regulated by putative tumor suppressor genes mapping to chromosome 6q16.3–q23. Translocations or deletions involving the long arm of chromosome 6 (6q) occur in more than 80% of metastatic melanomata. Loss of heterozygosity of 6q16.3–q23 in melanoma is frequently associated with loss of *kiss1* RNA expression [Shirasaki et al. 2001] and tumor invasiveness.

BRMS-1 acts as a metastasis suppressor gene product in melanoma. *brms1* mRNA expression is high in melanocytes, reduced in early stage melanoma, and barely detectable in advanced or metastatic melanoma cells.

Inherited melanoma syndromes. Certain heritable malignant and premalignant conditions are characterized by an increased risk of developing primary melanoma, a higher incidence of multiple primary melanomata, and an earlier age at onset. Heritable malignant melanoma comprises about 5% of malignant melanoma cases.

– The incidence of cutaneous malignant melanoma (CMM, dysplastic nevus syndrome) may cluster in families. In a proportion of the families with melanoma, the susceptibility is determined by defects in high penetrance genes. The most common one of these is *cdkn2A* (*cyclin-dependent kinase inhibitor 2A*), located at 9p21, which codes for the cell cycle control and senescence protein $P16^{INK4a}$. Around 55% of families with four or more melanoma cases have *cdkn2A* mutations, but only 15% of families with two or three cases. Families carrying mutations in this gene are located in Europe, the United States, and Australia. It is estimated that the penetrance in *cdkn2A* is higher in geographic areas with higher levels of UV exposure, such as Australia. Dysplastic nevi are generally larger than ordinary moles and have irregular and indistinct borders. Their color frequently is not uniform and ranges from pink to dark brown. They usually are flat, but parts may be raised above the skin surface.
– Inactivating germline point mutations in *cdkn2A*, encoding the tumor suppressors $P16^{INK4a}$ and

$P14^{ARF}$, predispose to the familial atypical multiple mole melanoma (FAMMM) syndrome. The mutations cause the amino acid substitutions M53V, M53I, G101W, G122V, or V126D. Familial atypical multiple mole melanoma-pancreatic carcinoma (FAMMPC) syndrome can also be caused by mutations in *cdkn2A*. Another, less common high penetrance gene in familial melanoma is *cdk4*. Clustering may also occur in families as a result of low penetrance genes such as *mc1R* and possibly polymorphisms in *egf* [Newton-Bishop 2003].

- Xeroderma pigmentosum (XP) involves DNA repair and replication deficiencies that predispose homozygous individuals to a 1,000-fold increased incidence of nonmelanoma and melanoma skin cancers. Melanoma in children or adolescents is rare. In a small fraction of these, predisposing conditions, including xeroderma pigmentosum or giant congenital melanocytic naevi, exist.
- Patients with nevoid basal cell carcinoma have an increased risk of developing melanoma.
- Melanoma may occur as a second primary tumor on the basis of retinoma or retinoblastoma [Korswagen et al. 2004].
- Neurocutaneous melanosis is a rare condition, associated with skin and meningeal pigmentation. The brains of patients have intraparenchymal melanin deposits, but no leptomeningeal abnormality. The disease is potentially highly malignant, and death usually occurs in early childhood.

15.2.2 Squamous Cell Carcinoma

Squamous cell carcinomata account for about 20% of nonmelanoma skin cancers. They usually develop in the epithelial layer of the skin, but may in rare cases originate in various mucous membranes of the body. Typical locations include the skin, the lips, inside the mouth, inside the throat, or in the esophagus. Squamous cell carcinomata are characterized by red, scaly skin that becomes an open sore (Figure 15.2.2.A). They are able to form distal metastases. Actinic keratosis is a precursor lesion of squamous cell carcinoma.

Molecular pathogenesis. Squamous carcinoma cells have a greatly increased number of EGFRs on their surfaces as compared with normal keratinocytes. Mutational loss of P16 and P53 are frequent early events in the development of squamous cell carcinomata. These mutations endow keratinocytes with extended replicative potential and predispose to neoplastic transformation. Cyclooxygenases contribute to skin carcinogenesis. Deficiency of the

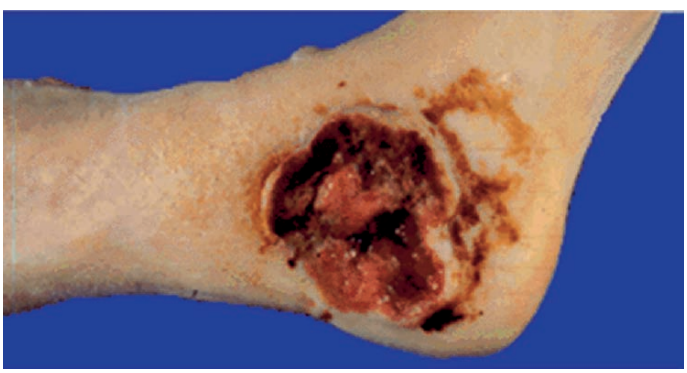


Figure 15.2.2.A. Squamous cell carcinoma (SCC). The picture depicts a large, ulcerating SCC at the ankle. A nonhealing ulcer with raised edges is the usual presentation of squamous carcinoma of skin. These are growth with a distinct tendency to be both locally invasive and to spread primarily to local lymph nodes and from there to other regions of the body. Squamous cell carcinomata of the skin can be slowly growing, relatively indolent tumors, or somewhat more rapidly growing tumors. There is a correlation between the prolonged exposure to UV radiation and incidence of SCC. It is particularly common in white-skinned individuals exposed to sunlight, as is the case in Australia. In addition, inherited disorders resulting in DNA repair defects, such as xeroderma pigmentosum, result in an increased incidence. Chronic underlying inflammation, such as a sinus draining into the skin surface, has also been associated with an increased frequency of SCC. UV radiation directly damages DNA. It also directly affects Langerhans cell activity (epidermal antigen presenting cells). HPV may play a role in carcinogenesis. Chemical agents can create DNA adducts. Squamous cell carcinomata represent 20% of nonpigmented malignancies of the skin, second only to basal cell carcinoma. They are somewhat more common in men than in women. [Reproduced from http://pathweb.uchc.edu. With permission.]

cox-1 or *cox-2* genes reduces the susceptibility to skin tumorigenesis. This is likely due to a premature onset of keratinocyte terminal differentiation.

– Keratoacanthomata are rapidly growing benign epithelial-derived neoplasms that may evolve into squamous cell carcinomata. During keratoacanthoma progression, there is an increase in the expression of VCAM (Vascular Cell Adhesion Molecule). Increased expression of VCAM and ICAM (Intercellular Adhesion Molecule) is concomitant with squamous cell carcinoma dedifferentiation [Melendez et al. 2003].
– Retinoids exert significant preventive and therapeutic effects against certain cancers. A retinoic acid-regulated gene product from oral squamous carcinoma is RAIG1 (Retinoic Acid Inducible Gene 1, RAI3). Its expression is rapidly and dose-dependently induced by all-trans-retinoic acid. *raig1* is expressed in several normal tissues, with the highest expression levels in fetal and adult lung. 2 *raig1* transcripts of 2.4 and 6.8 kb likely result from the alternative use of different polyadenylation sites. RAIG1 is predicted to be a 357 amino acid protein with a calculated molecular mass of 40.3 kD. It contains seven transmembrane domains, which is a signature motif of the G-Protein-Coupled Receptor superfamily, and a potential *N*-linked glycosylation site [Cheng and Lotan 1998]. RAIG1 may protect from transformation by supporting cell differentiation. Oral squamous cell carcinomata with lymph node metastasis exhibit increased expression levels of *mmp-1*, *mmp-3*, *upa*, *integrin α3*, *paxillin*, *tenascin-C*, and *il-6* transcripts as compared to carcinomata without lymph node metastasis. The elevated levels of MMP-1, MMP-3, and UPA originate in components of the neoplastic stroma, particularly in mononuclear eosinophilic inflammatory cells [Nagata et al. 2003].
– Squamous carcinoma of the anal canal is a relatively uncommon tumor. Most of these carcinomata develop from squamous epithelium, but some arise from the transitional zone between the columnar epithelium of the rectum and the squamous epithelium of the anal canal. The latter type is called cloacogenic carcinoma (transitional carcinoma). Anal canal cancers have chromosomal abnormalities. They include frequent rearrangements involving the long arm of chromosome 11 (del11q22-qter) or rearrangements of chromosome 3 (del 3p22) [Muleris et al. 1987].
– Epidermolysis bullosa is characterized by fragile skin with frequent blistering. Dystrophic epidermolysis bullosa predisposes to highly aggressive metastatic squamous cell carcinoma. This recessive disease is caused by mutations in the *collagen VII α chain* (*col7A1*) {3p21.3}, which results in the deposition of its NC1 fragment. Anchorage of the epidermis to the dermis depends on the interaction of Laminin-5 with Fibronectin III-like repeats within the NC1 domain of Collagen VII. Keratinocytes from patients that carry mutations, which abrogate the deposition of Collagen VII do not develop invasive squamous cell carcinoma. Collagen VII has a central glycine-rich, triple helical domain, with noncollagenous domains at its ends. The NH_2-terminal noncollagenous NC1 domain is essential for the development of invasiveness by squamous cell carcinoma.

Metastasis. Tetraspanins are important metastasis suppressor molecules in squamous neoplasms. Several Tetraspanin family members are involved in metastasis suppression, an effect that may be related to their association with β_1 Integrins. The Integrin $\alpha_3\beta_1$ can suppress the malignant conversion of epidermal squamous cell papillomata to carcinomata through a mechanism that may entail CD81. In squamous cell carcinoma of the head and neck, reduced CD9 expression is associated with high grade and low disease-free survival. CD82 inhibits metastasis formation by squamous neoplasms.

Genetic predisposition. Howel–Evans syndrome (inherited palmoplantar keratoderma) is associated with an increased risk for squamous cell carcinoma. While skin changes manifest during childhood, cancer may appear decades later. The cancer risk is likely based on the involvement of genes that are closely linked to the genetic defect responsible for the palmoplantar keratoderma.

15.2.3 Basal Cell Carcinoma

Basal cell carcinoma (basalioma, BCC) is a locally invasive tumor that rarely forms distal metastases (Figure 15.2.3.A). It is composed of cells that have the appearance of the basal layer of the epidermis and a characteristic stroma. Basal cell carcinoma tends to grow slowly and invade locally, eventually leading to ulceration. It most frequently occurs on sun-exposed areas, such as the face and upper trunk, and is rare on

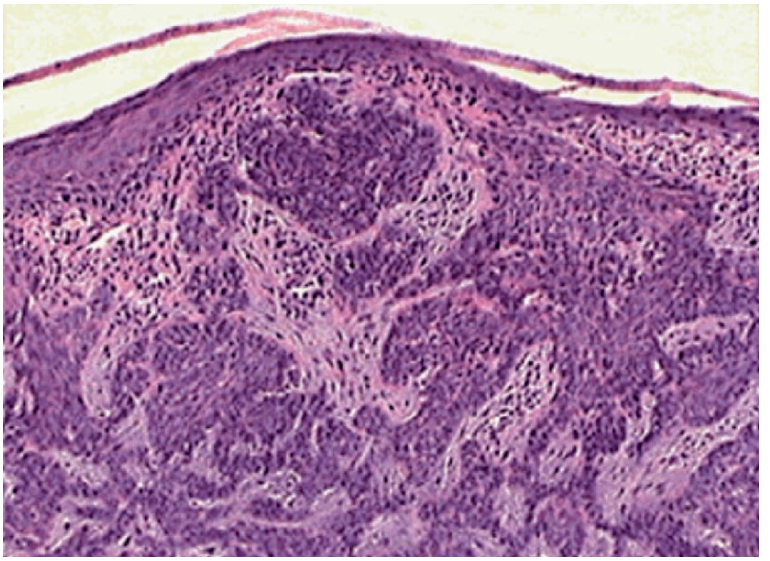


Figure 15.2.3.A. Basal cell carcinoma. Shown are multiple small, irregularly shaped islands of proliferating basal cells with a tendency to form finger-like projections in all directions. The lesions are lined by a single layer of basal cells around the periphery with a distinct tendency to palisading (cells in the periphery line up with their nuclei with the long access perpendicular to the basement membrane and approximately parallel to each other). The small dark staining cuboidal basal cells with vesicular nuclei and basophilic cytoplasm comprise the rest of this lesion. The presence of these cuboidal cells that resemble the normal basal cells of stratified squamous epithelium is a distinctive feature of a basal cell carcinoma. A distinction from squamous cell carcinomata is the absence of a stratum spinosum (spiny cell layer). Macroscopically, the neoplasm consists of pearly white nodules of varying size, often with a central area of ulceration and telangiectatic blood vessels around them. Basal cell carcinomata are common growths particularly in older light-skinned individuals, which arise due to sun exposure causing direct damage to DNA by UV radiation. Basal cell carcinomata occur mostly on the face and are more frequent in men in most locations, except on the legs where they occur more frequently in women. They are slowly growing tumors that can be locally invasive and destructive, but usually do not metastasize to distant sites and are nonlethal. However, if treatment is delayed of a basalioma around the eye or ear, it may invade into the brain. [Reproduced from http://pathweb.uchc.edu. With permission.]

the palms and soles. Basal cell carcinoma has the histologic appearance of proliferating undifferentiated basaloid keratinocytes, which may reflect the expansion of abnormally differentiated stem cells.

Virtually all basal cell carcinomata have dysregulated SHH signaling. SHH pathway signaling is important in hair growth. Its lack results in arrested hair follicle development. The uncontrolled activation of the SHH pathway can induce follicular tumors [Oro et al. 1997]. A high incidence of basal cell carcinomata around a nuclear testing site in Kazakhstan may be associated with loss of heterozygosity of chromosome 9q22.3. This region contains the basal cell carcinoma susceptibility genes *patched* (*ptch*) and *xeroderma pigmentosa group A complementing gene* (*xpa*) [Iwata et al. 2004]. Mutations of *p53* are present in about half of all cases [Rady et al. 1992]. Reductions in E-Cadherin and BCL-2 occur in the aggressive, infiltrative type of basal cell carcinoma.

Basal cell carcinoma is the least differentiated form in a spectrum of follicular tumors, including trichoblastomata, trichoepitheliomata, and cylindromata. Trichoepitheliomata shows a greater degree of HH pathway target gene induction than basal cell carcinomata [Vorechovsky et al. 1997]. Cylindromata are associated with mutations in *cylindromatosis-1* (*cyld1*) {16q12–q13}, distinct from the HH pathway [Bignell et al. 2000].

Hereditary basal cell carcinoma. Basal cell nevus syndrome (Gorlin syndrome, Gorlin–Goltz syndrome) is an inherited multisystem disorder, in which multiple basal cell carcinomata are associated with palmoplantar pits, characteristic facies, and a variety of skeletal, soft tissue, ocular, neurologic, and endocrine abnormalities. The condition predisposes to basal cell carcinoma, medulloblastoma, and rhabdomyosarcoma. The inheritance is autosomal dominant, with variable penetrance. The underlying genetic defect is a loss of the tumor suppressor *patched* (*ptc*) {9q22.3} [Hahn et al. 1996; Johnson et al. 1996].

15.2.4 Other Tumors

Dermatofibrosarcoma protuberans is a rare, slowly growing, infiltrating dermal neoplasm of intermediate malignancy, made up of spindle-shaped tumor cells that are often positive for CD34. It is subdivided into
– Classical dermatofibrosarcoma protuberans
– Giant cell fibroblastoma
– Bednar tumor
– Adult superficial fibrosarcoma
– The granular cell variant of dermatofibrosarcoma protuberans.

Cytogenetically, the cells are characterized by either supernumerary ring chromosomes, which are derived from chromosome 22 and contain low level-amplified sequences from 17q22-qter and 22q10–q13.1, or t(17;22), that are most often unbalanced. Both the rings and linear der(22) contain a specific fusion of *col1A1* with *pdgfB*, which is occasionally cryptic, associated with complex chromosomal rearrangements. The breakpoint localization in *pdgfB* is very constant, placing exon 2 under the control of the *col1A1* promoter. In contrast, the *col1A1* breakpoint is variably located within the exons of the α-helical coding region (exons 6–49). PDGF-B may act as a mitogen by autocrine stimulation of the PDGFR [Sirvent et al. 2003].

Merkel cells are nondendritic, nonkeratinocytic epithelial clear cells, normally located in the epidermis and dermis. They are of likely neuroendocrine origin and function as specific slowly adapting sensory touch receptors. Merkel cell carcinoma [Toker 1972] is a high-grade malignant tumor, with a strong tendency for local recurrence. Lymph node metastasis is frequent and visceral metastasis is common. This neoplasm shares clinical, histopathological, and genetic features with small cell lung carcinoma. Merkel cell carcinomata may be characterized by a loss of RB {13q14.1–q14.2}. Loss of heterozygosity on chromosome 1p occurs frequently and several tumor suppressor genes on 1p are likely to play a role in the development of this tumor type. There is frequent allelic loss at 10q23, but low incidence of *pten* mutations. Merkel cell carcinomata are grouped into classic and variant tumors.

Pilomatricoma (pilomatrixoma, epithelioma calcificans of Malherbe) is a common, benign skin tumor. It can occur at any age and without a difference in risk between males and females. Pilomatricomata typically arise in the head and neck area. The tumors originate from the hair follicle matrix cells, whose development depends on β-Catenin signaling [Chan et al. 1999; van Genderen et al. 1994]. A high frequency of *ctnnβ1* (*β-catenin*) {3p22–p21.3} mutations occurs in pilomatricomata. Most of these tumors possess mutations affecting the NH_2-terminal segment of β-Catenin. This domain is normally involved in the phosphorylation-dependent, Ubiquitin-mediated degradation of the protein. Consistent with their cells of origin, nuclear LEF-1 is expressed in the dividing tumor cells.

Mutations in the tumor suppressor *cyld1* (*cylindromatosis gene 1*) {16q12–q13} predispose to cylindromata, which occur mostly in the head and neck area. The *cyld1* gene is composed of 20 exons, of which 3 in the 5′ portion are untranslated. It extends over approximately 56 kb of genomic DNA. The *cyld1* gene is expressed in fetal brain, testis, and skeletal muscle, and at a lower level in adult brain, leukocytes, liver, heart, kidney, spleen, ovary, and lung. Alternative splicing may delete the untranslated exons 3 and 7. The minus 7 exon splice variant is absent from the kidneys. The CYLD1 protein contains 956 amino acids, with the minus exon 7 splice variant encoding 953 amino acids. CYLD1 contains three CAP-GLY (cytoskeletal-associated protein-glycine-conserved) domains, which characterize proteins that coordinate the attachment of organelles to microtubules. It also has sequence homology to the catalytic domain of Ubiquitin COOH-terminal Hydrolases. *cyld-1* encodes a regulator of protein degradation in the NF-κB pathway [Biggs et al. 1996]. A familial predisposition can cause familial cylindromatosis (turban tumor syndrome) [Bignell et al. 2000].

Skin tumor predisposition syndromes. In certain familial predisposition syndromes, skin tumors may arise together with internal growths.
– Dyskeratosis congenita (DKC, Zinsser–Cole–Engeman syndrome, Hoyeraal–Hreidarsson syndrome) is a rare inherited disorder that is characterized by early death from bone marrow failure or pulmonary complications. The disease involves a multisystem failure, affecting predominantly tissues with a high proliferation rate: skin, mucous membranes, and bone marrow. The mucosal leucoplakia can transform into spinocellular carcinoma. Other carcinomata can develop during the third decade of life. The underlying defect

is a germline mutation of *dkc1* {Xq28}, which encodes Dyskerin, a 514 amino acid protein. Dyskerin is the nucleolar Pseudouridine Synthase component of the box H/ACA small nucleolar RNAs. DKC1 plays a role in ribosomal RNA synthesis and in ribosome biogenesis. It also interacts with the RNA component of Telomerase. Chromosome instability could be linked to increased telomere shortening due to an alteration in Telomerase-dependent telomere maintenance. Further, Dyskerin may be a centromere or microtubule protein and, if mutated, could give rise to abnormalities in chromosome segregation and consequent malignant predisposition.

– Muir–Torre syndrome represents the association of sebaceous skin tumors with internal malignancy. Approximately 15% of female patients with Muir–Torre syndrome develop endometrial cancer. The disease may be a manifestation of the Lynch type-2 cancer syndrome (cancer family syndrome), caused by mutations of *msh2* or *mlh1*.
– The Gardner syndrome, a variant of familial adenomatous polyposis (FAP), is a skin-polyposis syndrome. It comprises familial adenomatous polyposis disorders in conjunction with extracolonic abnormalities, such as epidermoid cysts and osteomata. Patients are at approximately 10–20% risk to develop mesenteric and intraperitoneal desmoid tumors, which is nearly 1,000-fold higher than the general population. Although desmoid tumors are benign, they are prone to recurrence. The underlying lesion is a defect within the *apc* gene.
– Peutz–Jegher syndrome is a skin-polyposis syndrome. It constitutes an autosomal dominant disorder characterized by melanocytic macules of the lips, buccal mucosa, and digits, multiple gastrointestinal hamartomatous polyps (especially of the upper jejunum), and an increased risk of various neoplasms. Approximately 50% of cases are due to mutations in the *stk11* (*serine/threonine kinase 11*) gene.
– Polyps of the stomach can occur in conjunction with the basal cell nevus syndrome.

15.3 COLORECTAL CANCER

The adult colon epithelium contains three differentiated cell types that arise from a multipotent stem cell, absorptive epithelial cells, enteroendocrine cells, and Goblet cells. Deviation from the normal maturation pathway during neoplastic transformation is likely to initiate in stem cells or their early descendants. Relatively undifferentiated tumors, which may have a higher proliferative potential, are often more aggressive than well-differentiated tumors. Intestinal cell differentiation is associated with G_1 arrest and suppression of CDK4 [Ding et al. 1998]. The expression of HLA-B and ATPase 6 is up-regulated, while the expression of Nucleophosmin (NPM) and Adenylosuccinate Lyase (ADSL) is down-regulated during differentiation. An approximately 900 bp message, termed *ict-1* (*immature colon carcinoma transcript-1*, *ds-1*, *digestion subtraction-1*), is more highly expressed in undifferentiated than in differentiated colon epithelial cells. The *ict1* transcript encodes a putative protein of 206 amino acids with a molecular mass of 24 kD. It is expressed in two forms of 25 and 20 kD. The major species of lower size may represent a processed form of the protein [van Belzen et al. 1995].

Multiple sequential genetic changes need to occur to ensure colorectal cancer evolution. Colorectal tumors arise from normal epithelium through increasingly more severe degrees of dysplasia, undergoing a sequence from aberrant crypt foci through adenomata to carcinomata. The Dukes classification of colorectal cancer defines groups A, B, and C, according to depth and extent of tumor invasion (Table 15.3.A).

Benign colon tumors. Benign colon tumors arise in three histopathologic forms, comprising adenomata,

Table 15.3.A. Dukes classification of colorectal cancer.

Dukes stage	TNM stage	Extent of invasion	5-Year survival rate (%)
A	T1N0M0 or T2N0M0	Mucosa	100
B1	T3N0M0	Muscularis propria	65
B2	T4N0M0	Serosa	50
C1	(Any T) N1M0	Muscularis propria and lymph nodes	40
C2	(Any T) N2M0 (Any T) N3M0	Serosa and lymph nodes	25
D	(Any T,N) M1	Distant metastases	5

hamartomata, and hyperplastic polyps (Figure 15.3.A). Pathogenetically, these tumors are distinct [Sweet et al. 2005].

– Adenomata may be initiated through germline mutations in *apc*. They undergo a progression to invasive cancers.
– Alternative routes are taken by hamartomatous polyps. Colonic hamartomata in adults are rare and tend to not become invasive. They are typically single, pedunculated, and localized predominantly in the rectosigmoid region.
– Serrated neoplasias (comprising hyperplastic polyps and sessile-serrated adenomata) may not have malignant potential. Hyperplastic polyps, often in the distal colon and rectum, arise in about 10% of people under the age of 50 and up to 50% of individuals between the ages of 50 and 70. Mutations in *smad4* may be involved. Serrated sessile adenomata have a greater propensity for the proximal colon. Activating somatic mutations of B-RAF, mostly V600E, are the initiating events in the progression from hyperplastic polyps to serrated sessile adenomata.

Molecular pathogenesis. Four basic pathogenetic groups of colon cancer comprise hereditary nonpolyposis colorectal cancers (HNPCC), which are characterized by sequence infidelity (microsatellite instability), cancers similar to familial adenomatous polyposis coli (FAP), where pathways associated with APC are defective and chromosome instability occurs, cancers caused by activating mutations of RAS, and cancers with the CpG island methylator phenotype (CIMP) (Figure 15.3.B). These mechanisms are not mutually exclusive and may amplify each other.

Some colorectal cancers are characterized by sequence infidelity (microsatellite instability). They are typically diploid, mucinous, high-grade tumors located in the proximal colon. In these tumors, germline loss-of-function mutations of the mismatch repair genes *msh2*, *mlh1*, *msh6*, and *pms2* are frequent. Consistently, sequence infidelity is a recessive trait. The major cause of *mlh1* inactivation is hypermethylation of tits promoter. MSH2 may be important in maintaining the integrity of

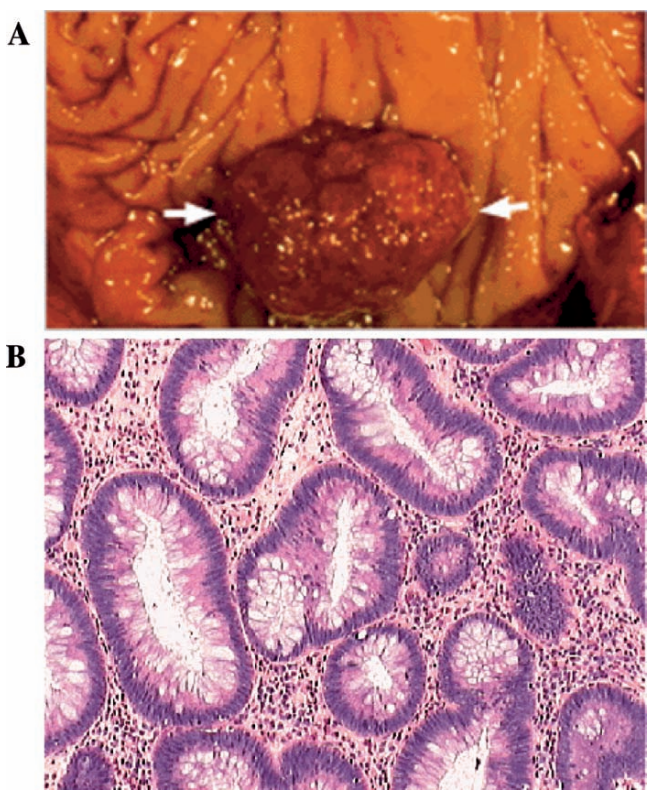


Figure 15.3.A. Colon adenoma. (A) The adenomatous polyp has a pink, cauliflower-like surface. The margins are very circumscribed, consistent with absence of invasion. (B) The glands are lined by dysplastic cells with crowded hyperchromatic nuclei. The gland outlines are intact without evidence of invasiveness. The pattern of round, gland-like spaces is called a tubular adenoma (adenomatous polyp). The dysplasia–adenoma–carcinoma sequence occurs in the setting of increasing loss of heterozygosity in genes involved in DNA repair and tumor suppression. Polyps are the manifestation of early stages of transformation. [Reproduced from http://pathweb.uchc.edu. With permission.]

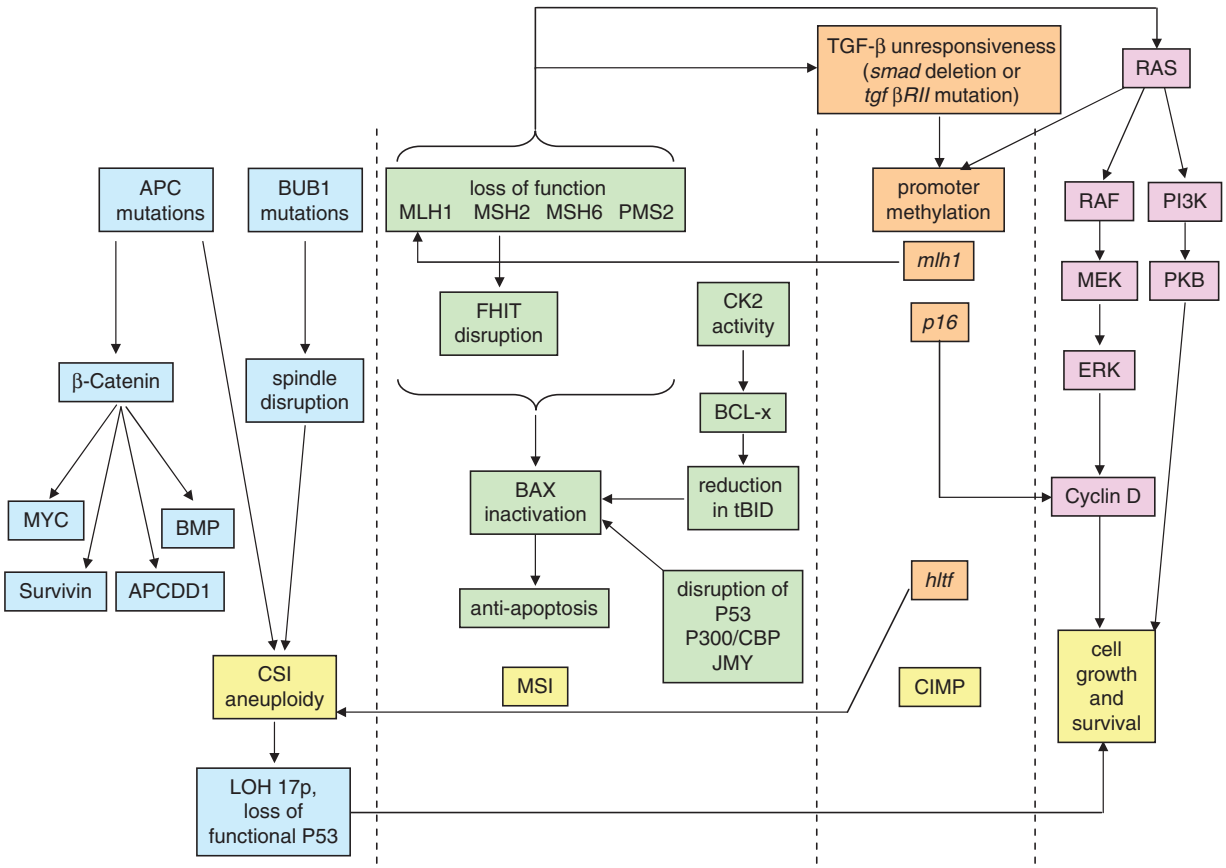


Figure 15.3.B. Molecular pathways associated with colon carcinogenesis. Four basic pathogenetic groups of colon cancer comprise those based on sequence infidelity (microsatellite instability), those where pathways associated with APC are defective and chromosome instability occurs, those caused by activating mutations of RAS, and those with the CpG island methylator phenotype. These mechanisms are not mutually exclusive and may amplify each other.

the common fragile locus associated with the *fhit* gene. Frequent loss of FHIT occurs in gastrointestinal carcinomata, and it can be a consequence of defects in MSH2. More than 50% of colon adenocarcinomata of the microsatellite mutator phenotype contain somatic frameshift mutations in an unstable tract of eight deoxyguanosines in the third exon within *bax*. Because these cancers do not normally contain mutations of P53 it is possible that loss-of-function mutations in BAX eliminate the selective pressure in regard to P53 inactivation. Loss of BAX, but not loss of P53, renders colorectal cancer cells completely resistant to TRAIL-induced apoptosis [Ravi and Bedi 2002]. BAK is expressed in normal gastrointestinal epithelium. Missense mutations in *bak* may occur in colorectal cancers. They are prevalent only in advanced stages of the disease.

To function efficiently as an activator of gene expression, P53 forms complexes with other transcriptional regulators, including P300/CBP. This interaction allows the acetylation of Histones that surround P53-binding sites and open up the chromatin. P300/CBP synergizes with JMY to enhance the P53-dependent transactivation of *bax*, which is important for apoptosis. Missense mutations in P300, and mutations that encode truncated P300, arise in colorectal primary tumors. In these cases, the second allele is frequently deleted, generating a loss of heterozygosity. Aberrant Histone acetylation, caused by the disruption of Histone Acetyl Transferase or Histone Deacetylase activity, may be associated with the development of colorectal cancer.

Many colorectal tumors without microsatellite instability exhibit a striking defect in chromosome segregation, resulting in gains or losses with every

cell division. This form of chromosomal instability reflects a continuing cellular dysfunction that persists throughout the lifetime of the tumor cell and is not simply related to chromosome number. Chromosomal instability has the characteristics of a dominant trait [Lengauer et al. 1997a,b]. It leads to aneuploidy, which is associated with 50–70% of colorectal cancers. Chromosome instability can arise through defects in pathways that depend on APC or BUB1. During mitosis, APC localizes to the ends of microtubules embedded in kinetochores and forms a complex with the checkpoint proteins BUB1 and BUB3. Malfunctions in these molecular interactions interfere with proper chromosome separation.

The gastrointestinal epithelium contains crypts and villi. Cell division occurs in the crypts and the cells gradually move up the crypt walls to the villi as they differentiate. WNT signaling maintains the crypt progenitor compartments and controls cell fate during the differentiation along the crypt-to-villus axis. The expression of the receptor tyrosine kinase MET is part of a genetic program controlled by the WNT pathway and it contributes to this maturation. In the normal intestinal epithelium, nuclear β-Catenin amounts are higher in the proliferative compartment at the base of the crypt than in the cells in the upper portions of the crypt. Conversely, cytoplasmic APC is markedly increased in the mature cells within the upper portions of the crypt. This expression pattern is in agreement with a role for β-Catenin signaling in the maintenance of stem cell properties and control of differentiation in the intestine. Mutations in the *apc* gene that produce a truncated polypeptide, which is unable to promote the degradation of β-Catenin, arise in up to 85% of sporadic colon cancers [Seeling et al. 1999]. These loss-of-function mutations of APC also lead to chromosomal instability when APC binding to the microtubules is compromised. This causes cells carrying a truncated *apc* gene to be defective in chromosome segregation [Kaplan et al. 2001].

The vast majority of *apc* (*adenomatous polyposis coli*) mutations occur in the mutation cluster region (exon 15, codons 1286–1513), and lead to COOH-terminal truncations of the APC protein. This results in a lack of Axin/Conductin-binding motifs, such as the SAMP repeats, and a lack of the variable number of 20 amino acid repeats that are associated with the down-regulation of intracellular β-Catenin. Normally, the APC/β-Catenin complex stimulates the breakdown of β-Catenin. Therefore, mutations that cause a loss of APC, or a loss of the portion of the APC protein that interacts with β-Catenin, can lead to a constitutive activation of TCF and unrestricted growth. This occurs through several mechanisms:

- MYC activation is central to the signal transduction through APC. The loss of *c-myc* regulation is common in carcinomata with deletions of the *apc* gene. *c-myc* is is up-regulated in about 30% of colorectal cancers, with amplification accounting for *c-myc* elevation in 25% of these tumors. Elevated expression of MYC is particularly associated with tumors of the distal colon.
- BMP4 is overexpressed and secreted by human cancer cells with mutant *apc* gene.
- The expression of *survivin* correlates inversely with the expression of APC. The gradual transformation of colorectal epithelium to carcinomata is associated with the progressive inhibition of apoptosis. Survivin is highly expressed in the majority of colorectal carcinomata, likely accounting for this phenomenon.
- The expression of *apcdd1* (*downregulated by apc1*) is directly regulated by the β-Catenin/TCF-4 complex. Elevated expression of APCDD1 promotes the proliferation of colonic epithelial cells and the molecule is frequently overexpressed in colorectal cancer.

apc mutations are typically accompanied by specific chromosomal defects. They include a complete loss of chromosome 18, a structural rearrangement of chromosome 17 leading most often to the loss of a short arm, and loss of part of 5q as reflected in loss of heterozygosity. Loss of chromosome 17p, nearly always associated with missense mutations in the remaining *p53* allele, occurs in about 75% of colon carcinomata, but infrequently in benign lesions, indicating that loss of *p53* is involved in late progression rather than in initiation [Fodde et al. 2001]. During the conversion of adenoma to early carcinoma in both familial adenomatous polyposis and in nonfamilial polyposis cases, both alleles of the *p53* gene are altered through mutation and loss of heterozygosity, which results in abnormal protein accumulation. Mutations in five hot spots (codons 175, 248, 282, 273, 245) account for over 40% of *p53* mutations in colorectal cancer. C→T transitions at CpG sites are the most prevalent mutations in the *p53* tumor suppressor gene in colon tumors and in the germline (Li–Fraumeni syndrome). All of the mutational hot spots are methylated to 5-methylcytosine,

which may be caused by spontaneous hydrolytic deamination of this base to thymine.

Expression of the NH_2-terminal region of the spindle checkpoint kinase BUB1, which contains the kinetochore association domain but lacks the checkpoint kinase domain, suppresses the spindle checkpoint. Mutations of *bub1* in a portion of colorectal cancers cause a dominant negative disruption of the spindle, leading to chromosome instability.

In over 40% of colon tumors, activation of *ras* oncogenes by point mutations (never *H-ras*, rarely *N-ras*, and most frequently *K-ras*) is causative. In *K-ras*, the activation typically occurs by a change in the coding properties of codons 12 or 13 [Delattre et al. 1989]. Codon 61 may also be affected, whereas amplifications or rearrangements do not typically occur. The frequency of *K-ras* mutations does not differ between proximal and distal cancers. Elevated levels of *ras* transcripts may be present in premalignant lesions, suggesting an early role for *ras* in transformation. A G12V (glycine to valine at codon 12) mutation of RAS is associated with aggressive disease and high risk of recurrence. FGFR signals through RAS. Activating mutations in the *fgfr3* gene can cause excessive RAS activity and are associated with colorectal cancer.

PI 3-K is a downstream target of RAS. Somatic mutations of *pik3ca* occur in colon cancer at a frequency around 30%. Somatic mutations of the gene for the P85α subunit (*pik3r1*), comprising deletions in the inter-SH2 region proximal to the S608 autoregulatory site, may contribute to colon cancers.

A common substitution polymorphism A870G in the *ccnd1* gene, which encodes Cyclin D_1, occurs in the conserved splice donor region of exon 4. It results in an altered mRNA transcript that encodes a protein with prolonged half-life. This allele is associated with colorectal cancer, and particularly with forms of the disease that result in severe morbidity and mortality [Le Marchand et al. 2003].

Protein phosphatases are negative regulators of kinase signaling. The *protein tyrosine phosphatase* superfamily of genes is composed of three main families.

- Classical receptor and nonreceptor tyrosine phosphatases
- Dual specificity phosphatases, which can dephosphorylate serine and threonine in addition to tyrosine
- Low-molecular weight phosphatases

A fraction of colon cancers is affected by nonsense, frameshift, or splice site mutations of protein tyrosine phosphatases *ptprf*, *ptprg*, *ptprt*, *ptpn3*, *ptpn13*, or *ptpn14*. They result in truncated proteins lacking phosphatase activity. These protein tyrosine phosphatases may normally act as tumor suppressor genes that negatively regulate growth factor-associated signal transduction [Wang et al. 2004]. Their loss of function predisposes to transformation.

Tumors of the CpG island methylator phenotype (CIMP) [Toyota et al. 2000] have a high degree of CpG island hypermethylation in tumor suppressor genes or in DNA repair genes, such as *p16* and *mlh1*. Expression of HLTF (Helicase-Like Transcription Factor), a member of the SWI/SNF family of chromatin remodeling enzymes, is lost in about 30% of colon cancers. This is due to promoter methylation [Moinova et al. 2002]. Hypermethylation is often a consequence of mutations in *K-ras* and *tgf-βrII*.

The loss of TGF-β control may be a proximal defect in the CpG island methylator phenotype. It is a critical event in colorectal carcinogenesis [Derynck et al. 2001], with over 75% of cases lacking responsiveness to this mechanism. This may occur through two principal mechanisms:

- Mutations of both alleles of *tgf-βRII* occur in the majority of tumors with microsatellite instability. They are located within a poly-A tract in the coding region, resulting in the expression of a truncated and nonfunctional receptor [Markowitz et al. 1995]. These mutations are late events associated with tumor progression.
- Allelic loss of 18q21 occurs in 60% of colorectal cancers, mostly those without microsatellite instability. Loss of heterozygosity (LOH) on chromosome 18 is associated with colorectal carcinomata (75%) and in advanced adenomata (45%), but only occasionally with earlier stage adenomata (10–15%). *dpc4* (*smad4*) {18q21.1} is deleted in up to one third of cases of sporadic colon cancers, while *smad2* {18q21}, or a closely linked gene, is deleted in the remaining tumors. SMAD2 and SMAD4 act as trimers with SMAD3 downstream in TGF-β signaling.

Colon cancer cell survival. Casein Kinase-2 is at the nexus of two signaling pathways that protect tumor cells from TRAIL (APO2L)-dependent apoptosis. It inhibits the TRAIL-induced, Caspase-8-mediated cleavage of BID, thereby reducing the formation of tBID. In addition, Casein Kinase-2 promotes the

NF-κB-mediated expression of BCL-X_L, which sequesters tBID and curtails its ability to activate BAX. Tumor cells with constitutive activation of Casein Kinase-2 therefore exhibit a high BCL-X_L to tBID ratio and fail to activate Caspase-9 or undergo apoptosis in response to TRAIL (Figure 15.3.C).

The cellular pools of arachidonic acid are tightly controlled through downstream enzymes, including Cyclooxygenases, which synthesize eicosanoids. Colon cancers often display perturbations in arachidonic acid metabolism, with frequently low expression levels of cytoplasmic PLA_2, elevated levels of Cyclooxygenase expression, and high prostaglandin E_2 production. While COX-2 and PGE_2 are associated with cancer cell survival, arachidonic acid induces a strong, ceramide-mediated apoptotic signal. Moreover, the high COX-2, low PLA_2 phenotype may arise in preneoplastic aberrant crypt foci from high risk patients. Colon cancer growth is favored when the intracellular arachidonic acid levels are suppressed, as is the case with loss of function of PLA_2 [Dong et al. 2005; Ilsley et al. 2005].

Metastasis. Progression of colon cancer is often associated with an elevated expression and activity of the SRC family tyrosine kinases. This is reflected in higher SRC activity in the tumor cells over that in the adjacent normal mucosa, with activation being linked to malignant potential. While there is some elevation of SRC activity in premalignant lesions and in adenomata, a progressive increase in c-SRC activity occurs as the tumor stage advances. A subset of advanced metastatic colon cancers harbor an activating mutation within the COOH-terminus of SRC, leading to the production of a transforming protein. A C→T transition mutation in *src* occurs in a fraction of colon cancers. It causes the truncation of SRC at tyrosine 530 and results in increased kinase activity. A truncating mutation in SRC at codon 531 arises in about 10% of cases of advanced colon cancer. SRC family tyrosine kinases are ordinarily in equilibrium between inactive and primed states by a balance of negative regulatory kinase CSK and its counteracting tyrosine phosphatases, both of which act on the regulatory COOH-terminal tyrosine of SRC family tyrosine kinases. Elevated CSK leads to decreased SRC family tyrosine kinase activation and is associated with increased cell–cell contacts mediated by E-Cadherin, decreased number of focal contacts and decreased cell invasiveness. These events occur depending on Integrin-mediated cell adhesion [Rengifo-Cam et al. 2004].

Metadherin (Metastasis Adhesion Protein, LYRIC) is a 579 amino acid cell surface molecule, which causes homing specifically to the lung vasculature when it is expressed on tumor cells. It is a putative CEACAM-1 (CD66, Biliary Glycoprotein-1, BGP-1) associated protein in colon carcinoma.

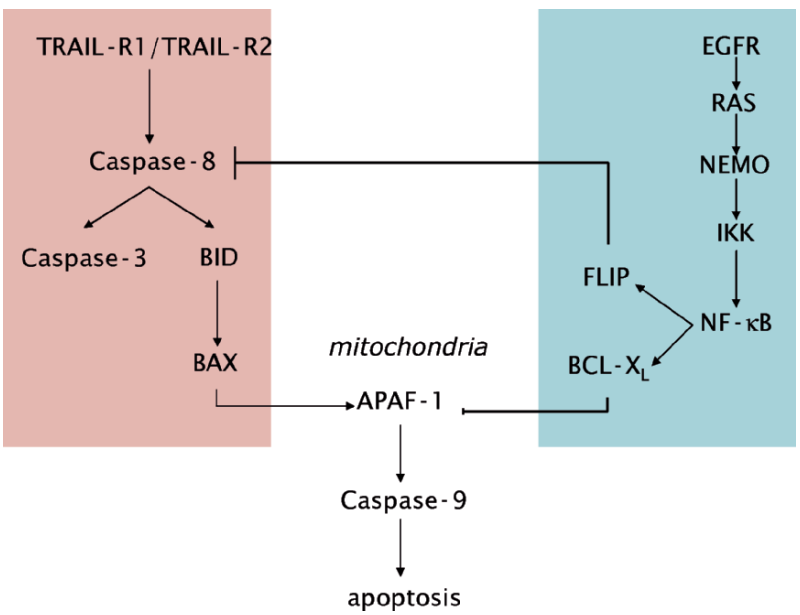


Figure 15.3.C. Regulation of apoptosis in colorectal cancer. The engagement of death receptors activates Caspase-8, which then activates Caspase-3 and BID. The induction of programmed cell death proceeds through the effects of BAX on the mitochondria. Excessive activities of EGFR or RAS, due to overexpression or activating mutations in colon cancer, induce NF-κB, which protects from apoptosis by transactivating the expression of BCL-X_L and FLIP. These proteins counteract the proapoptotic pathway.

Hepatic metastasis is one of the major complications in colon cancer. Osteopontin substantially contributes to colon cancer cell invasion and liver metastasis [Wai et al. 2005]. Osteopontin is consistently differentially expressed in conjunction with colon cancer progression, and its levels correlate with advancing tumor stage [Agrawal et al. 2002]. Some splice variants of CD44 act as receptors for Osteopontin. Colon cancer-associated changes in CD44 include alterations in glycosylation and splicing, both of which correlate with metastatic potential. Increased mucosal expression of the oncofetal blood group antigen TF (Thomsen-Friedenreich, β1-3 *N*-acetylgalactosamine α-galactose) in colon cancer is predominantly carried on high molecular weight splice variants of CD44 and allows the binding of specific lectins to the colorectal epithelium [Singh et al. 2001].

The gastrointestinal tract is lined by a layer of mucus comprised of highly glycosylated Mucin proteins. Genetic deficiency in the most abundant secreted gastrointestinal Mucin MUC2 alters crypt morphology and maturation, leading to invasive adenocarcinomata and rectal cancers. Thus, MUC2 is involved in the suppression of colorectal carcinogenesis [Velcich et al. 2002]. Colon cancers typically produce a skewed profile of Mucins. Cancer cells with high Mucin production invade basement membranes to a greater extent than cancer cells with low Mucin production [Schwartz et al. 1992], possibly accounting for the role of Mucins in colon cancer metastasis. Liver metastases of colorectal carcinoma express increased levels of Mucin forms that contain sialyl-dimeric Lewis^X antigen and sialosyl-Tn. These Mucin antigens are predictors of poor prognosis [Hoff et al. 1989; Itzkowitz et al. 1990]. Selectins are receptors for sialyl-Lewis^X. They contribute to colon cancer dissemination.

The *dcc* gene {18q21.3} is expressed in most normal tissues, including the colonic mucosa. Loss of heterozygosity on chromosome 18 is associated with about 75% of colorectal cancers. Loss or somatic mutations of *dcc* occur in colorectal cancers with intact *smad4* [Barbera et al. 2000]. Associated mutations include a homozygous deletion of the 5′ end, a point mutation within an intron, and DNA insertions within a 170 bp fragment immediately downstream of one of the exons. Loss of heterozygosity of *dcc* in colorectal cancer is associated with both liver metastasis and lymph node metastasis.

The levels of Decorin, a leucine-rich proteoglycan capable of regulating matrix assembly and cell proliferation, are markedly elevated in the stroma of colon carcinomata.

Genetic predisposition to colorectal cancer. Colorectal cancer due to hereditary diseases comprises approximately 5% of the overall colorectal cancer burden. Various hereditary cancer syndromes involve colon cancer.

– Juvenile polyposis is an autosomal dominant gastrointestinal syndrome associated with inactivating mutations in the BMP (Bone Morphogenetic Protein) pathway. Some patients, comprising 20–30% of cases, have mutations in the gene that encodes BMP Receptor 1A (BMPR1A) {10q22.3}. Early onset disease, possibly at ages 3–5, is often caused by mutations in *edg* (*endoglin*). Endoglin is a homodimeric transmembrane glycoprotein and a component of the TGF-β complex. Endoglin contributes to angiogenesis, which may facilitate the growth of polyps. Germline mutations of *smad4* {18q21.1} exist in a subset of patients. This variant of the syndrome often includes hemorrhagic teleangiectasias. The juvenile polyps can be isolated or multiple. Their histology, with normal epithelium but hypertrophic lamina propria, and their natural history suggest that they are hamartomata, which are unlikely to be precancerous. Patients bear 50–200 polyps, usually in the rectosigmoid region of the gut. The polyps are subject to chronic inflammation. They contain cystic spaces, lined by columnar epithelium and surrounded by abundant stroma. Patients with juvenile polyposis syndrome have an increased risk of developing gastrointestinal polyps and colorectal cancer.
– familial adenomatous polyposis (FAP) is an autosomal dominant disorder, which typically leads to colorectal cancer in early adulthood secondary to extensive adenomatous polyps of the colon (Figure 15.3.D). Polyps also develop in the upper gastrointestinal tract and malignancies may occur in other sites. The disease is caused by mutations of the *apc* gene {5q21}. Most of these mutations accumulate in the central region, which is called the mutation cluster region (MCR), and result in the expression of a COOH-terminally truncated protein. Without surgical intervention, malignant transformation of some of the polyps by the third to fourth decade of life is virtually certain. In rare cases, familial adenomatous

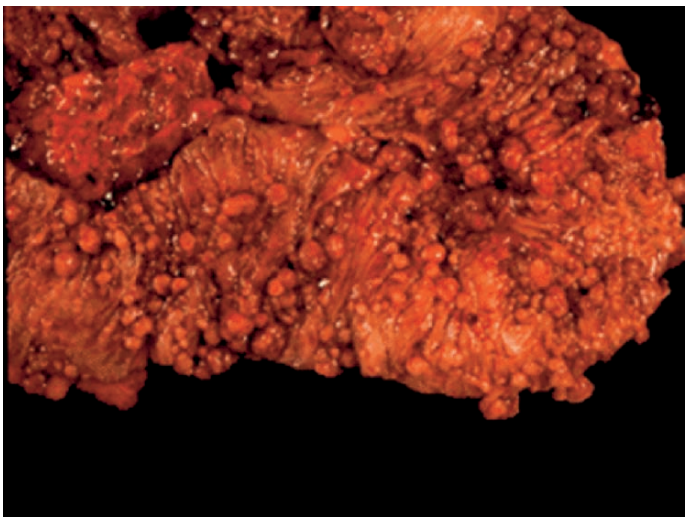


Figure 15.3.D. Adenomatous polyposis coli (APC). The opened colon displays a mucosal surface covered with numerous small- to medium-sized polyps. The onset of polyp formation in patients with familial adenomatous polyposis (FAP, APC) is usually after the age of 15. Colorectal carcinomata arise beginning 10–15 years after the occurrence of visible polyps. In an APC kindred, the risk of carrying the gene drops to 4% if no polyps have developed by age 30. FAP is caused by an inherited, autosomal dominant mutation of *apc* on chromosome 5q21. The exact mutation at the apc locus is unique for each kindred, but variable among kindreds. If the mutation arises in the proximal four exons of the gene, a form with limited manifestations results, called attenuated APC (AAPC). The greatest number of polyps grows consequent to mutations in the midpoint of exon 15. While in the presence of specific mutations in the apc gene polyposis is inevitable, during childhood the mucosa shows no signs of polyposis for a number of years. There is evidence of abnormal proliferative activity in the mucosa of APC patients as well as in areas of adenomatous proliferation. A number of other abnormalities, involving Mucin formation, cell adherence, and chromosome instability are also present. [Reproduced from http://pathweb.uchc.edu. With permission.]

polyposis may occur in conjunction with extracolonic tumors, including brain cancer, thyroid cancer, pancreatoblastoma, uterine adenocarcinoma, hepatocellular adenoma, or adrenocortical adenoma. Congenital hypertrophy of the retinal pigment epithelium is present in about 80% of families. Their mutations are typically located between codons 457 and 1,444 of the *apc* gene.

– Gardner syndrome [Gardner 1951] represents a genetic connection between the dysfunctional regulation of epithelial and mesenchymal cells. It comprises familial adenomatous polyposis disorders in conjunction with extracolonic abnormalities such as epidermoid cysts and osteomata. The patients are at approximately 10–20% risk to develop mesenteric and intraperitoneal desmoid tumors, which is nearly 1,000-fold higher than the general population. Although desmoid tumors are benign, they are prone to recurrence. The underlying lesion is a defect within the *apc* gene. Hence, Gardner syndrome is a phenotypic variant of familial adenomatous polyposis.
– Turcot syndrome is a rare, autosomal recessive disorder, characterized by the development of primary neuroepithelial tumors of the central nervous system and numerous adenomatous colorectal polyps. Patients with Turcot syndrome who develop medulloblastomata harbor germline *apc* mutations. Patients with glioblastoma multiforme and colorectal tumors have replication errors characteristic of hereditary non-polyposis colorectal cancer. They are caused by germline mutations in the mismatch repair genes *mlh1* or *pms2*.
– Hereditary non-polyposis colorectal cancer (HNPCC) becomes manifest mostly in the proximal colon. The mean age of onset of cancer is 45 years. The tumors are poorly differentiated, predominantly right sided, synchronous and metachronous, and frequently indolent. The syndrome is characterized by microsatellite instability. In these tumors, germline loss-of-function mutations of the mismatch repair genes *msh2*, *mlh1*, *msh3*, and *msh6* are frequent. A large genomic deletion of *msh2*, encompassing exons 1 through 6 [Lynch et al. 2004], and two specific mutations in *mlh1* [Moisio et al. 1996] are common in certain geographic areas due to founder effects. Since defects in *msh2* account for as

many as 60% of cases of hereditary nonpolyposis colon cancer, and defects in *mlh1* play a role in up to 30%, defects in these two genes can account for the vast majority of all cases. Hereditary non-polyposis colorectal cancer is subdivided into three clinical forms, Lynch type 1, Lynch type 2, and Muir–Torre Syndrome. Lynch type-1 syndrome is limited to colorectal manifestations. In Lynch type-2 syndrome, there is an increased risk for endometrial, ovarian, small bowel, and hepatobiliary cancers, as well as transitional cell tumors of the renal pelvis and ureter. Muir-Torre syndrome is furthermore associated with sebaceous adenomata and keratoacanthomata.

- The MYH-adenomatous polyposis syndrome (autosomal recessive colorectal polyposis) manifests with multiple polyposis of the colon and rectum. It is caused by homozygous mutations in the *myh* (*mut YH*) gene {1p34.3–p32.1}. MYH acts in base excision repair. Its Adenine Glycosylase activity is significantly reduced by the predisposing mutations. This may compromise DNA sequence fidelity and cause transforming genetic changes.
- Peutz–Jeghers syndrome is an autosomal dominant disorder characterized by melanocytic macules of the lips, buccal mucosa, and digits, multiple gastrointestinal hamartomatous polyps (especially of the upper jejunum), and an increased risk of various neoplasms. Approximately 50% of cases are due to mutations in the *stk11* (*serine/threonine kinase 11*) gene.
- Germline *pten* {10q23.31} mutations cause Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome (Bannayan–Zonana syndrome, BZS), Lhermitte–Duclos disease (LD, cerebelloparenchymal disorder VI), and Proteus syndrome. Hyperplastic–dysplastic changes in the prostate, skin, and colon are characteristic of all four syndromes. Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome share the clinical characteristics of hamartomatous polyps of the gastrointestinal tract and mucocutaneous lesions. Cowden syndrome [Lloyd and Dennis 1963] includes hamartomatous polyps predominantly of the colon. Their likelihood for full transformation is low.
- Hereditary mixed polyposis syndrome is characterized by atypical juvenile polyps, colonic adenomata, and colorectal carcinomata. The patients may also have an increased propensity to develop inflammatory and metaplastic polyps. The disease is caused by mutations in *crac1* (*colorectal adenoma and carcinoma 1*, *hereditary mixed polyposis syndrome 1*, *hmps1*) {15q15.3–q22.1} [Tomlinson et al. 1999; Jaeger et al. 2003].
- Birt–Hogg–Dube syndrome (fibrofolliculomata with trichodiscomata and acrochordons) is a rare inherited genodermatosis characterized by hair follicle hamartomata, kidney tumors, and spontaneous pneumothorax. It is caused by mutations in *folliculin* (*flcn*) {17p11.2}. The condition is associated with a risk for intestinal polyposis [Rongioletti et al. 1989].

Environmental predisposition to colorectal cancer. The role of the colon in alimentation potentially exposes it to exogenous carcinogens, which at the later stage of digestion may be highly concentrated. This constitutes a particular importance for metabolic enzymes in the detoxification of carcinogens or activation of procarcinogens. There is a relationship between the distribution of polymorphic variants of various metabolic enzymes and the susceptibility to colorectal cancer [Fettman et al. 1991].

- Chemical carcinogens often require metabolic activation in order to be able to bind to DNA and contribute to cancer causation. The Cytochrome P450 1A2 (CYP1A2) *F allele is involved in the metabolic activation of polycyclic aromatic hydrocarbons and may be associated with an increased risk of colon cancer. There is a positive association between the development of colorectal cancer and the mutant homozygous genotype in *msp1* polymorphism of *cyp1A1* gene among Japanese in Hawaii [Kiyohara 2000].
- Cytosolic Glutathione S-Transferases (GST) form a superfamily consisting of four distinct families, named α, μ, π, and θ. A member of the μ class gene family (*gstm1*) is polymorphic and is only expressed in 55–60% of individuals. The risk for cancer of the proximal colon is increased about twofold in carriers of the *gstm1* null allele [Zhong et al. 1993]. Furthermore, the age of onset of colon cancer may be affected by alleles of the *gstm1* and *gstt1* genes [Chenevix-Trench et al. 1995].
- Exposure to aromatic amines can play a role in the etiology of colorectal cancer. Aromatic and heterocyclic amines require metabolic activation to electrophilic intermediates that initiate carcinogenesis. The acetylator phenotype is based on a common genetic trait that results from the presence of several alleles of the genes coding for

Arylamine *N*-Acetyl Transferase (*nat1* and *nat2*). These polymorphisms are associated with several disease states, including colorectal cancer. For colon cancer induced by heterocyclic amines, in which *N*-acetylation is negligible and *O*-acetylation is an activation step, the rapid acetylator phenotype is at higher risk [Lang et al. 1986]. Individuals that are both rapid acetylators and exhibit a high Cytochrome P450 1A2 activity appear to have an even higher risk of colorectal cancer.

– Aldehyde Dehydrogenase 2 (ALDH2) has a polymorphism, E487K, which is closely linked with the phenotypic loss of enzyme activity. Alcohol consumption is a risk factor for colorectal cancer and the risk can be enhanced in *aldh2* heterozygotes [Yokoyama et al. 1998; Murata et al. 1999].
– Low folate intake, particularly if combined with high alcohol consumption, is associated with an increased risk for colorectal cancer. 5,10-MethyleneTetrahydrofolate Reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate, required for purine and thymidine synthesis, to 5-methyl-tetrahydrofolate, the primary circulatory form of folate necessary for methionine synthesis. Common functional polymorphisms, C667T (alanine to valine) or A1298C, in the gene *mthfr*, lead to reduced enzyme activity and are associated with an increased risk for colorectal cancer. However, *mthfr* polymorphisms are not associated with the risk for colonic adenomata. The impact of the MTHFR polymorphism is modulated by dietary factors. Although the valine/valine genotype (667 T/T) of MTHFR is associated with a reduced risk for colorectal cancer, the protection is negated by low folate intake or high alcohol consumption, both of which compromise the dietary methyl supply. When dietary methyl supply is high, homozygous individuals with the valine/valine MTHFR may be at reduced risk of colorectal cancer, probably because high activity of the enzyme is suited to prevent imbalances of nucleotide pools during DNA synthesis, preventing potentially oncogenic alterations in DNA methylation [Chen et al. 1996]. Loss of heterozygosity of *mthfr* occurs in a fraction of colon carcinomata [Pereira et al. 1999].
– The enzyme Cystathionine β-Synthase reduces homocysteine levels and thus may protect against colorectal cancers. The *cbs* gene has a variant 844ins68 that is linked with increased activity. This variant may be involved in the development of colorectal cancers with aberrant DNA methylation and microsatellite instability [Shannon et al. 2002].

Chronic inflammation (inflammatory bowel disease) may be a pathogenetic factor in colon cancer. Patients with ulcerative colitis are at elevated risk. KLF6 (Krüppel-Like Factor 6) is a ubiquitous zinc finger tumor suppressor. It is inactivated by loss or mutation in most sporadic colorectal cancers and most colorectal cancers occurring on the basis of inflammatory bowel disease. *klf6* mutations are present in tumors with either microsatellite or chromosomal instability and are more common in the presence of wild-type *apc*. The cancer-derived KLF6 mutants neither suppress growth nor induce *p21*. The deregulation of *klf6* by a combination of allelic imbalance and mutation may play a role in the development of colorectal cancers [Reeves et al. 2004].

Carcinoma of the small bowel. In contrast to colon cancer, primary carcinoma of the small bowel is an uncommon type of tumor, with an incidence of 0.5–0.8 per 100,000 per year. Although most cases are adenocarcinomata, rare cases of sarcomatoid carcinoma occur [Robey-Cafferty et al. 1989]. Chronic inflammation, Peutz–Jeghers syndrome, and hereditary non-polyposis colorectal cancer constitute lifetime risk factors. Mutations in *K-ras* and *p53* are present in about 50% of cases.

15.4 CANCERS OF THE UPPER DIGESTIVE TRACT

15.4.1 Esophageal Cancer

Heavy alcohol consumption increases the risk for esophageal cancer. High levels of dietary nitrate may result in potentially carcinogenic levels of nitric oxide in the epithelium of the lower esophagus. Cigarette smoking is linked to the development of both histologic types, squamous cell cancers, and adenocarcinomata. In patients with cancer of the esophagus, obstruction of the esophageal lumen is the most significant cause of morbidity. Progressive dysphagia with decreased oral intake, malnutrition, and in some cases, pulmonary aspiration is the sequelae of esophageal obstruction. In approximately 15% of patients with esophageal carcinoma, the development of an esophagopulmonary fistula

is a major complication. Metastasis to the liver is possible (Figure 15.4.1.A).

Genetic alterations associated with neoplasms of the esophagus include allelic losses at chromosomes 3p, 5q, 9p, 9q, 13q, 17p, 17q, and 18q. Missense mutations of *p53* [Hollstein et al. 1990; Casson et al. 1991], deletions of *rb* [Montesano et al. 1996; Roncalli et al. 1998]. The two principal histologic forms are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma.

The epidemiology of squamous cell carcinoma of the esophagus (Figure 15.4.1.B) shows considerable geographic diversity. A tumor-specific missense mutation in *wwox*, associated with loss of heterozygosity in the other allele, is common in esophageal squamous cell carcinoma. Somatic mutations in the putative tumor suppressor *rnf6* (*ring finger protein 6*) also occur [Lo et al. 2002]. Loss of heterozygosity of the *p53* locus on 17p13 arise in high-grade dysplasias, which are precursor lesions to squamous cell carcinomata. Loss of heterozygosity at 9q31 is often associated with esophageal squamous cell carcinomata. A candidate tumor suppressor gene in this region is *dec1* (*deleted in esophageal cancer 1*) [Nishiwaki et al. 2000].

In esophageal squamous carcinoma, amplification of 11q21–q23 frequently occurs. This leads to the overexpression of the antiapoptotic gene *ciap1* (*hiap2*, *mihb*, *birc2*). Amplifications of *cyclin* D_1 and *c-myc* also commonly arise.

The single confirmed genetic abnormality that is associated with a 25% lifetime incidence of squamous cell cancer of the esophagus is tylosis A [Maillefer and Greydanus 1999]. The locus for tylosis with esophageal cancer (TOC, keratosis palmaris et plantaris) [Clarke and McConnell 1954; Howel-Evans et al. 1958] maps to chromosome 17q25, a region frequently deleted in sporadic squamous cell esophageal tumors [Risk et al. 2002].

Barrett esophagus is the abnormal growth of intestinal type cells above the gastroesophageal border, often in the context of gastroesophageal reflux disease (GERD). Goblet cells containing acidic Mucin are a hallmark of the intestinal metaplasia. The condition poses a 30- to 125-fold elevated risk for the development of adenocarcinoma, which makes gastro-esophageal reflux disease a significant risk factor for esophageal adenocarcinoma. Tumor progression from Barrett esophagus is characterized by alterations in *p53* and *cdkn2A*. Aneuploidy is a frequent occurrence. Deletions involving chromosome 3p arise in 60–100% of esophageal adenocarcinomata [Krishnadath et al. 1995]. This may reflect defects in FHIT. The promoter region of *apc* is frequently hypermethylated

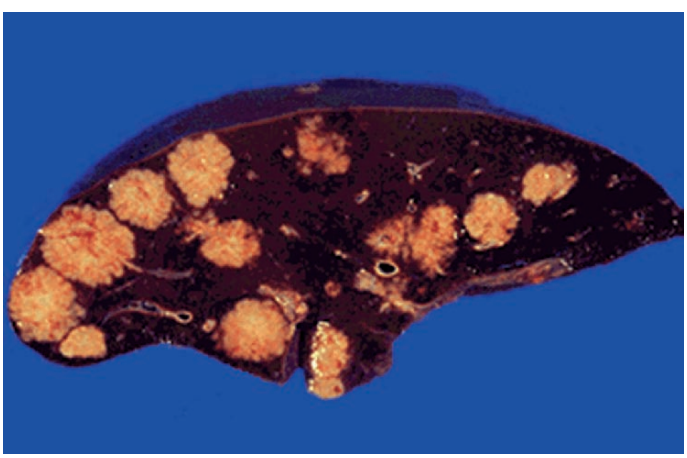


Figure 15.4.1.A. Metastatic esophageal carcinoma. Cut surface of a liver with multiple foci of metastatic tumor. Note the brownish color of the normal liver parenchyma and the white–tan color of the tumor deposits. Histologically, the tumor recapitulates the appearance of the primary lesion. The liver parenchyma between the tumor nodules shows evidence of compression and often of an inflammatory, predominantly lymphocytic infiltrate. The liver is a frequent site of metastatic spread, particularly from the gastrointestinal tract, the lungs, and the breasts. Implantation on the surface of the liver may occur in ovarian malignancies. Metastases often tend to be multiple, nodular, and clearly demarcated from the rest of the liver tissue. Very often, the growth of the tumor outstrips its blood supply with the development of central necrosis and hemorrhage. [Reproduced from http://pathweb.uchc.edu. With permission.]

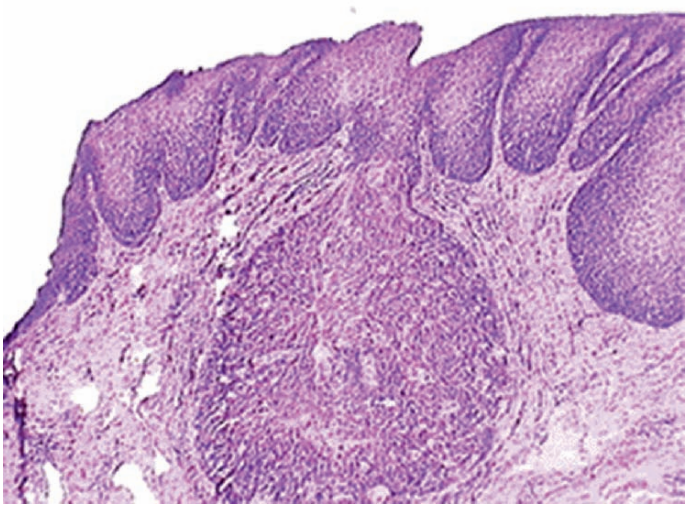


Figure 15.4.1.B. Esophageal squamous cell carcinoma (ESCC). A single focus of early invasive carcinoma is seen. The benign surface epithelium adjacent to the tumor shows little dysplasia in this field, but elsewhere contained extensive carcinoma in situ. Note the total disorganization of the tumor cells as opposed to the orderly maturation of the adjoining benign epithelium. Invasive squamous carcinoma of the esophagus is the end result of a progression through increasingly severe degrees of dysplasia to carcinoma in situ to invasive carcinoma. The common link among genetic, dietary, and environmental factors is a chronic inflammatory state associated with an increased turnover of epithelial cells. Dysphagia and weight loss are the most common symptoms. The trachea is the adjacent tissue most frequently involved as tracheoesophageal fistulas form in up to 30% of cases. They cause symptoms of aspiration, such as pneumonia or coughing. Other regional tissues involved are the bronchi and the aorta. Environmental factors strongly linked to the development of esophageal dysplasia and squamous carcinoma include in the United States and Europe alcohol and smoking, in China nitrosamine-containing foods, fungal contamination of foods, vitamin deficiency, and essential metal deficiency. There is also evidence that HPV may play a role. Chronic esophagitis (nonreflux) contributes causatively. The only known genetic predisposition occurs in hereditary tylosis, an autosomal dominant symmetrical keratosis of the palms and soles. Because of the striking pockets of high incidence, it is possible that hereditary or racial predisposition may augment the carcinogenic effect of environmental factors. [Reproduced from http://pathweb.uchc.edu. With permission.]

in esophageal adenocarcinoma [Kawakami et al. 2000]. Loss of heterozygosity of this locus is also common. The prevalence of truncating mutations in *apc* in this malignancy is, however, low.

15.4.2 Gastric Cancer

Forms of gastric cancer, based on histology, include diffuse, intestinal, and mixed gastric carcinomata. They come about by activation of different carcinogenic pathways. Mixed gastric carcinomata, composed of well differentiated and poorly differentiated components, exhibit some of the molecular events that cause diffuse and intestinal gastric cancers.

Chronic infection with *Helicobacter pylori* poses a substantial risk of gastric cancer. This bacterial infection may be a strong trigger for the hyperplasia of TERT (Telomerase Reverse Transcriptase) expressing precursor cells in intestinal metaplasia. Individuals with polymorphisms in the promoter region of type I (T_H1) cytokines that are associated with high expression are particularly susceptible to developing gastric cancer consecutive to infection with *Helicobacter pylori*. The elevated cytokines increase the probability of hypochlorhydria, gastric atrophy, and consecutive distal gastric adenocarcinoma. Achlorhydria is characterized by a lack of gastric acid production. It leads to increased sensitivity to carcinogens, such as acetaldehyde. Further, the HLA allele DQA1*0102 increases the risk of atrophic gastritis and intestinal-type gastric adenocarcinoma.

APC pathway. Activating mutations of *cdh1*, the gene encoding E-Cadherin, are associated with diffuse-type gastric cancer, and germline mutations in *E-cadherin* constitute a predisposition for this cancer. Mutation or loss of *E-cadherin* is preferentially involved in the development of poorly differentiated

gastric cancers. Mutations in *β-catenin* are more frequent in intestinal forms than in diffuse gastric cancer. About 20% of gastric adenomata with *apc* mutations transform to carcinomata.

The Trefoil Factors TFF1, TFF2, and TFF3 are secreted peptides with trefoil domains (40 amino acid motifs that contain three disulfide bonds) and COOH-terminal dimerization domains. The TFF1 tumor suppressor protein exerts protective functions. Its absence may lead to intestinal mucosal barrier defects, accompanied by a local lymphoproliferative response. The loss of *tff1* may be one step in gastric transformation. It can occur through deletion, missense mutation, or promoter hypermethylation [Katoh 2003]. *tff1* (*pS2*, *bcei*) {21q22.3} and *tff2* (*sp*, *sml1*) {21q22.3} mRNAs are frequently down-regulated in intestinal-type gastric cancer. TFF3 (Intestinal Trefoil Factor, ITF) {21q22.3} is constitutively expressed in epithelial tissues, including the gastrointestinal tract. By perturbing the complexes of E-Cadherin, β-Catenin, and their associated proteins, TFF3 modulates epithelial cell adhesion, migration, and survival [Efstathiou et al. 1998]. The de novo pathway for carcinogenesis of well-differentiated intestinal gastric cancer involves loss of heterozygosity and abnormal expression of the *p73* gene that is responsible for the development of foveolar (located in the pits that mark the openings of the gastric glands) gastric cancers with TFF1 expression.

Krüppel-like Factor-4 (KLF-4, GKLF) is a zinc finger transcription factor expressed predominantly in terminally differentiated epithelial cells. It is a transcriptional activator of the tumor suppressor gene $p21^{CIP1/WAF1}$. KLF-4 also interacts with β-Catenin and represses β-Catenin-mediated gene expression. Histidine Decarboxylase (HDC) catalyzes the conversion of histidine to histamine, which plays important roles in allergic responses, inflammation, and gastric acid secretion. KLF-4 binds to the Gastrin responsive elements in the *hdc* promoter and represses its gene expression [Ai et al. 2004]. A lack of KLF-4 may sensitize to inflammatory stimuli and thus increase the risk of transformation. Whereas KLF-4 is highly expressed in epithelial tissues, including the gut, its expression is lost in primary and metastatic gastric tumors, and this loss is associated with poor survival. Promoter hypermethylation and hemizygous deletion contribute to the down-regulation of *klf-4* expression [Wei et al. 2005] (Figure 15.4.2.A).

TGF-β signaling. Loss of the gene that encodes the TGF-β Receptor occurs in gastric cancer, suggesting that TGF-β signaling induces a major tumor suppressor pathway in the stomach. Reduced *tgf-β type I receptor* expression is frequently associated with an advanced stage of intestinal type gastric cancer.

The Runt family of transcription factors associate with SMADs to transmit signals from the TGF-β Receptor. *runx3* may play a role as a tumor suppres-

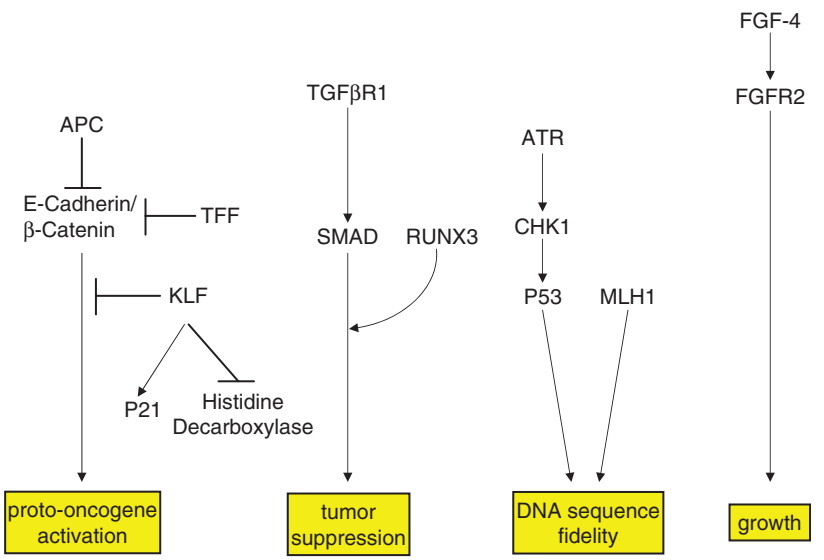


Figure 15.4.2.A. Molecular pathways of gastric carcinogenesis. Four principal pathways are associated with gastric cancer. They involve WNT signaling/APC/β-Catenin, TGF-β signaling/SMAD, checkpoint control/ATR/CHK1/P53, and FGFR signaling. Their dysregulation affects various mechanisms of carcinogenesis, including proto-oncogene activation, tumor suppressor gene inactivation, and loss of DNA sequence fidelity.

sor in gastric carcinomata, because it controls cell proliferation in the gastric epithelium. In cancer, the gene is mutated, deleted, or down-regulated by hypermethylation [Li et al. 2002]. In the absence of RUNX3, gastric epithelial cells are not responsive to the apoptosis-inducing effects of TGF-β.

Genetic instability. Sporadic gastric cancer is often characterized by microsatellite instability, which is most frequently caused by hypermethylation of *mlh1* [Ogata et al. 2001]. Genetic instability and hyperplasia of TERT expressing stem cells precede the occurrence of replication errors, DNA hypermethylation, and the expression of abnormal transcripts, all of which accumulate in at least 30% of incomplete intestinal metaplasias. Stomach tumors with microsatellite instability have a high frequency of mutations in ATR, while mutations in CHK1 arise with lower frequency. Therefore, inhibition of the ATR→CHK1 DNA damage response pathway may be involved in gastric carcinogenesis.

BAK (BCL-2 Antagonist Killer) is expressed in normal gastrointestinal epithelium. Missense mutations in *bak* may occur in gastric cancers. They arise only in advanced stages of the disease.

Tumor suppressor genes located on chromosomes 17p and 18q are critically involved in the development of most gastric cancers. Loss of heterozygosity at chromosome 17p and mutation or loss of *p53* are preferentially involved in the development of poorly differentiated gastric cancers. The transformation from precancerous lesions to intestinal-type gastric cancer comes about through *p53* mutation and loss of heterozygosity. It may be associated with reduced expression of *p27*, or elevated expression of *cyclin E*.

FGF Receptor. Overexpression of FGFR 2 in undifferentiated gastric cancers is a sign of poor prognosis. A point mutation S267P and a splice site mutation 940-2A→G of the *fgfr 2* gene also occur in gastric carcinoma. Furthermore, abundance of the ligand FGF-4 can contribute to transformation in stomach cancer.

Metastasis. The dissemination of gastric cancer is genetically controlled. It may be based on the loss of metastasis suppressors, on the expression of homing receptors and their associated signaling molecules, or on the secretion of extracellular matrix degrading enzymes.

Gastric cancer metastasis primarily targets the peritoneal cavity, but it may reach the mesothelium. Invasion of the mesothelium involves tumor-induced mesothelial apoptosis. Micrometastases in lymph nodes are frequently present at early stages of gastric cancer.

Loss of DCC expression is an important factor in the progression of gastrointestinal cancers. The types of mutations that occur include a homozygous deletion of the 5′ end, a point mutation within one of the introns, and DNA insertions within a 170 bp fragment immediately downstream of one of the exons. The loss of DCC function may be rather selective for gastrointestinal cancer metastasis.

Reduced *nm23* expression is frequently associated with an advanced stage of intestinal-type gastric cancer. Gene amplification of *K-sam* (the *fibroblast growth factor receptor 2* gene pre-mRNA can be spliced by using either the *K-sam* exon or the *bek* exon) and *c-met*, in conjunction with reduced *nm23* confer progression, metastasis, and diffusely productive fibrosis.

Peritoneal carcinomatosis is a frequent form of disease progression in gastrointestinal cancer. Peritoneal tumor cell adhesion depends on adhesion molecules, such as CD44 and β_1 Integrins. The expression of VCAM-1 in gastric carcinoma is closely related to tumor progression. AGER expression is associated with invasion and metastasis. AGER expressing cancer cells tend to be distributed at the invasive front of primary tumors and are present in all metastatic foci in lymph nodes.

The gene product IQGAP functionally links components of the cytoskeleton with cell adhesion molecules. It acts in signal transduction that regulates cell morphology and cell movement. IQGAP is up-regulated in diffuse gastric cancer.

In gastric cancer, the presence in the bone marrow of disseminated tumor cells that express UPAR (Urokinase-type Plasminogen Activator Receptor) correlates with an unfavorable prognosis [Heiss et al. 1995]. Cathepsin D is expressed in more than 50% of gastric cancers. It can be produced by the tumor cells or by the stromal cells. Cathepsin D may support tissue invasion and its abundance is a poor prognostic indicator. Furthermore, tumors that are positive for Cathepsin D expression have an increased risk of recurrence.

Matrix Metalloproteinases, such as MMP-7, facilitate stromal invasion. MMP-9 is also related to gastric cancer metastasis. Stromal cells release this MMP, which affects tumor aggressiveness. Furthermore, MMP-9 is up-regulated by Thrombospondin-1 in gastric cancer. A single nucleotide polymorphism at position –1306 in the *mmp-2* promoter sequence generates (C allele) or destroys (T allele) a SP-1 site and thus affects gene expression. Bearers of the CC genotype of the *mmp-2* promoter have a threefold increased risk for gastric adenocarcinoma, likely due to elevated MMP-2 expression levels.

The expression of Cycloxygenase-2 (COX-2) is elevated in gastric adenocarcinomata compared to nonneoplastic mucosa. COX-2 overexpression correlates with loss of *tff1*.

15.4.3 Other Tumors

Gastrointestinal stromal tumors (GISTs) likely arise from cells of the Cajal lineage. They are mesenchymal tumors (sarcomata) characterized by the absence of muscle or Schwann cell markers. In more than 85% of cases, the transforming oncoprotein is the receptor tyrosine kinase c-KIT. Among the tumors that lack *c-kit* mutations, many have activating mutations in their *pdgfr* α. Both types of damage lead to identical activation of downstream signaling intermediates and cytogenetic changes [Heinrich et al. 2003]. About 30% of gastrointestinal stromal tumors express HIF-1α, which is a marker of poor prognosis and correlates with tumor size, liver metastasis, VEGF expression, and microvessel density [Takahashi et al. 2003]. The prognosis of gastrointestinal stromal tumors correlates with the levels of KI-67 expression, but not with CD34 or Desmin.

Oral cancer, cancerous tissue growing in the mouth, is one of the most common types of cancer in the world. Smoking and other tobacco use are associated with 70–80% of oral cancer cases. The smoke and heat from cigarettes, cigars, or pipes exert transforming damage to the mucous membranes. The use of chewing tobacco or snuff is an oral cancer risk because it establishes direct contact between the tobacco and the mucous membranes. A similar hazard is the high prevalence of betel quid chewing in many Asian cultures. Heavy alcohol consumption is also a high risk activity for this type of cancer. The synergistic damaging effect of heavy smoking and drinking leads to a greatly increased risk. Oral cancers may originate in any of the tissues of the mouth. They comprise

– Adenocarcinoma derived from a salivary gland
– Lymphoma from tonsillar or other lymphoid tissue
– Melanoma from the pigment producing cells of the oral mucosa
– Squamous cell carcinoma (most common form) originating in the tissues that line the mouth and lips

Oral cancers tend to originate in precancerous leukoplakias (mouth ulcers). Promoter methylation and inactivation of tumor suppressor genes plays a role in oral squamous cell carcinoma [Ha and Califano 2006]. Inactivation of the CDK Inhibitor P27 is a critical event for the G_1 to S transition in the cell cycle. It occurs through ubiquitination by the F-box complex SCF and subsequent degradation in the 26S proteasome. The down-regulation of P27 is associated with poor prognosis in oral squamous cell carcinoma. Furthermore, SKP2 and CKS1, the specific recognition factors for P27 ubiquitination, have oncogenic properties [Kudo et al. 2005]. Genes that are overexpressed in oral cancer include *caspase-1*, *stat-1*, *cox-2*, and *pleiotrophin*.

Ameloblastoma is a rare, benign tumor of the odontogenic epithelium, often appearing in the upper or lower jaw. While these tumors are rarely malignant or metastatic and progress slowly, the resulting lesions can cause severe abnormalities of the face and jaw. The three main clinical subtypes of ameloblastoma are unicystic, multicystic, and peripheral. Ameloblastomata are often associated with the presence of unerupted teeth. Symptoms include painless swelling, facial deformity, pain, loose teeth, ulcers, and gum disease.

15.5 PANCREATIC CARCINOMA

Pancreatic cancers nearly always originate from the ductal epithelium and are therefore adenocarcinomata. Because most tumors arise in the pancreas head they bear the potential of obstructing the common bile duct and main pancreatic duct, resulting in stenosis. Patients usually present with increasing abdominal pain, weight loss, and in 10–20% with painless jaundice.

Pancreatic carcinogenesis follows a sequence of dysplasia→carcinoma in situ→invasive carcinoma (Figure 15.5.A). Pancreatic duct hyperplasia occurs

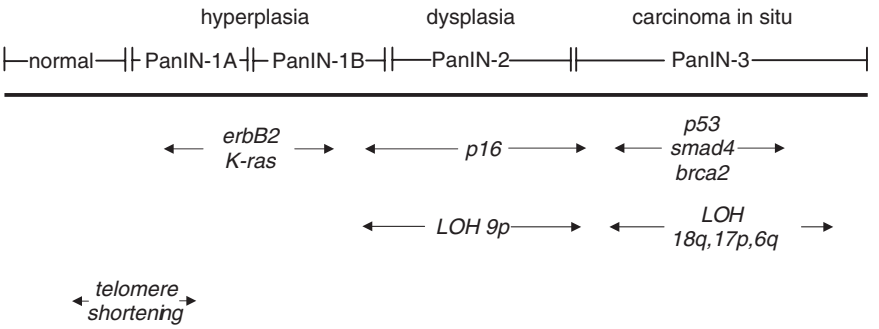


Figure 15.5.A. Multistage pancreas carcinogenesis. Sequential genetic defects are associated with the progression of precancerous pancreatic lesions. They involve defects of individual genes, chromosomal losses, and telomere shortening. PanIN = pancreas intraepithelial neoplasia.

in close to 10% of the population and may represent a precursor lesion to cancer [Sommers et al. 1954]. Pancreatic intraepithelial neoplasms (PanINs, small size, incidental duct lesions) progress to invasive ductal adenocarcinomata. Overexpression of Cyclin D_3 and Cyclin A occurs early in pancreatic intraepithelial neoplasia, and they are common in cancerous cells. Infiltrating adenocarcinoma of the exocrine pancreatic duct is the most frequent histologic subtype of pancreatic cancer. In many cases of atypical papillary lesions in pancreatic ducts, intraductal growth of an invasive cancer ensues. This phenomenon, cancerization of the ducts, may account for the markedly atypical papillary intraductal proliferations adjacent to infiltrating adenocarcinomata. The nonneoplastic pancreatic parenchyma adjacent to infiltrating ductal adenocarcinoma is also afflicted by inflammation, fibrosis, loss of acinar cells, and small duct like metaplasia of acinar cells.

Molecular carcinogenesis. Several functionally related genes, including *ras, cyclin* D_1 (*ccnd1*), *smad4* (*dpc4*, *madH4*), *p53*, $p21^{CIP1/WAF1}$ (*cdkn1A*), $p16^{ink4a}$ (*cdkn2*), and *armet* are frequently affected in cancer of the pancreas. This suggests that gain-of-function alterations in the RAS pathway or the TGF-β pathway are involved in pancreatic adenocarcinogenesis. In addition, alterations in P53 or Cyclins affect final common pathways of growth control. The mutation profiles of *p53* and *K-ras* in pancreatic cancer have a high proportion of G→A transitions. This suggests the involvement of nitrosamines or alkylating agents in pancreatic carcinogenesis. Consistently, individuals with deficient repair of DNA alkylation products have an increased risk for pancreatic cancer.

The *K-ras* gene {12p13} is mutated in many precursor lesions, as well as in the vast majority of pancreatic adenocarcinomata. Missense mutations in the *K-ras* gene arise in over 80% of pancreatic cancers [Almoguera et al. 1988]. Specifically, point mutations in codons 12, 13, or 61 of the *K-ras2* gene occur in 75–95% of pancreatic cancers, and also in foci of the precursor lesion pancreatic intraepithelial neoplasia. Specific amino acid substitutions at the susceptible sites then alter its GDP/GTP-binding domain and result in retention of GTP by blocking its hydrolysis to GDP. This produces a constitutively active K-RAS protein. The risk for mutations in *K-ras* is higher in smokers than in nonsmokers.

ERBB2 expression may provide early pancreatic neoplasms with a growth advantage over adjacent nonneoplastic epithelium. Elevated levels of ERBB2 are an early molecular alteration that can occur in up to 70% of the tumors. The affected lesions may represent intraepithelial neoplasias with the potential for subsequent invasion and metastasis [Day et al. 1996].

Homozygous deletions at 18q21.1 in about 20% of pancreatic cancers affect the *smad4* gene. The loss of SMAD4 is likely a late event in transformation. Physiologically, SMAD4 (DPC4) induces $P15^{INK4b}$ (CDNK2B) to inhibit the CDK4 interaction with Cyclin D. Its inactivation promotes the progression through the G_1 to S restriction point. TGF-β expression may be a positive prognostic factor in pancreatic adenocarcinoma because it induces cell cycle arrest through SMAD4 and $P15^{INK4b}$.

Interactions between Cyclins and CDKs are essential for cell cycle progression. They are positively regulated by the RAS pathway and negatively controlled by the TGF-β pathway. Cyclin D_3 overexpression arises early in pancreatic intraepithelial neoplasia and is prevalent in 90–100% of high-grade

pancreatic intraepithelial neoplasias and ductal cancer. Cyclin A overexpression also occurs early and reaches 50–100% of high-grade pancreatic intraepithelial neoplasias and cancer. Cyclin E overexpression is associated with 20–25% of high-grade pancreatic intraepithelial neoplasias and with 75% of ductal carcinoma. Overexpression of the interacting kinases CDK2 and CDK4 arises in early pancreatic intraepithelial neoplasias and progressively increases to reach 60–75% in carcinoma [Al-Aynati et al. 2004]. *cdc25B* is overexpressed in pancreatic ductal adenocarcinoma in comparison with the normal pancreas.

p53 mutations {17p13} occur in at least of 50% of cases of pancreatic ductal adenocarcinoma. Low-grade duct lesions have no apparent loss of P53 function, suggesting that defects of P53 occur late in transformation. Fifty percent of pancreatic cancer-derived cells have deletions of both exons 1 and 2 of the *cdkn2A* gene {9p21}. Furthermore, an additional 30% of pancreatic cancer cells harbor point mutations or microdeletions in this gene [Liu et al. 1995]. Loss of function of $P16^{INK4a}$ or $P14^{ARF}$ also occurs through homozygous deletion, point mutations, or hypermethylation.

An ATG→AGG transversion or deletion in codon 50 of the *armet* (*arginine-rich mutated in early stage tumors*, *arp*) gene occurs in a fraction of pancreatic tumors. These changes eliminate a methionine residue and give rise to an uninterrupted string of AGG repeats. [Shridhar et al. 1997]. ARMET may play a causal role as an oncogene.

Endocrine effects. Gastrointestinal Peptides, including Bombesin, Cholecystokinin, Gastrin, and Neurotensin stimulate DNA synthesis and cell proliferation. They exert their biological effects through G-Protein-Coupled Receptors. Bombesin (BN, Gastrin-Releasing Polypeptide, GRP) is secreted by pancreas carcinoma. The tumor cells also have a high level expression of specific Bombesin Receptors, which implies the possibility of autocrine stimulation. Furthermore, Neurotensin Receptors are widely expressed on pancreas carcinomata.

Metastasis. Netrin-1 is involved in pancreatic morphogenesis and tissue remodeling and plays a role in the regulation of duct cell and fetal islet cell migration. Loss of expression of the Netrin receptor DCC is an important factor in the progression of pancreatic adenocarcinoma. A highly reduced or absent expression of DCC occurs in all poorly differentiated or undifferentiated pancreatic tumor cells [Hohne et al. 1992].

Plasminogen Activators, TPA and UPA, play an important role in tumor cell invasion. The Plasminogen Activator Receptors (PARs) and Annexin II are localized in the basolateral membrane of pancreas tumor cells. Thrombin enhances the adhesion of pancreatic adenocarcinoma to extracellular matrix proteins and to endothelial cells. The effect is mediated through the Thrombin Receptor PAR-1, which is present on pancreas cancer cells.

Pancreatic colloid carcinoma. Intraductal papillary mucinous neoplasm (IPMN) is one of the premalignant lesions of the pancreas that can progresses to carcinoma. These growths of the pancreas constitute a unique pathological entity with an overall incidence of consecutive invasive malignancy of 20%. The tumor progresses from adenoma to invasive carcinoma. As in pancreatic adenocarcinoma, intraductal mucinous tumors are frequently initiated by mutations in *K-ras*. Loss of $P16^{INK4A}$ and SMAD4 expression occur more frequently in the intraductal carcinoma alone, or with associated invasive carcinoma, compared to adenoma [Biankin et al. 2002]. Intraductal papillary mucinous neoplasms (large size, intraductal tumors with ductal dilation) are often associated with colloid carcinoma.

Hereditary pancreas cancer syndromes. Beside spontaneous cases, pancreatic carcinoma occurs in the context of certain inherited disorders. Because pancreatic adenocarcinoma is uncommon, but not rare, some case clustering in families may be coincidental. Alternatively, since environmental carcinogens (nitrosamines, alkylating agents) can predispose to pancreatic adenocarcinoma, some familial clustering may result from shared environmental risk factors rather than from genetic predisposition. The cases of families that clearly have a genetically determined increased risk of pancreatic adenocarcinoma are estimated to account for 3–10% of all pancreatic adenocarcinomata [Lynch et al. 1996].

– In family clusters of pancreatic cancer, three or more generations may be afflicted with pancreatic adenocarcinoma, some in association with non-insulin-dependent diabetes mellitus. There is

evidence for an autosomal dominant mode of transmission in families with up to four affected siblings afflicted by pancreatic adenocarcinoma. The age presentation is comparable to that of sporadic pancreatic adenocarcinoma with tumors developing relatively late in life.
– Inherited and somatic mutations of the Fanconi complementation group genes *fancC* and *fancG* are present in young onset pancreatic cancer [Hill 1976; van der Heijden et al. 2003]. *brca2* is a Fanconi anemia gene, which conveys an inherited risk for breast, ovarian, and pancreatic cancer for individuals carrying a single mutated allele. Germline mutations in *brca2* are the most common inherited alterations identified in familial pancreatic cancer [Murphy et al. 2002].
– Striking pancreatic involvement may be part of von Hippel-Lindau syndrome. This can become manifest in pancreatic cysts or in exocrine pancreas insufficiency. In affected kindreds, carcinoma of the pancreas can occur in about 10% of cases.
– Peutz–Jeghers syndrome is caused by a defect in *stk11* {19p13.3}. It is inherited in autosomal dominant fashion and almost always leads to pancreas cancer, typically manifest in adenocarcinomata.
– Patients with hereditary pancreatitis have a high risk of pancreatic cancer several decades after the initial onset of pancreatitis. A paternal inheritance pattern increases the probability of developing pancreatic cancer [Lowenfels et al. 1997]. Hereditary pancreatitis can be caused by mutations in *prss1* (*cationic trypsinogen*), *cftr* (*cystic fibrosis transmembrane conductance regulator*), or *spink1* (*serine protease inhibitor, Kazal type 1*).

In additional inherited diseases pancreatic cancer is infrequent, but it can constitute a part of the syndrome.
– Ataxia telangiectasia (Louis–Barr syndrome) is an autosomal recessive disorder, caused by mutations in *atm*. Although this leads to frequent leukemia, cases of breast cancer, gastric cancer, or pancreas cancer can occur.
– The FAMMM syndrome (familial atypical mole malignant melanoma syndrome) is inherited in an autosomal dominant fashion and predisposes to the development of multiple malignant melanomata. Mutations in the *cdkn2A* gene (which encodes $P16^{INK4a}$ and $P14^{ARF}$) can result in pancreatic cancer in association with melanoma. Some kindreds have germline mutations in this gene, which is also frequently altered in sporadic pancreatic adenocarcinomata.
– In hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome 2), pancreatic tumors arise, but they are much less frequent than the characteristic colon cancers, which define this syndrome.

15.6 HEPATOBILIARY TUMORS

The neoplastic transformation of hepatocytes results from an accumulation of genetic damage during repetitive cellular proliferation that occurs in the injured liver in response to tissue damage (Figure 15.6.A). Hepatocellular carcinomata exhibit a high degree of genetic heterogeneity, suggesting that multiple molecular pathways may be involved in the genesis of subsets of hepatocellular neoplasms [Coleman 2003]. The major risk factors responsible for the development of hepatocellular carcinoma (HCC) are
– Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a high risk factor for cirrhosis and hepatocellular carcinoma. In patients, HBV is present in body fluids and secretions. In developed countries, the virus is most commonly transmitted sexually or via intravenous drug use. Occupational exposure and perinatal transmission do occur, but are rare. Hepatocellular carcinomata from HBV carriers often contain clonally propagated viral DNA integrations. HBV integration is accompanied by a deletion of at least 13.5 kb of cellular sequences on chromosome 11p13–11p14. This leaves only a single copy of the remaining cellular allele [Rogler et al. 1985].
– Aflatoxin is a naturally occurring mycotoxin produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are among the most potent mutagenic and carcinogenic substances known. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered to be the most toxic. The exposure to aflatoxin B1 causes a mutation G249T in *p53*.
– Cirrhosis of any etiology, including alcoholic cirrhosis and cirrhosis associated with genetic liver diseases, increases the risk of ensuing carcinoma.
– Androgens and estrogens contribute to the risk for liver cancer. The liver is morphologically and functionally modulated by sex hormones. Hepatocellular carcinoma is predominant in males and there is associated with a worse prognosis than in females. Some hepatocellular carcinomata are

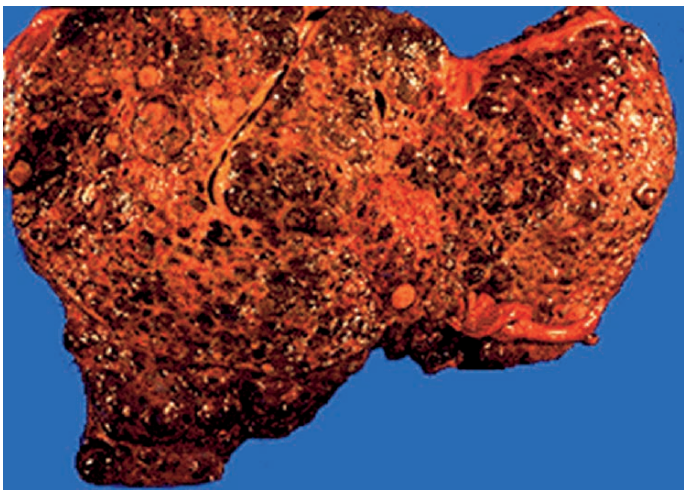


Figure 15.6.A. Hepatocellular carcinoma. Cross section of the liver showing advanced stage cirrhosis. The larger paler nodules are separate primary foci of hepatocellular carcinoma (HCC). The multifocality indicates a field effect of precancerous changes, widespread dysplasia in advanced cirrhosis. In general, hepatocellular carcinomata take the forms of a unifocal large mass, multifocal lesions with numerous nodules, or diffusely infiltrative growths. In all cases, the hepatocellular lesion is clearly distinguishable from the rest of the liver parenchyma. Even unifocal large masses are often associated with small satellite nodules. The nodules of hepatocellular carcinomata can be bile stained if the liver cells retain sufficient differentiation to make bile. Hepatitis B virus (HBV) infection is strongly linked to the prevalence of hepatocellular carcinoma. Other correlations exist with the ingestion of aflatoxin and a background of cirrhosis. This may be associated with repeated necrosis and regeneration of the liver. Primary carcinomata of the liver are extremely uncommon in Western Europe and represent around 1% of all reported cancers. However, in many parts of the Orient, particularly in areas where viral hepatitis is common, primary cancers of the liver can represent up to 40% of all reported malignancies. Males predominate over females at a ratio of 8:1. The outlook for hepatocellular carcinomata is not good with death occurring within 6 months of diagnosis. [Reproduced from http://pathweb.uchc.edu. With permission.]

androgen dependent, but others may depend on estrogen or on both hormones.

– Inherited metabolic disorders, such as *α-1-antitrypsin* deficiency [Eriksson et al. 1986], hemochromatosis [Deugnier et al. 1993], and tyrosinemia contribute to the risk. Homozygous *α1-antitrypsin* (*protease inhibitor 1*, *pi1*, *antielastase*) deficiency, which has an incidence between 1 in 1,600 and 1 in 2,000 live births, is the most common genetic cause of liver disease in children, and is also associated with hepatocellular carcinoma in adults.

These noxious influences drastically alter the matrix and microenvironment of the liver. The initial hepatocellular alterations that precede the appearance of hepatocellular carcinoma include foci of phenotypically altered hepatocytes and, subsequently, dysplastic hepatocytes that form foci and nodules [Thorgeirsson and Grisham 2002]. Specific molecular alterations characterize the precancerous liver.

– During most of the long preneoplastic stage, elevated expression of TGF-α and IGF-2 is responsible for accelerated hepatocyte proliferation. This results from the persistent production of these cytokines by inflammatory cells that infiltrate the damaged liver.
– Expression of DNA Methyl Transferases, which catalyze the methylation and demethylation of CpG groups, is increased in a fraction of livers affected with chronic hepatitis and cirrhosis. Both DNMT1 and DNMT3a are strongly up-regulated in hepatocellular carcinomata. *S*-Adenosylmethionine Synthase and Glycine *N*-Methyl Transferase, which augment the hepatocellular pool of methyl groups available for methylation reactions, also are expressed at elevated levels.
– Microsatellite instability occurs in hepatocytes in some forms of chronic hepatitis or cirrhosis.
– Nucleolar proteins regulate cell proliferation and growth by controlling ribosome biosynthesis and P53 functions. The gene product of *nucleostemin* is a nuclear protein that contains a NH_2-terminal basic domain, which specifies its nuclear localization and interaction with P53, and contains two GTP binding motifs. Nucleostemin is expressed in embryonic stem cells and primitive bone marrow cells. At the onset of differentiation, it is abruptly silenced. Nucleostemin is involved in regulating the proliferation of liver cancer cells.

Molecular pathways. Hepatocarcinogenesis proceeds through overactivation of pathways associated with IGF-2, TGF-α, WNT, and steroids. Transcripts from the *cyclin* gene family members *cdc20* and *cdk4*, as well as *myb* homologs are consistently elevated in hepatocellular carcinoma (Figure 15.6.B).

In liver cancer cells, MYC activity prevents the differentiation into hepatocytes and biliary cells forming bile duct structures. MYC is associated with the expression of the tumor marker α-Fetoprotein and the suppression of the liver cell marker Cytokeratin-8. Inactivation of MYC leads to dormancy and uncovers the pluripotent capacity of the tumors to differentiate into normal cellular lineages and tissue structures, while retaining their latent potential to become cancerous [Shachaf et al. 2004].

HCCA2 (YAP) is not expressed in normal adult liver tissue, but it is expressed in about 80% of hepatocellular carcinomata. The 467 amino acid, 51 kD protein contains two SH3-binding domains, 2 *N*-glycosylation sites, 6 *N*-myristoylation sites, and numerous phosphorylation sites, but it does not have a transmembrane domain, signal peptide, or targeting sequences. 1.8 kb and 2.5 kb transcripts of the gene exist in all adult tissues except the liver. Expression is also present in fetal liver, lung, brain, and spleen. The HCCA2 protein is localized in the cytoplasm of liver cancer tissues, but not in surrounding nontumor hepatocytes [Wang et al. 2001b].

IGF signaling proceeds through the RAS and PI 3-K pathways. It provides a growth stimulus to hepatocytes.

Chromosome 11p15 is subject to imprinting. In the liver, the monoallelic expression of *igf-2* due to genomic imprinting is relaxed during the postnatal period, resulting in biallelic expression thereafter. Abnormal imprinting, involving 11p15, is frequent in hepatocarcinoma. This leads to a change in expression of genes present on the maternal or paternal chromosome. As compared with normal liver tissue, significant alteration of expression commences in 1 allele of the *cdkn1C*, *slc22A1L* (*orctl2*, *Beckwith-Wiedemann region 1A*, *bwr1A*), and *igf-2* genes. Extreme allelic expression imbalance, leading to the restoration of monoallelic *igf-2* expression from the paternal allele, occurs in most hepatocellular carcinomata. Loss of maternal specific methylation of the *Kvdmr1* gene {11p15.5} in hepatocellular carcinoma correlates with abnormal expression of CDKN1C and IGF-2, suggesting a function for KvDMR1 as a long range imprinting factor active in adult tissues [Schwienbacher et al. 2000].

The Forkhead Box m1b (FOXm1b) transcription factor is essential for the development of hepatocellular carcinoma. In the absence of FOXm1b, resistance to hepatocellular carcinoma development is associated with nuclear accumulation of the cell cycle inhibitor $P27^{KIP1}$ and reduced expression of the CDK1 activator CDC25B. The tumor suppressor $P14^{ARF}$ inhibits FOXm1b [Kalinichenko et al. 2004].

The expression of *survivin* is under the control of the β-Catenin pathway. Survivin promotes cell proliferation in hepatocellular carcinoma by releasing $p21^{WAF1/CIP1}$ from CDK4, thus facilitating cell

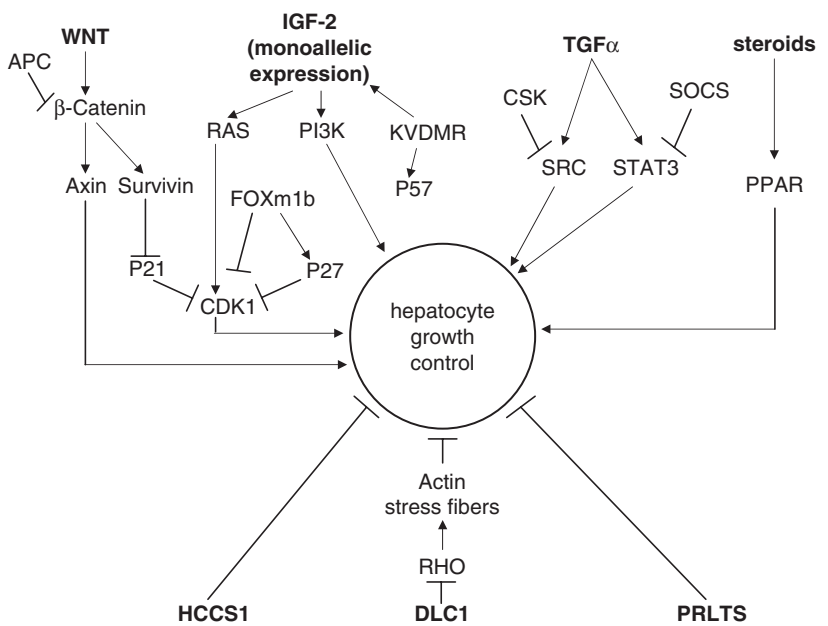


Figure 15.6.B. Hepatocellular carcinogenesis. The dysregulation of growth control is an early step in transformation. This may come about through a loss of function of negative regulators or through a gain of function of positive regulators. Four principal pathways to accelerated growth include signal transduction through WNT, IGF-2, TGF-α, and steroids. Three tumor suppressor pathways in the liver are associated with HCCS1, DLC1, and PRLTS.

cycle progression. The expression of *survivin* correlates with poor prognosis in patients with hepatocellular carcinoma.

TGF-α is a member of the EGF family. It transduces signals through SH2 domains, SRC, and STAT. A functional loss of negative regulators of SRC can lead to transformation. The kinase CSK, a negative regulator of SRC, is reduced in hepatocellular carcinoma as compared to normal liver tissue, and this altered expression correlates with enhanced SRC activity.

Hepatocellular carcinoma harbors persistently active STAT3 in association with hypermethylation, and hence suppression, of *socs1*, which encodes a negative regulator of STAT activity.

Activation of WNT signaling through mutations in *β-catenin* contributes to the development of hepatocellular carcinoma. Most of these are point mutations. Less frequent are mutations in the tumor suppressor gene *axin-1*, which arise in about 10% of cases. They comprise predominantly missense mutations and are present in cancer cells in conjunction with loss of heterozygosity of the other allele. *axin-2* is rarely mutated [Taniguchi et al. 2002]. The consequence of these defects is often an accumulation of β-Catenin in the nucleus.

Steroid hormones induce cell growth through nuclear receptors. Hepatocytes are sensitive to nuclear receptor signaling. Many phthalate ester plasticizers are classified as peroxisome proliferators. The exposure to some phthalates increases hepatocyte peroxisome expansion and cellular proliferation, as well as an increased incidence of hepatocellular tumors. Most effects of peroxisome proliferators are mediated by the nuclear receptors PPAR-α, PPAR-β, and PPAR-γ, which may play a critical role in events leading to liver cancer.

Loss of tumor suppression. Loss of heterozygosity on chromosome 17p13.3 is frequent in hepatocellular carcinoma. This may reflect the loss of the *hccs1* tumor suppressor [Zhao et al. 2001]. HCCS1 is a mitochondrial protein.

dlc1 (*deleted in liver cancer 1*) {8p22–p21.3} is a candidate tumor suppressor gene that is frequently deleted in hepatocellular carcinoma. Loss of heterozygosity for this gene occurs. In most tissues, including liver, a major 7.5 kb and a minor 4.5 kb transcript are expressed. The encoded protein contains 1,091 amino acids and shares high sequence similarity with RHO-GAP [Yuan et al. 1998].

prlts (*PDGF-Receptor β-like tumor suppressor*) is physiologically expressed as a 1.6 kb transcript at low levels in colon, lung, and liver. A 600 kb region on chromosome 8p22–p21.3 is commonly deleted in hepatocellular carcinoma. This causes a loss of *prlts*. Somatic missense and frameshift mutations of *prlts* may also arise in hepatocellular carcinoma.

Metastasis. The high mortality of hepatocellular carcinoma is mainly a result of intrahepatic metastases. The gene expression signature of primary hepatocellular carcinomata with accompanying metastases is very similar to that of their corresponding metastases, implying that the genes favoring metastatic progression are initiated in the primary tumors.

osteopontin is overexpressed in metastatic hepatocellular carcinoma and tumor invasiveness is dependent on Osteopontin [Gotoh et al. 2002; Ye et al. 2003].

Loss of heterozygosity for the long arm of chromosome {16q}, the location of several *cadherins*, including *cdh1* and *cdh13*, occurs in hepatocellular cancer. It increases the propensity for invasiveness.

Genetic predisposition. Several genes underlie familial hepatocellular carcinoma and sporadic (acquired) hepatocellular carcinoma, including *p53*, *met*, and *ctnnb1* (*β-catenin*).

Hepatocellular carcinoma is rare in children. Its occurrence is almost always a manifestation of familial adenomatous polyposis or its variant, Gardner syndrome. The underlying defects are a mutation in *apc*.

Childhood hepatocellular carcinoma may arise in the context of Silver–Russell syndrome (SRS) [Chitayat et al. 1988]. Silver–Russell syndrome is characterized by congenital hemihypertrophy, low birth weight, short stature, and elevated urinary Gonadotrophins. There are characteristic facial features, including a triangular shape with a broad forehead and small pointed chin [Silver et al. 1953; Russell 1954].

GSTs (Glutathione S-Transferases) are a family of enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. The expression of GST π is markedly upregulated in the initial phase of chemical hepatocarcinogenesis [Higashi et al. 2004]. Thus, the exposure to carcinogens induces the expression of a detoxifying enzyme, which then protects the liver cells from transformation. Hypermethylation of *gstp1* (*glutathione S-transferase π1*, *gst3*) {11q13} is

selectively connected to liver cancer. This may reflect the accumulation of carcinogenic agents in the liver in the absence of this enzyme.

Other liver tumors. Intrahepatic cholangiocarcinomata arise from large bile ducts within the liver parenchyma. Their incidence is high in Laos and Northern Thailand, where chronic parasitic infection is the most significant risk factor.

– G→A transitions in *p53* are often associated with forms that grow slowly and remain noninvasive.
– Mutations in codon 12 of *K-ras* are associated with the periductal infiltrating form of the disease.

Hepatoblastoma is a rare malignant tumor of the liver that occurs in children at an average age of 2–3 years. There is an association between very low birth weight and the development of hepatoblastoma. Although most cases of hepatoblastoma are sporadic, the incidence is highly elevated in patients with familial adenomatous polyposis coli, who carry germline mutations of the *apc* gene. In about 50% of sporadic hepatoblastomata mutations in *ctnnb1* affect exon 3, which encodes the degradation targeting box of β-Catenin. Most of these mutations are deletions. This leads to the accumulation of cytoplasmic as well as nuclear β-Catenin.

Loss of heterozygosity of chromosome 11p15 occurs in children with hepatoblastoma and in hepatoblastoma patients with Beckwith–Wiedemann syndrome. The localization of the *insulin-like growth factor 2* and *h19* genes on chromosome 11p15 suggests that their overexpression is associated with the somatic overgrowth of Beckwith–Wiedemann syndrome and the development of embryonal tumors, including hepatoblastoma.

Cancers of the gall bladder occur most frequently in native and Hispanic Americans. Gall stones are a risk factor. *p53* mutations are associated with the majority of gall bladder cancers.

Cancer of the extrahepatic bile duct may arise secondary to sclerosing cholangitis, coledochal cysts, or chronic infections. There is a high frequency of *K-ras* mutations.

15.7 RENAL CANCER

15.7.1 Renal Cell Carcinoma

Smoking, obesity, long-term use of phenacetin and acetaminophen, presence of kidney stones, and exposure to cadmium, thorotrast, petroleum products, and other industrial chemicals are risk factors for developing renal cancer. Membrane transporter molecules are paramount to the excretion of toxins through the kidneys. Their activity determines the exposure of kidney cells to carcinogens. The transmembrane transporter *abcb1* influences the susceptibility to develop renal epithelial tumors. The polymorphism C3435T is associated with ABCB1 expression levels and modulates disease risk. Especially, T and TT carriers are at risk for developing nonclear cell renal cell carcinoma, including papillary and chromophobe renal cell carcinoma as well as oncocytic adenomata [Siegsmund et al. 2002].

Chromosome instability is common in renal cancers. About one third of renal cell carcinomata are associated with 9p loss of heterozygosity, homozygous deletion of 9p21–22, or selective deletion of 9q. Among the target tumor suppressor genes, *p16* is within the deleted region. Consistently, the expression of P16 is often low in renal cell carcinomata [Cairns et al. 1995; Grady et al. 2001].

Renal cell carcinoma shares molecular features and regulatory mechanisms with renal regeneration and repair. The majority of the genes (75%) expressed in these distinct states are concordantly regulated. They reflect the orchestrated processes of cell proliferation and immune response. The discordant gene signature comprises genes of morphogenesis, glycolysis, HIF signaling, and IGF-1 signaling, which reflect the intrinsic pathologic nature of renal cell carcinoma [Riss et al. 2006].

Molecular pathways. Histologic forms of renal cell carcinoma (hypernephroma, adenocarcinoma of the kidney) include clear cell, chromophil (papillary), chromophobe, and collecting duct carcinoma (Bellini duct tumor). The underlying defect is often chromosome instability, but sequence infidelity in the RAS pathway may also occur.

Clear cell carcinoma of the kidney is characterized by a loss of chromosome 3p, reciprocal translocations between chromosomes 3 and 8, or gains of 5q. This tumor often metastasizes to the thyroid. In this case, most patients experience progressive disease with further spread and growth of metastasis resulting in death, frequently within 1 year of diagnosis. However, some patients have a protracted clinical course, living for several years with slowly progressive disease. The expression of *vascular cell*

adhesion molecule-1 (*vcam-1*) on the tumor cells is the gene most predictive for survival, with high levels of VCAM-1 indicating a good prognosis. VCAM-1 is a cell surface glycoprotein that interacts with the Integrin $\alpha_4\beta_1$ (Very-Late Antigen-4, VLA-4). In the normal kidney, the cells of Bowman capsule have high expression of VCAM-1 [Vasselli et al. 2003]. Clear cell renal carcinomata are characterized by inactivation of both copies of the *vhl* (*von Hippel-Lindau*) tumor suppressor gene by mutation (50% of cases) or by hypermethylation (10–20% of cases). The inactivation of the *vhl* gene mediates the overexpression of VEGF, results in high vascularity, and drives the malignant phenotype.

Chromophil renal tumors, which account for 15–20% of renal carcinomata, occur in both sporadic and familial forms. Histologically, vascularized connective tissue stalks are surrounded by neoplastic cells. Malignant papillary renal carcinomata are characterized by trisomy of the chromosomes 7, 16, or 17, and in men by loss of the Y chromosome.

Oncogenic missense mutations of the tyrosine kinase domain of *met* occur in papillary renal cell carcinomata. MET is activated or overexpressed in almost every case of differentiated chromophil renal tumors. Point mutations in the kinase domain convert MET to an oncogenic receptor. Such mutants are catalytically highly active, which correlates with more efficient MET autophosphorylation and phosphorylation of its substrates. The constitutive binding of c-SRC to the cytoplasmic domain of the MET M1268T mutant in renal papillary carcinomata, elevates c-SRC phosphorylation and activity. MET M1268T also phosphorylates substrates of the cytosolic kinase c-ABL, whereas wild-type MET does not. The expression of MET M1268T induces β-Catenin tyrosine phosphorylation and accumulation, induces constitutive activation of the transcription factor TCF, which acts in concert with β-Catenin in the nucleus and increases the expression of the β-Catenin/TCF target genes *myc* and *cyclin* D_1. RAN-BPM (RAN-BP9) is an interacting protein of MET and the interaction can be strengthened by HGF stimulation. RAN-BPM interacts with the tyrosine kinase domain of MET through its SPRY domain. RAN-BPM is not a guanine exchange protein, but it stimulates RAS activation by recruiting SOS. This induces GTP–RAS association and ERK phosphorylation and elevates *serum response element* (*sre*) expression, indicating that RAN-BPM can activate the RAS→ERK→SRE pathway [Wang et al. 2002].

Cromophobe renal cell cancer is characterized by clear or eosinophilic cytoplasm. It may have losses of chromosomes 1, 2, 6, 10, 13, 17, or 21. The receptor tyrosine kinase c-KIT (CD117) is virtually always highly expressed.

Collecting duct carcinoma is a usually poorly circumscribed and centrally necrotic tumor located in the medulla. It contains hobnail cells and desmoplasic stroma. Losses of chromosomes 1, 6, 14, 15, and 22 are common.

Protection from apoptosis. Proline Oxidase induces apoptosis in a pathway that mediates the formation of reactive oxygen species, the activation of Calcineurin, and the induction of the transcription factor NF-AT. Proline Oxidase expression is absent or reduced, compared to normal tissue, in a fraction of primary renal cell carcinomata. *Proline oxidase* is induced by P53. A lack of its expression in renal cancers may reflect P53 loss-of-function mutations [Maxwell and Rivera 2003; Rivera and Maxwell 2005].

Metastasis. Thyroid metastases are rare clinical entities, with the kidney being the most common primary site of origin. Metastatic colonization of the thyroid usually occurs in conjunction with dissemination to other sites in the body [Wood et al. 2004].

The VHL tumor suppressor is inactivated in many sporadic renal cancers. The gene product is required for the assembly of a proper extracellular Fibronectin matrix. This is evidenced in VHL-deficient cells by a delayed maturation of the Integrin β_1 chain, which is essential for the formation of β_1 fibrillar adhesions. This phenomenon may account for the increased motility of some renal cancer cells lacking VHL.

High FGF-2 levels in renal carcinoma correlate with a poor disease outcome [Nanus et al. 1993]. FGF-2 may contribute to tumor progression through its potent angiogenic activity.

Thymidine Kinase has a key role in the complementary or alternative salvage pathway of pyrimidine synthesis. The level of Thymidine Kinase activity correlates with the stage and grade of renal cell carcinoma. This suggests that Thymidine Kinase may be associated with the malignant potential of renal carcinoma cells [Mizutani et al. 2003].

Paraneoplastic syndromes. Paraneoplastic manifestations are present in up to 20% of patients with renal cell carcinoma and may cause the first clinical symptoms. Renal cell carcinoma cells elaborate proteins that serve as mediators of endocrine as well as nonendocrine paraneoplastic syndromes. These tumors may ectopically produce PTHrP (Parathyroid Hormone-related Protein) or Erythropoietin. Other paraneoplastic manifestations include hypercalcemia, cachexia, fever, hepatic dysfunction, anemia, and amyloidosis. Most paraneoplastic syndromes associated with renal cell cancer remit after resection of the primary tumor or after the treatment of metastatic sites [Gold et al. 1996].

15.7.2 Childhood Renal Cancer

Renal cancer in childhood may occur as a manifestation of inherited diseases.

– The von Hippel-Lindau (VHL) syndrome is an autosomal dominant familial cancer syndrome caused by deletions or mutations in the *vhl* tumor suppressor gene on chromosome 3p25. The disease is characterized by highly vascularized tumors. Von Hippel-Lindau syndrome predisposes to a variety of malignant and benign neoplasms, most frequently hemangioblastomata, but also renal tumors and pancreatic tumors. The cardinal features of von Hippel-Lindau syndrome are angiomata of the retina and hemangioblastomata of the cerebellum and spinal cord. The combination of hypertension with angioma may lead to subarachnoid hemorrhage. Polycythemia may be due either to hemangioblastoma of the cerebellum or to hypernephroma. Hemangiomata of the adrenals, lungs, and liver, and multiple cysts of the pancreas and kidneys, may arise in some instances. Clear cell renal cell carcinoma is a frequent cause of death, occurring in up to 70% of patients with von Hippel-Lindau syndrome. Pheochromocytomata occur in association with specific alleles. The pheochromocytoma may lead to hypercalcemia.
– Hereditary papillary renal carcinoma is characterized by the development of multiple, bilateral papillary renal tumors [Zbar et al. 1994]. The pattern of inheritance is consistent with autosomal dominant transmission with reduced penetrance.
– Birt–Hogg–Dube syndrome (fibrofolliculomata with trichodiscomata and acrochordons) is a rare inherited genodermatosis characterized by lung cysts and spontaneous pneumothorax, intestinal polyposis, hair follicle hamartomata, and kidney tumors. The renal neoplasias are typically chromophobe and oncocytic hybrid tumors. Chromophobe renal cancers in this syndrome express HIF-2α with weaker expression of HIF-1α. The syndrome is caused by mutations in *folliculin* (*flcn*, *bhd*) {17p11.2} [Rongioletti et al. 1989].

Childhood renal cancers typically occur on the basis of specific chromosomal translocations. They often involve the MITF/TFE subfamily of transcription factors. Some share the underlying genetic defect with alveolar soft tissue sarcoma.

– The MITF/TFE subfamily of basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors consists of TFE-3, TFE-B, and TFE-C, as well as MITF-A, MITF-H, MITF-M, and MITF-C. They can form both homodimers and heterodimers. Papillary renal carcinomata resulting from *tfe3* translocation primarily occur in children and young adults, arising typically before 30 years, and are more frequent in females than males. Xp11.2 translocations that result in fusions involving the gene for the transcription factor TFE3 lead to the formation of a distinctive neoplasm that shares the identical gene fusion as alveolar soft tissue sarcoma. Specifically, the translocation t(X;1)(p11.2;q21.2) results in the fusion of *prcc* (*papillary renal cell carcinoma*) at 1q21.2 to the *tfe3* gene at Xp11.2. Through this fusion, reciprocal translocation products are formed, which are both expressed in the nuclei of papillary renal cell carcinomata. PRCC is ubiquitously expressed in normal adult and fetal tissues and encodes a protein of 491 amino acids with a proline-rich domain. TFE3 is a member of the basic helix-loop-helix leucine zipper family of transcription factors with the ability to bind to μE3 elements in the *immunoglobin heavy chain* intronic enhancer. The fusion of the NH_2-terminal region of the PRCC protein to the entire TFE3 protein appears to be accompanied by the complete loss of normal *tfe3* transcripts [Sidhar et al. 1996; Weterman et al. 1996]. The resulting gene fusion generates excessively active transcriptional activators [Weterman et al. 2000], which are sufficient to cause transformation [Weterman et al. 2001a]. Papillary renal cell carcinomata with the translocation (X;1) (p11;q21) are defective in their mitotic checkpoint. The mitotic checkpoint protein MAD-2B interacts with PRCC. The PRCC–TFE3 fusion protein retains the MAD-2B-binding domain, but the functional interaction is

impaired [Weterman et al. 2001b]. These tumors express RCC and CD10, whereas the expression of Cytokeratins is inconsistent.

– A translocation t(X;1)(p11.2;p34) leads to the expression of a PSF–TFE3 fusion protein. Unlike wild-type TFE3 or PSF, which are nuclear proteins, PSF–TFE3 is targeted to the endosomal compartment. PSF–TFE3 sequesters wild-type TFE3, as well as P53 in the extranuclear compartment. The fusion protein acts through exporting TFE3, P53 and possibly other factors from the nucleus to the cytoplasm for degradation, leading to a transformed phenotype [Mathur et al. 2003].
– A *clathrin heavy chain-tfe3* fusion results from t(X;17)(p11.2;q23) in a renal carcinoma. The fusion transcript joins the 5′ exons of *cltc* {17q23} to the 3′ exons of *tfe3*. The CLTC–TFE3 product retains the nuclear localization and DNA-binding domains of TFE3, but lacks the multimerization domain of CLTC [Argani et al. 2003].
– Renal carcinomata associated with the t(X;17)(p11.2;q25), fusing *aspl* with *tfe3*, share this defect with a subset of alveolar soft tissue sarcomata, with the distinction that the translocation is balanced in renal cancers [Argani et al. 2001].
– A X chromosome inversion, inv(X)(p11.2;q12), results in the fusion of the *nonO* (*p54nrb*) gene to *tfe3* in renal tumors. NonO is probably involved in RNA splicing.
– In t(6;11)(p21;q13) containing renal cell carcinomata, the *tfe-B* gene on chromosome 6 is fused to the *alpha* gene on chromosome 11. The *alpha-tfe-B* fusion gene contains all *tfe-B* coding exons linked to 5′ upstream regulatory sequences of *alpha*. This effects a promoter substitution and results in a dramatic up-regulation of TFE-B protein levels, thereby severely unbalancing the nuclear ratios of the MITF/TFE subfamily members [Kuiper et al. 2003]. The basic helix-loop-helix leucine zipper subfamily of transcription factors may play a critical role in the regulation of renal cell growth; therefore, their dysregulation predisposes to transformation.
– A reciprocal translocation, t(14;15)(q11;q24), may be the sole cytogenetic aberration in classic congenital mesoblastic nephroma [Sawyer et al. 1996]. Congenital mesoblastic nephromata contain abundant levels of *igf-2*, but not *wt1* message [Sharifah et al. 1995]. The cellular variant of congenital mesoblastic nephroma, but not the classic variant, bears the same chromosome aberration t(12;15)(p13;q25) and ETV6–NTRK3 fusion protein as infantile fibrosarcoma, a tumor with which it shares morphologic and clinical features [Knezevich et al. 1998; Rubin et al. 1998].
– In the t(3;8)(p14.2;q24.1) translocation, a 200–300 kb region of 3p14.2, including the fragile site locus FRA3B, is homozygously deleted. This may cause renal cancers. Even in patients with nonhereditary renal cell carcinomata, a loss of alleles at loci on the short arm of chromosome 3 is common, suggesting that critical genes for kidney differentiation and growth control map to this site.
– An inherited chromosomal translocation, t(3;8)(p21;q24), predisposes to renal cancer [Cohen et al. 1979]. This may involve the activation of the *myc* proto-oncogene {8q24}.

Wilms tumor. Wilms tumor (nephroblastoma) is a common malignant renal tumor of childhood (Figure 15.7.2.A). It usually arises during the first 5 years of life, affecting approximately 1 in 10,000 children in this age group, and can occur unilaterally or bilaterally. The tumor origin is embryonic. It manifests when condensed metanephric mesenchymal cells (blastema) of the developing kidney fail to differentiate properly. The tumor has a premalignant state, termed the nephrogenic rests [Beckwith et al. 1990]. In Wilms tumor, *insulin-like growth factor 2* is highly elevated as compared with the adjacent, nontransformed kidney. The levels of FGF-2 in the urine of children with Wilms tumor correlate with disease stage and tumor grade [Lin et al. 1995].

– Mutations of *wt1* are present in only 10% of sporadic Wilms tumor, and constitutive mutations within the *wt1* gene {11p13} occur at a low frequency in bilateral Wilms tumor. *wt1* gene alterations include truncations, missense mutations, and deletions. Patients with the two most frequent nonsense mutations, R362X and R390X, suffer from very early onset. WT1 is a transcription factor and also has the ability to bind RNA. A downstream target in the WT1 pathway is the transcription factor PAX-2, which is active during the mesenchyme-to-epithelium transition in early kidney development and in Wilms tumor. *amphiregulin* is a transcriptional target of WT1. Amphiregulin modulates the growth of tumor cells through ligation of the EGFR.
– Loss of heterozygosity may occur on the Wilms tumor locus *wt2* (*multiple tumor-associated chromosome region 1*, *mtacr1*), which maps to chromosome 11p15.5. This is a chromosome location

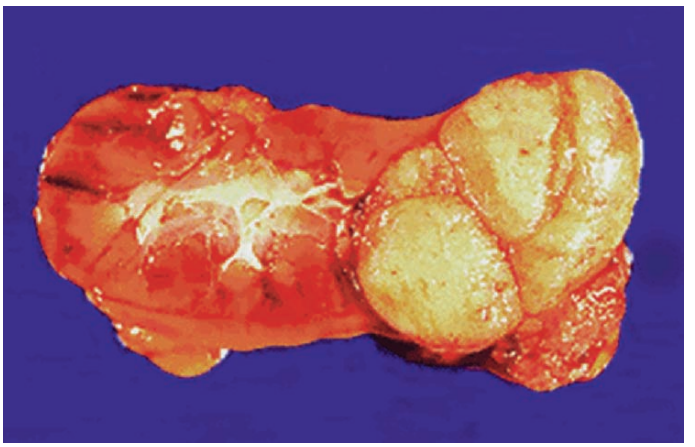


Figure 15.7.2.A. Wilms tumor. The tumor involves the lower pole of this kidney. Several seemingly separate tumor nodules have molded against each other to form the overall mass. The classic Wilms tumor is tripartite showing areas that are blastemal (nondescript undifferentiated small round cells), areas that show epithelial differentiation (tubules, glomeruloid structures) and areas that show stromal differentiation (spindle shaped cells, skeletal muscle). Mitotic figures are usually plentiful. Evidence is strong for a genetic and developmental etiology. The tumor may arise as a result of failure of blastemal tissue to differentiate into normal renal structures. The clinical presentation is that of a large abdominal mass in a child, usually 1–5 years old. The neoplasm is aggressive and metastasizes widely. At the time of detection, Wilms tumors are usually large and dwarf the native kidney. Irregular areas of hemorrhage and necrosis may be present. [Reproduced from http://pathweb.uchc.edu. With permission.]

that is subject to genomic imprinting, and *wt2* is controlled in this manner.

– Relevant tumor suppressor genes may lie on chromosomes 16q and 1p. Loss of heterozygosity for these regions occurs in 17% and 11% of Wilms tumors, respectively. Patients classified by tumor-specific loss of 16q, which contains *wt3*, have significantly worse survival rates.
– A familial Wilms tumor predisposition gene, *wt4* (*familial Wilms tumor 1*, *fwt1*) is located on chromosome 17q12–q21. It leads to transformation somewhat later (average age 5 years) than sporadic Wilms tumors (average age 3–4 years) [Rahman et al. 1996].
– Abnormalities involving chromosome 7p in Wilms tumor, including an interstitial deletion of 7q14 in some cases, reflect the presence of a relevant tumor suppressor gene, *wt5* (*wtsl*, *pou6F2*) {7p14–p13}. A constitutional balanced translocation t(1;7)(q42;p15) can cause Wilms tumor. Trisomy of 7q may occur in conjunction with this translocation [Wilmore et al. 1994; Perotti et al. 2004].
– Somatic mutations in *glypican-3* {Xq26} are associated with a fraction of Wilms tumors [White et al. 2002]. Glypicans (GRIPS) constitute a major family of cell surface proteoglycans. The Glypicans are anchored to the peripheral membrane through glycosylphosphatidylinositol linkage.

Wilms tumors are mostly sporadic [Breslow and Beckwith 1982], germline mutations cause fewer than 5% of all cases. However, the risk of Wilms tumor is increased in association with several congenital malformation syndromes.

– Children with Wilms tumor may have associated anomalies, including aniridia, hemihypertrophy, cryptorchidism, and hypospadias (WAGR syndrome). It occurs in association with an interstitial deletion of chromosome 11p13 [Miller et al. 1964; Riccardi et al. 1978]. The hemizygous deletion of the *pax6* gene {11p13} is responsible for aniridia associated with WAGR syndrome, whereas hemizygous deletion of the *wt1* gene {11p13} is associated with the genitourinary system malformations of this syndrome and with predisposition to the development of Wilms tumor in about 30% of cases.
– Beckwith-Wiedemann syndrome (BWS syndrome, Exomphalos–Macroglossia–Gigantism syndrome, EMG syndrome) is an overgrowth syndrome that predisposes to various pediatric malignancies. It may lead to Wilms' tumor in about 8% of cases through the loss of imprinting of *igf-2* {11p15.5}. This is caused by an expanded population of nephrogenic precursor cells [Beckwith et al. 1990]. Loss of $p57^{KIP2}$ (*cdkn1C*) underlies a fraction of cases of Beckwith-Wiedemann syndrome. $p57^{KIP2}$ is genomically imprinted, the

maternal allele being preferentially expressed while the paternally inherited allele is methylated and transcriptionally repressed. A microdeletion involving the entire *lit1* gene {11p15} causes silencing of $p57^{KIP2}$ when inherited maternally, and results in Beckwith-Wiedemann syndrome. A fraction of Beckwith-Wiedemann syndromes is also due to mutations in *nsd1*. The embryonal tumors hepatoblastoma, rhabdomyosarcoma, and Wilms tumor occur with increased frequency.
- Constitutive mutations within the *wt1* gene {11p13} lead to Wilms tumor, combined with pseudohermaphroditism and nephropathy, in the rare Denys–Drash syndrome [Denys et al. 1967; Drash et al. 1970]. The glomerular nephropathy, caused by diffuse mesangial sclerosis, is the most consistent feature of this condition and leads to end stage renal disease. The underlying hot spot mutation is WT1 R394W/Q/L. This mutation in zinc finger III is sufficient to abrogate DNA binding by the WT1 protein to a high affinity site. The risk of developing Wilms' tumor is about 90%.
- Perlman syndrome [Liban and Kozenitzky 1970; Perlman et al. 1973] comprises nephromegaly with renal dysplasia, macrosomia, cryptorchism, and multiple facial abnormalities. It predisposes to Wilms tumor [Henneveld et al. 1999]. Death frequently occurs during infancy and may be due to hyperinsulinism.

15.7.3 Pheochromocytoma

Pheochromocytomata are tumors of the adrenal medulla, originating from chromaffin cells. They secrete excessive amounts of catecholamines, usually epinephrine and norepinephrine. This causes symptoms of sympathetic nervous system hyperactivity, including elevated heart rate, elevated blood pressure, palpitations, anxiety often resembling that of a panic attack, diaphoresis, and headaches. Malignant hypertension may be a cause of death. Pheochromocytomata occur most often during young adult to midlife. Less than 10% of these tumors are invasive. About 10% of cases are familial and occur as part of
- Multiple endocrine neoplasia type 2 (MEN-2)
- von Hippel-Lindau syndrome
- Neurofibromatosis type 1 (NF-1)

In MEN-2, the overrepresentation of mutant RET (Rearranged During Transfection) in selected adrenomedullary cells may be an important mechanism in initiating the formation of a pheochromocytoma. In von Hippel-Lindau syndrome, pheochromocytoma development may occur by a *vhl* germline mutation and wild-type allelic deletion.

Paragangliomata (extraadrenal pheochromocytomata) are normally benign, chromaffin cell tumors arising from the sympathetic nervous system. They account for approximately 15% of all pheochromocytomata. Paragangliomata may be sporadic or hereditary. The hereditary variant characteristically manifests itself between the second and third decade of life, whereas the sporadic variant becomes manifest beyond the fourth decade. The familial forms occur in patients with germline mutations in genes that encode subunits of the mitochondrial complex II. Somatic mutations in these genes are often associated with sporadic paraganglioma [Martin et al. 1988; Niemann and Mueller 2000, Niemann et al. 2003]. Complex II (Succinate-Coenzyme Q Reductase, Succinate Dehydrogenase) of the respiratory chain, which is specifically involved in the oxidation of succinate, carries electrons from FADH to Coenzyme Q. It consists of four polypeptides, the flavoprotein, the iron sulfur protein subunit, and the integral membrane protein subunits SDHC and SDHD. They are products of the genes *sdhB* {11p36–35}, *sdhC* {1q21}, and *sdhD* {11q23}. These genes are encoded by the nucleus, not by mitochondrial DNA.

The Carney triad comprises gastric leiomyosarcoma, functioning extraadrenal paraganglioma, and pulmonary chondroma. The gastric tumor may metastasize. The syndrome affects mostly young women [Carney et al. 1977].

In a familial syndrome, paraganglioma may arise in conjunction with gastric stromal sarcoma. The condition is likely inherited in an autosomal dominant manner with incomplete penetrance. The paragangliomata are multicentric, the gastric stromal sarcomata are multifocal. The average age of the patients is 23 years [Carney and Stratakis 2002].

15.7.4 Rare Renal Tumors

Oncocytomata are epithelial tumors composed of large eosinophilic cells with small, round nuclei that have large nucleoli. They likely arise from the intercalated cells of collecting ducts. About 5% of all tumors derived from the tubular epithelium are renal oncocytomata. These tumors are always benign. c-KIT expression is a hallmark of oncocytoma.

Angiomyolipoma of the kidney may occur in conjunction with lymphangioleiomyomatosis (LAM). The disease primarily affects women. The lymphangioleiomyomatosis originates from the invasion of lung tissue by smooth muscle cells. They penetrate airways, blood vessels, and lymph vessels, leading to cyst formation and airflow obstruction. The underlying genetic defect is in *tsc*, and some patients display multiple symptoms of tuberous sclerosis.

Clear cell sarcoma of the kidney is a monomorphous neoplasm of early childhood, with a propensity for bone metastases, brain metastases, and early death. Although histologically quite distinctive, epithelial, myxoid and other variants can be misdiagnosed as Wilms tumor. There is no evidence for consistent genetic gains or losses. The high frequency of loss of imprinting for *igf-2* (around 45%), but not for the *snrpn* and *h19* loci, is comparable with that in Wilms tumors. IGF-2 is a potent growth factor and may play a role in the development or progression of clear cell sarcoma of the kidneys. Sporadic gain of 1q, loss of 10q, loss of terminal 4p, chromosome 19 loss, or chromosome 19p gain are associated with this neoplasm [Schuster et al. 2003].

Rhabdoid tumor of the kidney is a rare and highly malignant tumor of childhood [Beckwith and Palmer 1978]. Its cells resemble myoblasts, and their cytoplasm is prominently acidophilic. There are no ultrastructural features of skeletal muscle, however, and the cell type of origin is unknown. Separate primary neuroectodermal tumors of the brain sometimes occur. Rhabdoid tumor of the kidney is characterized by deletion of the *snf5* (*ini1*, *smarcB1*) {22q11} gene, which links it to other rhabdoid tumors of infancy that arise in the soft tissue and brain.

15.8 BLADDER CANCER

Most cases of bladder cancer probably result from the exposure to environmental carcinogens, most commonly cigarette smoke. Similarly, exposure to aromatic amines, which is common in dye manufacturing, constitutes a risk factor for bladder cancer. In Africa, squamous cell carcinoma of the bladder has a high incidence among the Fellaheen of Egypt and the Africans of Mozambique, Zimbabwe, and Zambia, all of which are countries where *Schistosoma haematobium* is endemic. While the association between schistosomiasis (bilharzia) and neoplasia is very strong [Cheever 1978; Gentile 1985], the underlying pathogenetic mechanisms are incompletely understood. *erbB2* (*her-2/neu*) is amplified in a fraction of schistosomiasis-associated bladder cancers.

In the Western hemisphere, most cancers arising in the bladder are transitional cell carcinomata (TCC). Some transitional cell carcinomata appear mixed with squamous features or a glandular component. In the Middle East and Africa, the most common form is squamous cell carcinoma. Less common pathologies are adenocarcinoma and small cell carcinoma. Distinct configurations of transitional cell carcinoma are papillary tumors and solid tumors. Most growths are papillary and low grade. They do not invade the muscularis propria of the bladder wall. Solid tumors typically are high grade and invasive. Two unique aspects of bladder cancer are multifocality and polychronotropism.

Molecular pathogenesis. Distinct molecular mechanisms underlie transitional cell carcinogenesis and squamous cell carcinogenesis. P53 is affected in most transitional cell and squamous cell carcinomata. The expression of a constitutively activated FGFR3 occurs in a large proportion of bladder cancers. The most frequent FGFR3 somatic mutation in epithelial tumors is S249C, affecting more than half of bladder cancers [Cappellen et al. 1999].

Aberrations of chromosome 9 are common in squamous cell carcinoma of the bladder. Superficial bladder cancer shows a high frequency of total or partial chromosome 9 losses. Deletions involving chromosome 9 represent the most frequent genetic change with an occurrence of 50–70%. This may reflect, in part, the loss of the tumor suppressor locus *cdkn2* {9p21}. Loss of heterozygosity at position 9q22.3, which harbors *patched* (*ptc*), is associated with highly recurrent tumors. PTC is a member of a signal transduction pathway and a tumor suppressor gene involved in basal cell carcinoma and may also play this role in superficial bladder cancer.

Chromosome 17 aberrations are frequent in transitional cell carcinoma, although deletion of 17p13, the region that contains the *p53* tumor suppressor gene, does not normally occur. Allelic loss of 11p and 17p is more frequent in high-grade tumors

[Sandberg and Berger 1994]. Loss of heterozygosity at 11p13 leads to deletion at the *cat* (*catalase*) locus in about 70% of bladder cancers, at the *wt1* (*Wilms tumor 1*) locus in about 50%, and at the *fshB* (*follicle stimulating hormone* β) locus in about 40% [Shipman et al. 1993].

In transitional cell carcinoma, EGF in the urine may enhance the dysregulation of cell growth. Therefore, the overexpression of EGFR on the tumor cells is related to higher grade and more aggressive disease.

In transitional cell carcinoma of the bladder, the genes overexpressed compared to normal bladder mucosa include protein tyrosine kinase-encoding genes, G-Protein-encoding genes, genes for second messengers, and *calmodulin*. Underexpressed genes in transitional cell carcinoma include apoptosis-related genes, protein tyrosine phosphatase encoding genes, *fibronectin*, *laminin*, and *collagen*.

Tumor cell survival. Circulating soluble CD95 (sFAS) antagonizes cell surface CD95 function and may interfere with immune surveillance against autologous tumors. Patients with bladder cancer on average have threefold elevated blood levels of soluble CD95, and higher levels of soluble CD95 correlate with reduced disease-free survival. This may be due to decreased antitumor activity by cytotoxic T-lymphocytes [Mizutani et al. 1998]. In addition, a high expression of CD95L by transitional cell carcinomata may contribute to immune escape through CD95-mediated killing of tumor reactive T-lymphocytes [Lee et al. 1999a,b].

A high incidence of *cd95* mutations exists in bladder cancer. The mutation G993A, which lies in the death domain, is a hot spot in this malignancy. Loss-of-function mutations of CD95 may arise in conjunction with loss of heterozygosity [Lee et al. 1999b].

Metastasis. The first step in bladder cancer dissemination is the invasion of the muscularis propria of the bladder wall. This process may continue with the infiltration of rectum or prostate. The spread of bladder cancer is often predominantly pelvic. Liver metastases are common, intestinal invasion may occur and poses a risk for an ileus.

Bladder cancer progression is associated with the loss of metastasis suppressor gene expression and the increased abundance of homing receptors that support cell motility. A decrease of E-Cadherin is a molecular marker for bladder cancer progression [Bringuier et al. 1993]. Likewise, low levels of NM23 are associated with tumor progression [Chow et al. 2000]. Splice variants of the homing receptor CD44 induce tumor cell dissemination. The expression of CD44 variants occurs during acquisition of invasiveness in bladder cancer [Chow et al. 2000].

Neovascularization is an important factor in tumor progression and metastasis. Various regulators of angiogenesis are aberrantly expressed in bladder cancers. CD147 (EMMPRIN, Basigin) is present on the surface of bladder cancer cells. These cells also express one or more VEGFR, which may participate in tumor progression.

PKC levels correlate with malignancy in bladder carcinoma [Schwartz et al. 1993]. Specific sequences within the Fibronectin molecule stimulate bladder cancer cell migration. These domains activate the PKC signal transduction pathway in invasive bladder cancer [Margolis et al. 1996].

Endocrine effects. G-CSF may be expressed in bladder cancer, which causes elevated G-CSF blood levels and leads to marked neutrophilia. This may present clinically as a paraneoplastic leukemoid reaction. G-CSF secretion is often associated with aggressive tumor growth and poor prognosis. In some cases, bladder cancer cells also express G-CSFR, and G-CSF stimulates their clonal growth in an autocrine fashion [Tachibana et al. 1995]. A fraction of bladder cancer cells may secrete G-CSF and PTHrP [Kamai et al. 1999; Ueno et al. 2000].

Environmental risk factors. Soluble products for excretion, including potential carcinogens, are concentrated highly in the bladder. Tobacco smoking and occupation are major risk factors of bladder cancer via exposure to polycyclic aromatic hydrocarbons and aromatic amines. This implies a role for drug-metabolizing gene products in modulating the risk for contracting bladder cancer.

– The slow *N*-acetylation genotype is a susceptibility factor in occupational and smoking-related bladder cancer. For cancers in which *N*-acetylation is a detoxification step, such as aromatic amine-related urinary bladder cancer, the NAT2 slow acetylator phenotype is at higher risk. Urinary bladder cancer risk is particularly high in the slowest NAT2 acetylator phenotype or genotype.

– The Gutathione Transferases GSTM1, GSTT1, and GSTP1 are involved in the detoxification of polycyclic aromatic hydrocarbon reactive metabolites. Null genotypes of *gstm1* and *gstt1* are associated with an increased risk of bladder cancer.
– A polymorphism of Glutathione Peroxidase places either a leucine or a proline at codon 198 of GPX-1. The GPX-1 P/L genotype, compared to the GPX1 P/P genotype, may significantly increase the risk of bladder cancer and may influence its disease status [Ichimura et al. 2004].
– Sulfotransferases (SULT) catalyze both the bioactivation and detoxification of a wide range of promutagens and procarcinogens. The *sult1A1* gene possesses a G→A polymorphism that results in an arginine to histidine amino acid substitution, and the 213 H allele has low activity and low thermal stability. Reduced bladder cancer risk is associated with the SULT1A1 213 H polymorphism [Zheng et al. 2003].
– DNA repair is an essential mechanism of tumor suppression. The frequency of the variant allele for an A/C polymorphism in exon 15 of *xpc* is significantly higher in patients with bladder cancer than in the healthy population. Variant allele genotypes in the DNA repair genes *xpg* and *nbs1* may also be associated with the occurrence of bladder cancer [Sanyal et al. 2004].

Genetic risk factors. Signal transduction intermediates in the RAS pathway are important determinants of cancer susceptibility. H-RAS, RAL-A, RAL-B, and RHO-GDI2, a regulator of RHO family members, participate in bladder cancer progression. Therefore, acquired mutations and inherited polymorphisms in the *H-ras* gene may modulate the risk of urinary bladder cancer. A frequent polymorphism, T81C, occurs in a wobble position. Individuals harboring the homozygous 81C genotype are at an increased risk of bladder cancer [Johne et al. 2003]. Furthermore, H-RAS-1 in bladder cancer may bear the activating mutation G12V.

A genetic predisposition to the development of bladder cancer is associated with Costello syndrome (Faciocutaneoskeletal syndrome, FCS syndrome). The condition comprises short stature, redundant skin of the neck, palms, soles, and fingers, curly hair, papillomata around the mouth and nostrils, and mental retardation. It is caused by the germline mutation G12V of H-RAS. In these patients, the incidence of bladder carcinoma is increased.

Benign bladder tumors. Papillomata are benign epithelial tumors. Activating point mutations in *fgfr3* are common in low-grade and low-stage bladder tumors. They are present in about 75% of urothelial papillomata [van Rhijn et al. 2002].

15.9 PROSTATE CARCINOMA

In 1853, J. Adams, a surgeon at The London Hospital, described the first case of prostate cancer [Adams 1853]. Heredity plays a role in the disease. Men with a father or brother affected are twice as likely to develop prostate cancer as men with no relatives affected. There is increasing risk with an increasing number of affected family members, such that men with two or three first-degree relatives affected have a 5- and 11-fold increased risk of developing prostate cancer, respectively [Steinberg et al. 1990]. Among races, African American men have the highest risk for prostate cancer development. They present with more advanced disease than Caucasians and have higher mortality rates, even when diagnosed at the same clinical stage. This may be caused by presence of significantly shorter CAG and GGC trinucleotide alleles of the *androgen receptor* among African Americans. Environmental factors also contribute to the cancer risk. The incidence of prostate cancer is highest in the Scandinavian countries, while Asian countries have the lowest rates. However, prostate cancer incidence and mortality rates in immigrants and their offspring soon approach those of their adopted homeland.

The heterologous morphologic lesions of prostatic epithelial dysplasia or atypia that occur at early stages of transformation are conventionally termed prostatic intraepithelial neoplasia (PIN). Gleason described a system for classifying prostate tumors, based on two levels of scoring, recognizing the heterogeneous differentiation in prostate carcinomata. The primary pattern of differentiation is assigned a Gleason grade of 1–5 based on the dominant morphology of the specimen and its departure from normal appearance. The secondary pattern is also assigned a grade [Gleason 1966]. While benign prostate hyperplasia typically originates from the transitional compartment of the prostate, prostate adenocarcinomata are derived from the posterior compartment (Figure 15.9.A).

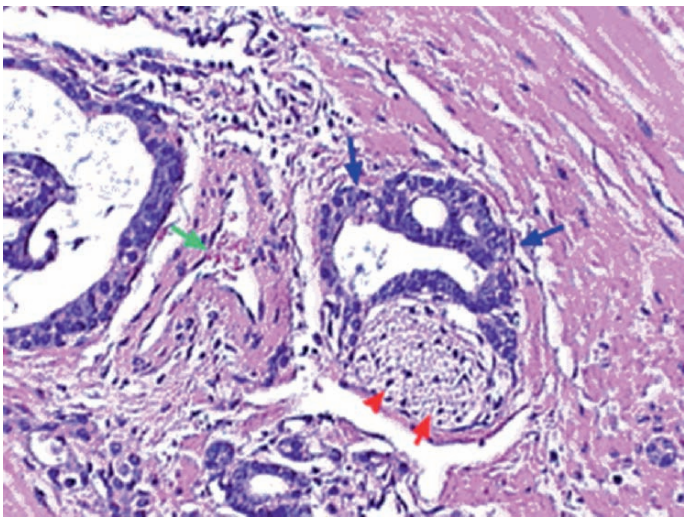


Figure 15.9.A. Prostate adenocarcinoma. This intermediate power photomicrograph of prostatic adenocarcinoma demonstrates perineural invasion. The red arrows point to a small nerve. The blue arrows illuminate a malignant gland immediately adjacent to the nerve which is within a perineural lymphatic. The green arrows show a blood vessel coursing with the nerve. Prostatic adenocarcinomata tend to arise peripherally rather than centrally. They are histologically diverse and many individual cases will have varying histologic patterns. Prostatic adenocarcinoma is a disease of older men, mostly over the age of 50. The incidence of latent prostatic adenocarcinoma may be 10% of men in their fifth decade and increases to 60% of men in their ninth decade. There are racial differences. Compared to the US white population, prostatic carcinoma has a higher incidence in the black population and a lower incidence in the oriental population. [Reproduced from http://pathweb.uchc.edu. With permission.]

Androgen pathways. Androgens are the primary growth hormones for prostate cells. Hence, upregulation of androgen signaling is an important pathogenetic mechanism of prostate cancer, and most prostate carcinomata are androgen dependent. Androgen independence (hormone refractory prostate cancer) may develop consecutive to missense mutations, which cause the Androgen Receptor (AR) to loose its ligand specificity. Frequently, there is not complete androgen independence, but increased ligand sensitivity.

– Hypersensitization to very low levels of androgen can be acquired as a consequence of gene amplification, resulting in elevated numbers of Androgen Receptors on the cell surface.
– Increased stability and enhanced nuclear localization of the Androgen Receptors can also cause a sensitization.
– Consecutive to missense mutations, the Androgen Receptor can loose its ligand specificity and promiscuously respond to a range of steroid hormones and pseudoandrogens. Receptors with the mutations T877A and L701H have increased affinity for glucocorticosteroids. The double mutant T877A, L701H, called AR^{CCR} (cortisol and cortisone responsive), has increased affinity for glucocorticosteroids. The H874Y mutation influences the binding of coactivator proteins by affecting the conformation of helix 12.
– High activity of 5α-Reductase may increase the local concentration of the ligand dihydrotestosterone, generate the phenotype of hypersensitization, and thus facilitate tumor progression.

The androgen-dependent growth pathways can be induced by increased transcriptional activity of the Androgen Receptor. This activity is regulated through interactions of the Androgen Receptor with various cofactors. Therefore, aberrant activity of the cofactors can contribute to transformation.

– CDC25B is up-regulated in high-grade and poorly differentiated prostate tumors, which are likely transiting to hormone independence. The coactivation of the Androgen Receptor by CDC25B may induce genes responsible for this progression. Therefore, CDC25B can promote neoplasia by coactivation to induce higher expression levels of Steroid Receptor target genes and by activating CDKs to enhance progression of the cell cycle and DNA reduplication.

– The cofactor ZIMP10 associates through its central region with the transactivation domain of the Androgen Receptor. In prostate cancer cells, ZIMP10 augments the transcriptional activity of the Androgen Receptor. It colocalizes with Androgen Receptor and SUMO-1 at replication foci throughout S phase and is capable of enhancing the sumolation of Androgen Receptor.

Androgen Receptor pathways that are activated by ligand-independent mechanisms can support the dysregulated growth of prostate cells. Mutations of signal transduction molecules associated with the Androgen Receptor may underlie this phenomenon. The receptor tyrosine kinase ERBB2 (HER-2/NEU) is consistently overexpressed in androgen-independent prostate cancer cells. ERBB2 can activate Androgen Receptor-dependent genes in the absence of Androgen Receptor ligands, but not in the absence of the Androgen Receptor. This process is mediated by MAPK (ERK), which phosphorylates the Androgen Receptor. Alternatively, PTEN is frequently inactivated in metastatic prostate cancers. PTEN normally inhibits PKB. In its absence, PKB can phosphorylate the Androgen Receptor on serines 213 and 791 and activate it. An alteration that occurs in approximately 60% of advanced prostate cancers is loss of heterozygosity at chromosome 10q23. It contains the tumor suppressor gene *pten* (*phosphatase and tensin homolog deleted on chromosome 10*).

Estrogens functionally antagonize androgens in the prostate. The prostate stroma expresses ERα and ERβ, and it may respond to estrogens by secreting growth-controlling substances into the prostate epithelium. Whereas the basal epithelial cells of the prostate express high levels of ERβ in healthy individuals, they diminish during tumor progression due to gene methylation.

TGF-β pathway. TGF-β regulates apoptosis in the prostate. TGF-β inhibits transcriptional activation mediated by Androgen Receptor. SMAD-3 specifically represses the transcriptional activation mediated by Androgen Receptor through a direct interaction between transcription activation domain of Androgen Receptor and the MH2 domain of SMAD-3. Through this mechanism, the TGF-β pathway regulates androgen signaling in prostate cancer [Hayes et al. 2001]. Normal transcripts and proteins of TGF-β Receptors are frequently lost in advanced prostate cancer cells. Aberrant functions of TGF-β Receptors I or II may contribute to tumor progression.

Although TGF-β inhibits the proliferation of normal prostate epithelial cells and functions as a tumor suppressor in early transformation, it acts as a tumor promoter in the later stages of tumor progression. While prostate cancer cells become resistant to TGF-β-induced growth inhibition and apoptosis, they retain TGF-β-mediated induction of extracellular matrix proteins, cell adhesion proteins, and proteases. Elevated expression of TGF-β in prostate cancer cells is associated with poor clinical outcome.

RNF11 (RING Finger Protein 11) can enhance TGF-β signaling through a direct association with SMAD4, the common signal transducer in the TGF-β, BMP, and Activin pathways. It interacts with the HECT-type E3 Ubiquitin Ligases NEDD4, AIP4, SMURF1, and SMURF2 [Azmi and Seth 2005]. RNF11 is highly expressed in prostate cancer.

MYC binds to SMAD-2 and SMAD-3 and represses the transcription of the $p15^{INK4B}$ gene, thus rendering cells unresponsive to the TGF-β-mediated inhibition of cell cycle progression. The *mxi-1* (*MAX-interacting protein-1*) tumor suppressor gene is involved in either the pathogenesis or the neoplastic evolution of some prostatic cancers. The MXI-1 protein negatively regulates MYC activity. *mxi-1* maps to chromosome 10q25, a region that is deleted in some cases of prostate cancer, and the retained allele may contain mutations [Eagle et al. 1995].

Loss of cell cycle control. Retinoids may be required for the normal differentiation of prostate epithelial cells. Retinoic acid inhibits the growth of prostate cells, possibly by activation of RB and modulation of Androgen Receptor pathways. Prostate cancer cells express abnormally low levels of the receptors RARβ and RXRβ. Prostate cancer cells are not able to metabolize significant amounts of retinal to retinyl esters. This lack of retinal esterification is associated with the absence of Lethicin:Retinol Acyltransferase protein expression [Guo et al. 2002]. Alterations in the *rb2* (*p130*) gene are also associated with prostate carcinoma. They may include loss of heterozygosity of chromosome 16q12.2.

Up-regulated expression of the zinc finger transcription factor *klf6* reduces cell proliferation and increases the levels of $P21^{CIP1/WAF1}$. KLF6 also mediates growth inhibition by inhibiting Cyclin D_1/CDK4 activity. Loss of heterozygosity of *klf6*

{10p15}, accompanied by mutations in the remaining allele, occur in prostate cancer [Narla et al. 2001]. Chromosome 10p is deleted in 50–60% of prostate cancers. A single nucleotide polymorphism in *klf6* causes the expression of a truncated gene product, which localizes into the cytoplasm. This is associated with an increased risk for prostate cancer [Narla et al. 2005].

Antiapoptosis. Overactivation of alternative survival pathways can relieve prostate cells of their dependence on androgen to protect them from apoptosis. BCL-2 is not normally expressed in the secretory epithelial cells of the prostate. Aberrant expression of this gene product in prostate cancer may enhance cell survival. BCL-X_L is also highly expressed in prostate cancer cells.

Endogenous Beclin-1 expression is frequently low in prostate carcinoma. This may reflect allelic deletions of *beclin-1*, but not *beclin-1* coding mutations. The *beclin-1* gene is monoallelically deleted in a fraction of prostate cancers. Loss of the *beclin-1* gene {17q21} leads to an increase in spontaneous tumors, consistent with a role for this gene as a tumor suppressor that mediates autophagic cell death.

Furthermore, hormone insensitive prostate cancer cells may escape programmed cell death by the secretion of high amounts of Osteoprotegerin.

DNA repair. Mismatch repair genes, including *msh2*, *mlh1*, *pms2*, and *pms1*, are often defective in prostate cancer.

A large proportion of prostate cancers carry fusions of the 5′ untranslated region of *tmprss2* to the genes for either of two ETS transcription factors, *erg* or *etv-1*. ERG and ETV-1 are components of a growth control pathway. The fusion places the regulatory sequence of the androgen-responsive *tmprss2* upstream of *erg* or *etv-2*. This leads to overexpression of ERG or ETV-1 [Tomlins et al. 2005].

Metabolism. The detoxifying enzyme GSTπ1 is expressed in normal prostatic epithelium, where it catalyzes the intracellular elimination of electrophilic compounds. It acts as a negative regulator of steroid hormone associated pathways. The gene for GST*π1* is often silenced by promoter methylation in steroid hormone-related tumors, including prostate cancer.

Metastasis. The aggressiveness of prostate cancer varies widely. Some tumors progress to invasive disease, whereas others stay latent for the remainder of an individual's lifetime. The stroma plays a role in this process. Primary prostatic epithelial cells migrate toward healthy prostatic stromal cells. In contrast, stromal cells from tumor tissue do not induce such migration [Hall et al. 2002]. Tumor progression in prostate cancer is also associated with specific genes expressed by the cancer cells.

The loss of metastasis suppressor genes is a factor in prostate cancer progression. *kai-1* (*prostate cancer antimetastasis gene*, *leukocyte surface antigen r2*, *sar2*, *cd82*, *suppressor of tumorigenicity*, *st6*) {1p11.2} encodes a member of a structurally unique family of leukocyte surface glycoproteins. KAI-1 is a protein of 267 amino acids, with four hydrophobic transmembrane domains and one large extracellular hydrophilic domain with three potential *N*-glycosylation sites. Its presence on the cell surface suppresses metastatic potential. The expression of this protein is reduced in metastatic prostate tumor cells.

Cadherins facilitate adhesion and suppress invasion. Loss of heterozygosity for the long arm of chromosome {16q}, the location of several *cadherins*, including *cdh1* and *cdh13*, occurs in prostate cancer. Furthermore, posttranslational truncation and inactivation of E-Cadherin may occur in prostate cancer.

Various signaling molecules, mostly associated with homing receptors, regulate invasive potential. Their dysregulation may lead to prostate cancer metastasis. An amplicon on chromosome 17 leads to overexpression of PRC17, a RAB GTPase Activating Protein (RAB-GAP), in 15% of all prostate cancers. The protein is overexpressed in about half of metastasizing prostate cancers [Pei et al. 2002].

A decreased expression of RKIP (RAF Kinase Inhibitor Protein) is associated with an invasive phenotype in prostate cancer, which is consistent with the possibility that RKIP is a metastasis suppressor gene. RKIP is a negative regulator of RAF signaling, G-Protein signaling, anf NF-κB activity. It promotes apoptosis and its loss may aid the survival of circulating cancer cells [Keller et al. 2004, 2005].

Extracellular proteins contribute to metastasis formation in various ways. Kallikrein-2 (Prostase) and Kallikrein-3 (PSA, Prostate-Specific Antigen), as well as Kallikrein-11 are markers for prostate

cancer progression. Kallikrein-3 is also implicated in the development of osteoblastic bone metastasis in prostate cancer through its support of interactions between prostate cancer cells and bone endothelial cells.

Vimentin is considered to be the intermediate filament of mesenchymal tissue and may serve as a tumor marker. Vimentin contributes to the invasive phenotype of prostate cancer.

Hereditary prostate cancers. Approximately 10% of all prostate cancers are hereditary with an autosomal dominant pattern.

- A susceptibility locus for prostate cancer on 1q24–q25 is designated *hpc-1* (*hereditary prostate cancer 1*, *prostate cancer susceptibility 1*, *pcs-1*, *prca1*) [Smith et al. 1996].
- There is a prostate cancer susceptibility locus on 1q42.2–q43, significantly distant from the locus on 1q24–q25. It is designated *pcap* (*predisposing for prostate cancer*, *prostate cancer susceptibility 2*, *pcs-2*) [Berthon et al. 1998]. *pcap* is the locus most frequently predisposing to hereditary prostate cancer in Southern and Western Europe.
- The location of a prostate cancer susceptibility gene, symbolized *hpcx* (*prostate cancer hereditary X-linked*, *prostate cancer susceptibility X-linked*, *pcsx*), is on chromosome Xq27–q28. This gene accounts for approximately 16% of hereditary prostate cancer cases [Xu et al. 1998].
- Differences in Steroid Hormone Receptors play an important role in the risk of prostate cancer. A germline variation in CAG repeat length within the *androgen receptor* gene is a significant predictor for aggressive prostate cancer. A shorter CAG repeat sequence in the *androgen receptor* gene predicts higher grade and advanced stage of prostate cancer at diagnosis, and metastasis and mortality from the disease [Giovannucci et al. 1997].

Other prostate tumors. Benign prostatic hyperplasia is characterized by a proportional increase in the size of the stromal compartment of the gland, involving alterations to extracellular matrix components. Some of these changes are associated with the activity and expression of TGF-β_1.

Versican (Chondroitin Sulfate Proteoglycan-2) is overexpressed in benign prostatic hyperplasia as well as in prostate cancer and potentially contributes to disease pathology. Some of the ADAMTS lineage of Metalloproteases possess Versican degrading properties and are potential regulators of proteoglycan accumulation. The major inhibitor of these proteases in the extracellular matrix is TIMP-3. The negative effect of TGF-β_1 on ADAMTS-1, ADAMTS-5, ADAMTS-9, and ADAMTS-15, coupled with increases in their inhibitor TIMP-3 may aid the accumulation of Versican in the stromal compartment of the prostate in benign prostatic hyperplasia and prostate cancer [Cross et al. 2005].

Most prostate cancers are adenocarcinomata. A subset of prostate carcinomata is composed predominantly of neuroendocrine cells and forms prostatic small cell neuroendocrine carcinoma. Prostate small cell carcinoma expresses a repertoire of genes that reflect characteristics of their neuroendocrine origin. *ascl1*, *ina*, and *sv2B* are potential molecular markers for small cell neuroendocrine tumors and for neuroendocrine cells of the prostate [Clegg et al. 2003].

15.10 TESTICULAR CANCER

Cancer of the testis is a relatively uncommon disease, accounting for approximately 1% of all cancers in males. The age-related incidence is bimodal with one peak between ages 15 and 35 years, due predominantly to embryonal carcinomata, and a second peak up to the age of 75 years, constituted mostly by seminomata. Cryptorchidism is the major identifiable risk factor associated with the development of testicular cancer. Patients with a history of unilateral testicular cancer are at risk for developing cancer in the other testicle.

In normal testis, the cell-type-restricted expression patterns of Cyclins are dominated by high levels of Cyclin D_3 in quiescent Leydig cells and the lack of any D-type Cyclins in the germ cells. Leydig cells are the only nontransformed mammalian cells that proliferate in the absence of these Cyclins. Most carcinoma in situ lesions gain expression of Cyclin D_2, but not D_1 or D_3. In contrast, invasive testicular tumors show variable positivity for Cyclins D_2 and D_3, but rarely D_1.

While *mgmt* {10q26} is rarely mutated in cancer, low levels of the MGMT protein are associated with testicular cancer. This may reflect reduced transcription due to altered gene methylation. The average serum levels of PTN, FGF-2, EGF, and VEGF

are elevated in patients with testicular cancer because of high secretion from the tumor. This occurs, from early stages on, in seminomatous as well as nonseminatous tumors.

In 90% of testicular tumors, including all histologic types and both gonadal and extragonadal presentation, isochromosome 12p is present. In contrast, del(12)(q13-q22) arises selectively in nonseminomatous germ cell tumors and mixed germ cell tumors, occurring in 44% of such lesions. Genes located in the affected areas of chromosome 12 include *mgf* and *kit*. KIT is expressed in a large fraction of seminomata, but rarely in nonseminomatous testicular cancers. In contrast, MGF is rarely expressed in seminomata and 50% of the nonseminomata [Murty et al. 1992]. Loss of heterozygosity for 3p or 11p arises in 40% of testicular cancers [Samaniego et al. 1990; Lothe et al. 1989].

Embryonal carcinoma. The most frequent germ cell neoplasm is embryonal carcinoma. It is a primitive tumor derived from totipotent germ cells. Risk factors for testicular germ cell tumors (TGCTs) include a history of undescended testis, testicular dysgenesis, infertility, previous testicular germ cell tumor, and a family history of the disease. Testicular germ cell tumor is associated with a characteristic series of abnormalities in the RB pathway, including the up-regulation of Cyclin D_2 and P27 and down-regulation of RB1 and the CDK inhibitors P16, P18, P14, and P21. The synergistic pattern in gene expressions of the RB pathway, characteristic of testicular germ cell tumors, is rare in other malignancies [von Eyben 2004]. *tgct-1* {Xq27} is a susceptibility gene for this cancer [Rapley et al. 2000]. A consistent chromosomal abnormality in invasive testicular germ cell tumors is a gain of the short arm of chromosome 12, mostly due to isochromosome formation of 12p. Klinefelter syndrome (47,XXY) is a risk factor for extragonadal germ cell tumors.

An initial precursor stage, intratubular germ cell neoplasia, is characterized by triploidization and by an up-regulation of KIT, ALPP, CCDN2, and ZNF354A, with a concomitant down-regulation of CDKN2D ($P14^{INK4d}$).

Seminoma. Seminomata are tumors that occur during the continued differentiation along germ cell lines. They are subclassified as either classic or spermatocytic. A somatic mutation of the co-SMAD gene *smad4* leads to the loss of SMAD4 protein function in seminomata. This mutational inactivation may affect the activity of several members of the TGF-β superfamily, such as TGF-β, Activin, Inhibin, and BMP.

Angiogenesis is essential for tumor growth and metastasis. VEGF is involved in increased angiogenic activity and disease progression in solid tumors. VEGF expression predicts metastasis formation in seminomata [Fukuda et al. 1999].

Teratocarcinoma. Teratocarcinomata are malignant tumors that contain elements of teratomata and embryonal carcinomata. Teratocarcinoma occurs during differentiation into somatic structures. They can form from germ cells in the testes. Extratesticular teratomata can arise from pathogenetically activated oocytes.

Choriocarcinoma. Choriocarcinomata are cancers that develop from germ cells, typically in younger men. They usually have a fast growth rate and high propensity for hematogenous dissemination, preferentially to the lungs, liver, and brain. Occasionally, the primary tumor grows so fast that necroses from insufficient blood supply, leaving behind only a small scar. The prognosis is poor.

Leydig cell tumor. Tumors arising from stromal tissue may originate from primitive gonadal mesenchyme and are subcategorized as Leydig cell tumors (interstitial cell tumors), Sertoli cell tumors, gonadoblastomata, and granulosa-theca cell tumors.

Adult male germ cell tumors exhibit pluripotency to differentiate into embryonic, extraembryonic, and somatic tissue types. Multiplication of chromosome 12p, manifested in i(12p) or tandem duplication of 12p, is a unique change in germ cell tumors. *Cyclin* D_2 maps to 12p and may play a causative role in transformation. Chromosomal deletions associated with germ cell tumors include 12q13, 12q22, and 5p15.1–15.2 [Murty and Chaganti 1998]. Loss of function of the tumor suppressor gene *rb1* play a key role in the process. During tumor progression, genetic alterations in the metastasis suppressor genes *dcc* and *nme-1* come into play.

15.11 OVARIAN CANCER

Reproductive and hormonal factors are main determinants of ovarian cancer risk. The incidence of ovarian cancer is relatively high in industrial countries,

where women on average have few children. The incidence is lower in Asia and Africa with higher fertility rates. Hormone replacement therapy increases the risk for ovarian cancer.

Epithelial ovarian cancers constitute 90% of all ovarian cancers. There are four major histologic types of epithelial ovarian carcinoma, mucinous, clear cell, serous, and endometrioid. Borderline mucinous ovarian tumors constitute a unique group characterized by higher epithelial cell proliferation and atypia than benign tumors, but lacking the stromal invasion characteristics of ovarian cancers. These tumors metastasize within the peritoneal cavity, but rarely result in death. Serous adenocarcinomata are highly aggressive tumors that develop from the precursor lesion endometrial intraepithelial carcinoma.

In epithelial ovarian cancers, two signaling pathways, involving RAS→MAPK and Phosphatidylinositol 3-Kinase→PKB, are particularly important. These pathways may interact at various levels. DNA repair is also frequently compromised in ovarian cancer.

A tumor marker for ovarian cancer is CA125. Genes overexpressed in clear cell ovarian carcinoma include *gpx3* (*glutathione peroxidase 3*), *glrx* (*glutaredoxin*), *lbp* (*lipopolysaccharide-binding protein*), *cspg2* (*chondroitin sulfate proteoglycan 2*), *igfbp1* (*IGF-binding protein 1*), *pthlh* (*parathyroid hormone-like hormone*), *tcf2* (*transcription factor 2*), *nid2* (*nidogen 2*), *lamb1* (*laminin β1*), *comp* (*cartilage oligomeric matrix protein*), *anxA4* (*annexin A4*), *ggt1* (*γ-glutamyl transferase 1*), *nnmt* (*nicotinamide N-methyl transferase*), *mal* (*T-cell differentiation protein*), *fxyd2* (*FXYD domain containing ion transport regulator 2*), and *rbp4* (*retinol-binding protein 4*) [Schwartz et al. 2002].

RAS pathway. The vast majority of mucinous ovarian adenocarcinomata have *K-ras* mutations. Their frequent occurrence implies that these mutations are an early event in the development of mucinous ovarian cancers.

Serous adenocarcinomata are a rare, highly aggressive form of ovarian cancer. The B-RAF mutation V600E is associated with 30% of low-grade and borderline serous carcinoma. The mutation likely occurs very early in transformation. High-grade tumors do not have B-RAF mutations.

The tumor suppressor ARHI (RAS Homolog Member I, NOEY-2) {1p31} is a GTPase with 50–60% amino acid homology to RAS and RAP. Whereas ARHI and RAS proteins share similar GTP/GDP-binding domains, they exert opposite functions. ARHI is consistently expressed in normal ovarian epithelial cells but is dramatically down-regulated in more then 70% of ovarian cancers.

PI 3-K pathway. The EGF Receptor (EGFR) family of tyrosine kinases play important roles in ovarian follicle development and in cell growth regulation of the ovarian surface epithelium. EGFRs are up-regulated in up to 70% of ovarian cancers. EGFRvIII (EGF Receptor variant III) is a constitutively active EGFR mutant that is expressed at a high frequency in ovarian cancer. It induced a reduced expression of E-Cadherin and increased levels of N-Cadherin, coupled with a gain of Vimentin expression, and a loss of the epithelial Keratins and Mucins. This suggests that EGFRvIII expression induces a mesenchymal phenotype in ovarian cancer, and thereby contributes to more aggressive disease [Zeineldin et al. 2006]. A fraction of ovarian cancers carry amplified *erbB2*. This correlates with poor survival.

Amplification of multiple members of the Phosphatidylinositol 3-Kinase pathway is a hallmark of serous epithelial ovarian cancers. The resultant activation of PI 3-K→PKB signaling in the tumor cells contributes to cell cycle progression and decreased apoptosis. The α subunit of *pi3k* is amplified in approximately 80% of ovarian cancers. Somatic mutations of the gene for the P85α subunit (*pik3r1*), comprising deletions in the inter-SH2 region proximal to the S608 autoregulatory site, may also contribute to ovarian cancers. PKB is a downstream signaling target of PI 3-K. *pkb2* is amplified in 12% of ovarian cancers, which are characterized by poor differentiation.

DNA repair. Chromosome instability is common in ovarian carcinoma. Almost half of all ovarian cancers have reduced expression of MAD2 and fail to activate spindle checkpoint control. Fragile sites may also be afflicted in ovarian cancers. *park2* expression is down-regulated in 60% of primary ovarian tumors. *park2* is located on the fragile site FRA6E. FRA9E is located on chromosome 9q32–33. The distal end of this fragile site contains *pappa* (*pregnancy-associated plasma protein A*), whose expression is frequently lost in ovarian cancer.

A high frequency of loss of heterozygosity on chromosome 6p, 6q, 17p, or 17q is associated with

ovarian cancers [Eccles et al. 1990; Saito et al. 1992]. Possibly relevant tumor suppressor genes on chromosome 17 include *p53* and *brca1*. The region of chromosome 17p13.3 that is deleted in 80% of all ovarian epithelial malignancies contains the potential tumor suppressor genes *ovca1* (*dph2l1*) and *ovca2* [Schultz et al. 1996]. The frequent loss of heterozygosity of chromosome 11q25 suggests that a tumor suppressor gene associated with sporadic ovarian cancer may reside at this locus.

Amplification at chromosome locus 11q13.5 is common in ovarian carcinomata. *rsf-1* (*hBXap*, *HBV X-associated protein*) is the only gene consistently overexpressed in all of the tumors harboring this amplification. This overexpression is associated with a significantly shorter overall survival. RSF-1 plays a role in chromatin remodeling and transcriptional regulation, and it functions as a Histone chaperone. The RSF-1/SNF2H complex (RSF complex) participates in chromatin remodeling by mobilizing nucleosomes in response to a variety of growth modifying signals and environmental cues [Shih et al. 2005].

Sequence fidelity may be compromised in ovarian carcinomata. Loss-of-function mutations in mismatch repair genes, such as *msh2*, predispose to ovarian cancer. Hypermethylation of the *mlh1* promoter results in microsatellite instability and can lead to ovarian tumors. Endometriosis may be a precursor of the endometrioid subtype of ovarian carcinoma. Endometrioid adenocarcinomata preferentially exhibit microsatellite instability. The activity of β-Catenin, a key component of the WNT signaling pathway, is deregulated in about 40% of ovarian endometrioid adenocarcinomata, usually as a result of *ctnnb1* (*β-catenin*) gene mutations.

Antiapoptosis. Serous adenocarcinomata have frequent *p53* mutations. Mutations in *p53* also occur in 20% of endometrioid adenocarcinomata, but are not prevalent in endometrial hyperplasias. Mutant P53 may be overexpressed in ovarian carcinoma. The overexpression of *p53* is associated with advanced stage and poor survival. Hence, alterations in *p53* are a relatively late event in transformation.

Endogenous Beclin-1 expression can be reduced in ovarian carcinoma. This may reflect allelic deletions of *beclin-1*, but not *beclin-1* coding mutations. The *beclin-1* gene is monoallelically deleted in 40–75% of sporadic ovarian cancers.

In some cases of ovarian cancer, TNF-α can induce a signal that leads to the death of these cells. However, many ovarian malignancies are resistant to the effects of TNF-α. In these cases, the extracellular signals transduced by death receptors are extinguished before the cascade of Caspases can be activated. The overexpression of FLIP, a protein that blocks the Caspase activity of FLICE, mediates this resistance.

Invasion and metastasis. Ovarian carcinoma can spread by direct extension to adjacent organs, and exfoliated tumor cells can be transported throughout the peritoneal cavity by the peritoneal fluid [Naora and Montell 2005]. The peritoneal cavity is the preferred site for metastasis. Ovarian cancer cells frequently express CXCR4. Its ligand CXCL12 is expressed by peritoneal mesothelial cells and is present in the ascites of ovarian cancer patients. The seeding of the peritoneal cavity is often followed by the formation of ascites. VEGF is an important factor for promoting ascites accumulation.

Lysophosphatidic acid (LPA) is a soluble factor associated with ascites formation and promotion of invasiveness by ovarian cancer cells. This phospholipid induces the secretion of UPA and the activation of MMPs. Lysophosphatidic acid also activates MEK-1, which facilitates the redistribution of Focal Adhesion Kinase (FAK) to focal adhesions. This localization of FAK is essential for the mediation of focal adhesion turnover, which is a rate-limiting step of cell migration [Naora and Montell 2005]. In ovarian cancer, lysophosphatidic acid induces the secretion of pro-MMP-2. This may depend on the up-regulation of Integrin β_1 expression by lysophosphatidic acid. β_1 Integrin clustering on the cell surface promotes the activation of pro-MMP-2 and the processing of Membrane Type-1 MMP.

Metastasis to the breast is possible [Recine et al. 2004]. Skin involvement is a late complication that occurs rarely in ovarian cancer patients. The prognosis after skin metastases is poor and the most important prognostic factor associated with survival is the interval time until cutaneous involvement [Cormio et al. 2003]. Isolated parenchymal splenic lesions are very rare and may occur as a late recurrence in epithelial ovarian cancer [Tserkezoglou et al. 2005].

Secreted proteases are important for ovarian cancer cell invasiveness. They may be induced in various ways.

– In ovarian carcinoma, the increased expression of type-IV Collagenases (MMP-2 and MMP-9) and the decreased expression of E-Cadherin are associated with increasing stage of the disease and with poor prognosis [Herrera et al. 2002].
– A correlation exists between the expression level of E1AF (ETS Variant 4, ETV4) and tumor progression in ovarian cancer. E1AF can activate the promoters of various *matrix metalloproteinases*, genes whose expression is associated with tumor cell invasion and metastasis.
– EGFR is overexpressed in up to 70% of ovarian carcinomata. Signaling through this receptor promotes the motility and invasiveness of ovarian carcinoma cells by stimulating the phosphorylation and translocation of Ezrin, a protein that links the Actin cytoskeleton to the plasma membrane. This alters cell–matrix adhesion by modulating the expression of Integrin α_6, a principal receptor for Laminin-1, and by activating MMP-9.
– The expression of Kallikrein-9 is regulated by steroid hormones in ovarian cancer cells. Kallikrein-9 constitutes a favorable prognostic marker in ovarian cancer [Yousef et al. 2001].

Aggressive, high-grade types of ovarian carcinoma show very high levels of activated STAT3. It is possible that STAT3 contributes to the motility of ovarian carcinoma cells, in addition to its ability to promote survival. STAT3 localizes not only to nuclei, but also to focal adhesions in ovarian cancer cells. There, it interacts with phosphorylated Paxillin and FAK. JAK/STAT signaling may contribute to ovarian cancer cell invasiveness [Silver et al. 2004].

Ovarian cancer predisposition syndromes. Ovarian cancer can arise on the basis of inherited predisposition. Mucinous carcinomata are underrepresented in familial ovarian cancer, as compared to sporadic ovarian cancer.
– In ovarian cancer-prone families, the susceptible tissue is not limited to the ovary, but includes other derivatives of the celomic epithelium, from which primary peritoneal neoplasms may arise, a condition referred to as familial peritoneal ovarian carcinomatosis. The embryologic derivatives of the ovary comprise gonadal ridges composed of mesodermal cells covered by coelomic epithelium. Patients with a hereditary predisposition to ovarian carcinoma may harbor the first germinal hit in both the epithelial cells of the ovary as well as their derivatives in the celomic mesothelium. These patients are then inordinately susceptible to carcinogenesis from somatic hits in these same tissues. [Lynch et al. 1986].
– Mutations in the *brca1* gene account for around 80% of families with the hereditary breast–ovarian cancer syndrome [Narod et al. 1994]. Most others (around 15%) are due to mutations in *brca2*. Patients with germline *brca1* mutations may develop papillary serous carcinoma of the peritoneum (PSCP), a malignancy that diffusely involves the peritoneal surfaces, while sparing or only superficially involving the ovaries. Papillary serous carcinoma of the peritoneum is histologically indistinguishable from serous epithelial ovarian carcinoma, and it may develop years after oophorectomy.
– Hereditary site-specific ovarian cancer represents a variant of the breast–ovarian cancer syndrome. The condition is inherited as an autosomal dominant trait with an early age of onset [Gallion and Smith 1994]. It is attributable to mutations in either *brca1* or *brca2*. A large Ashkenazi Jewish kindred with eight cases of ovarian carcinoma was characterized by a 185delAG mutation of the *brca1* gene [Liede et al. 1998].
– Tumorigenesis based on inherited mutations of *brca1* displays variable penetrance. In this context, the *H-ras-1* variable number of tandem repeats (VNTR) polymorphism, located 1 kb downstream of the *H-ras-1* proto-oncogene is a possible genetic modifier of cancer penetrance in individuals with *brca1* mutations. Individuals, who have rare alleles of the variable number of tandem repeats polymorphism, have an increased risk of ovarian cancer. Hence, a modifying gene may have an effect on the penetrance of an inherited cancer syndrome [Phelan et al. 1996].
– In some forms of hereditary non-polyposis colorectal cancer, the endometrium and ovaries, as well as the pancreas also have high incidence of cancer. These forms of hereditary non-polyposis colorectal cancer are referred to as Lynch syndrome 2.
– Peutz–Jeghers syndrome is an autosomal dominant disorder characterized by melanocytic macules of the lips, buccal mucosa, and digits, multiple gastrointestinal hamartomatous polyps, and an increased risk of various neoplasms. Ovarian tumors occur in the Peutz–Jeghers syndrome [Christian et al. 1964]. They are characteristically of granulosa cell type. The disorder is due to mutations in the *serine/threonine kinase 11* (*stk11*, *lkb1*) gene.

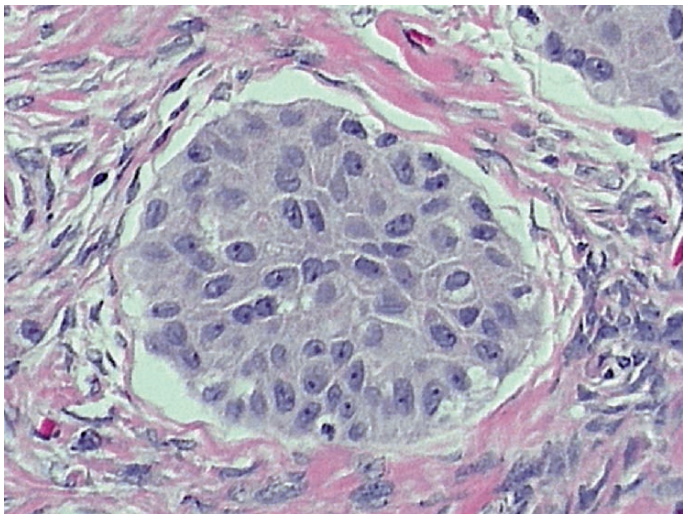


Figure 15.11.A. Brenner tumor. The center of the image is an epithelial nest in a Brenner tumor. The cells have very distinct margins. The nuclei are oval with definite micronucleoli. There is no pleomorphism or mitotic activity. The cells do not organize around a lumen. The growth consists of nests or cysts of cells that resemble the urothelium. They are generally bland and contain benign nuclei. Brenner tumors are solid or cystic. These tumors are virtually always benign. The malignant counterpart, which is rare, resembles transitional cell carcinoma of bladder. Brenner tumors are usually asymptomatic until achieve large size. Brenner tumors arise in premenopausal - or postmenopausal women. In 10% of cases they are bilateral. [Reproduced from http://pathweb.uchc.edu. With permission.]

– Ovarian cancer arises in the basal cell nevus syndrome [Berlin et al. 1966]. The disorder results from mutations in the *ptch* gene. Ovarian fibromata are frequent.
– Ovarian cancer is associated with cases of gonadal dysgenesis, in which XY cells are present [Vergote et al. 1983] and in Turner syndrome [Goldberg and Scully 1967].

Other tumors. Brenner tumors (**Figure 15.11.A**) are uncommon tumors that are part of the surface epithelial–stromal tumor group of ovarian neoplasms. Histologically, there are nests of transitional-type epithelial cells with longitudinal nuclear grooves (coffee bean nuclei) lying in abundant fibrous stroma. Ninety percent are unilateral. The tumors can vary in size from less than 1 cm to 30 cm. The majority of these tumors are benign. They are solid and sharply circumscribed. Borderline and malignant Brenner tumors are possible but each is rare.

Transitional cell carcinoma is the malignant variant of Brenner tumors. It is a very rare entity, in which neoplastic transitional epithelial cells are present in the ovary. Histologically, it is characterized by multilayered epithelium with papillary folds and solid nests that invade into the stroma.

Granulosa cell tumors are ovarian stromal tumors that represent 5% of all malignant ovarian neoplasms. They exhibit many morphological, biochemical, and hormonal features of proliferating preovulatory granulosa cells. The cells of these tumors generate estrogen and Inhibin, and they express FSH Receptors. The excessive estrogen serum levels may lead to paraneoplastic syndromes. The incidence of granulosa cell tumors ranges from 0.4 to 1.7 per 100,000. Eighty percent of the patients with advanced tumors die from recurrent disease [Fuller and Chu 2004].

Although Peutz–Jeghers syndrome is associated with an increased risk for granulosa cell tumor, neither allele loss at the disease locus 19p13.3 nor mutations in the *stk11* (*lkb1*) gene are associated with the sporadic forms of these tumors.

15.12 ENDOMETRIAL CARCINOMA

Unopposed stimulation of the endometrium by estrogens is the classic etiologic factor associated with the development of endometrial cancer. Two types of endometrial carcinoma are distinguished with respect to biology and clinical course. Type-I carcinoma is related to hyperestrogenism by association with endometrial hyperplasia, frequent expression of Estrogen Receptors and Progesterone Receptors, and younger age. In contrast, type-II carcinoma is unrelated to estrogen, associated with atrophic endometrium, frequently lacks Estrogen Receptors and Progesterone Receptors, and occurs at older age. Histologically, endometrioid and mucinous carcinomata are considered type I, serous and clear cell carcinomata are type II.

Different genetic pathways are active in the development of type I and type II carcinoma. The most frequent genetic alteration in type I carcinoma is PTEN inactivation by mutation, followed

by sequence infidelity (microsatellite instability), and mutations of *K-ras* or *β-catenin. pten* inactivation, *K-ras* mutations, and loss of sequence fidelity are early events, occurring in a subset of atypical endometrial hyperplasia, whereas *p53* mutation is a late event, during progression in about 10–20% of endometrioid carcinomata. In type II carcinoma, *p53* mutation is the most frequent genetic alteration, followed by inactivation of P16 and E-Cadherin, and amplification of *erbB2* (*her2/neu*). Aneuploidy can arise [Evans and Podratz 1996]. *p53* mutation occurs in endometrial intraepithelial carcinoma, the putative precursor of serous carcinoma. Due to the identification of these genetic pathways, the current histological classification of endometrial carcinoma is molecular based [Lax 2004].

PTEN. Somatic, genetic, and epigenetic inactivation of PTEN is involved in as high as 93% of sporadic endometrial carcinomata, irrespective of their microsatellite status, and can occur in the earliest precancers. Somatic mutations in *pten* account for around 50% of cases. They mostly result in protein inactivation and, as with germline mutations, recurrent somatic mutations arise in CpG dinucleotides. Loss of PTEN function leads to overactivity of the PI 3-K→PKB pathway, resulting in excessive growth.

K-RAS. The RAS-dependent growth pathway can be excessively activated in endometrial cancer by amplification of *K-ras* (around 5% of cases) or by mutation (25%), most frequently a point mutation in the hot spot of codon 12. There may be geographic variations in the frequency of *K-ras* mutations with an incidence of about 10% in Americans and 20–30% in Japanese endometrial cancers. *K-ras* mutations are associated with endometrial hyperplasias, and therefore likely represent an early event in the development of endometrial carcinomata.

APC pathway. β-Catenin acts as a negative regulator of the APC pathway. *β-catenin* mutations arise in endometrioid carcinoma (type I). The transcription factor MYC is a downstream target in the APC pathway. It induces cell cycle progression. The amplification of *c-myc* (around 20%) is common in endometrial carcinoma.

Amplification of *cyclin E* (*ccne*) occurs in a fraction of endometrial carcinomata (15%). The overexpression of Cyclin E in this cancer may also be due to mutation and loss of heterozygosity in *cdc4*, which codes for the F-box protein that targets phosphorylated Cyclin E for proteosomal degradation.

DNA sequence fidelity. A form of mutagenesis by insertion or deletion in repetitive elements is specifically associated with endometrial carcinomata. Approximately 20% of endometrial cancers display microsatellite instability, which is a reflection of mutations in mismatch repair genes, that may affect *msh2*, *msh3*, *msh6*, and *mlh1* [Simpkins et al. 1999]. Microsatellite instability in endometrial carcinogenesis occurs late in the transition from complex hyperplasia to carcinoma. It is preceded by progressive inactivation of *mlh1* through promoter hypermethylation. The ensuing loss of MLH1 protein expression leads to microsatellite instability, diploid tumors, and lack of P53 overexpression. In contrast, *msh2* methylation is infrequent in endometrial carcinomata.

Frequent loss of chromosome 10q sequences in endometrial cancers is consistent with the involvement of a tumor suppressor gene. The afflicted region, associated with grade 1 cancer, is 10q25–10q26. The genetic alterations suggest that disruption of the DNA mismatch repair system plays an important role in the course of endometrial carcinogenesis [Peiffer et al. 1995].

The endometrioid adenocarcinomata that exhibit microsatellite instability shows a stepwise progressive accumulation of secondary mutations in oncogenes and tumor suppressor genes that contain short tandem repeats in their coding sequences.

Steroid hormones. The nuclear receptor for progesterone is widely expressed in uterine cancer. The loss of Progesterone Receptor expression is associated with more advanced disease. Furthermore, a loss of expression of both Progesterone Receptor and E-Cadherin at an advanced stage is associated with the increased expression of the metastasis gene products CD44 and CSPG2 (Versican) [Hanekamp et al. 2003]. The presence of the chaperone HSP27 is correlated with the degree of tumor differentiation as well as with the presence of Estrogen Receptors and Progesterone Receptors.

The expression of CYP1B1 is predominantly extrahepatic with high amounts in the endometrium. CYP1B1 is important in steroid metabolism because it catalyzes the 4′ hydroxylation of estradiol.

Polymorphisms in *cyp1B1* modulate the risk for endometrial cancer.

Familial predisposition. Familial predisposition for endometrial carcinoma exists.
– Endometrial carcinoma is the most frequent extracolonic cancer in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome, type 2) [Vasen et al. 1990].
– The common T16189C transition within the D loop region of the mitochondrial chromosome is associated with type II diabetes, with dilated cardiomyopathy, and with endometrial cancer [Liu et al. 2003].

15.13 Cervical Carcinoma

Cervical cancer is the second most common cancer in women, although 80% of the world's cervical cancers occur in less developed countries. This cancer affects about 16 per 100,000 women per year and causes death in about 9 per 100,000 per year. Risk factors for cervical cancer include HPV infection, smoking, HIV infection, chlamydia infection, dietary factors, oral contraceptives, multiple pregnancies, low socioeconomic status, use of the hormonal drug diethylstilbestrol (DES), and a family history of cervical cancer.

Cervical cancer is detectable early by screening utilizing the Pap smear and microscopic evaluation. Premalignant dysplastic changes are characterized as cervical intraepithelial neoplasia. Clinically, the cancer may present with vaginal bleeding, but symptoms are often absent until advanced stages have been reached. In late-stage disease, metastases may be present intra-abdominally and in the lungs.

The histologic types of cervical carcinoma include:
– Squamous cell carcinoma
– Adenocarcinoma
– Adenosquamous carcinoma
– Neuroendocrine carcinoma
– Variant or mixed forms

P63 is a member of the P53 family of proteins. It is normally present in cervical epithelial cells. The expression of P63 sharply distinguishes squamous cell carcinoma ($P63^+$) from adenocarcinoma ($P63^-$) (Figure 15.13.A). Large cell, poorly differentiated carcinomata can be

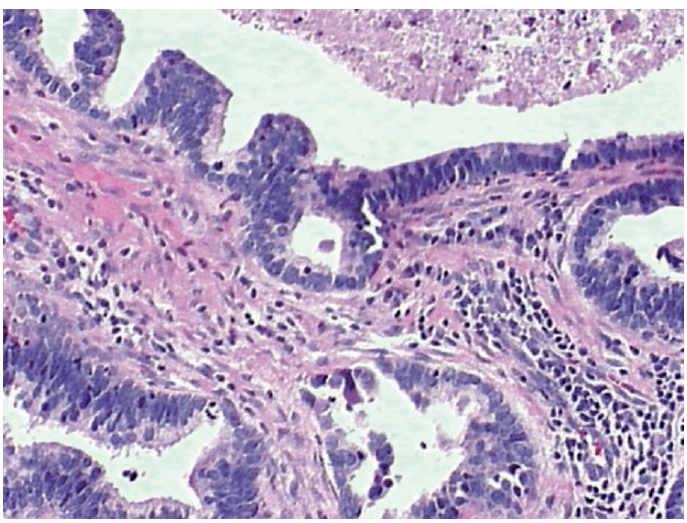


Figure 15.13.A. Cervical adenocarcinoma. The glands are lined by cells with large hyperchromatic nuclei and scant cytoplasm. The large gland lumen at the top of the image shows necrotic debris. The stroma contains lymphocytes and plasma cells. Multiple layers of neoplastic cells can be seen lining the glands. The appearance of adenocarcinomata may be fungating or may be ulcerated, but is often deeply infiltrative. Necrosis and hemorrhage are common. The tumor is composed of glands with cells containing Mucin vacuoles. Adenocarcinomata account for up to 10% of carcinomata of the cervix. They are associated with infection by human papilloma virus infection, especially type 18. Integration of HPV genetic material into the host genome leads to inactivation of P53. These tumors are a consequence of sexually transmitted diseases particularly affecting young women, increased in cigarette smokers, with an age peak at 40–45 years. [Reproduced from http://pathweb.uchc.edu. With permission.]

distinguished as putative glandular (glassy cell) or squamous (lymphoepithelial like or spindle cell) types based on the expression of P63. Neuroendocrine tumors are Chromogranin positive and show no or low levels of P63 expression [Wang et al. 2001a].

Human papillomavirus (HPV) is a major pathogenetic factor in cervical cancer. The oncogenic HPV types (HPVs 16, 18, 45, and 58) can be present as episomes or may integrate into the human chromosomes. The integration of HPV DNA into the genome occurs early in cervical cancer development and is probably an important event in malignant transformation [Meisels and Fortin 1976; zur Hausen 1977]. Infection with HPV requires the availability of basal layer cells, which is usually given in microlesions of skin or mucosa. In the basal layer cells, the loss of the viral gene *e2* together with the expression of the early viral genes *e5*, *e6*, and *e7* results in enhanced proliferation. A frequent characteristic of HPV-infected cervical epithelial cells is the loss of viral *e2* gene expression as a consequence of the viral DNA integration into the cellular genome. The integration of the HPV genome usually disrupts the *e2* gene open reading frame. It eliminates the E2-dependent suppression of E6 and E7 protein synthesis. In the absence of the viral E6 and E7 oncoproteins, the P53 and RB pathways are activated, G_1 cell cycle arrest sets in, followed by the induction of cellular senescence.

E5 enhances cell growth by

– Forming complexes with the EGFR and the PDGFR. The sustained activation of the PDGFR, caused by binding to E5, accelerates the proliferation of the transformed cells.
– Preventing apoptosis in response to DNA damage.

E6 and E7 are consistently expressed in malignant tissues. Only the E6 and E7 gene products of high-risk HPV types are able to immortalize and transform human cells. E7

– Interacts with and blocks RB
– May block the functions of $P21^{CIP1/WAF1}$ and $P27^{KIP1}$
– Activates *cyclin E* and *cyclin A* expressions
– Induces centriole amplification, which leads to aneuploidy

E6

– Targets P53 for degradation in the proteasome
– Activates Telomerase
– Inhibits the degradation of SRC family kinases

E6 is impaired by $P16^{INK4A}$. E7 bypasses this inhibition by directly activating Cyclin E and Cyclin A. E6 prevents E7-induced apoptosis by degrading P53 and BAK. The *p53* gene is polymorphic at codon 72, encoding either proline or arginine. The arginine form of P53 is much more susceptible to degradation by E6 than the proline form. Patients with cervical cancer have an overrepresentation of homozygosity for the P53 arginine allele compared with the normal population [Storey et al. 1998].

In HPV-infected cervical cancer cells, Endothelin-1 and its receptor Endothelin Receptor A are overexpressed. The Endothelin A Receptor is a G-Protein-coupled receptor that mediates mitogenesis by Endothelin-1. Their coexpression supports an autocrine growth response. This receptor–ligand pair also mediates cervical cancer neoangiogenesis.

Expression of a constitutively activated FGFR3 occurs in a small proportion of cervical cancers. A FGFR3 somatic mutation in epithelial tumors is S249C [Cappellen et al. 1999; Wu et al. 2000]. Signaling pathways induced by constitutively active FGFR3 may converge with PYK-2 pathways to provide maximal activation of STAT-5B, resulting in protection from apoptosis [Meyer et al. 2004].

Metabolism. Increased glucose uptake and utilization is exhibited by malignant cells. The principal mechanism, by which transformed cells achieve this is the overexpression of the Glucose Transporter protein family. Glucose Transporter-1 (GLUT1, SLC2A1) is overexpressed in squamous cell carcinoma of the cervix. The expression levels correlate with tumor grade, but not with the progression from preneoplastic lesions, suggesting that GLUT1 is a late marker of cellular transformation. The possible relationship between GLUT1 expression and tumor blood supply suggests that malignant cells may have an adaptive ability to compensate for a compromised microenvironment [Mendez et al. 2002].

Invasion. Predictors for tumor progression are the depth of stromal invasion, lesion depth, and nodal involvement. The elevated expression of NEGF-2 (Midkine) and VEGF are associated with the progression of cervical cancer. MMPs are important for the digestion of extracellular matrix components during invasion. In cervical cancer, active MMP-2 associates with the tumor cell membrane. There, it forms a complex with Integrin $\alpha_V\beta_3$, MT1-MMP, and TIMP-2. This leads to the activation of MMP-2 in the cervical cancer cell membrane and may have a role in dissemination [Mitra et al. 2003].

Sialic acids and their derivatives are ubiquitous at the terminal positions of the oligosaccharides of glycoproteins. Their transfer from cystidine-5-monophospho-*N*-acetyl neuraminic acid (CMP-NeuAc) to the terminal position of the carbohydrates on glycoproteins and glycolipids is catalyzed by a family of Sialyl Transferases. Cervical squamous carcinoma cells express altered sialoglycoconjugates on the plasma membrane. This is due to enhanced Sialyl Transferase activity during tumor invasion and metastasis. A combination of enhanced *st6gal I* mRNA expression and decreased mRNA expression for *st3gal I*, *st3gal III*, and *st3gal IV* may be important in cervical cancer progression and lymph node metastasis [Wang et al. 2001, 2002; Chen et al. 2002].

Neurologic complications are rare in cervical cancer and virtually nonexistent in stage 0 disease. Metastatic neurologic complications are more common than nonmetastatic complications. Lumbosacral plexopathy, caused by retroperitoneal lymph node metastases, is the most common neurologic complication [Saphner et al. 1989].

15.14 BREAST CANCER

Risk factors. Early menarche, late menopause, postmenopausal hormone replacement therapy, exposure to xenoestrogens, and nulliparity increase breast cancer risk, while early full-term pregnancy has a protective effect. Some of this variation may be due to the modification of reproductive risk factors, such as age of menarche, by diet.

Factors associated with increased estrogen production, such as obesity and increased dietary fat, elevate the risk for breast cancer, especially in postmenopausal women. There is a strong correlation between fat intake and breast cancer rates. A change from a traditional Oriental diet to a Western diet increases the risk of breast cancer, likely reflecting the high fat content of the Western diet [Armstrong and Doll 1975; Doll and Peto 1981]. Abundant consumption of ω-6 polyunsaturated fatty acids (PUFAs) stimulates several stages in the development of mammary cancer, from an increase in oxidative DNA damage to effects on cell proliferation, and from free estrogen levels to hormonal catabolism. In contrast, fish oil derived ω-3 fatty acids and monounsaturated fatty acids, such as oleic acid (ω-9), may prevent cancer by influencing the activity of enzymes and proteins related to intracellular signaling and cell proliferation [Bartsch et al. 1999].

Weight gain after menopause increases the risk of breast cancer [Eliassen et al. 2006] by about 50% through increasing the serum concentration of estradiol. After menopause, when the ovarian production of estrogens has ceased, the circulating estrogens are synthesized in the stromal cells of the adipose tissue through the enzymatic aromatization of androstendione to yield estrone and then estradiole. In obese women, there is an elevation in postmenopausal plasma estrogen concentrations, which may be associated with an increased breast cancer risk [Rose et al. 2004].

Obesity and Insulin resistance often occur together. They are risk factors for breast cancer, particularly among postmenopausal women. They are also associated with late-stage disease and poor prognosis. This may be due, in part, to increased levels of Adipocytokine production in obesity or Insulin resistance. Adipocytokines [Funahashi et al. 1999] are a group of cytokines that are produced exclusively or substantially by fat cells. Some Adipocytokines may act directly on breast cancer cells to stimulate their proliferation. Most Adipocytokines also mediate an increase in angiogenesis [Rose et al. 2004].

Women with denser breasts have a higher risk of developing breast cancer than women with less dense breasts [Wolfe 1976]. The connective tissue in dense breasts contains high amounts of Collagen, which has a rapid turnover. Fibroblasts in dense breasts also secrete more IGF-1, which may act as a growth factor for breast epithelial cells. It is possible that the fibroblasts in dense breasts facilitate the transformation of epithelial cells with genetic damage. A dense layer of adipose tissue fibroblasts may possess particularly high levels of Aromatase and exert estrogenic stimulation through a paracrine mechanism.

The luminal epithelial cells are responsible for most breast tumors. A proportion of breast cancers have myoepithelial cell characteristics. Women who have a history of fibrocystic changes with atypical ductal hyperplasia have 4.5 times elevated risk for breast cancer.

Histology. Breast cancers have varied histopathologic appearances, including tubular, colloid

(mucinous), and papillary. Papillary carcinoma of the breast is a rare entity.

Early stages of transformation form lesions called carcinoma in situ. It is characterized by the proliferation of malignant epithelial cells within lobules or ducts, which are surrounded by an intact basement membrane. In situ breast tumors are distinguishable as ductal carcinoma in situ (DCIS) (Figure 15.14.A) and lobular carcinoma in situ (LCIS), according to whether the lesion involves the ducts in the terminal duct lobular unit, or in small ducts and lobules. Forms of breast cancer include:

– DCIS occurs at low grade (cribriform patterns without nuclear atypia of pleomorphism, mitosis, or necrosis), intermediate grade (solid patterns with moderate nuclear atypia, necrosis possible), and high grade (marked nuclear pleomorphism, necrosis, mitoses). Low-grade DCIS includes cribriform DCIS (common type is characterized by ducts filled with uniformly appearing cells surrounding small lumina, favorable prognosis if completely excised) and papillary carcinoma in situ (rare form, composed of fibrovascular stalks, lined by stratified epithelial cells, absence of myoepithelial cells in the papillary processes). High-grade DCIS may be a comedocarcinoma (most common type, characterized by loosely cohesive cells, high nuclear grade, mitoses, and central necrosis, frequently expresses *erbB2* and *p53*). Other forms are micropapillary DCIS (variant epithelial tufts project into the duct lumen, cells are small and uniform, a myoepithelial cell layer is usually present), apocrine DCIS (containing abundant granular eosinophilic cytoplasm similar to apocrine metaplasia, but with complex architectural patterns and necrosis), clear cell DCIS (cells with optically empty or vacuolated cytoplasm), and Signet-ring cell DCIS.

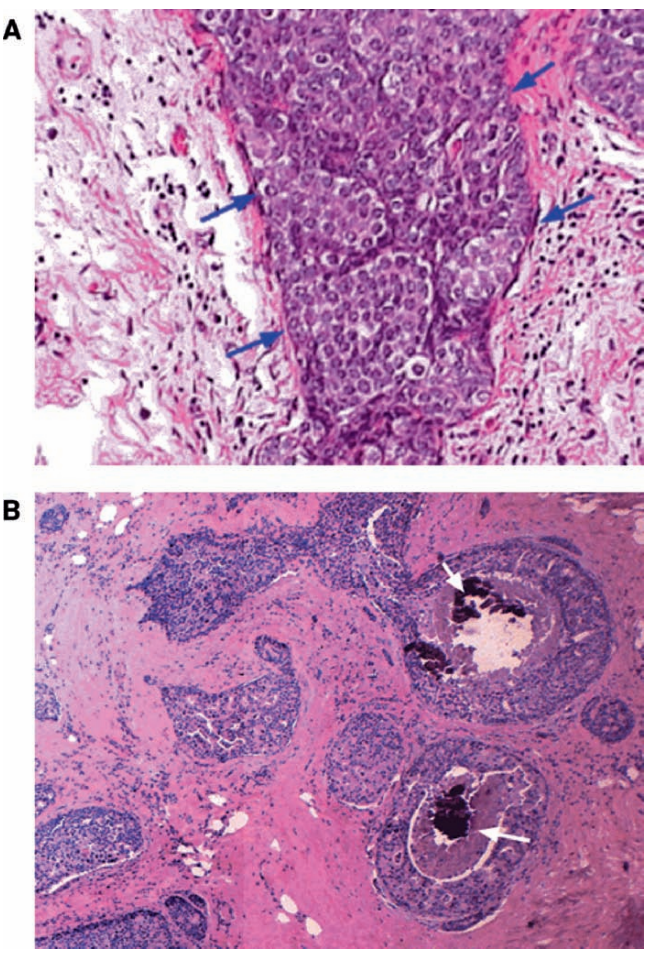


Figure 15.14.A. Breast intraductal carcinoma. (A) The blue arrows point to the basement membrane of a large duct that is completely filled by intraductal carcinoma (DCIS). The cells filling the duct are monotonous and monomorphic. At higher power they show features of malignancy. The adjacent stroma contains scattered lymphocytes. There are no myoepithelial cells visible. (B) This lower power view shows ducts filled with cells and with necrotic debris. The arrow points to calcifications within the necrotic debris. Common histologic changes in breast carcinoma include a loss of the typical bilayered epithelium, enlarged round to oval nuclei with nucleoli, and a loss of polarity towards the lumen. Furthermore, intraductal carcinomata may be associated with microcalcifications within the lumina. Fibrocystic change can be present. In the comedo variant, the cysts (dilated ducts) are filled with granular, yellow to white material. The risk for breast cancer is elevated with increasing age, obesity, in women of low parity and who had their first child after the age of 30, in women with a history of atypical hyperplasia, and in women whose mother or sibling has been afflicted with breast cancer. [Reproduced from http://pathweb.uchc.edu. With permission.]

– Infiltrating ductal carcinoma (IDC) is the most common type of breast cancer, accounting for about 65% of infiltrating breast tumors. Tumor grade, degree of anaplasia, and lymphovascular invasion are significant prognostic parameters. In general, high-grade tumors have large, pleomorphic nuclei, with macronucleoli, irregularities of the nuclear membrane, and frequent mitoses, while low-grade tumors have small, uniform nuclei, inconspicuous nucleoli, and rare mitoses. Histologic and nuclear grade coincide in most IDC. Forms of IDC are tubular carcinoma (less than 1% of all breast carcinomata, usually less than 2 cm in diameter, composed of regular, rounded, or angulated tubules, scattered in a fibrous stroma, without any lobular arrangement), mucinous (colloid) carcinoma (approximately 2% of breast carcinomata, usually contains substantial mucinous material in the cells as well as in the stroma, tumors are soft, gelatinous, and with well-demarcated, but pushing borders), medullary carcinoma (a rare form of breast cancer, accounting for less than 5% of all cases, presence of solid sheets or nests of relatively poorly differentiated cells, surrounded by a mantle of plasma cells and lymphocytes, sometimes with germinal centers present), papillary carcinoma (comprises about 1–2% of breast carcinomata, invasive pattern is predominantly in the form of papillary structures), metaplastic carcinoma (epithelial elements undergo metaplastic changes to a nonglandular, most often squamous or heterologous pattern, frequent lung metastases), inflammatory carcinoma (large, erythematous and painful mass, purple discoloration of the skin, lymphatic dermal involvement).
– Colloid carcinoma (mucinous carcinoma, variant of IDC, very good prognosis, contain lakes of mucin with islands of relatively bland tumor cells).
– LCIS (lesion is not clinically apparent, but is frequently multicentric and multifocal, the tumors express Estrogen Receptors and Progesterone Receptors, the neoplastic cells are small, uniform, and have clear or eosinophilic cytoplasm and round regular nucleus).
– Invasive lobular carcinoma (ILC) (ill-defined mass, which is in part due to the diffuse tumor growth pattern, there is a frequent lack of calcifications, the tumors are composed of small uniform cells with a low mitotic rate).
– Paget disease of the nipple (1–5% of breast carcinomata, presence of large cells with abundant pale cytoplasm and large, atypical nuclei in the surface epithelium of the nipple, redness, and eczematoid changes of the nipple).
– Inflammatory breast cancer (dermal lymphatic carcinomatosis) of the breast (characterized by skin redness and warmth, a visible erysipeloid margin, and induration of the underlying breast).

Molecular pathology. Breast cancer arises through uncontrolled up-regulation of Estrogen Receptor pathways, EGFR pathways, or WNT pathways (Figure 15.14.B).

Most breast cancers depend on estrogen signaling for growth promotion. The exposure to estradiol (E_2) is an important risk factor for the genesis and evolution of breast tumors. About one third of the cases of breast cancer in postmenopausal women are hormone dependent. In these patients, estrogens are synthesized in extraglandular tissues from adrenal precursors and enter the plasma to produce moderately high estrone and estradiol levels. However, the levels of estrogen in breast tumor tissue are an order of magnitude higher than plasma levels, reflecting estrogen production in the tumor. There are two active pathways:
– The Aromatase pathway transforms androgens into estrogens. Aromatase (Estrogen Synthetase, CYP19A1) is a Cytochrome P450. It catalyzes three consecutive hydroxylation reactions, converting C19 androgens to aromatic C18 estrogenic steroids. Aromatase synthesizes estrogens from adrenal steroids. Breast tumors produce high levels of Aromatase activity, and certain polymorphisms of the gene encoding this enzyme represent high risk factors.
– The Sulfatase pathway regulates the conversion of estrone sulfate into estrone. Estrogen Sulfotransferase sulfonates estrogens to yield inactive estrogen sulfates, whereas Steroid Sulfatase hydrolyzes estrone sulfate to estrone. The final step of steroidogenesis is the conversion of the weak estrone to the potent estradiol by the action of a reductive 17β-Hydroxysteroid Dehydrogenase 1 activity. In breast cancer, Estrogen Sulfotransferase is present in the carcinoma and in the intratumoral stromal cells and is inversely correlated with tumor size and lymph node status. Steroid Sulfatase is present only in

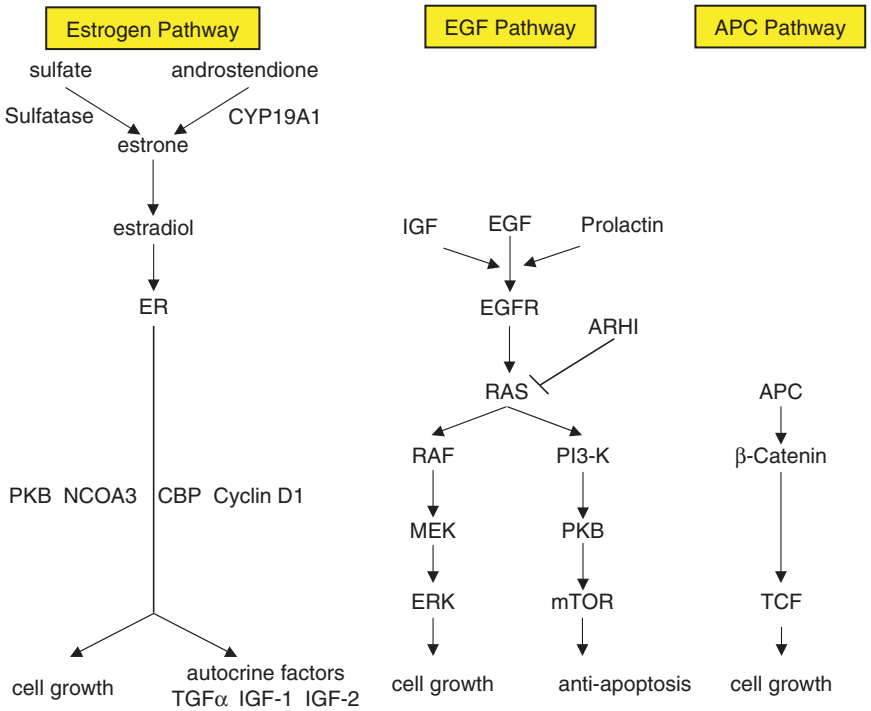


Figure 15.14.B. Molecular pathways to breast cancer. Defects in three major pathways, associated with estrogen signaling, EGF signaling, and the APC pathway, can lead to uncontrolled growth and transformation of breast epithelial cells.

the carcinoma cells and is correlated with tumor size [Suzuki et al. 2003]. In most tumors, 17β-Hydroxysteroid Dehydrogenase is also present. Mammary carcinomata can synthesize estradiol from circulating estrogen precursor and this local conversion is biologically important. The estrone sulfate→estrone→estradiol pathway is quantitatively more important than the pathway involving androstenedione→estrone→estradiol [Santen et al. 1986; Pasqualini 2004].

Structural modifications of the Estrogen Receptor regulate its activity. Estrogen independence can be acquired through certain Estrogen Receptor mutations, which result in increased sensitivity to the ligand, such Y537D. Posttranslational modifications can result in ligand-independent activation of the Estrogen Receptor. PKB phosphorylates the Estrogen Receptor on serine 167. The expression and activity of all three PKB family members is increased in breast cancer and often involves gene amplification [Bellacosa et al. 1995]. Furthermore, the negative regulator of PKB, PTEN, is inactivated in a fraction of breast cancers [Rhei et al. 1997].

Increased expression of the coactivator proteins that mediate Estrogen Receptor activity leads to estrogen independence.

- NCOA3 (AIB1) is a coactivator that directly binds nuclear receptors and stimulates their transcriptional activities. By recruiting CBP and PCAF, NCOA3 plays a central role in creating a multi-subunit coactivator complex. NCOA3 may be overexpressed due to amplification in breast cancer.
- CBP is mutated in several epithelial cancers, including those of the breast. These mutations cause a loss of function. The Estrogen Receptor missense mutation L303R alters the response to the coactivator CBP.
- *Cyclin D_1* is overexpressed in about 50% of all breast cancers. Cyclin D_1 can interact directly with the Estrogen Receptor and enhance its transcriptional activity.

There are at least two types of Estrogen Receptor-expressing breast cancers, referred to as luminal A and luminal B. While both express genes dependent on ER and GATA3, luminal B tumors often also express ERBB1, ERBB2, or Cyclin E_1, and have less favorable outcomes.

In Estrogen Receptor bearing breast cancer cells, the expression and secretion of autocrine growth factors, including TGF-α and IGF-2, are stimulated by estrogens and inhibited by antiestrogens. In

breast cancer cells that lack Estrogen Receptors, the secretion of these factors is not estrogen regulated. The effects of IGF-1 and IGF-2, potent mitogens and survival factors for breast epithelial cells, are mediated primarily through the IGF-1 Receptor, which is significantly overexpressed and highly activated in many breast tumors.

About 30% of all breast cancers are positive for ERBB2. EGFR (HER-1) and ERBB2 (HER-2, NEU) are frequently amplified or overexpressed in breast cancer. Gene amplification correlates with increased mRNA expression. Overexpression is inversely correlated with Estrogen Receptor expression and is reflective of poorly differentiated tumors with high growth rates. On average, the prognosis is worse for patients with ERBB2 overexpressing tumors than for patients with normal ERBB2 expression. The levels of COX-2 are increased in ERBB2 overexpressing breast cancer cells, and high levels of COX-2 are predictors of reduced survival [Ristimaki et al. 2002]. *mln64* (*steroidogenic acute regulatory protein-related gene*), *grb7* (*growth factor receptor bound protein 7*), *pnmt* (*phenylethanolamine N-methyltransferase*), *psmb3* (*proteasome subunit, ß type 3*), *rpl19* (*ribosomal protein L19*), and *nrlD1* (*nuclear receptor sub-family 1, group D, member 1*) are coexpressed with ERBB2 in breast cancer. These genes map within the same chromosomal location as the ERBB2 gene. Coexpression and colocalization indicate that the amplification of *erbB2* includes the chromosomal region {17q22–q24} harboring these genes [Dressman et al. 2003]. In some breast cancers, the *erbB2* gene is activated by a point mutation, V664E, which may result in cell transformation.

EGFR signals through the RAS→RAF→MEK→ERK, and RAS→PI 3-K→PKB pathways. Hyperactive RAS can promote breast cancer growth and development. In some cases, this is mediated by elevated active RAS-GTP levels that may be due, in part, to ERBB2 activation. Minisatellites are unstable repetitive sequences of DNA that are present throughout the genome. The highly polymorphic *H-ras-1* minisatellite locus immediately downstream from the proto-oncogene consists of four common progenitor alleles and multiple rare alleles, which apparently derive from mutations of the progenitors. Mutant alleles of this *H-ras-1* minisatellite locus represent a major risk factor for breast cancer. Although the relative risk associated with the presence of one rare allele is moderate, the aggregate prevalence of this class of mutant alleles implies an important attributable risk of 1 in 11 cancers of the breast [Krontiris et al. 1993]. In contrast, mutated forms of RAS are associated with fewer than 5% of breast cancers.

ARHI (RAS Homolog Member I) {1p31} encodes a 26 kD GTPase with 50–60% amino acid homology to RAS and RAP. ARHI and RAS proteins share similar GTP/GDP-binding domains, but exert opposite functions. ARHI is a tumor suppressor in the RAS superfamily, which is consistently expressed in normal breast epithelial cells but is dramatically downregulated in more then 70% of breast cancers. *arhi* is imprinted with methylation of the three CpG islands in the maternal allele of normal cells and expression only from the paternal allele. Loss of *arhi* expression can occur through a genetic event, with loss of heterozygosity arising in 40% of breast cancers; but it can also occur through epigenetic mechanisms, including DNA methylation, Histone deacetylation, Histone methylation, and transcriptional regulation. Changes in chromatin that silence *arhi* may be driven by methylation-dependent and methylation-independent pathways [Yu et al. 2003].

Cyclin D_1 plays an essential role in RAS-induced carcinogenesis. The levels of Cyclin D are elevated in up to 50% of breast cancers. This may be due to amplification of chromosome 11q13, the region in which the *cyclin* D_1 (*ccnd1*, *prad1*) gene is located. Gain-of-function mutations of Cyclin D_1 are also possible.

Transcription factors that are downstream targets in the RAS pathway can be excessively activated in breast carcinomata.

- ETS family transcription factors are activated through the RAS pathway. Increased levels of *ets-1* transcripts in breast tumors predict a poor prognosis, and *pea-3* group members are overexpressed in metastatic breast cancer. Their activity induces the transcription of *matrix metalloproteinases* and *icam-1*, and may be sufficient to generate a metastatic phenotype. ETS transcription factors, particularly PEA-3, enhance the transcriptional activation of *osteopontin* by β-Catenin and LEF-1 as well as by c-JUN. This may play a role in breast cancer dissemination.
- MYC is a downstream signal transduction target for RAS and for HIF-1. *myc* amplification arises in a proportion of breast cancers. Amplification is primarily associated with infiltrating ductal carci-

nomata and poorly differentiated tumors. It is a predictor of high risk for relapse and death. *myc* amplification does not appear to be an early event in breast carcinogenesis because it rarely is present in premalignant lesions. In breast cancers, *myc* may also be overexpressed in the absence of amplification.

– Alterations in P53 are common in breast cancer. P53 may be sequestered in the cytoplasm and be prevented from entering the nucleus, which impairs the activation of G_1 arrest. This mode of P53 inactivation can arise in the absence of mutations. Cytoplasmic P53 is abundant in normal lactating breast tissue, implying that this mechanism is employed in specific physiological situations to permit transient cell proliferation [Moll et al. 1992].
– PNRC (Proline-Rich Nuclear Receptor Coregulatory Protein) is a nuclear receptor coactivator that interacts with nuclear receptors through an SH3-binding motif located in its COOH-terminus. GRB2 decreases the coactivator activity of PNRC for nuclear receptors, and PNRC suppresses GRB2-mediated RAS→ERK activation. The interaction between PNRC and GRB2 constitutes a cross-talk mechanism between nuclear receptor-mediated and RAS-mediated pathways. PNRC expression may be significantly reduced in breast cancer tissue as compared to surrounding noncancer tissue [Zhou et al. 2004].

The pathway PI 3-K→PKB→mTOR can be activated by RAS. It is critical for the regulation of cellular proliferation and survival. This sequence is aberrantly up-regulated in some breast cancers. Cell cycle control occurs through the binding of Cyclin to CDKs that stimulate G_1 progression. In cancers with constitutively active PKB, the CDK Inhibitor $P27^{KIP1}$ is inactivated by three mechanisms:

– PKB decreases $P27^{KIP1}$ by increasing its proteolysis.
– PKB mediates a reduction of $p27^{KIP1}$ transcription.
– PKB phosphorylates $P27^{KIP1}$ and impairs its nuclear import. The exclusion of $P27^{KIP1}$ from the nucleus leads to a poor patient outcome in breast cancer.

The EGFR can act as a nodal point for the interaction of various growth factor receptors.

– EGFR signaling can be activated indirectly by IGF-1. IGF-1 partially mediates its survival effect on mammary epithelial cells through transactivation of the EGFR and the consequent stimulation of MAPK. Whereas, either IGF-1 or EGF can induce the expression of early G_1 type Cyclins in mammary cells, IGF-1 is specifically required to traverse the G_1/S checkpoint.
– Prolactin signaling and EGFR signaling may synergize in breast cancer progression. Signaling through Prolactin Receptors proceeds through the JAK/STAT pathway. The constitutive activation of the STAT3 oncogene product and mutation of the *p53* tumor suppressor frequently occur in breast cancer. Expression of wild-type P53, but not mutant P53, diminishes the phosphorylation of STAT3, reduces STAT3 DNA-binding activity, and inhibits STAT3-dependent transcriptional activity.

The APC→β-Catenin→TCF signaling pathway is commonly disturbed during mammary oncogenesis. One of the main differences between lobular breast cancers and ductal carcinomata is the presence of inactivating *E-cadherin* gene mutations in lobular breast cancers. In contrast, truncating mutations of APC are rare in breast cancers.

MUC1 is aberrantly overexpressed in breast cancer. Like E-Cadherin and APC, MUC1 contains an SXXXXXSSL site that is responsible for direct binding to β-Catenin. The interaction between MUC1 and β-Catenin may play a role in the adhesion of breast epithelial cells.

Antiapoptosis. *bcl-2* is a gene frequently overexpressed in breast cancer. It is associated with an unfavorable prognosis. Endogenous Beclin-1 expression is frequently low in breast carcinoma. This may reflect allelic deletions of *beclin-1*, but not *beclin-1* coding mutations. The *beclin-1* gene is monoallelically deleted in 40–75% of cases of sporadic breast cancer. Frameshift mutations of *bax* that lead to a loss of expression, and mutations in the BH domains that result in a loss of BAX functions occur in breast cancer. They render the affected tumor cells resistant to apoptosis. Reduced BAX expression may be associated with shorter patient survival in breast adenocarcinoma. Missense mutations in *dr4* (*trail receptor 1*) and *dr5* (*trail receptor 2*), caused by single nucleotide substitution, may occur [Shin et al. 2001].

ATM phosphorylates CTIP upon ionizing radiation. This may modulate the BRCA1-mediated regulation of the DNA damage response gene *gadd45*, thus providing a potential link between ATM deficiency and breast cancer. Two *atm* mutations that yield dominant negative inhibitors cosegregate with breast cancer. Due to this mechanism, the mutations appear to have a dominant nature [Chenevix-Trench et al. 2002]. Transcription of *gadd45* (*growth arrest and DNA damage*

inducible gene 45 kD, *DNA damage inducible transcript 1*, *ddit1*) plays a role in P53-induced apoptosis. The nuclear protein GADD45 may mediate genotoxic stress or BRCA1-induced apoptosis via activation of JNK or $P38^{MAPK}$.

Life span extension. Overcoming replicative senescence is an important factor in carcinogenesis. P53 is phosphorylated on serines 33 and 46 by $P38^{MAPK}$. This sensitizes affected cells to oncogenic RAS. PPM1D blocks the $P38^{MAPK}$-mediated phosphorylation and prevents the senescence response that protects cells from the oncogenic effects of RAS. The phosphatase PPM1D is amplified in over 10% of breast tumors.

Mammary epithelial stem cells age under the influence of TGF-β. This diminishes the pool of cells that are prone to transformation. Elevated expression of TGF-β in mammary epithelial cells is associated with a reduced susceptibility to cancer.

– The expression of *tgf-β* depends in part on the transcription factor EGR-1, which markedly reduces growth in the mammary gland.
– RNF11 (RING Finger Protein 11) interacts with the HECT-type E3 Ubiquitin Ligases NEDD4, AIP4, SMURF1, and SMURF2. It can enhance TGF-β signaling through a direct association with SMAD4. RNF11 is highly expressed in breast cancer.
– EDD is an HECT domain E3 Ligase. The *edd* gene is frequently overexpressed in breast cancer, implying a potential role in cancer progression.

Invasion and metastasis. The preferred site for breast cancer metastasis are the regional lymph nodes (Figure 15.14.C), bones [Paget 1889], and lungs, followed by the brain. The ovaries can also be target organs for dissemination. In rare cases, breast cancer may metastasize to the thyroid.

The expression of *rarα1*, *rho-B*, *keratins 18* and *19*, *gata-3*, and *igf-bp1* is elevated in noninvasive compared to invasive breast carcinoma. In contrast, *mt1-mmp, pai-1*, *c-jun*, *fra-1*, *vimentin*, *osteonectin*, *tsp1*, and *collagen VI α-1* are increased in highly invasive breast carcinomata. Distinct from primary breast cancers without detectable micrometastases, primary breast cancers that have disseminated to the bone marrow lack the expression of the metastasis suppressor genes *kiss-1*, *nme3*, and *nme4*. The genes that are associated with bone marrow micrometastases include those for extracellular matrix remodeling, cytoskeleton plasticity, and signal transduction through the JAK/STAT and HIF-1α pathways. Members of the RAS pathway for signal transduction are downregulated. The expression of Cytokeratins is reduced in patients with bone marrow micrometastases [Woelfle et al. 2003]. The gene expression signature associated with lymph node metastasis is distinct from the gene expression signature associated with bone marrow micrometastasis.

CXCL8 (Interleukin-8, IL-8) is a member of the CXC Chemokine family. IL-8 is physiologically secreted by osteoclasts. The elevated expression of IL-8 by breast carcinoma correlates with bone metastasis. The formation of breast cancer bone metastases may depend on their ability to create a microenvironment which resembles that of osteoclasts, in part through

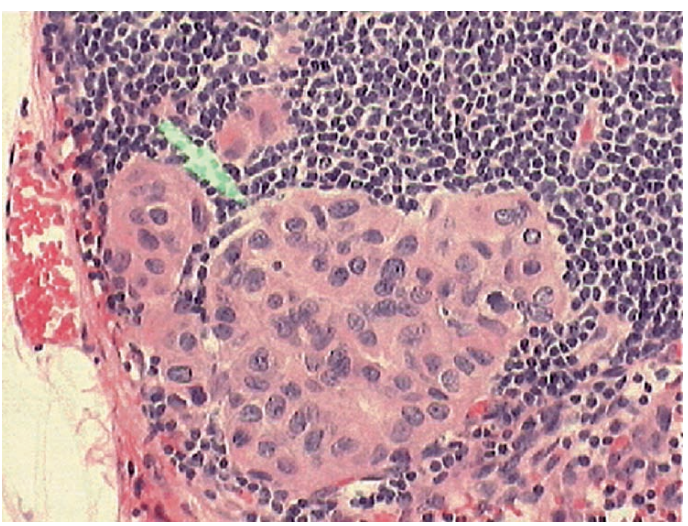


Figure 15.14.C. Metastatic adenocarcinoma. The slide shows a portion of a lymph node, with a nest of metastatic breast tumor. The histology resembles an infiltrating carcinoma of breast with the tumor locating close to the capsule of the lymph node. This is a common early site for implantation of metastatic cancers, since they enter the lymph node through afferent lymphatics, which empty into the marginal sinus just below the capsule. Subsequently, the entire lymph node can become involved. The lymph node may be of normal size or significantly enlarged. Depending on the rate of growth of the tumor, there can be necrosis, cystic change, or hemorrhage. In extreme cases, a necrotic node with the center appearing as a white liquefying material may result. The presence of metastatic deposits in draining lymph nodes generally worsens the prognosis. In some malignancies, particularly those of internal viscera, lymph node metastases may be the first indication of a tumor. [Reproduced from http://pathweb.uchc.edu. With permission.]

the release of IL-8. The Chemokine receptors CXCR4 and CCR7 are highly expressed on breast cancer cells and their metastases, but not, or at low levels, on normal breast cells. Their ligands, CXCL12 (SDF-1α) and CCL21 (6Ckine), exhibit peak levels of expression in organs that represent target tissues for metastasis formation, including lymph nodes, bone marrow, lungs, and liver. In particular, CCL21 may be important for homing to secondary lymphoid organs. The engagement of the Chemokine Receptors on breast cancer cells by their cognate ligands triggers Actin polymerization, pseudopodia formation, and directed migration. A neutralization of the interaction between CXCL12 and CXCR4 leads to a significant reduction of lymph node and lung metastases [Mueller et al. 2001]. The secreted protein SLIT-2 engages its receptor ROBO and inhibits CXCR4-mediated migration. *slit-2* {4p15.2} is frequently inactivated in breast cancers because of hypermethylation of its promoter region [Prasad et al. 2004].

Metastatic breast cancers express elevated levels of *osteopontin* and *activin βA* [Reinholz et al. 2002]. Osteopontin gene expression in breast carcinoma cells may be a downstream consequence of PKB activation. Osteopontin supports the migration but not the growth of these cells [Zhang et al. 2003]. The expression of the cytokine Osteopontin is necessary and may be sufficient for the formation of metastases by breast cancer. High levels of Osteopontin in the disease are an adverse prognostic factor [Singhal et al. 1997; Rudland et al. 2002].

The exposure of breast carcinoma cells to estrogens induces the expression of *upa* and *tpa*. Osteopontin, produced by the breast tumor cells, also up-regulates *upa* expression in an autocrine fashion. This activates Plasminogen, which is required for the degradation of Collagen I during bone metastasis by breast cancer. Plasminogen Activator Receptors are expressed in breast carcinoma, but not in normal breast tissue. Breast cancer cell invasion is promoted by the activation of PAR-1 in cooperation with the Integrin $\alpha_v\beta_5$.

Breast cancer cells can colonize bone because they activate genetic programs that mediate cell–cell communications with osteoblasts and osteoclasts. The plasticity of the bone structure is maintained by a balance between osteoblastic and osteoclastic activities. This balance is regulated by various signals between these cell types. Important interactions in the regulation of bone resorption include the induction and activation of osteoclastogenesis, induced by binding of RANK Ligand on osteoblasts to RANK on osteoclast progenitors, and the suppression of osteoclastogenesis, mediated by binding of RANKL to soluble Osteoprotegerin, which interferes with the engagement of RANK. Osteoprotegerin is a member of the TNFR family and a negative regulator of osteoclast function. Bone metastases of breast cancer typically express RANKL and thus activate bone resorption.

Selectins contribute to breast cancer dissemination. CD24 serves as a counterreceptor for P-Selectin on breast carcinoma cells under flow conditions. The interaction of tumor cells with P-Selectin via CD24 may be an important adhesion mechanism in breast cancer metastasis.

Most epithelial Mucins belong to the MUC family. In the normal polarized epithelium, Mucins are expressed exclusively on the apical domain. Likewise, soluble Mucins are secreted selectively into the lumen. The disturbance of the tissue architecture in cancer allows Mucins to be expressed on all aspects of the cells and, following excretion, to enter the extracellular space and body fluids. The Polymorphic Epithelial Mucin (PEM, MUC1), the product of the *muc1* gene, is aberrantly glycosylated in breast cancer. MUC1 expressed by breast cancer cells contains more sialylated *O*-glycans and has a lower GlcNAc content than that expressed by normal cells. This results in exposure of normally cryptic peptide epitopes [Brockhausen et al. 1995].

Metadherin is a cell surface molecule that causes homing specifically to the lung vasculature. Breast cancers express high levels of Metadherin and in some cases initially disseminate to the lungs [Brown and Ruoslahti 2004].

The loss of metastasis suppressor molecules plays a role in breast cancer dissemination. Affected molecules include Cadherins, BRMS-1, and Kallikrein-3.

Loss of heterozygosity for the long arm of chromosome {16q}, the location of several *cadherins*, including *cdh1* and *cdh13*, occurs in breast carcinoma. The transcription factor TWIST is a master regulator of embryonic morphogenesis. It plays an essential role in metastasis. Ectopic TWIST expression in highly metastatic mammary carcinoma cells specifically induces their ability to metastasize from the mammary gland to the lung. It results in a loss of E-Cadherin-mediated cell–cell adhesion, activation of mesenchymal markers, and induction of cell motility, suggesting that TWIST contributes to metastasis by promoting epithelial–mesenchymal transition. A high

level of *twist* expression is correlated with invasive lobular carcinoma, which is a strongly infiltrating tumor type associated with the loss of E-Cadherin expression [Yang et al. 2004]. MTA3 is an estrogen-dependent component of the MI-2/NURD transcriptional corepressor complex. Estrogen Receptor signaling up-regulates MTA3 levels to negatively modulate the Snail-mediated repression of E-Cadherin. This may account for epithelial–mesenchymal transition in breast cancers that do not express Estrogen Receptors [Fujita et al. 2003]. High microvessel density in the primary tumors is concurrent with low expression of NME-1 and E-Cadherin and reflects a poor prognosis.

brms-1 {11q13.1–q13.2} is a metastasis suppressor gene, which may inhibit the dissemination of breast cancer [Seraj et al. 2000]. Chromosome 11q is often lost in late-stage breast cancers and regions near 11q13 are among the most commonly affected by amplifications and deletions associated with breast cancer progression.

Polymorphisms in the promoter regions of *klk* genes may impact their transcriptional regulation, which in turn influences the outcome of cancer. Patients with breast cancer, who are homozygous for the G allele in position – 158 of the *klk3* gene have elevated concentrations of Kallikrein-3 in their tumors and increased overall survival compared to patients homozygous for the A allele [Bharaj et al. 2000].

Familial breast cancer. About 10% of breast cancer cases cluster in families. Features characteristic of familial, versus sporadic, breast cancer are younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of disease among men [Hall et al. 1990].

– Fewer than 5% of breast cancers are hereditary, with BRCA-1 and BRCA-2 [Miki et al. 1994; Wooster et al. 1995] being responsible for the majority of familial breast cancer syndromes. Inherited breast cancer is linked to *brca1* {17q21} in an estimated 50% of families, to *brca2* {13} in 30% of families, and to neither gene in close to 20%, suggesting the existence of other predisposition genes [Ford et al. 1998] (Figure 15.14.D). *brca1* is composed of 24 exons, 22 of which are coding. The gene product is a 1,863 amino acid nuclear phosphoprotein that contains a RING finger motif for interaction with BARD1, two BRCT domains to activate transcription during DNA repair, a RAD51-binding domain, a Granin motif,

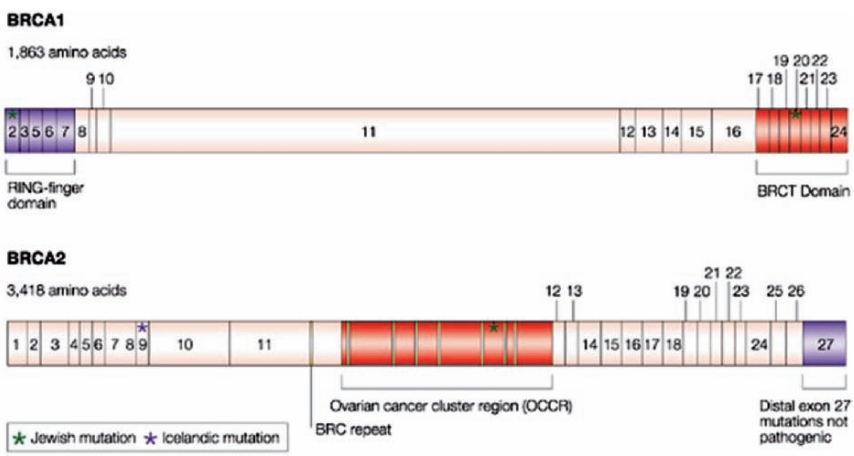


Figure 15.14.D. Structures of BRCA1 and BRCA2. BRCA1 functions as a transcription factor that is involved in the regulation of Estrogen Receptor activity and is related to the control of estrogen-induced proliferation of breast tissue. BRCA1 also contributes to homologous recombination and repair of transcription coupled, oxidation-induced DNA damage, as well as to chromatin remodeling. In cell cycle checkpoint control, BRCA1 interacts with RB, ESF1, and P53. The RING finger domain of BRCA1 is a protein–protein interaction domain that mediates binding to BARD1 (BRCA1-Associated RING Domain-1). The COOH-terminal BRCT domain is necessary for DNA repair and transcriptional activation. The highlighted mutations (denoted by *) are the two common founder mutations in Ashkenazi Jews.

BRCA2 functions in homologous recombination and chromatin remodeling. In cell cycle checkpoint control, it interacts with P53. Exon 11 of BRCA2 contains both the ovarian cancer cluster region (OCCR) and eight BRC repeats, which are required for binding to RAD51. Mutations in this region are associated with a greater risk of ovarian cancer and a lower risk of breast cancer than are mutations outside this region. The highlighted mutations (denoted by *) are the common founder mutations in Ashkenazi Jews and in Iceland. [Reproduced from Narod 2002. With permission from Macmillan.]

and a nuclear localization signal. BRCA1 can be phosphorylated by ATM, ATR, or CHK2. In BRCA1-expressing cells, the protein levels and kinase activity of the CDC2/Cyclin B complex are suppressed by BRCA1-dependent activation of CHK1, which down-regulates CDC25C. CDC25C is then unable to activate CDC2 by dephosphorylation on tyrosine 15, thus implementing cell cycle arrest. GADD45 is a 165 amino acid nuclear protein that can mediate genotoxic stress or BRCA1-induced apoptosis via activation of JNK or $P38^{MAPK}$. BRCA2 spans 27 exons, 26 of which are coding. The expressed nuclear phosphoprotein has 3,418 amino acids and contains an NH_2-terminal transcriptional activation domain, eight copies of BRC repeats in the central domain, and a COOH-terminal nuclear localization signal. BRCA2 binds to the transcriptional comodulator P/CAF. In both cases of hereditary breast cancers, based on dysfunctional BRCA-1 or dysfunctional BRCA-2, a mutated copy of either gene is inherited and a second mutation (usually loss of heterozygosity in breast tissue) has to occur in order for the breast cancer to develop. Most mutations are frameshift or missense mutations that cause premature protein truncations, although large deletions can also arise. The RING heterodimer BRCA1/BARD1 is a Ubiquitin Ligase. It is inactivated by the BRCA1 mutation C61G, which predisposes to breast cancer. Alternatively, BRCA-1 may be involved in large genomic rearrangements, including deletions and duplications, which are caused by recombination between Alu repeats. A drastic reduction in *brca1* gene expression is characteristic of aggressive sporadic breast carcinoma. This may be caused by the binding of overexpressed HMGA1b protein to the *brca1* promoter, resulting in transcriptional inhibition [Baldassarre et al. 2003]. Breast epithelial cells with deleted or mutant *brca1* are unable to arrest at the G_2/M checkpoint in response to γ-irradiation, because the BRCA1-dependent activation of CHK1 cannot take place. The loss of BRCA1 function also compromises chromosome stability. About 80% of invasive breast cancers have amplified centrosomes. Centrosome size and number correlate with chromosomal instability, because centrosome amplification drives chromosomal instability in breast cancer. BRCA2-deficient cells exhibit aneuploidy and structurally aberrant chromosomes. This is due to impaired completion of cell division by cytokinesis. The impeded cell separation is accompanied by abnormalities in Myosin II organization during the late stages in cytokinesis. BRCA2 may have a role in regulating these events, as it localizes to the cytokinetic midbody [Daniels et al. 2004]. Among Ashkenazi Jewish women, the *brca1* 185delAG mutation is associated with an estimated 20% risk for breast cancer [Offit et al. 1996]. The *brca2* mutation 6174delT, with a frequency of 1.3% in the Ashkenazi Jewish population, predisposes to breast cancer. The 999del5 mutation of *brca2*, which is relatively frequent in the Icelandic population, leads to high prevalence of breast cancer [Johannesdottir et al. 1996].

- Mutations in the *brca1* and *brca2* genes account for most families with the hereditary breast–ovarian cancer syndrome.
- Li–Fraumeni syndrome is a familial predisposition factor for the early onset of breast cancer. Most mutations are of missense type and located in the central region of the gene, comprising the exons 5–8. Many breast tumors associated with this disease have inherited CG to TA transitions within the central region of *p53*. Mutations in *p53* are rare in other familial breast cancer syndromes.
- PTEN hamartoma-tumor syndromes are caused by germline mutations in *pten*. These loss-of-function mutations are associated with a high incidence of breast cancer [Liaw et al. 1997].
- In familial ductal breast cancer, loss of heterozygosity on chromosome 13 is common. This region is associated with the tumor suppressor gene *brcd-1* (*breast cancer, ductal, 1*; *breast cancer, ductal, suppressor-1*; *bcds1*). Alternatively, loss of *brcd-2* (*bcds2*) on chromosome 1p36 may play a role.
- Peutz–Jegher syndrome, due to mutations in the *stk11* gene, is associated with an elevated incidence of breast cancer.

Rare breast tumors. Basal-like breast cancers typically express genes characteristic of the basal epithelial cell layer, including Cytokeratins 5, 6, and 17. They loose the expression of CBP [Dietze et al. 2005], and are also characterized by the loss of mutations in *p53*, and by the frequent absence of Estrogen Receptors, Progesterone Receptors, or ERBB2. Two pathways lead to transformation.

- A lack of functioning P53 is followed by a loss of CBP, which leads to a loss of *laminin 5* expression and inability to undergo apoptosis.
- The lack of CBP compromises the ability of BRCA1 to repair DNA, resulting in possibly transforming mutations.

The patient population is typically between the ages of 30 and 40 years. Having given birth before the age of 30 is not protective of this form of breast cancer. Basal-like tumors have a higher prevalence among premenopausal African American women compared to postmenopausal African American patients and to non-African Americans. This may account for the overall poor prognosis of breast cancer in this population.

Secretory breast carcinoma is a rare subtype of infiltrating ductal carcinoma. It expresses the ETV6–NTRK3 fusion protein that often arises in pediatric mesenchymal cancers. The underlying gene fusion encodes a chimeric tyrosine kinase with potent transforming activity. ETV6–NTRK3 expression occurs in secretory breast carcinoma, but not in other ductal carcinomata [Tognon et al. 2002].

Some breast tumors have mixed histologic appearance.

– Mammary carcinosarcoma is a heterogenous malignant tumor, in which both epithelial and mesenchymal elements originate from a single totipotent stem cell (high-grade malignant phylloides tumor with high-grade mammary carcinoma). Mammary carcinosarcoma is associated with the mutation G12D in H-RAS.
– Phylloides tumor (phyllodes tumor) originates in the connective tissue of the breast. It is a rare fibroepithelial breast tumor that occasionally has unpredictable clinical behavior. Phylloides tumor accounts for 0.3–1% of all breast tumors. It occurs mostly in women in their fifth or sixth decade of life, and occasionally in women younger than 20 years. In rare cases, it may occur in males with gynecomatatia. There are benign and malignant forms of phylloides tumors. Osseous and chondroid metaplasias of the stroma and the epithelium occur. The growth of the tumor extends sometimes over several years; periodic remission and sudden growth are typical.
– Adenomyoepithelioma has a growth patten, in which myoepithelial cells encircle glands. The tumor cells express Myosin.

Benign tumors of the breast comprise fibrocystic breast disease, and intraductal papilloma. Fibroadenomata are the most common benign breast tumors (Figure 15.14.E). Their presence does not pose a cancer risk.

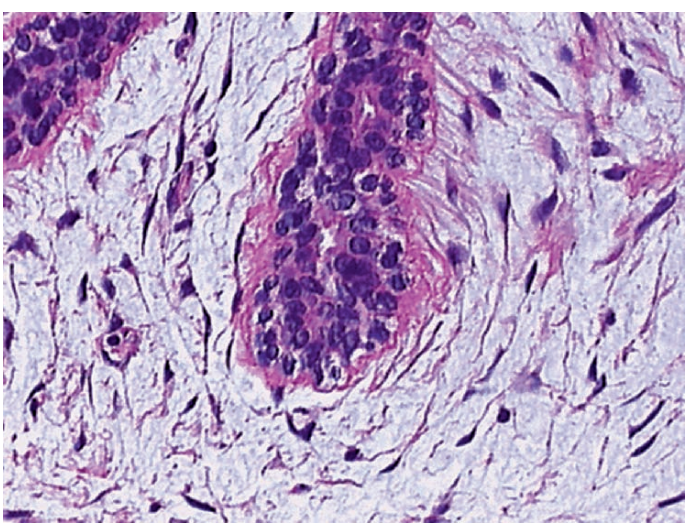


Figure 15.14.E. Breast fibroadenoma. Fibroadenoma derives from intralobular stroma, with the stromal component of the lesion frequently being clonal. The stroma contains numerous fibroblasts and palely eosinophilic cytoplasm. The glandular spaces are lined by typical duct epithelium and may be compressed by fibrous proliferation. The ductal portion of the neoplasm in the center of the photograph has an intact basement membrane, myoepithelial cell layer, and duct epithelial layer, although the lumen is compressed. The stroma is loose without dense Collagen and contains spindly fibroblasts. Broad birefringent Collagen bands are typically absent until the lesion involutes. Fibroadenoma is the most common benign neoplasm in the breast. The tumor is encapsulated and mobile. It is more frequent in women under the age of 30 and tends to regress with menopause. Juvenile fibroadenoma arises in teenagers and may grow to a large size. [Reproduced from http://pathweb.uchc.edu. With permission.]

Male breast cancer is rare. It represents about 1% of cancers in men. Loss of heterozygosity on chromosome 11q13 is associated with about two thirds of cases. Androgens suppress the growth of breast cancer cells. Consistently, a polymorphic polyglutamine repeat in the *androgen receptor* {Xq11–q12}, which determines the level of transcriptional activity, may be protective against breast cancer. Mutations in the *androgen receptor*, primarily at codons 607 and 608 in the second zinc finger of the DNA-binding domain, occur.

15.15 THYROID CANCER

Thyroid nodules are common, and their frequency increases with age. In over 35% of people, thyroid cancer exists in a subclinical form and is only detected during autopsy [VanderLaan 1947; Harach et al. 1985]. Radiation exposure, familial predisposition, or spontaneous mutations may result in thyroid carcinoma. Most often thyroid cancer is detected as a nodule of the neck. Other symptoms may include pain, changes in the voice, hypothyroidism, or hyperthyroidism.

Molecular pathogenesis. The most frequent histologic type is papillary thyroid cancer. Less common forms comprise follicular, medullary, and anaplastic cancers.

Thyroid papillary cancers are associated with activating mutations of genes associated with the RET or TRK tyrosine kinase receptor pathways.

– Characteristic chromosomal rearrangements link the promoter and NH_2-terminal domains of unrelated genes to the COOH-terminal domain of RET {10q11.2}. Constitutive kinase activity may be conferred to RET by a chromosomal translocation that forms the chimeric *ret-ptc* (*papillary thyroid cancer*) oncogene and occurs in 30% of papillary thyroid carcinomata. The RET-PTC1 rearrangement is the result of an inversion of chromosome 10 inv(10)(q11.2q21), which causes the fusion of the tyrosine kinase domain of the RET gene product with a section of the H4 gene product {10q21}. In a substantial fraction of normal thyroid cells, the *ret* and *h4* genes are in close proximity to one another. The association of these regions may be important in thyroid cell differentiation [Nikiforova et al. 2000]. The chromosomal translocation t(10;17)(q11.2;q23) juxtaposes the tyrosine kinase domain of the *ret* proto-oncogene to a 5′ portion of *pkar1α* (*regulatory subunit of c-AMP-dependent protein kinase*, *tse1*, *tissue-specific extinguisher-1*) on chromosome 17, leading to the formation of the chimeric transforming gene product RET-PTC2 [Sozzi et al. 1994]. Another form of rearrangement fuses the *ret* proto-oncogene with a portion of the *ele1* gene, generating RET-PTC3 [Bongarzone et al. 1994]. The constitutive activation of RET, resulting from these translocations, promotes the activity of SHC, an intermediate in the RAS pathway, which then proceeds through RAF. A somatic mutation of B-RAF, V599E (T1796A), is the most common genetic change in papillary thyroid carcinoma, occurring in around 35%. This activating mutation mimics phosphorylation in the activation segment by proximal insertion of an acidic residue. Mutations in *ras* or *B-raf* or the *ret-ptc* translocation, in toto accounting for 66% of thyroid papillary cancers, do not occur together, consistent with their operation through the same signaling pathway [Kimura et al. 2003]. The constitutive activation of the RET-PTC→RAS→BRAF signal transduction pathway may be sufficient for papillary thyroid carcinogenesis. Activating mutations in any one of these components can induce transformation.
– Rearrangements of the tyrosine kinase receptor TRK (NTRK1, High-Affinity Nerve Growth Factor Receptor) occur with low frequency (5–25%). TRK-T1 is created by an intrachromosomal rearrangement that juxtaposes the 5′ end of the *tpr* gene to the *trk* tyrosine kinase domain. The resulting hybrid mRNA contains 598 nucleotides of *tpr* and 1,148 nucleotides of *trk* [Greco et al. 1992]. An illegitimate recombination that inverts a part of the long arm of chromosome 1 places the 611 base pairs *ntrk1* intron upstream of the transmembrane domain and the *tpm3* (*tropomyosin 3*) intron between exons 7 and 8 encodes a chimeric protein of 70 kD, which is constitutively phosphorylated on tyrosine [Butti et al. 1995]. Reciprocal *ntrk1-tpm3* transcripts exist in tumors with *tpm3-ntrk1* rearrangement, indicating an intrachromosomal balanced reciprocal inversion.

The PAX-8 (Paired Box-8) protein is localized in the nucleus of normal adult thyroids and adult papillary thyroid cancers, but not of undifferentiated thyroid cancers. In a fraction of pediatric thyroid cancers, PAX-8 is cytoplasmic and this is associated with reduced disease-free survival. Psammoma bodies are

often associated with papillary carcinomata of the thyroid. Osteopontin appears to play an important role in the development of psammoma bodies. In this setting, Osteopontin is produced by macrophages.

Oncocytic carcinomata (Hurthle cell carcinomata, HCC, Askanazy cell carcinomata) are commonly considered a subgroup of follicular thyroid carcinomata. However, they harbor *ret* hybrid oncogenes in the majority of cases. Therefore, a papillary origin of these tumors is possible [Cheung et al. 2000; Chiappetta et al. 2002].

Follicular adenomata are benign thyroid tumors. Follicular carcinomata are rare thyroid malignancies. While histologically similar, follicular adenomata and carcinomata display strikingly distinct gene expression patterns [Barden et al. 2003]. The expression levels of *tef3* (*trefoil factor 3*) mRNA are significantly decreased in follicular carcinomata, compared to follicular adenomata, especially in widely invasive types and those with evident metastases. There are two genetic origins of thyroid follicular carcinomata.

– Activating mutations of *ras* are typically present in thyroid follicular neoplasms. Oncogenic RAS may act as a mutator gene product in thyroid cancers through sustained MEK→ERK activation, which may lead to inappropriate phosphorylation of components of the kinetochore complex, presumably causing centrosome amplification, chromosome misalignment, and chromosomal instability. Thyroid follicular neoplasms commonly have aneuploidy. This accelerates the progression to a malignant state [Fagin 2002].
– The chromosomal rearrangement t(2;3)(q13;p25), generating *pax8-pparγ*, occurs in thyroid follicular carcinomata. It results in fusion of the DNA-binding domains of the thyroid transcription factor PAX8 to domains A–F of the Peroxisome Proliferator-Activated Receptor γl [Kroll et al. 2000].

Thyroid medullary cancer is a rare neoplasm that arises from the parafollicular C-cells. It occurs in a sporadic form, or less commonly in a hereditary form as part of multiple endocrine neoplasia syndromes types 2A and 2B.

– Germline activation of the *ret* gene, attributable to specific point mutations, causes the hereditary form of thyroid medullary carcinoma.
– Allelic losses in thyroid medullary cancers occur at a frequency of about 45% and mostly affect the tumor suppressor genes *nf2* and *p53*. The frequency of allelic losses is higher in tumors that recur. Basic helix-loop-helix transcription factors of the MASH family are regulators of development in the neural crest. MASH-1 (ASCL-1) is highly expressed in medullary thyroid cancer.

Calcitonin and Chromogranin A are secreted by the thyroidal C-cells and constitute markers of medullary carcinomata. These tumors also produce Carcinoembryonic Antigen.

Due to the endocrine activity of medullary thyroid carcinoma, a major clinical symptom may be diarrhea. Flushing episodes may occur, particularly if the tumors are sizeable or liver metastasis is present. The prognosis is affected by the age of the patient.

Anaplastic thyroid cancer has a very poor prognosis with near 100% mortality. This is due to its aggressiveness and resistance to conventional cancer treatments. Anaplastic thyroid cancer rapidly invades surrounding tissues, including the trachea.

Antiapoptosis. Amplification of the chromosome locus 2p21 occurs in about 30% of thyroid neoplasms. This causes a rearrangement and amplification of the *pkcε* gene and results in the overexpression of a chimeric truncated *pkcε* mRNA, coding for the NH_2-terminal amino acids 1–116 of the enzyme form, fused to an unrelated sequence. Cells expressing the truncated PKCε are resistant to apoptosis. This is associated with higher BCL-2 levels, a marked impairment in P53 stabilization, and a reduced expression of BAX [Knauf et al. 1999].

A point mutation in PKCα is expressed in a subpopulation of thyroid neoplasms. It is located at position 294 of the protein, in the V3 region, leading to a substitution of a negatively charged aspartic acid by an apolar glycine. In response to the exposure to phorbol ester, wild-type PKCα translocates mainly to the plasma membrane, but mutant PKCα translocates mainly to the perinuclear region. The cells that express mutant PKCα display dysregulated growth.

Invasion. Papillary thyroid cancers metastasize to the regional lymph nodes. Parapharyngeal metastases arising from papillary thyroid cancer are rare. Sites of spread for medullary thyroid carcinoma include the regional lymph nodes, the mediastinal lymph nodes, the liver, the lungs, and the bones.

Thyroid cancer is generally circumscribed by a fibrous capsule, which is constituted of Collagens I and III. Secreted proteases of the MMP family are necessary for cancer cells to penetrate the capsule and disseminate. The *mmp-1* gene is not expressed in the cancer cells, but in the fibrous capsules of papillary carcinomata. It may play an important role in the invasion by thyroid cancer [Kameyama 1996]. The concentrations of MMP-2 and MMP-9 in the serum differentiate malignant from benign thyroid tumors.

Plasma VEGF is elevated in thyroid cancer patients as compared to healthy plasma [Lin et al. 2003]. VEGF expression in thyroid tumors is a marker for invasiveness [Klein et al. 2001].

Rare thyroid tumors. Activating mutations in the TSH Receptor or in the associated Guanine Nucleotide Stimulatory Factor α subunit ($G_s\alpha$) lead to thyroid hyperplasia and hyperthyroidism, but not to thyroid carcinoma. This is likely attributable to the importance of TSH signaling in more differentiated cells of the thyroid gland, which are resistant to malignant transformation.

Mucoepidermoid carcinoma is a rare malignancy of the thyroid. This tumor may arise from solid cell nests of the ultimobranchial apparatus or from follicular epithelium. The tumor is frequently low grade, but it may invade regional lymph nodes and the transverse cervical vein [Bhandarkar et al. 2005].

There is an association between papillary thyroid carcinoma and familial adenomatous polyposis [Rohaizak et al. 2003]. The cribriform-morular variant of papillary thyroid carcinoma is a rare histologic subtype of papillary thyroid cancer that shows a combination of growth patterns including cribriform and spindle cell areas. The thyroid cancer with this unique histology may occur in patients with familial adenomatous polyposis. The underlying genetic defects are mutations in the *apc* or *ctnnb1* (encodes for β-Catenin) genes.

In a familial predisposition syndrome, thyroid adenomata may occur in conjunction with ovarian arrhenoblastomata [Jensen et al. 1974; O'Brien and Wilansky 1981]. Arrhenoblastoma is a rare ovarian stromal neoplasm that secretes testosterone. This leads to progressive masculinization, anovulation and amenorrhea, acne and hirsutism, and voice changes.

Fewer than 3% of all thyroid tumors are lymphomata, squamous cell carcinomata, and sarcomata.

15.16 THYMIC CANCER

Thymoma incidence peaks between the ages of 40 and 60, with about equal risks for men and women. About one third of cases present with symptoms that include chest pain, cough, shortness of breath, dysphagia (difficulty in swallowing), and hoarseness of the voice. If the tumor compresses the upper caval vein it may cause vena cava superior syndrome. One third of cases manifest through the symptoms of paraneoplastic autoimmune diseases. One third of cases are detected serendipidously in X-rays of the chest.

According to histologic appearance, WHO type A (epithelial, spindle cell, medullary), type B1 (lymphocyte rich, lymphocytic, predominantly cortical, organoid), type B2 (cortical), type B3 (epithelial, atypical, squamoid, well-differentiated thymic carcinoma), type AB (mixed), and type C thymoma (thymic carcinoma) are distinguished. The histologic characterization of thymic carcinomata may distinguish keratinizing or nonkeratinizing squamous cell carcinoma, lymphoepithelioma-like carcinoma, neuroendocrine carcinoma, adenosquamous carcinoma, mucoepidermoid carcinoma, clear cell carcinoma, adenocarcinoma, basaloid carcinoma, and sarcomatoid carcinoma.

Molecular pathogenesis. Receptor tyrosine kinases play important roles in thymic tumorigenesis. Gene amplification of *egfr* correlates with WHO histologic type, invasion, and advanced clinical stage, but not with tumor size and outcome. EGFR expression is more common in thymomata than in thymic carcinomata. Hyperploidy occurs more frequently in thymomata associated with myasthenia gravis than in other thymomata, but there is no difference in *egfr* gene amplification. In contrast to normal thymus glands and thymomata, thymic carcinomata frequently express the tyrosine kinase receptor KIT (CD117) [Henley et al. 2004; Pan et al. 2004].

The oncogenic potential of COT is associated with thymomata. In correspondence with the broad effector specificity of COT, COT-dependent transformation requires P38, SAPK, and ERK5.

The pathology of thymic transformation may involve chromosome abnormalities. They include the

translocations t(15;22)(p11;q11), t(14;20)(q24;p13), the deletion del(6)(p22p25), and ring chromosome 6.

Metastasis. Malignant thymomata generally metastasize to the pleura, kidneys, bones, liver, or brain.

Paraneoplastic syndromes. Thymomata may be associated with paraneoplastic syndromes. Often, these are various neurological disorders. Autoantibodies directed to antigens in the central nervous system and muscles are present in the sera of such patients. These antibodies usually have high affinity and specificity for the intact conformation of their cognate antigen. Some are directed to cell surface antigens, and are directly pathogenic, while others are specific for intracellular antigens. Thymomata often generate large numbers of T-lymphocytes that are sensitized to self epitopes in the thymoma. Both cytolytic and helper T-lymphocytes may be induced to specific peptides in thymomata and then move to the periphery, where they can persist. Tissue damage or inflammation may lead to reactivation and cross-priming of the cytolytic T-cells to recognize epitopes presented by muscle or central nervous system tissue. In conjunction with helper T-cell and B-cell activation, clinical symptoms occur [Vincent and Willcox 1999]. Isaac neuromyotonia secondary to thymoma is caused by antibodies to potassium channels. About 25–50% of myasthenia gravis cases are associated with a thymoma.

Good syndrome is constituted by the association between the presence of a thymoma and immunodeficiency [Good 1954]. In patients with thymoma the incidence of hypogammaglobulinemia is 6–11%. Anemia is present in more than half of the patients with Good syndrome.

Acquired pure red cell aplasia (PRCA) is a type of anemia that affects the erythrocyte precursors, but not the leukocyte precursors. The disease has an autoimmune origin and may be a manifestation of thymoma.

Familial thymoma. Familial aggregation of thymoma occurs at a frequency of 0.1–0.4 per 100,000. The chromosome translocation t(14;20)(q24;p12) may be causative for some of these. They affect the tumor suppressor gene product RAD51L1, which is involved in the recombinational repair of DNA double strand breaks [Matani and Dristsas 1973; Nicodeme et al. 2005].

15.17 HEAD AND NECK CANCER

15.17.1 Nasopharyngeal Carcinoma

Worldwide, the incidence rate of nasopharyngeal carcinoma is less than 1 per 100,000. However, it is among the leading causes of death in the Guangdong Province and several other Southern Chinese districts. The incidence rate per 100,000 for males is over 40 in the Cantonese-speaking population in the Guangdong Province. Nasopharyngeal carcinoma is more common in men than in women by a factor of 2–3, its incidence increases with age and plateaus at 50–60 years. The two major predisposing factors for contracting nasopharyngeal carcinoma are Epstein-Barr virus (EBV) and the consumption of salted fish. A genetic component also influences the risk for nasopharyngeal cancer. The incidence rises as Chinese genes are introduced into an area. Also, first-generation Chinese–Americans have a higher risk of developing this lesion than the overall US rate.

The types of malignancy occurring in the nasopharynx include squamous cell carcinoma, lymphoma, salivary gland malignancy, and sarcoma. Squamous cell carcinoma is by far the most common type. There are three types of nasopharyngeal squamous cell carcinoma,

- Type I is a keratinizing form, which is EBV negative (25% of cases); it has a poor prognosis with 10% survival after 5 years.
- Type II is nonkeratinizing and poorly differentiated (15% of cases), the 5-year survival rate is about 50%.
- Type III is nonkeratinizing and undifferentiated (60% of cases); the 5-year survival rate is about 50%.

Early clinical symptoms of nasopharyngeal carcinoma are most commonly unilateral hearing loss from a middle ear effusion or a neck mass resulting from regional spread. Large or exophytic lesions may cause nasal obstruction or epistaxis. Moreover, tumor growth may affect adjacent cranial nerves and cause symptoms according to its interference with their functions:

- Facial pain based on trigeminal nerve involvement
- Diplopia (double vision) with isolated abducens nerve injury
- Ophthalmoplegia (paralysis of the extraocular muscles) consecutive to involvement of the cranial nerves III, IV, and VI
- Xerophthalmia (dry eyes) due to involvement of the greater superficial petrosal nerve

– Horner syndrome (ptosis, miosis, enophthalmus) with injury to the cervical sympathetic chain.

Molecular pathogenesis. All undifferentiated nasopharyngeal carcinomata are EBV positive, they are monoclonal with regard to EBV, and virtually all cells in each tumor contain EBV DNA and proteins. The expression of the EBV protein LMP-1 (Latent Membrane Protein 1) may be a suitable marker for classification. LMP-1 functions as a constitutively active TNFR, engaging signaling pathways that include the persistent activation of NF-κB, MAPKs, JNK, P38, the JAK/STAT pathway, and the small RHO-GTPases.

Alterations of genes such as *Ras association domain family 1A* (*rassf1A*), $p16^{INK4A}$, $p14^{ARF}$ suggest that multiple cellular pathways are dysregulated in nasopharyngeal carcinoma cells.

Genetic predisposition. Polymorphisms in specific metabolic enzyme genes may influence the susceptibility to nasopharyngeal carcinoma. GSTM1 detoxifies benzo[a]pyrene and together with Cytochrome P450 2E1 (CYP2E1) is responsible for the metabolic activation of nitrosamines. Alterations of *gstm1* and *cyp2E1* are associated with an increased risk of nasopharyngeal carcinoma.

Genetic susceptibility to nasopharyngeal carcinoma is caused by some alleles of the *hla-Bw46* locus [Lu et al. 1990], mutations in the *p53* gene, and a major susceptibility locus on chromosome 4p15.1–q12 (*npca1*; *nasopharyngeal carcinoma 1*) [Feng et al. 2002].

15.17.2 Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma (HNSCC) develops from premalignant lesions such as leukoplakia, dysplasia, erythroplakia, or lichen planus (Figure 15.17.2.A). The incidence of head and neck squamous cell carcinoma increases with age and patients are typically older than 50 years. Its primary etiologic agent is exposure to tobacco. Consistently, the levels of Aryl Hydrocarbon Hydroxylase, the enzyme that activates polycyclic aromatic hydrocarbons (the major carcinogens in cigarette smoke), are elevated in laryngeal cancer patients.

Molecular pathogenesis. *ing1* {13q34} encodes a 33 kD, 294 amino acid protein that displays the

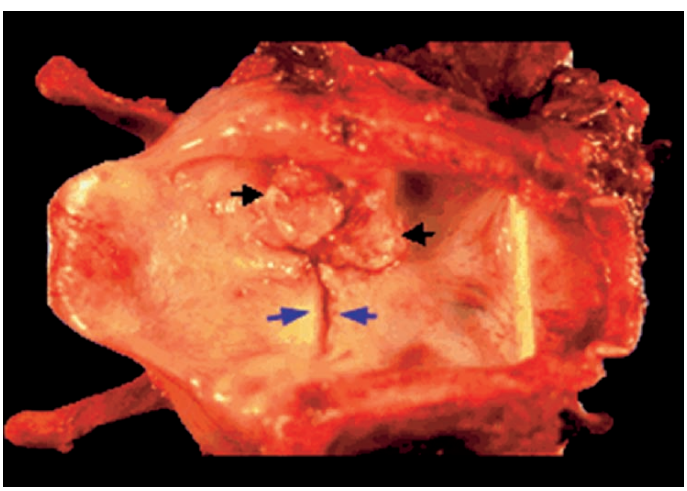


Figure 15.17.2.A. Head and neck squamous cell carcinoma (SCC). View of the larynx opened posteriorly. The blue arrows point to a normal left vocal cord; the black arrows show a granular tan neoplasm obliterating the right vocal cord. Squamous cell carcinomata are granular, gray white, exophytic, or ulcerated neoplasms. The patients present with hoarseness or airway obstruction. All of these tumors initially spread to the regional lymph nodes, and eventually to distant sites such as the lungs. There is extensive morbidity and mortality due to local disease with airway obstruction, hemorrhage, or infection. The prognosis is best for glottic neoplasms. Usually afflicted are men with a long cigarette smoking history, generally over 50 years. In some cases, the human papilloma virus genome may be present in the tumor cells. Asbestosis constitutes a cofactor. [Reproduced from http://pathweb.uchc.edu. With permission.]

characteristics of a tumor suppressor gene. Four splice variants of the gene differ in their 5′ regions. The proteins encoded by variants A (279 amino acids), C (235 amino acids), and D (294 amino acids) share a common 233 amino acid COOH-terminus, but differ in their NH_2-termini. Variant B encodes an NH_2-terminally truncated protein of 210 amino acids. Consistent with its role as a growth regulator, $P33^{ING1}$ is located in the nucleus. In tumor tissue of squamous cell carcinoma of the head and neck, a change of TGC to TCC in exon 2 of the *ing1* gene results in a C215S substitution. The cysteine substituted is 1 of 7 composing the C4HC3 motif. This change may affect the PHD finger and break the three-dimensional structure of the ING1 protein, leading to a loss of function. A change of AAC to AGC in exon 2 results in a N216S mutation. This mutation may affect the conformation of the zinc finger domain in the ING1 protein. In tumor tissue from squamous cell carcinoma of the head and neck, a GCC to GAC change in exon 2 results in an A192D substitution. This mutation may affect the nuclear localization signal and ultimately interfere in the accumulation of ING1 protein in the nucleus [Gunduz et al. 2000].

Overexpression of EGFR occurs in up to 90% of head and neck squamous cell carcinomata. STATs may be downstream targets of EGFR signaling. Constitutively, activated STATs exist in a wide variety of cancers, including almost all head and neck cancers.

Loss of heterozygosity is frequent among chromosomes 3p, 17p, 13q, 11q, 6p, 9p, and 14q. Fractional or entire DNA loss of chromosome 3p is associated with 97% and amplifications of 11q13 region with 70% of primary head and neck squamous cell carcinomata. The amplification of the 11q13 region is correlated with high grade, late stage, aneuploid tumors, poor prognosis, recurrence, and distant metastasis. The oncogenes present in 11q13 include *int-2*, *hst-1*, *cyclin* D_1, *ems-1*. The *int-2* gene encodes a member of the FGF family and is amplified in about 50% of patients. The dysregulation of *cyclin* D_1 expression takes place early during the tumorigenesis process in about 35% of head and neck squamous cell carcinomata and enables subsequent *cyclin* D_1 gene amplification. This is associated with reduced survival. The overexpression of *cyclin* D_1 also correlates with recurrence [Michalides et al. 1995].

The 8p21 region is a frequent site of translocations in head and neck tumors. Alternatively, mutations of the *tnfrsf10B* (*dr5*, *trailR2*) gene {8p22–p21} can cause squamous cell carcinoma of the head and neck. TNFRSF10B engages a FADD-dependent and Caspase-dependent apoptotic pathway. Its loss of function may protect the affected cells from apoptosis.

Extension of life span. Inactivation of the senescence and tumor suppressor gene *p16* may occur in up to 66% of head and neck squamous cell carcinomata. PTEN is associated with replicative senescence. Mutations in *pten* occur in a small number of cases of squamous cell carcinoma of the head and neck.

Metastasis. The regional lymph nodes are almost always the first site of metastasis. The development of nodal metastasis is a major adverse event for patients with squamous carcinomata of the upper aerodigestive tract, reducing survival by approximately 50%. The incidence of nodal metastases varies with the primary site and size of the carcinoma, with carcinomata of the hypopharynx and nasopharynx showing a high incidence of 65–85%. Supraglottic laryngeal carcinomata have a higher incidence of nodal metastasis than glottic carcinomata, and T1-2 carcinomata of the oral tongue and floor of mouth have a lower incidence of nodal metastasis than do T3-4 carcinomata [Helliwell 2001].

Semaphorin 4D (SEMA4D) regulates axonal growth cone guidance in the developing central nervous system through its receptor Plexin-B1. It is highly expressed in head and neck squamous cell carcinomata at both the protein and message level. The high levels of SEMA4D form a cell surface pattern in invading islands of transformed epithelial cells, but not in normal and noninvasive dysplastic epithelium, which is similar to malignant cells from prostate, colon, breast, and lung cancer tissues. When shed from the cancer cells, SEMA4D stimulates endothelial cell migration [Basile et al. 2006].

While the gene expression profile is very similar between the primary and metastatic tumor cells, the expression levels of *mta-1* are reduced compared to

the primary tumors. MTA-1 is a component of the NuRD complex and contributes to controlling epithelial–mesenchymal transition. Its suppression in metastatic sites suggests that MTA-1 is needed only at early stages of dissemination [Roepman et al. 2006].

Genetic predisposition. A family history of head and neck squamous cell carcinoma is a predisposing factor for this malignancy [Copper et al. 1995; Foulkes et al. 1995].

REFERENCES

Adams J. 1853. The case of scirrhous of the prostate gland with corresponding affliction of the lymphoid glands in the lumbar region of the pelvis. The Lancet 1:393.

Agrawal D, Chen T, Irby R, Quackenbush J, Chambers AF, Szabo M, Cantor A, Coppola D, Yeatman TJ. 2002. Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. The Journal of the National Cancer Institute 94:513–521.

Ahrendt SA, Decker PA, Alawi EA, Zhu Y, Sanchez-Cespedes M, Yang SC, Haasler GB, Kajdacsy-Balla A, Demeure MJ, Sidransky D. 2001. Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. Cancer 92:1525–1530.

Ai W, Liu Y, Langlois M, Wang TC. 2004. Kruppel-like factor 4 (KLF4) represses histidine decarboxylase gene expression through an upstream Sp1 site and downstream gastrin responsive elements. The Journal of Biological Chemistry 279:8684–8693.

Al-Aynati MM, Radulovich N, Ho J, Tsao MS. 2004. Overexpression of G1-S cyclins and cyclin-dependent kinases during multistage human pancreatic duct cell carcinogenesis. Clinical Cancer Research 10:6598–6605.

All-Ericsson C, Girnita L, Seregard S, Bartolazzi A, Jager MJ, Larsson O. 2002. Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target. Investigative Ophthalmology and Visual Science 43:1–8.

Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. 1988. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 53:549–554.

Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, Reuter VE, Garvin AJ, Perez-Atayde AR, Fletcher JA, Beckwith JB, Bridge JA, Ladanyi M. 2001. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. American Journal of Pathology 159:179–192.

Argani P, Lui MY, Couturier J, Bouvier R, Fournet JC, Ladanyi M. 2003. A novel CLTC-TFE3 gene fusion in pediatric renal adenocarcinoma with t(X;17)(p11.2;q23). Oncogene 22:5374–5378.

Armstrong B, Doll R. 1975. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. International Journal of Cancer 15:617–631.

Azmi P, Seth A. 2005. RNF11 is a multifunctional modulator of growth factor receptor signalling and transcriptional regulation. European Journal of Cancer 41:2549–2560.

Bailey-Wilson JE, Amos CI, Pinney SM, Petersen GM, de Andrade M, Wiest JS, Fain P, Schwartz AG, You M, Franklin W, Klein C, Gazdar A, Rothschild H, Mandal D, Coons T, Slusser J, Lee J, Gaba C, Kupert E, Perez A, Zhou X, Zeng D, Liu Q, Zhang Q, Seminara D, Minna J, Anderson MW. 2004. A major lung cancer susceptibility locus maps to chromosome 6q23-25. American Journal of Human Genetics 75:460–474.

Baldassarre G, Battista S, Belletti B, Thakur S, Pentimalli F, Trapasso F, Fedele M, Pierantoni G, Croce CM, Fusco A. 2003. Negative regulation of BRCA1 gene expression by HMGA1 proteins accounts for the reduced BRCA1 protein levels in sporadic breast carcinoma. Molecular and Cellular Biology 23:2225–2238.

Barbera VM, Martin M, Marinoso L, Munne A, Carrato A, Real FX, Fabre M. 2000. The 18q21 region in colorectal and pancreatic cancer: independent loss of DCC and DPC4 expression. Biochimica et Biophysica Acta 1502:283–296.

Barden CB, Shister KW, Zhu B, Guiter G, Greenblatt DY, Zeiger MA, Fahey TJ III. 2003. Classification of follicular thyroid tumors by molecular signature: results of gene profiling. Clinical Cancer Research 9:1792–1800.

Bartsch H, Nair J, Owen RW. 1999. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis 20:2209–2218.

Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. 2000. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiology, Biomarkers and Prevention 9:3–28.

Basile JR, Castilho RM, Williams VP, Gutkind JS. 2006. Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proceedings of the National Academy of Sciences USA 103:9017–9022.

Beckwith JB, Kiviat NB, Bonadio JF. 1990. Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumor. Pediatric Pathology 10:1–36.

Beckwith JB, Palmer NF. 1978. Histopathology and prognosis of Wilms tumors: results from the First National Wilms' Tumor Study. Cancer 41:1937–1948.

Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, Ferrandina G, Benedetti Panici P, Mancuso S, Neri G, Testa JR. 1995. Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. International Journal of Cancer 64:280–285.

Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A. 1998. Association between lung cancer and microsomal epoxide hydrolase genotypes. Cancer Research 58:5291–5293.

Berlin NI, Van Scott EJ, Clendenning WE, Archard HO, Block JB, Witkop CJ Jr, Haynes HA. 1966. Basal cell nevus syndrome. Annals of Internal Medicine 64:403–421.

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wohr G, Latil A, Millasseau P, Mellah I, Cohen N, Blanche H, Bellane-Chantelot C, Demenais F, Teillac P, Le Duc A, de Petriconi R, Hautmann R, Chumakov I, Bachner L, Maitland NJ, Lidereau R, Vogel W, Fournier G, Mangin P, Cussenot O, et al. 1998. Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43. American Journal for Human Genetics 62:1416–1424.

Bhandarkar ND, Chan J, Strome M. 2005. A rare case of mucoepidermoid carcinoma of the thyroid. American Journal of Otolaryngology 26:138–141.

Bharaj B, Scorilas A, Diamandis EP, Giai M, Levesque MA, Sutherland DJ, Hoffman BR. 2000. Breast cancer prognostic significance of a single nucleotide polymorphism in the proximal androgen response element of the prostate specific antigen gene promoter. Breast Cancer Research and Treatment 61:111–119.

Biankin AV, Biankin SA, Kench JG, Morey AL, Lee CS, Head DR, Eckstein RP, Hugh TB, Henshall SM, Sutherland RL. 2002. Aberrant $p16^{INK4A}$ and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut 50:861–868.

Biggs PJ, Chapman P, Lakhani SR, Burn J, Stratton MR. 1996. The cylindromatosis gene (cyld1) on chromosome 16q may be the only tumour suppressor gene involved in the development of cylindromas. Oncogene 12:1375–1377.

Bignell GR, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R, Green H, Brown C, Biggs PJ, Lakhani SR, Jones C, Hansen J, Blair E, Hofmann B, Siebert R, Turner G, Evans DG, Schrander-Stumpel C, Beemer FA, van Den Ouweland A, Halley D, Delpech B, Cleveland MG, Leigh I, Leisti J, Rasmussen S. 2000. Identification of the familial cylindromatosis tumour-suppressor gene. Nature Genetics 25:160–165.

Bongarzone I, Butti MG, Coronelli S, Borrello MG, Santoro M, Mondellini P, Pilotti S, Fusco A, Della Porta G, Pierotti MA. 1994. Frequent activation of ret protooncogene by fusion with a new activating gene in papillary thyroid carcinomas. Cancer Research 54:2979–2985.

Breslow NE, Beckwith JB. 1982. Epidemiological features of Wilms' tumor: results of the National Wilms' Tumor Study. The Journal of the National Cancer Institute 68:429–436.

Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA. 1993. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer Research 53:3241–3245.

Brockhausen I, Yang JM, Burchell J, Whitehouse C, Taylor-Papadimitriou J. 1995. Mechanisms underlying aberrant glycosylation of MUC1 mucin in breast cancer cells. European Journal of Biochemistry 233:607–617.

Bromen K, Pohlabeln H, Jahn I, Ahrens W, Jockel KH. 2000. Aggregation of lung cancer in families: results from a population-based case-control study in Germany. American Journal of Epidemiology 152:497–505.

Brown DM, Ruoslahti E. 2004. Metadherin, a cell surface protein in breast tumors that mediates lung metastases. Cancer Cell 5:365–374.

Butti MG, Bongarzone I, Ferraresi G, Mondellini P, Borrello MG, Pierotti MA. 1995. A sequence analysis of the genomic regions involved in the rearrangements between TPM3 and NTRK1 genes producing TRK oncogenes in papillary thyroid carcinomas. Genomics 28:15–24.

Cairns P, Tokino K, Eby Y, Sidransky D. 1995. Localization of tumor suppressor loci on chromosome 9 in primary human renal cell carcinomas. Cancer Research 55:224–227.

Cappellen D, De Oliveira C, Ricol D, Gil Diez de Medina S, Bourdin J, Sastre-Garau X, Chopin D, Thiery JP, Radvanyi F. 1999. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nature Genetics 23:18–20.

Carney JA, Sheps SG, Go VLW, Gordon H. 1977. The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. The New England Journal of Medicine 296:1517–1518.

Carney JA, Stratakis CA. 2002. Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. American Journal of Medical Genetics 108:132–139.

Casson AG, Mukhopadhyay T, Cleary KR, Ro JY, Levin B, Roth JA. 1991. p53 gene mutations in Barrett's epithelium and esophageal cancer. Cancer Research 51:4495–4499.

Chan EF, Gat U, McNiff JM, Fuchs E. 1999. A common human skin tumour is caused by activating mutations in β-catenin. Nature Genetics 21:410–413.

Chan KH, Vernino S, Lennon VA. 2001. ANNA-3 anti-neuronal nuclear antibody: marker of lung cancer-related autoimmunity. Annals of Neurology 50:301–311.

Cheever AW. 1978. Schistosomiasis and neoplasia. The Journal of the National Cancer Institute 61:13–18.

Chen CL, Lee WL, Tsai YC, Yuan CC, Ng HT, Wang PH. 2002. Sialyltransferase family members and cervix squamous cell carcinoma. European Journal of Gynaecologic Oncology 23:514–518.

Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. 1996. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Research 56:4862–4864.

Cheng Y, Lotan R. 1998. Molecular cloning and characterization of a novel retinoic acid-inducible gene that encodes a putative G protein-coupled receptor. The Journal of Biological Chemistry 273:35008–35015.

Chenevix-Trench G, Spurdle AB, Gatei M, Kelly H, Marsh A, Chen X, Donn K, Cummings M, Nyholt D, Jenkins MA, Scott C, Pupo GM, Dork T, Bendix R, Kirk J, Tucker K, McCredie MR, Hopper JL, Sambrook J, Mann GJ, Khanna KK. 2002. Dominant negative ATM mutations in breast cancer families. The Journal of the National Cancer Institute 94:205–215.

Chenevix-Trench G, Young J, Coggan M, Board P. 1995. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. Carcinogenesis 16:1655–1657.

Cheung CC, Ezzat S, Ramyar L, Freeman JL, Asa SL. 2000. Molecular basis of Hurthle cell papillary thyroid carcinoma. The Journal of Clinical Endocrinology and Metabolism 85:878–882.

Chiappetta G, Toti P, Cetta F, Giuliano A, Pentimalli F, Amendola I, Lazzi S, Monaco M, Mazzuchelli L, Tosi P, Santoro M, Fusco A. 2002. The RET/PTC oncogene is frequently activated in oncocytic thyroid tumors (Hurthle cell adenomas and carcinomas), but not in oncocytic hyperplastic lesions. The Journal of Clinical Endocrinology and Metabolism 87:364–369.

Chitayat D, Friedman JM, Anderson L, Dimmick JE. 1988. Hepatocellular carcinoma in a child with familial Russell-Silver syndrome. American Journal of Medical Genetics 31:909–914.

Chow NH, Liu HS, Chan SH. 2000. The role of nm23-H1 in the progression of transitional cell bladder cancer. Clinical Cancer Research 6:3595–3599.

Christian CD, McLoughlin TG, Cathcart ES, Eisenberg MM. 1964. Peutz-Jeghers syndrome associated with functioning ovarian tumor. The Journal of the American Medical Association 190:935–938.

Clark WH Jr, Ainsworth AM, Bernardino EA, Yang CH, Mihm CM Jr, Reed RJ. 1975. The developmental biology of primary human malignant melanomas. Seminars in Oncology 2:83–103.

Clarke CA, McConnell RB. 1954. Six cases of carcinoma of the oesophagus occurring in one family. British Medical Journal 2:1137–1138.

Clegg N, Ferguson C, True LD, Arnold H, Moorman A, Quinn JE, Vessella RL, Nelson PS. 2003. Molecular characterization of prostatic small-cell neuroendocrine carcinoma. Prostate 55:55–64.

Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS. 1979. Hereditary renal-cell carcinoma associated with chromosomal translocation. The New England Journal of Medicine 301:592–595.

Coleman WB. 2003. Mechanisms of human hepatocarcinogenesis. Current Molecular Medicine 3:573–588.

Copper MP, Jovanovic A, Nauta JJ, Braakhuis BJ, de Vries N, van der Waal I, Snow GB. 1995. Role of genetic factors in the etiology of squamous cell carcinoma of the head and neck. Archives of Otolaryngology – Head and Neck Surgery 121:157–160.

Cormio G, Capotorto M, Vagno GD, Cazzolla A, Carriero C, Selvaggi L. 2003. Skin metastases in ovarian carcinoma: a report of nine cases and a review of the literature. Gynecologic Oncology 90:682–685.

Cross NA, Chandrasekharan S, Jokonya N, Fowles A, Hamdy FC, Buttle DJ, Eaton CL. 2005. The expression and regulation of ADAMTS-1, -4, -5, -9, and -15, and TIMP-3 by TGFβ1 in prostate cells: relevance to the accumulation of versican. Prostate 63:269–275.

Daniels MJ, Wang Y, Lee M, Venkitaraman AR. 2004. Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. Science 306:876–879.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. 2002. Mutations of the BRAF gene in human cancer. Nature 417:949–954.

Day JD, Digiuseppe JA, Yeo C, Lai-Goldman M, Anderson SM, Goodman SN, Kern SE, Hruban RH. 1996. Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. Human Pathology 27:119–124.

de Lange R, Dimoudis N, Weidle UH. 2003. Identification of genes associated with enhanced metastasis of a large cell lung carcinoma cell line. Anticancer Research 23:187–194.

Delattre O, Olschwang S, Law DJ, Melot T, Remvikos Y, Salmon RJ, Sastre X, Validire P, Feinberg AP, Thomas G. 1989. Multiple genetic alterations in distal and proximal colorectal cancer. The Lancet 2:353–356.

Denkins Y, Reiland J, Roy M, Sinnappah-Kang ND, Galjour J, Murry BP, Blust J, Aucoin R, Marchetti D. 2004. Brain metastases in melanoma: roles of neurotrophins. Neuro-oncology 6:154–165.

Denys P, Malvaux P, van den Berghe H, Tanghe W, Proesmans W. 1967. Association d'un syndrome anatomo-pathologique de pseudohermaphrodisme masculin, d'une tumeur de Wilms, d'une nephropathie parenchymateuse et d'un mosaicisme XX/XY. Archives Francaises de Pediatrie 24:729–739.

De Oliveira WR, Rady PL, Grady J, Hughes TK, Neto CF, Rivitti EA, Tyring SK. 2004. Association of p53 arginine polymorphism with skin cancer. International Journal for Dermatology 43:489–493.

Derynck R, Akhurst RJ, Balmain A. 2001. TGF-β signaling in tumor suppression and cancer progression. Nature Genetics 29:117–129.

Deugnier YM, Guyader D, Crantock L, Lopez J-M, Turlin B, Yaouanq J, Jouanolle H, Campion J-P, Launois B, Halliday JW, Powell LW, Brissot P. 1993. Primary liver cancer in genetic hemochromatosis: a clinical, pathological, and pathogenetic study of 54 cases. Gastroenterology 104:228–234.

Dietze EC, Bowie ML, Mrozek K, Caldwell LE, Neal C, Marjoram RJ, Troch MM, Bean GR, Yokoyama KK, Ibarra CA, Seewaldt VL. 2005. CREB-binding protein regulates apoptosis and growth of HMECs grown in reconstituted ECM via laminin-5. The Journal of Cell Science 118(Pt 21):5005–5022.

Ding QM, Ko TC, Evers BM. 1998. Caco-2 intestinal cell differentiation is associated with G_1 arrest and suppression of CDK4. American Journal of Physiology 275:C1193–C1200.

Doll R, Peto R. 1981. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. The Journal of the National Cancer Institute 66:1191–1308.

Dong M, Johnson M, Rezaie A, Ilsley JN, Nakanishi M, Sanders MM, Forouhar F, Levine J, Montrose DC, Giardina C, Rosenberg DW. 2005. Cytoplasmic phospholipase A_2 levels correlate with apoptosis in human colon tumorigenesis. Clinical Cancer Research 11:2265–2271.

Drash A, Sherman F, Hartmann WH, Blizzard RM. 1970. A syndrome of pseudohermaphroditism, Wilms' tumor, hypertension, and degenerative renal disease. The Journal of Pediatrics 76:585–593.

Dressman MA, Baras A, Malinowski R, Alvis LB, Kwon I, Walz TM, Polymeropoulos MH. 2003. Gene expression profiling detects gene amplification and differentiates tumor types in breast cancer. Cancer Research 63:2194–2199.

Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV. 1995. Mutation of the MXI1 gene in prostate cancer. Nature Genetics 9:249–255.

Eccles DM, Cranston G, Steel CM, Nakamura Y, Leonard RCF. 1990. Allele losses on chromosome 17 in human epithelial ovarian carcinoma. Oncogene 5:1599–1601.

Efstathiou JA, Noda M, Rowan A, Dixon C, Chinery R, Jawhari A, Hattori T, Wright NA, Bodmer WF, Pignatelli M. 1998. Intestinal trefoil factor controls the expression of the adenomatous polyposis coli-catenin and the E-cadherin–catenin complexes in human colon carcinoma cells. Proceedings of the National Academy of Sciences USA 95:3122–3127.

Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE. 2006. Adult weight change and risk of postmenopausal breast cancer. The Journal of the American Medical Association 296:193–201.

Eriksson S, Carlson J, Velez R. 1986. Risk of cirrhosis and primary liver cancer in alpha-1-antitrypsin deficiency. The New England Journal of Medicine 314:736–739.

Evans MP, Podratz KC. 1996. Endometrial neoplasia: prognostic significance of ploidy status. Clinical Obstetrics and Gynecology 39:696–706.

Fagin JA. 2002. Branded from the start – distinct oncogenic initiating events may determine tumor fate in the thyroid. Molecular Endocrinology 16:903–911.

Feng B-J, Huang W, Shugart YY, Lee MK, Zhang F, Xia J-C, Wang H-Y, Huang T-B, Jian S-W, Huang P, Feng Q-S, Huang L-X, Yu XJ, Li D, Chen LZ, Jia WH, Fang Y, Huang HM, Zhu JL, Liu XM, Zhao Y, Liu WQ, Deng MQ, Hu WH, Wu SX, Mo HY, Hong MF, King MC, Chen Z, Zeng YX. 2002. Genome-wide scan for familial nasopharyngeal carcinoma reveals evidence of linkage to chromosome 4. Nature Genetics 31:395–399.

Fettman MJ, Butler RN, McMichael AJ, Roberts-Thomson IC. 1991. Metabolic phenotypes and colorectal neoplasia. Journal of Gastroenterology and Hepatology 6:81–89.

Fodde R, Smits R, Clevers H. 2001. *APC*, signal transduction and genetic instability in colorectal cancer. Nature Reviews Cancer 1:55–67.

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BAJ, Gayther SA, Birch JM, Lindblom A, Stoppa-Lyonnet D, Bignon Y, Borg A, Hamann U, Haites N, Scott RJ, Maugard CM, Vasen H, Seitz S, Cannon-Albright LA, Schofield A, Zelada-Hedman M, and the Breast Cancer Linkage Consortium. 1998. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. American Journal of Human Genetics 62:676–689.

Foulkes WD, Brunet JS, Kowalski LP, Narod SA, Franco EL. 1995. Family history of cancer is a risk factor for squamous cell carcinoma of the head and neck in Brazil: a case-control study. International Journal of Cancer 63:769–773.

Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA. 2003. MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 113:207–219.

Fukuda S, Shirahama T, Imazono Y, Tsushima T, Ohmori H, Kayajima T, Take S, Nishiyama K, Yonezawa S, Akiba S, Akiyama S, Ohi Y. 1999. Expression of vascular endothelial growth factor in patients with testicular germ cell tumors as an indicator of metastatic disease. Cancer 85:1323–1330.

Fuller PJ, Chu S. 2004. Signalling pathways in the molecular pathogenesis of ovarian granulosa cell tumours. Trends in Endocrinology and Metabolism 15:122–128.

Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S, Matsuzawa Y. 1999. Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. Internal Medicine 38:202–206.

Gallion HH, Smith SA. 1994. Hereditary ovarian carcinoma. Seminars in Surgical Oncology 10:249–254.

Gardner EJ. 1951. A genetic and clinical study of intestinal polyposis, a predisposing factor for carcinoma of the colon and rectum. American Journal for Human Genetics 3:167–176.

Gentile JM. 1985. Schistosome related cancers: a possible role for genotoxins. Environmental Mutagenesis 7:775–785.

Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Dahl D, Brufsky A, Talcott J, Hennekens CH, Kantoff PW. 1997. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proceedings of the National Academy of Sciences USA 94:3320–3323.

Gleason DF. 1966. Classification of prostatic carcinomas. Cancer and Chemotherapy Reports 50:125–128.

Gold PJ, Fefer A, Thompson JA. 1996. Paraneoplastic manifestations of renal cell carcinoma. Seminars in Urologic Oncology 14:216–222.

Goldberg MB, Scully AL. 1967. Gonadal malignancy in gonadal dysgenesis: papillary pseudomucinous cystadenocarcinoma in a patient with Turner's syndrome. Journal of Clinical Endocrinology and Metabolism 27:341–347.

Good RA. 1954. Agammaglobulinaemia-a provocative experiment of nature. Bulletin of the University of Minnesota 26:1–19.

Gotoh M, Sakamoto M, Kanetaka K, Chuuma M, Hirohashi S. 2002. Overexpression of osteopontin in hepatocellular carcinoma. Pathology International 52:19–24.

Grady B, Goharderakhshan R, Chang J, Ribeiro-Filho LA, Perinchery G, Franks J, Presti J, Carroll P, Dahiya R. 2001. Frequently deleted loci on chromosome 9 may harbor several tumor suppressor genes in human renal cell carcinoma. Journal of Urology 166:1088–1092.

Greco A, Pierotti MA, Bongarzone I, Pagliardini S, Lanzi C, Della Porta G. 1992. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene 7:237–242.

Gross G, Hagedorn M, Ikenberg H, Rufli T, Dahlet C, Grosshans E, Gissmann L. 1985. Bowenoid papulosis. Presence of human papillomavirus (HPV) structural antigens and of HPV 16-related DNA sequences. Archives of Dermatology 121:858–863.

Gunduz M, Ouchida M, Fukushima K, Hanafusa H, Etani T, Nishioka S, Nishizaki K, Shimizu K. 2000. Genomic structure of the human ING1 gene and tumor-specific mutations detected in head and neck squamous cell carcinomas. Cancer Research 60:3143–3146.

Guo X, Knudsen BS, Peehl DM, Ruiz A, Bok D, Rando RR, Rhim JS, Nanus DM, Gudas LJ. 2002. Retinol metabolism and lethicin:retinol acyltransferase levels are reduced in cultured human prostate cancer cells and tissue specimens. Cancer Research 62:1654–1661.

Ha PK, Califano JA. 2006. Promoter methylation and inactivation of tumour-suppressor genes in oral squamous-cell carcinoma. Lancet Oncology 7:77–82.

Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. 1996. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85:841–851.

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King M-C. 1990. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250:1684–1689.

Hall JA, Maitland NJ, Stower M, Lang SH. 2002. Primary prostate stromal cells modulate the morphology and migration of primary prostate epithelial cells in type 1 collagen gels. Cancer Research 62:58–62.

Hanekamp EE, Gielen SC, Smid-Koopman E, Kuhne LC, de Ruiter PE, Chadha-Ajwani S, Brinkmann AO, Grootegoed JA, Burger CW, Huikeshoven FJ, Blok LJ. 2003. Consequences of loss of progesterone receptor expression in development of invasive endometrial cancer. Clinical Cancer Research 9:4190–4199.

Harach HR, Franssila KO, Wasenius VM. 1985. Occult papillary carcinoma of the thyroid: a "normal" finding in Finland. Cancer 56:531–538.

Hayes SA, Zarnegar M, Sharma M, Yang F, Peehl DM, ten Dijke P, Sun Z. 2001. SMAD3 represses androgen receptor-mediated transcription. Cancer Research 61:2112–2118.

Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. 2003. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299:708–710.

Heiss MM, Allgayer H, Gruetzner KU, Funke I, Babic R, Jauch KW, Schildberg FW. 1995. Individual development and uPA-receptor expression of disseminated tumour cells in bone marrow: a reference to early systemic disease in solid cancer. Nature Medicine 1:1035–1039.

Helliwell TR. 2001. Molecular markers of metastasis in squamous carcinomas. Journal of Pathology 194:289–293.

Henley JD, Cummings OW, Loehrer PJ Sr. 2004. Tyrosine kinase receptor expression in thymomas. The Journal of Cancer Research and Clinical Oncology 130:222–224.

Henneveld HT, van Lingen RA, Hamel BC, Stolte-Dijkstra I, van Essen AJ. 1999. Perlman syndrome: four additional cases and review. American Journal of Medical Genetics 86:439–446.

Herrera CA, Xu L, Bucana CD, Silva EG, Hess KR, Gershenson DM, Fidler IJ. 2002. Expression of metastasis-related genes in human epithelial ovarian tumors. International Journal of Oncology 20:5–13.

Herzog CR, Devereux TR, Pittman B, You M. 2002. Carcinogenic induction directs the selection of allelic losses in mouse lung tumorigenesis. Cancer Research 62:6424–6429.

Higashi K, Hiai H, Higashi T, Muramatsu M. 2004. Regulatory mechanism of glutathione S-transferase P-form during chemical hepatocarcinogenesis: old wine in a new bottle. Cancer Letters 209:155–163.

Hill RD. 1976. Familial cancer on a Scottish island. British Medical Journal 2:401–402.

Hoff SD, Matsushita Y, Ota DM, Cleary KR, Yamori T, Hakomori S, Irimura T. 1989. Increased expression of sialyldimeric LeX antigen in liver metastases of human colorectal carcinoma. Cancer Research 49:6883–6888.

Hohne MW, Halatsch ME, Kahl GF, Weinel RJ. 1992. Frequent loss of expression of the potential tumor suppressor gene DCC in ductal pancreatic adenocarcinoma. Cancer Research 52:2616–2619.

Hollstein MC, Metcalf RA, Welsh JA, Montesano R, Harris CC. 1990. Frequent mutation of the p53 gene in human esophageal cancer. Proceedings of the National Academy of Sciences USA 87:9958–9961.

Howel-Evans W, McConnell RB, Clarke CA, Sheppard PM. 1958. Carcinoma of the oesophagus with keratosis palmaris et plantaris (tylosis): a study of two families. Quarterly Journal of Medicine 27:413–429.

Ichimura Y, Habuchi T, Tsuchiya N, Wang L, Oyama C, Sato K, Nishiyama H, Ogawa O, Kato T. 2004. Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. Journal of Urology 172:728–732.

Ilsley JN, Nakanishi M, Flynn C, Belinsky GS, De Guise S, Adib JN, Dobrowsky RT, Bonventre JV, Rosenberg DW. 2005. Cytoplasmic phospholipase A_2 deletion enhances colon tumorigenesis. Cancer Research 65:2636–2643.

Itzkowitz SH, Bloom EJ, Kokal WA, Modin G, Hakomori S, Kim YS. 1990. Sialosyl-Tn: a novel mucin antigen associated with prognosis in colorectal cancer patients. Cancer 66:1960–1966.

Iwata K, Takamura N, Nakashima M, Alipov G, Mine M, Matsumoto N, Yoshiura K, Prouglo Y, Sekine I, Katayama I, Yamashita S. 2004. Loss of heterozygosity on chromosome 9q22.3 in microdissected basal cell carcinomas around the Semipalatinsk Nuclear Testing Site, Kazakhstan. Human Pathology 35:460–464.

Jaeger EE, Woodford-Richens KL, Lockett M, Rowan AJ, Sawyer EJ, Heinimann K, Rozen P, Murday VA, Whitelaw SC, Ginsberg A, Atkin WS, Lynch HT, Southey MC, Debinski H, Eng C, Bodmer WF, Talbot IC, Hodgson SV, Thomas HJ, Tomlinson IP. 2003. An ancestral Ashkenazi haplotype at the HMPS/CRAC1 locus on 15q13-q14 is associated with hereditary mixed polyposis syndrome. American Journal of Human Genetics 72:1261–1267.

Jensen RD, Norris HJ, Fraumeni JF Jr. 1974. Familial arrhenoblastoma and thyroid adenoma. Cancer 33:218–223.

Jhappan C, Noonan FP, Merlino G. 2003. Ultraviolet radiation and cutaneous malignant melanoma. Oncogene 22:3099–3112.

Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, Johannsson OT, Borg A, Ingvarsson S, Easton DF, Egilsson V, Barkardottir RB. 1996. High prevalence of the 999del5 mutation in icelandic breast and ovarian cancer patients. Cancer Research 56:3663–3665.

Johne A, Roots I, Brockmoller J. 2003. A single nucleotide polymorphism in the human H-ras proto-oncogene determines the risk of urinary bladder cancer. Cancer Epidemiology, Biomarkers and Prevention 12:68–70.

Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH Jr, Scott MP. 1996. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272:1668–1671.

Kalinichenko VV, Major ML, Wang X, Petrovic V, Kuechle J, Yoder HM, Dennewitz MB, Shin B, Datta A, Raychaudhuri P, Costa RH. 2004. Foxm1b transcription factor is essential for development of hepatocellular carcinomas and is negatively regulated by the p19ARF tumor suppressor. Genes and Development 18:830–850.

Kamai T, Arai G, Takagi K. 1999. Granulocyte colony-stimulating factor and parathyroid hormone related protein producing bladder cancer. The Journal of Urology 161:1565–1566.

Kameyama K. 1996. Expression of MMP-1 in the capsule of thyroid cancer – relationship with invasiveness. Pathology Research and Practice 192:20–26.

Kaplan KB, Burds AA, Swedlow JR, Bekir SS, Sorger PK, Nathke IS. 2001. A role for the adenomatous polyposis coli protein in chromosome segregation. Nature Cell Biology 3:492–432.

Katoh M. 2003. Trefoil factors and human gastric cancer (Review). International Journal of Molecular Medicine 12:3–9.

Kawakami K, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, Demeester TR, Eads C, Laird PW, Ilson DH, Kelsen DP, Harpole D, Moore MB, Danenberg KD, Danenberg PV, Meltzer SJ. 2000. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. The Journal of the National Cancer Institute 92:1805–1811.

Kawashima K, Shikama H, Imoto K, Izawa M, Naruke T, Okabayashi K, Nishimura S. 1988. Close correlation between restriction fragment length polymorphism of the L-MYC gene and metastasis of human lung cancer to the lymph nodes and

other organs. Proceedings of the National Academy of Sciences USA 85:2353–2356.

Keller ET, Fu Z, Brennan M. 2005. The biology of a prostate cancer metastasis suppressor protein: Raf kinase inhibitor protein. Journal of Cellular Biochemistry 94:273–278.

Keller ET, Fu Z, Yeung K, Brennan M. 2004. Raf kinase inhibitor protein: a prostate cancer metastasis suppressor gene. Cancer Letters 207:131–137.

Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. 2003. High prevalence of *BRAF* mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Research 63:1454–1457.

Kiyohara C. 2000. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of colorectal cancer. The Journal of Epidemiology 10:349–360.

Klein M, Vignaud JM, Hennequin V, Toussaint B, Bresler L, Plenat F, Leclere J, Duprez A, Weryha G. 2001. Increased expression of the vascular endothelial growth factor is a pejorative prognosis marker in papillary thyroid carcinoma. The Journal of Clinical Endocrinology and Metabolism 86:656–658.

Knauf JA, Elisei R, Mochly-Rosen D, Liron T, Chen XN, Gonsky R, Korenberg JR, Fagin JA. 1999. Involvement of protein kinase Cε (PKCε) in thyroid cell death. A truncated chimeric PKCε cloned from a thyroid cancer cell line protects thyroid cells from apoptosis. The Journal of Biological Chemistry 274:23414–23425.

Knezevich SR, Garnett MJ, Pysher TJ, Beckwith JB, Grundy PE, Sorensen PH. 1998. ETV6-NTRK3 gene fusions and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. Cancer Research 58:5046–5048.

Korswagen LA, Moll AC, Imhof SM, Schouten-van Meeteren AY. 2004. A second primary tumor in a patient with retinoma. Ophthalmic Genetics 25:45–48.

Krishnadath KK, Tilanus HW, van Blankenstein M, Hop WC, Teijgeman R, Mulder AH, Bosman FT, van Dekken H. 1995. Accumulation of genetic abnormalities during neoplastic progression in Barrett's esophagus. Cancer Research 55:1971–1976.

Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA. 2000. PAX8-PPARγl fusion oncogene in human thyroid carcinoma. Science 289:1357–1360.

Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N. 1993. An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. The New England Journal of Medicine 329:517–523.

Kudo Y, Kitajima S, Ogawa I, Miyauchi M, Takata T. 2005. Down-regulation of Cdk inhibitor p27 in oral squamous cell carcinoma. Oral Oncology 41:105–116.

Kuiper RP, Schepens M, Thijssen J, van Asseldonk M, van den Berg E, Bridge J, Schuuring E, Schoenmakers EF, van Kessel AG. 2003. Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. Human Molecular Genetics 12:1661–1669.

Lang NP, Chu DZ, Hunter CF, Kendall DC, Flammang TJ, Kadlubar FF. 1986. Role of aromatic amine acetyltransferase in human colorectal cancer. Archives of Surgery 121:1259–1261.

Lax SF. 2004. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Archiv 444:213–223.

Lee SH, Lee JY, Park WS, Kim SY, Jang JJ, Yoo NJ. 1999a. Transitional cell carcinoma expresses high levels of Fas ligand in vivo. BJU International 83:698–702.

Lee SH, Shin MS, Park WS, Kim SY, Dong SM, Pi JH, Lee HK, Kim HS, Jang JJ, Kim CS, Kim SH, Lee JY, Yoo NJ. 1999b. Alterations of Fas (APO-1/CD95) gene in transitional cell carcinomas of urinary bladder. Cancer Research 59:3068–3072.

Le Marchand L, Seifried A, Lum-Jones A, Donlon T, Wilkens LR. 2003. Association of the cyclin D1 A870G polymorphism with advanced colorectal cancer. The Journal of the American Medical Association 290:2843–2848.

Le Marchand L, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, Lau AF. 1998. Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. Cancer Research 58:4858–4863.

Lengauer C, Kinzler KW, Vogelstein B. 1997a. Genetic instability in colorectal cancers. Nature 386:623–627.

Lengauer C, Kinzler KW, Vogelstein B. 1997b. DNA methylation and genetic instability in colorectal cancer cells. Proceedings of the National Academy of Sciences USA 94:2545–2550.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2002. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109:113–124.

Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R. 1997. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nature Genetics 16:64–67.

Liban E, Kozenitzky IL. 1970. Metanephric hamartomas and nephroblastomatosis in siblings. Cancer 25:885–888.

Liede A, Tonin PN, Sun CC, Serruya C, Daly MB, Narod SA, Foulkes WD. 1998. Is hereditary site-specific ovarian cancer a distinct genetic condition? American Journal of Medical Genetics 75:55–58.

Lin RY, Argenta PA, Sullivan KM, Adzick NS. 1995. Diagnostic and prognostic role of basic fibroblast growth factor in Wilm's tumor patients. Clinical Cancer Research 1:327–331.

Lin SY, Wang YY, Sheu WH. 2003. Preoperative plasma concentrations of vascular endothelial growth factor and matrix metalloproteinase 9 are associated with stage progression in papillary thyroid cancer. Clinical Endocrinology 58:513–518.

Liu Q, Yan Y-X, McClure M, Nakagawa H, Fujimura F, Rustgi AK. 1995. MTS-1 (CDKN2) tumor suppressor gene deletions are a frequent event in esophagus squamous cancer and pancreatic adenocarcinoma cell lines. Oncogene 10:619–622.

Liu VWS, Wang Y, Yang H-J, Tsang PCK, Ng T-Y, Wong L-C, Nagley P, Ngan HYS. 2003. Mitochondrial DNA variant 16189T-to-C is associated with susceptibility to endometrial cancer. Human Mutations 22:173–174.

Lloyd KM, Dennis M. 1963. Cowden's disease: a possible new symptom complex with multiple system involvement. Annals of Internal Medicine 58:136–142.

Lo HS, Hu N, Gere S, Lu N, Su H, Goldstein AM, Taylor PR, Lee MP. 2002. Identification of somatic mutations of the RNF6 gene in human esophageal squamous cell carcinoma. Cancer Research 62:4191–4193.

Lothe RA, Fossa SD, Stenwig AE, Nakamura Y, White R, Borresen A-L, Brogger A. 1989. Loss of 3p or 11p alleles is associated with testicular cancer tumors. Genomics 5:134–138.

Lowenfels AB, Maisonneuve P, DiMagno EP, Elitsur Y, Gates LK Jr, Perrault J, Whitcomb DC. 1997. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. Journal of the National Cancer Institute 89:442–446.

Lu S, Day NE, Degos L, Lepage V, Wang P-C, Chan S-H, Simons M, McKnight B, Easton D, Zeng Y, de-The G. 1990. Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region. Nature 346:470–471.

Lynch HT, Bewtra C, Lynch JF. 1986. Familial peritoneal ovarian carcinomatosis: a new clinical entity. Medical Hypotheses 21:171–177.

Lynch HT, Coronel SM, Okimoto R, Hampel H, Sweet K, Lynch JF, Barrows A, Wijnen J, van der Klift H, Franken P, Wagner A, Fodde R, de la Chapelle A. 2004. A founder mutation of the MSH2 gene and hereditary nonpolyposis colorectal cancer in the United States. The Journal of the American Medical Association 291:718–724.

Lynch HT, Smyrk T, Kern SE, Hruban RH, Lightdale CJ, Lemon SJ, Lynch JF, Fusaro RM, Ghadirian P. 1996. Familial pancreatic cancer: a review. Seminars in Oncology 23:251–275.

Maillefer RH, Greydanus MP. 1999. To B or not to B: is tylosis B truly benign? American Journal of Gastroenterology 94:829–834.

Malats N, Camus-Radon AM, Nyberg F, Ahrens W, Constantinescu V, Mukeria A, Benhamou S, Batura-Gabryel H, Bruske-Hohlfeld I, Simonato L, Menezes A, Lea S, Lang M, Boffetta P. 2000. Lung cancer risk in nonsmokers and GSTM1 and GSTT1 genetic polymorphism. Cancer Epidemiology, Biomarkers and Prevention 9:827–833.

Margolis EJ, Choi JC, Shu WP, Liu BC. 1996. Specific sequences of fibronectin activate the protein kinase C signal transduction pathway in invasive bladder cancer. Cancer Letters 100:163–168.

Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M, Willson JKV. 1995. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science 268:1336–1338.

Martin JJ, Van de Vyver FL, Scholte HR, Roodhooft AM, Cevterick C, Martin L, Luyt-Houwen JEM. 1988. Defect in succinate oxidation by isolated muscle mitochondria in a patient with symmetrical lesions in the basal ganglia. Journal of Neurological Science 84:189–200.

Matani A, Dristsas C. 1973. Familial occurrence of thymoma. Archives of Pathology 95:90–91.

Mathur M, Das S, Samuels HH. 2003. PSF-TFE3 oncoprotein in papillary renal cell carcinoma inactivates TFE3 and p53 through cytoplasmic sequestration. Oncogene 22:5031–5044.

Maxwell SA, Rivera A. 2003. Proline oxidase induces apoptosis in tumor cells, and its expression is frequently absent or reduced in renal carcinomas. The Journal of Biological Chemistry 278:9784–9789.

McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, Lin YL, Ramaswamy S, Avery W, Ding HF, Jordan SA, Jackson IJ, Korsmeyer SJ, Golub TR, Fisher DE. 2002. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell 109:707–718.

Meisels A, Fortin R. 1976. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. Acta Cytologica 20:505–509.

Mendez LE, Manci N, Cantuaria G, Gomez-Marin O, Penalver M, Braunschweiger P, Nadji M. 2002. Expression of glucose transporter-1 in cervical cancer and its precursors. Gynecologic Oncology 86:138–143.

Melendez ND, Smoller BR, Morgan M. 2003. VCAM (CD-106) and ICAM (CD-54) adhesion molecules distinguish keratoacanthomas from cutaneous squamous cell carcinomas. Modern Pathology 16:8–13.

Meyer AN, Gastwirt RF, Schlaepfer DD, Donoghue DJ. 2004. The cytoplasmic tyrosine kinase Pyk2 as a novel effector of fibroblast growth factor receptor 3 activation. The Journal of Biological Chemistry 279:28450–28457.

Michalides R, van Veelen N, Hart A, Loftus B, Wientjens E, Balm A. 1995. Overexpression of cyclin D1 correlates with recurrence in a group of forty-seven operable squamous cell carcinomas of the head and neck. Cancer Research 55:975–978.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rostock P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71.

Miller RW, Fraumeni JF, Manning MD. 1964. Association of Wilms' tumor with aniridia, hemihypertrophy and other congenital anomalies. The New England Journal of Medicine 270:922–927.

Mitra A, Chakrabarti J, Chattopadhyay N, Chatterjee A. 2003. Membrane-associated MMP-2 in human cervical cancer. Journal of Environmental Pathology, Toxicology and Oncology 22:93–100.

Mizutani Y, Wada H, Yoshida O, Fukushima M, Nakao M, Miki T. 2003. Significance of thymidine kinase activity in renal cell carcinoma. Journal of Urology 169:706–709.

Mizutani Y, Yoshida O, Bonavida B. 1998. Prognostic significance of soluble Fas in the serum of patients with bladder cancer. The Journal of Urology 160:571–576.

Moinova HR, Chen WD, Shen L, Smiraglia D, Olechnowicz J, Ravi L, Kasturi L, Myeroff L, Plass C, Parsons R, Minna J, Willson JK, Green SB, Issa JP, Markowitz SD. 2002. HLTF gene silencing in human colon cancer. Proceedings of the National Academy of Sciences USA 99:4562–4567.

Moisio AL, Sistonen P, Weissenbach J, de la Chapelle A, Peltomaki P. 1996. Age and origin of two common MLH1 mutations predisposing to hereditary colon cancer. American Journal of Human Genetics 59:1243–1251.

Moll UM, Riou G, Levine AJ. 1992. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. Proceedings of the National Academy of Sciences USA 89:7262–7266.

Montesano R, Hollstein M, Hainaut P. 1996. Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. International Journal of Cancer 69:225–235.

Moy RL, Eliezri YD, Nuovo GJ, Zitelli JA, Bennett RG, Silverstein S. 1989. Human papillomavirus type 16 DNA in

periungual squamous cell carcinomas. Journal of the American Medical Association 261:2669–2673.

Mueller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56.

Muleris M, Salmon R-J, Girodet J, Zafrani B, Dutrillaux B. 1987. Recurrent deletions of chromosomes 11q and 3p in anal canal carcinoma. International Journal of Cancer 39:595–598.

Murata M, Tagawa M, Watanabe S, Kimura H, Takeshita T, Morimoto K. 1999. Genotype difference of aldehyde dehydrogenase 2 gene in alcohol drinkers influences the incidence of Japanese colorectal cancer patients. Japanese Journal of Cancer Research 90:711–719.

Murphy KM, Brune KA, Griffin C, Sollenberger JE, Petersen GM, Bansal R, Hruban RH, Kern SE. 2002. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. Cancer Research 62:3789–3793.

Murty VV, Chaganti RS. 1998. A genetic perspective of male germ cell tumors. Seminars in Oncology 25:133–144.

Murty VVVS, Houldsworth J, Baldwin S, Reuter V, Hunziker W, Besmer P, Bosl G, Chaganti RSK. 1992. Allelic deletions in the long arm of chromosome 12 identify sites of candidate tumor suppressor genes in male germ cell tumors. Proceedings of the National Academy of Sciences USA 89:11006–11010.

Nagata M, Fujita H, Ida H, Hoshina H, Inoue T, Seki Y, Ohnishi M, Ohyama T, Shingaki S, Kaji M, Saku T, Takagi R. 2003. Identification of potential biomarkers of lymph node metastasis in oral squamous cell carcinoma by cDNA microarray analysis. International Journal of Cancer 106:683–689.

Nanus DM, Schmitz-Drager BJ, Motzer RJ, Lee AC, Vlamis V, Cordon-Cardo C, Albino AP, Reuter VE. 1993. Expression of basic fibroblast growth factor in primary human renal tumors: correlation with poor survival. The Journal of the National Cancer Institute 85:1597–1599.

Naora H, Montell DJ. 2005. Ovarian cancer metastasis: integrating insights from disparate model organisms. Nature Reviews Cancer 5:355–366.

Narla G, Difeo A, Reeves HL, Schaid DJ, Hirshfeld J, Hod E, Katz A, Isaacs WB, Hebbring S, Komiya A, McDonnell SK, Wiley KE, Jacobsen SJ, Isaacs SD, Walsh PC, Zheng SL, Chang BL, Friedrichsen DM, Stanford JL, Ostrander EA, Chinnaiyan AM, Rubin MA, Xu J, Thibodeau SN, Friedman SL, Martignetti JA. 2005. A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. Cancer Research 65:1213–1222.

Narla G, Heath KE, Reeves HL, Li D, Giono LE, Kimmelman AC, Glucksman MJ, Narla J, Eng FJ, Chan AM, Ferrari AC, Martignetti JA, Friedman SL. 2001. KLF6, a candidate tumor suppressor gene mutated in prostate cancer. Science 294:2563–2566.

Narod S, Tonin P, Lynch H, Watson P, Feunteun J, Lenoir G. 1994. Histology of BRCA1-associated ovarian tumours. The Lancet 343:236.

Narod S. 2002. Modifiers of risk of hereditary breast and ovarian cancer. Nature Reviews Cancer 2:115.

Newton-Bishop JA. 2003. The genetics of susceptibility to melanoma. Pigment Cell Research 16:578–579.

Nicodeme F, Geffroy S, Conti M, Delobel B, Soenen V, Grardel N, Porte H, Copin M-C, Lai J-L, Andrieux J. 2005. Familial occurrence of thymoma and autoimmune diseases with the constitutional translocation t(14;20)(q24.1;p12.3). Genes, Chromosomes and Cancer 44:154–160.

Niemann S, Mueller U. 2000. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nature Genetics 26:268–270.

Niemann S, Mueller U, Engelhardt D, Lohse P. 2003. Autosomal dominant malignant and catecholamine-producing paraganglioma caused by a splice donor site mutation in SDHC. Human Genetics 113:92–94.

Nikiforova MN, Stringer JR, Bpough R, Medvedovic M, Fagin JA, Nikiforov YE. 2000. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science 290:138–141.

Nishiwaki T, Daigo Y, Kawasoe T, Nakamura Y. 2000. Isolation and mutational analysis of a novel human cDNA, DEC1 (deleted in esophageal cancer 1), derived from the tumor suppressor locus in 9q32. Genes, Chromosomes and Cancer 27:169–176.

Nyormoi O, Bar-Eli M. 2003. Transcriptional regulation of metastasis-related genes in human melanoma. Clinical and Experimental Metastasis 20:251–263.

O'Brien PK, Wilansky DL. 1981. Familial thyroid nodulation and arrhenoblastoma. American Journal of Clinical Pathology 75:578–581.

Offit K, Gilewski T, McGuire P, Schluger A, Hampel H, Brown K, Swensen J, Neuhausen S, Skolnick M, Norton L, Goldgar D. 1996. Germline BRCA1 185delAG mutations in Jewish women with breast cancer. The Lancet 347:1643–1645.

Ogata S, Tamura G, Endoh Y, Sakata K, Ohmura K, Motoyama T. 2001. Microsatellite alterations and target gene mutations in the early stages of multiple gastric cancer. The Journal of Pathology 194:334–340.

Oro AE, Higgins KM, Hu Z, Bonifas JM, Epstein EH Jr, Scott MP. 1997. Basal cell carcinomas in mice overexpressing sonic hedgehog. Science 276:817–821.

Paget S. 1889. The distribution of secondary growths in cancer of the breast. The Lancet 1:99–101.

Pan CC, Chen PC, Chiang H. 2004. KIT (CD117) is frequently overexpressed in thymic carcinomas but is absent in thymomas. The Journal of Pathology 202:375–381.

Pasqualini JR. 2004. The selective estrogen enzyme modulators in breast cancer: a review. Biochimica et Biophysica Acta 1654:123–143.

Pei L, Peng Y, Yang Y, Ling XB, Van Eyndhoven WG, Nguyen KC, Rubin M, Hoey T, Powers S, Li J. 2002. PRC17, a novel oncogene encoding a Rab GTPase-activating protein, is amplified in prostate cancer. Cancer Research 62:5420–5424.

Peiffer SL, Herzog TJ, Tribune DJ, Mutch DG, Gersell DJ, Goodfellow PJ. 1995. Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. Cancer Research 55:1922–1926.

Pereira P, Stanton V, Jothy S, Tomlinson IP, Foulkes WD, Rozen R. 1999. Loss of heterozygosity of methylenetetrahydrofolate reductase in colon carcinomas. Oncology Reports 6:597–599.

Perlman M, Goldberg GM, Bar-Ziv J, Danovitch G. 1973. Renal hamartomas and nephroblastomatosis with fetal gigantism: a familial syndrome. The Journal of Pediatrics 83:414–418.

Perotti D, De Vecchi G, Testi MA, Lualdi E, Modena P, Mondini P, Ravagnani F, Collini P, Di Renzo F, Spreafico F, Terenziani M, Sozzi G, Fossati-Bellani F, Radice P. 2004. Germline mutations of the POU6F2 gene in Wilms tumors with loss of heterozygosity on chromosome 7p14. Human Mutation 24:400–407.

Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BA, Cannon-Albright L, Larsson C, Goldgar DE, Narod SA. 1996. Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nature Genetics 12:309–311.

Prasad A, Fernandis AZ, Rao Y, Ganju RK. 2004. Slit protein-mediated inhibition of CXCR4-induced chemotactic and chemoinvasive signaling pathways in breast cancer cells. The Journal of Biological Chemistry 279:9115–9124.

Rady P, Scinicariello F, Wagner RF Jr, Tyring SK. 1992. p53 mutations in basal cell carcinomas. Cancer Research 52:3804–3806.

Rahman N, Arbour L, Tonin P, Renshaw J, Pelletier J, Baruchel S, Pritchard-Jones K, Stratton MR, Narod SA. 1996. Evidence for a familial Wilms' tumour gene (FWT1) on chromosome 17q12-q21. Nature Genetics 13:461–463.

Rapley EA, Crockford GP, Teare D, Biggs P, Seal S, Barfoot R, Edwards S, Hamoudi R, Heimdal K, Fossa SD, Tucker K, Donald J, Collins F, Friedlander M, Hogg D, Goss P, Heidenreich A, Ormiston W, Daly PA, Forman D, Oliver TD, Leahy M, Huddart R, Cooper CS, Bodmer JG, Easton DF, Stratton MR, Bishop DT. 2000. Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours. Nature Genetics 24:197–200.

Ravi R, Bedi A. 2002. Sensitization of tumor cells to Apo2 ligand/TRAIL-induced apoptosis by inhibition of casein kinase II. Cancer Research 62:4180–4185.

Recine MA, Deavers MT, Middleton LP, Silva EG, Malpica A. 2004. Serous carcinoma of the ovary and peritoneum with metastases to the breast and axillary lymph nodes: a potential pitfall. American Journal of Surgical Pathology 28:1646–1651.

Reeves HL, Narla G, Ogunbiyi O, Haq AI, Katz A, Benzeno S, Hod E, Harpaz N, Goldberg S, Tal-Kremer S, Eng FJ, Arthur MJ, Martignetti JA, Friedman SL. 2004. Kruppel-like factor 6 (KLF6) is a tumor-suppressor gene frequently inactivated in colorectal cancer. Gastroenterology 126:1090–1103.

Reinholz MM, Iturria SJ, Ingle JN, Roche PC. 2002. Differential gene expression of TGF-β family members and osteopontin in breast tumor tissue: analysis by real-time quantitative PCR. Breast Cancer Research and Treatment 74:255–269.

Rengifo-Cam W, Konishi A, Morishita N, Matsuoka H, Yamori T, Nada S, Okada M. 2004. Csk defines the ability of integrin-mediated cell adhesion and migration in human colon cancer cells: implication for a potential role in cancer metastasis. Oncogene 23:289–297.

Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI, Boyd J. 1997. Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. Cancer Research 57:3657–3659.

Riccardi VM, Sujansky E, Smith AC, Francke U. 1978. Chromosomal imbalance in the aniridia-Wilms' tumor association: 11p interstitial deletion. Pediatrics 61:604–610.

Risk JM, Evans KE, Jones J, Langan JE, Rowbottom L, McRonald FE, Mills HS, Ellis A, Shaw JM, Leigh IM, Kelsell DP, Field JK. 2002. Characterization of a 500 kb region on 17q25 and the exclusion of candidate genes as the familial tylosis oesophageal cancer (TOC) locus. Oncogene 21:6395–6402.

Riss J, Khanna C, Koo S, Chandramouli GV, Yang HH, Hu Y, Kleiner DE, Rosenwald A, Schaefer CF, Ben-Sasson SA, Yang L, Powell J, Kane DW, Star RA, Aprelikova O, Bauer K, Vasselli JR, Maranchie JK, Kohn KW, Buetow KH, Linehan WM, Weinstein JN, Lee MP, Klausner RD, Barrett JC. 2006. Cancers as wounds that do not heal: differences and similarities between renal regeneration/repair and renal cell carcinoma. Cancer Research 66:7216–7224.

Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J. 2002. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Research 62:632–635.

Rivera A, Maxwell SA. 2005. The p53-induced gene-6 (proline oxidase) mediates apoptosis through a calcineurin-dependent pathway. The Journal of Biological Chemistry 280:29346–29354.

Robey-Cafferty SS, Silva EG, Cleary KR. 1989. Anaplastic and sarcomatoid carcinoma of the small intestine: a clinicopathologic study. Human Pathology 20:858–863.

Roche J, Drabkin HA. 2001. The role of semaphorins in lung cancer. Clinical Lung Cancer 3:145–150.

Roepman P, de Jager A, Groot Koerkamp MJ, Kummer JA, Slootweg PJ, Holstege FC. 2006. Maintenance of head and neck tumor gene expression profiles upon lymph node metastasis. Cancer Research 66:11110–11114.

Rogler CE, Sherman M, Su CY, Shafritz DA, Summers J, Shows TB, Henderson A, Kew M. 1985. Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. Science 230:319–322.

Rohaizak M, Jasmi AY, Ismail MA, Munchar MJ, Meah FA. 2003. Thyroid carcinoma in patients with familial adenomatous polyposis. Asian Journal of Surgery 26:183–185.

Roncalli M, Bosari S, Marchetti A, Buttitta F, Bossi P, Graziani D, Peracchia A, Bonavina L, Viale G, Coggi G. 1998. Cell cycle-related gene abnormalities and product expression in esophageal carcinoma. Laboratory Investigations 78:1049–1057.

Rongioletti F, Hazini R, Gianotti G, Rebora A. 1989. Fibrofolliculomas, tricodiscomas and acrochordons (Birt-Hogg-Dube) associated with intestinal polyposis. Clinical and Experimental Dermatology 14:72–74.

Rose DP, Komninou D, Stephenson GD. 2004. Obesity, adipocytokines, and insulin resistance in breast cancer. Obesity Reviews 5:153–165.

Rubin BP, Chen CJ, Morgan TW, Xiao S, Grier HE, Kozakewich HP, Perez-Atayde AR, Fletcher JA. 1998. Congenital mesoblastic nephroma t(12;15) is associated with ETV6-NTRK3 gene fusion: cytogenetic and molecular relationship to congenital (infantile) fibrosarcoma. American Journal of Pathology 153:1451–1458.

Rudland PS, Platt-Higgins A, El-Tanani M, De Silva Rudland S, Barraclough R, Winstanley JH, Howitt R, West CR. 2002. Prognostic significance of the metastasis-associated protein osteopontin in human breast cancer. Cancer Research 62:3417–3427.

Rüdlinger R, Grob R, Yu YX, Schnyder UW. 1989. Human papillomavirus-35-positive bowenoid papulosis of the anogenital area and concurrent human papillomavirus-35-positive verruca with bowenoid dysplasia of the periungual area. Archives of Dermatology 125:655–659.

Russell A. 1954. A syndrome of intra-uterine-dwarfism recognizable at birth with cranio-facial dysostosis, disproportionate short arms, and other anomalies (5 examples). Proceedings of the Royal Society of Medicine 47:1040–1044.

Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K, Nakamura Y. 1992. Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. Cancer Research 52:5815–5817.

Samaniego F, Rodriguez E, Houldsworth J, Murty VVVS, Ladanyi M, Lele KP, Chen Q, Dmitrovsky E, Geller NL, Reuter V, Jhanwar SC, Bosl GJ, Chaganti RSK. 1990. Cytogenetic and molecular analysis of human male germ cell tumors: chromosome 12 abnormalities and gene amplification. Genes, Chromosomes and Cancer 1:289–300.

Sandberg AA, Berger CS. 1994. Review of chromosome studies in urological tumors. II. Cytogenetics and molecular genetics of bladder cancer. Journal of Urology 151:545–560.

Santen RJ, Leszczynski D, Tilson-Mallet N, Feil PD, Wright C, Manni A, Santner SJ. 1986. Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. Annals of the New York Academy of Science 464:126–137.

Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkstrom H, Larsson P, Kumar R, Hemminki K. 2004. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 25:729–734.

Saphner T, Gallion H, Nagell JR, Kryscio R, Patchell RA. 1989. Neurologic complications of cervical cancer. A review of 2261 cases. Cancer 64:1147–1151.

Sawyer JR, Miller JP, Roloson GJ. 1996. A novel reciprocal translocation (14;15)(q11;q24) in a congenital mesoblastic nephroma. Cancer Genetics and Cytogenetics 88:39–42.

Schultz DC, Vanderveer L, Berman DB, Hamilton TC, Wong AJ, Godwin AK. 1996. Identification of two candidate tumor suppressor genes on chromosome 17p13.3. Cancer Research 56:1997–2002.

Schuster AE, Schneider DT, Fritsch MK, Grundy P, Perlman EJ. 2003. Genetic and genetic expression analyses of clear cell sarcoma of the kidney. Laboratory Investigations 83:1293–1299.

Schwartz B, Bresalier RS, Kim YS. 1992. The role of mucin in colon-cancer metastasis. International Journal of Cancer 52:60–65.

Schwartz DR, Kardia SL, Shedden KA, Kuick R, Michailidis G, Taylor JM, Misek DE, Wu R, Zhai Y, Darrah DM, Reed H, Ellenson LH, Giordano TJ, Fearon ER, Hanash SM, Cho KR. 2002. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. Cancer Research 62:4722–4729.

Schwartz GK, Jiang J, Kelsen D, Albino AP. 1993. Protein kinase C: a novel target for inhibiting gastric cancer cell invasion. The Journal of the National Cancer Institute 85:402–407.

Schwienbacher C, Gramantieri L, Scelfo R, Veronese A, Calin GA, Bolondi L, Croce CM, Barbanti-Brodano G, Negrini M. 2000. Gain of imprinting at chromosome 11p15: a pathogenetic mechanism identified in human hepatocarcinomas. Proceedings of the National Academy of Sciences USA 97:5445–5449.

Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM. 1999. Regulation of β-catenin signaling by the B56 subunit of protein phosphatase 2A. Science 283:2089–2091.

Seraj MJ, Samant RS, Verderame MF, Welch DR. 2000. Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. Cancer Research 60:2764–2769.

Shachaf CM, Kopelman AM, Arvanitis C, Karlsson A, Beer S, Mandy S, Bachmann MH, Borowsky AD, Ruebner B, Cardiff RD, Yang Q, Bishop JM, Contag CH, Felsher DW. 2004. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature 431:1112–1117.

Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. 2002. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 50:520–524.

Sharifah NA, Yun K, McLay J. 1995. Insulin-like growth factor II gene expression by congenital mesoblastic nephroma. Diagnostic Molecular Pathology 4:279–285.

Shih IeM, Sheu JJ, Santillan A, Nakayama K, Yen MJ, Bristow RE, Vang R, Parmigiani G, Kurman RJ, Trope CG, Davidson B, Wang TL. 2005. Amplification of a chromatin remodeling gene, Rsf-1/HBXAP, in ovarian carcinoma. Proceedings of the National Academy of Sciences USA 102:14004–14009.

Shin MS, Kim HS, Lee SH, Park WS, Kim SY, Park JY, Lee JH, Lee SK, Lee SN, Jung SS, Han JY, Kim H, Lee JY, Yoo NJ. 2001. Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. Cancer Research 61:4942–4946.

Shipman R, Schraml P, Colombi M, Raefle G, Ludwig CU. 1993. Loss of heterozygosity on chromosome 11p13 in primary bladder carcinoma. Human Genetics 91:455–458.

Shirasaki F, Takata M, Hatta N, Takehara K. 2001. Loss of expression of the metastasis suppressor gene *KiSS1* during melanoma progression and its association with LOH of chromosome 6q16.3-q23. Cancer Research 61:7422–7425.

Shridhar V, Rivard S, Wang X, Shridhar R, Paisley C, Mullins C, Beirnat L, Dugan M, Sarkar F, Miller OJ, Vaitkevicius VK, Smith DI. 1997. Mutations in the arginine-rich protein gene (ARP) in pancreatic cancer. Oncogene 14:2213–2216.

Sidhar SK, Clark J, Gill S, Hamoudi R, Crew AJ, Gwilliam R, Ross M, Linehan WM, Birdsall S, Shipley J, Cooper CS. 1996. The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene PRCC to the TFE3 transcription factor gene. Human Molecular Genetics 5:1333–1338.

Siegsmund M, Brinkmann U, Schaffeler E, Weirich G, Schwab M, Eichelbaum M, Fritz P, Burk O, Decker J, Alken P, Rothenpieler U, Kerb R, Hoffmeyer S, Brauch H. 2002. Association of the P-glycoprotein transporter MDR1(C3435T) polymorphism with the susceptibility to renal epithelial tumors. Journal of the American Society of Nephrology 13:1847–1854.

Silver DL, Naora H, Liu J, Cheng W, Montell DJ. 2004. Activated signal transducer and activator of transcription (STAT) 3: localization in focal adhesions and function in ovarian cancer cell motility. Cancer Research 64:3550–3558.

Silver HK, Kiyasu W, George J, Deamer WC. 1953. Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. Pediatrics 12:368–376.

Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich AJ, Palazzo JP, Fishel R, Goodfellow PJ. 1999. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Human Molecular Genetics 8:661–666.

Singh R, Campbell BJ, Yu LG, Fernig DG, Milton JD, Goodlad RA, FitzGerald AJ, Rhodes JM. 2001. Cell surface-expressed Thomsen-Friedenreich antigen in colon cancer is predominantly carried on high molecular weight splice variants of CD44. Glycobiology 11:587–592.

Singhal H, Bautista DS, Tonkin KS, O'Malley FP, Tuck AB, Chambers AF, Harris JF. 1997. Elevated plasma osteopontin in metastatic breast cancer associated with increased tumor burden and decreased survival. Clinical Cancer Research 3:605–611.

Sirvent N, Maire G, Pedeutour F. 2003. Genetics of dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment. Genes, Chromosomes and Cancer 37:1–19.

Smith JR, Freije D, Carpten JD, Gronberg H, Xu J, Isaacs SD, Brownstein MJ, Bova GS, Guo H, Bujnovszky P, Nusskern DR, Damber J-E, Bergh A, Emanuelsson M, Kallioniemi OP, Walker-Daniels J, Bailey-Wilson JE, Beaty TH, Meyers DA, Walsh PC, Collins FS, Trent JM, Isaacs WB. 1996. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371–1374.

Sommers SC, Murphy SA, Warren S. 1954. Pancreatic duct hyperplasia and cancer. Gastroenterology 27:629–640.

Sozzi G, Bongarzone I, Miozzo M, Borrello MG, Blutti MG, Pilotti S, Della Porta G, Pierotti MA. 1994. A t(10;17) translocation creates the RET/PTC2 chimeric transforming sequence in papillary thyroid carcinoma. Genes, Chromosomes and Cancer 9:244–250.

Spugnardi M, Tommasi S, Dammann R, Pfeifer GP, Hoon DS. 2003. Epigenetic inactivation of RAS association domain family protein 1 (RASSF1A) in malignant cutaneous melanoma. Cancer Research 63:1639–1643.

Steinberg GD, Carter BS, Beaty TH, Childs B, Walsh PC. 1990. Family history and the risk of prostate cancer. Prostate 17:337–347.

Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. 1998. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 393:229–234.

Sturm RA. 2002. Skin colour and skin cancer -MC1R, the genetic link. Melanoma Research 12:405–416.

Suzuki T, Nakata T, Miki Y, Kaneko C, Moriya T, Ishida T, Akinaga S, Hirakawa H, Kimura M, Sasano H. 2003. Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. Cancer Research 63:2762.

Sweet K, Willis J, Zhou XP, Gallione C, Sawada T, Alhopuro P, Khoo SK, Patocs A, Martin C, Bridgeman S, Heinz J, Pilarski R, Lehtonen R, Prior TW, Frebourg T, Teh BT, Marchuk DA, Aaltonen LA, Eng C. 2005. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. The Journal of the American Medical Association 294:2465–2473.

Tachibana M, Miyakawa A, Tazaki H, Nakamura K, Kubo A, Hata J, Nishi T, Amano Y. 1995. Autocrine growth of transitional cell carcinoma of the bladder induced by granulocyte-colony stimulating factor. Cancer Research 55:3438–3443.

Takahashi R, Tanaka S, Hiyama T, Ito M, Kitadai Y, Sumii M, Haruma K, Chayama K. 2003. Hypoxia-inducible factor-1α expression and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncology Reports 10:797–802.

Taniguchi K, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, Nagorney DM, Burgart LJ, Roche PC, Smith DI, Ross JA, Liu W. 2002. Mutational spectrum of β-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. Oncogene 21:4863–4871.

Thorgeirsson SS, Grisham JW. 2002. Molecular pathogenesis of human hepatocellular carcinoma. Nature Genetics 31:339–346.

Tognon C, Knezevich SR, Huntsman D, Roskelley CD, Melnyk N, Mathers JA, Becker L, Carneiro F, MacPherson N, Horsman D, Poremba C, Sorensen PH. 2002. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2:367–376.

Toker C. 1972. Trabecular carcinoma of the skin. Archives of Dermatology 105:107–110.

Tokuhata GK, Lilienfeld AM. 1963. Familial aggregation of lung cancer in humans. Journal of the National Cancer Institute 30:289–312.

Tomizawa Y, Adachi J, Kohno T, Yamaguchi N, Saito R, Yokota J. 1998. Identification and characterization of families with aggregation of lung cancer. Japanese Journal of Clinical Oncology 28:192–195.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM. 2005. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310:644–648.

Tomlinson I, Rahman N, Frayling I, Mangion J, Barfoot R, Hamoudi R, Seal S, Northover J, Thomas HJ, Neale K, Hodgson S, Talbot I, Houlston R, Stratton MR. 1999. Inherited susceptibility to colorectal adenomas and carcinomas: evidence for a new predisposition gene on 15q14-q22. Gastroenterology 116:789–795.

Toyota M, Ohe-Toyota M, Ahuja N, Issa JP. 2000. Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype. Proceedings of the National Academy of Sciences USA 97:710–715.

Tschentscher F, Prescher G, Horsman DE, White VA, Rieder H, Anastassiou G, Schilling H, Bornfeld N, Bartz-Schmidt KU, Horsthemke B, Lohmann DR, Zeschnigk M. 2001. Partial deletions of the long and short arm of chromosome 3 point to two tumor suppressor genes in uveal melanoma. Cancer Research 61:3439–3442.

Tserkezoglou A, Kontou S, Hatjieleftheriou G, Nikolaidou ME, Plataniotis G, Apostolikas N, Magiakos G. 2005. Solitary parenchymal splenic recurrence of ovarian adenocarcinoma: a case report and review of the literature. Anticancer Research 25:1471–1476.

Ueno M, Ban S, Ohigashi T, Nakanoma T, Nonaka S, Hirata R, Iida M, Deguchi N. 2000. Simultaneous production of granulocyte colony-stimulating factor and parathyroid hormone-related protein in bladder cancer. International Journal of Urology 7:72–75.

van Belzen N, Diesveld MPG, van der Made ACJ, Nozawa Y, Dinjens WNM, Vlietstra R, Trapman J, Bosman FT. 1995. Identification of mRNAs that show modulated expression during colon carcinoma cell differentiation. European Journal of Biochemistry 284:843–848.

van der Heijden MS, Yeo CJ, Hruban RH, Kern SE. 2003. Fanconi anemia gene mutations in young-onset pancreatic cancer. Cancer Research 63:2585–2588.

VanderLaan W. 1947. The occurrence of carcinoma of the thyroid gland in autopsy material. The New England Journal of Medicine 237:221–222.

van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R. 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes and Development 8:2691–2703.

van Rhijn BW, Montironi R, Zwarthoff EC, Jobsis AC, van der Kwast TH. 2002. Frequent FGFR3 mutations in urothelial papilloma. The Journal of Pathology 198:245–251.

Vasen HF, Offerhaus GJ, den Hartog Jager FC, Menko FH, Nagengast FM, Griffioen G, van Hogezand RB, Heintz AP. 1990. The tumour spectrum in hereditary non-polyposis colorectal cancer: a study of 24 kindreds in the Netherlands. International Journal of Cancer 46:31–34.

Vasselli JR, Shih JH, Iyengar SR, Maranchie J, Riss J, Worrell R, Torres-Cabala C, Tabios R, Mariotti A, Stearman R, Merino M, Walther MM, Simon R, Klausner RD, Linehan WM. 2003. Predicting survival in patients with metastatic kidney cancer by gene-expression profiling in the primary tumor. Proceedings of the National Academy of Sciences USA 100:6958–6963.

Velcich A, Yang WC, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L. 2002. Colorectal cancer in mice deficient in the mucin Muc2. Science 295:1726–1729.

Vergote I, Dieleman V, Becquart D, Buytaert P. 1983. Dysgerminoma of the ovary in association with XY gonadal dysgenesis. European Journal of Obstetrics, Gynecology, and Reproductive Biology 14:385–391.

Vincent A, Willcox N. 1999. The role of T-cells in the initiation of autoantibody responses in thymoma patients. Pathology Research and Practice 195:535–540.

von Eyben FE. 2004. Chromosomes, genes, and development of testicular germ cell tumors. Cancer Genetics and Cytogenetics 151:93–138.

Vorechovsky I, Unden AB, Sandstedt B, Toftgard R, Stahle-Backdahl M. 1997. Trichoepitheliomas contain somatic mutations in the overexpressed PTCH gene: support for a gatekeeper mechanism in skin tumorigenesis. Cancer Research 57:4677–4681.

Vucic D, Stennicke HR, Pisabarro MT, Salvesen GS, Dixit VM. 2000. ML-IAP, a novel inhibitor of apoptosis that is preferentially expressed in human melanomas. Current Biology 10:1359–1366.

Wai PY, Mi Z, Guo H, Sarraf-Yazdi S, Gao C, Wei J, Marroquin CE, Clary B, Kuo PC. 2005. Osteopontin silencing by small interfering RNA suppresses in vitro and in vivo CT26 murine colon adenocarcinoma metastasis. Carcinogenesis 26:741–751.

Wang D, Li Z, Messing EM, Wu G. 2002. Activation of Ras/Erk pathway by a novel MET-interacting protein RanBPM. The Journal of Biological Chemistry 277:36216–36222.

Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Ng HT, Tsai YC, Yuan CC. 2002. Expression of sialyltransferase family members in cervix squamous cell carcinoma correlates with lymph node metastasis. Gynecologic Oncology 86:45–52.

Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Tsai YC, Yuan CC. 2001. Altered mRNA expression of sialyltransferase in squamous cell carcinomas of the cervix. Gynecologic Oncology 83:121–127.

Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Ng HT, Tsai YC, Yuan CC. 2002. Expression of sialyltransferase family members in cervix squamous cell carcinoma correlates with lymph node metastasis. Gynecologic Oncology 86:45–52.

Wang TY, Chen BF, Yang YC, Chen H, Wang Y, Cviko A, Quade BJ, Sun D, Yang A, McKeon FD, Crum CP. 2001a. Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: a study of 250 cases. Human Pathology 32:479–486.

Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, van der Heijden MS, Parmigiani G, Yan H, Wang TL, Riggins G, Powell SM, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. 2004. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science 304:1164–1166.

Wang Z-X, Wang H-Y, Wu M-C. 2001b. Identification and characterization of a novel human hepatocellular carcinoma-associated gene. British Journal of Cancer 85:1162–1167.

Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. 1998. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19:275–280.

Wei D, Gong W, Kanai M, Schlunk C, Wang L, Yao JC, Wu TT, Huang S, Xie K. 2005. Drastic down-regulation of Krüppel-like factor 4 expression is critical in human gastric cancer development and progression. Cancer Research 65:2746–2754.

Weterman MAJ, van Groningen JJM, den Hartog A, Geurts van Kessel A. 2001a. Transformation capacities of the papillary renal cell carcinoma-associated PRCCTFE3 and TFE3PRCC fusion genes. Oncogene 20:1414–1424.

Weterman MAJ, van Groningen JJM, Tertoolen L, van Kessel AG. 2001b. Impairment of MAD2B-PRCC interaction in mitotic checkpoint defective t(X;1)-positive renal cell carcinomas. Proceedings of the National Academy of Sciences USA 98:13808–13813.

Weterman MAJ, Wilbrink M, Geurts van Kessel A. 1996. Fusion of the transcription factor TFE3 gene to a novel gene, PRCC, in t(X;1)(p11;q21)-positive papillary renal cell carcinomas. Proceedings of the National Academy of Sciences USA 93:15294–15298.

Weterman MJ, van Groningen JJM, Jansen A, Geurts van Kessel A. 2000. Nuclear localization and transactivating capacities of the papillary renal cell carcinoma-associated TFE3 and PRCC (fusion) proteins. Oncogene 19:69–74.

Whang-Peng J, Kao-Shan CS, Lee EC, Bunn PA, Carney DN, Gazdar AF, Minna JD. 1982. Specific chromosome defect associated with human small-cell lung cancer: deletion 3p(14–23). Science 215:181–182.

White GRM, Kelsey AM, Varley JM, Birch JM. 2002. Somatic glypican 3 (GPC3) mutations in Wilms' tumour. British Journal of Cancer 86:1920–1922.

Wilmore HP, White GFJ, Howell RT, Brown KW. 1994. Germline and somatic abnormalities of chromosome 7 in Wilms' tumor. Cancer Genetics and Cytogenetics 77:93–98.

Woelfle U, Cloos J, Sauter G, Riethdorf L, Jaenicke F, van Diest P, Brakenhoff R, Pantel K. 2003. Molecular signature associated with bone marrow micrometastasis in human breast cancer. Cancer Research 63:5679–5684.

Wolf G, Elez R, Doermer A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger HM, Rubsamen-Waigmann H, Strebhardt K. 1997. Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. Oncogene 14:543–549.

Wolfe JN. 1976. Risk for breast cancer development determined by mammographic parenchymal pattern. Cancer 37:2486–2492.

Wood K, Vini L, Harmer C. 2004. Metastases to the thyroid gland: the Royal Marsden experience. European Journal of Surgical Oncology 30:583–588.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. 1995. Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792.

Wu R, Connolly D, Ngelangel C, Bosch FX, Munoz N, Cho KR. 2000. Somatic mutations of fibroblast growth factor receptor 3 (FGFR3) are uncommon in carcinomas of the uterine cervix. Oncogene 19:5543–5546.

Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, Wilkens E, Bujnovszky P, Bova GS, Walsh P, Isaacs W, Schleutker J, Matikainen M, Tammela T, Visakorpi T, Kallioniemi O-P, Berry R, Schaid D, French A, McDonnell S, Schroeder J, Blute M, Thibodeau S, Grönberg H, Emanuelsson M, Damber J-E, Bergh A, Jonsson B-A, Smith J, Bailey-Wilson J, Carpten J, Stephan D, Gillanders E, Amundson I, Kainu T, Freas-Lutz D, Baffoe-Bonnie A, Van Aucken A, Sood R, Collins F, Brownstein M, Trent J. 1998. Evidence for a prostate cancer susceptibility locus on the X chromosome. Nature Genetics 20:175–179.

Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. 2004. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927–939.

Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, Li Y, Robles AI, Chen Y, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. 2003. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nature Medicine 9:416–423.

Yokoyama A, Muramatsu T, Ohmori T, Yokoyama T, Okuyama K, Takahashi H, Hasegawa Y, Higuchi S, Maruyama K, Shirakura K, Ishii H. 1998. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. Carcinogenesis 19:1383–1387.

Yousef GM, Kyriakopoulou LG, Scorilas A, Fracchioli S, Ghiringhello B, Zarghooni M, Chang A, Diamandis M, Giardina G, Hartwick WJ, Richiardi G, Massobrio M, Diamandis EP, Katsaros D. 2001. Quantitative expression of the kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. Cancer Research 61:7811–7818.

Yu Y, Fujii S, Yuan J, Luo RZ, Wang L, Bao J, Kadota M, Oshimura M, Dent SR, Issa JP, Bast RC Jr. 2003. Epigenetic regulation of ARHI in breast and ovarian cancer cells. Annals of the New York Academy of Science 983:268–277.

Yuan B-Z, Miller MJ, Keck CL, Zimonjic DB, Thorgeirsson SS, Popescu NC. 1998. Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. Cancer Research 58:2196–2199.

Zbar B, Tory K, Merino M, Schmidt L, Glenn G, Choyke P, Walther MM, Lerman M, Linehan WM. 1994. Hereditary papillary renal cell carcinoma. Journal of Urology 151:561–566.

Zeineldin R, Rosenberg M, Ortega D, Buhr C, Chavez MG, Stack MS, Kusewitt DF, Hudson LG. 2006. Mesenchymal transformation in epithelial ovarian tumor cells expressing epidermal growth factor receptor variant III. Molecular Carcinogenesis. 45(11):851–860.

Zhang G, He B, Weber GF. 2003. Growth factor signaling induces metastasis genes in transformed cells: molecular connection between Akt kinase and osteopontin in breast cancer. Molecular and Cellular Biology 23:6507–6519.

Zhao X, Li J, He Y, Lan F, Fu L, Guo J, Zhao R, Ye Y, He M, Chong W, Chen J, Zhang L, Yang N, Xu B, Wu M, Wan D, Gu J. 2001. A novel growth suppressor gene on chromosome 17p13.3 with a high frequency of mutation in human hepatocellular carcinoma. Cancer Research 61:7383–7387.

Zheng L, Wang Y, Schabath MB, Grossman HB, Wu X. 2003. Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case-control study. Cancer Letters 202:61–69.

Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK. 1993. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 14:1821–1824.

Zhou D, Chen B, Ye JJ, Chen S. 2004. A novel crosstalk mechanism between nuclear receptor-mediated and growth factor/Ras-mediated pathways through PNRC-Grb2 interaction. Oncogene 23:5394–5404.

Zhou W, Thurston SW, Liu G, Xu LL, Miller DP, Wain JC, Lynch TJ, Su L, Christiani DC. 2001. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. Cancer Epidemiology, Biomarkers and Prevention 10:461–466.

zur Hausen H. 1977. Human papilloma viruses and their possible role in squamous cell carcinomas. Current Topics in Microbiology and Immunology 78:1–30.

CHAPTER 16

HEMATOLOGIC MALIGNANCIES

The term leukemia (white blood) refers to the transformation of cells of the erythroid or lymphoid lineages. The leukemia cells are predominantly present in the circulation.

– In acute leukemia, the bone marrow cells do not mature properly. These immature cells continue to reproduce and crowd out normal cells. Normally, blasts constitute less than 5% of the bone marrow. In leukemia, these blasts do not mature and multiply continuously, eventually constituting 30–100% of the bone marrow.
– In chronic leukemia the cells have a mature appearance, but do not function normally. The affected cells have extended live spans, accumulate, and crowd out normal bone marrow cells. The disease progresses slowly.

Lymphomata may originate in some of the same lineages as leukemias, but they form solid masses predominantly in the lymphoid organs. The group of cancers under the general term lymphoma is quite broad. The distinctions among the various types of lymphoma are based on the respective characteristics of the transformed cells.

Chromosome aberrations, mostly translocations, and viral infections are frequent causes for hematologic malignancies. The clonal development of T- and B-lymphocytes is physiologically associated with DNA recombination, and chromosome translocations in B- and T-cell neoplasia may reflect the aberrant activation of normal translocation processes [Tsujimoto et al. 1985]. Other pathogenetic origins for lymphoma are less common. They include occupational factors (farming, pesticide application, floor milling), exposure to certain chemicals (pesticides, herbicides, solvents), and immune deficiency. Connective tissue disorders (Sjögren syndrome, rheumatoid arthritis, chronic lymphocytic thyroiditis, systemic lupus erythematosus) are also associated with an increased risk for lymphoma. There is an increased incidence in gastrointestinal lymphomata in patients with celiac sprue and inflammatory bowel disease. Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is frequently associated with *Helicobacter pylori* infection.

Certain endemic geographical factors influence the development of lymphoma in specific regions. They may reflect genetic, environmental, or lifestyle predisposition.

– In Africa, the incidence of Burkitt lymphoma is 5.5–7.5 per 100,000.
– Follicular lymphomata are rather common in North America and Europe, but are rare in the Caribbean, Africa, China, Japan, and the Middle East.
– Adult T-cell lymphoma, due to human T-cell leukemia virus 1 (HTLV-1) infection, occurs commonly in Japan and in the Caribbean.
– Heavy chain disease is a disorder of B-lymphocytes that is characterized by diffuse thickening of the small intestine caused by a lymphoplasmacytic infiltrate with the secretion of incomplete IgA heavy chains. This clinicopathologic entity is rare in individuals other than those of Mediterranean ethnicity.

16.1 B-CELL NEOPLASMS

About 95% of lymphomata are of B-cell origin. B-lymphocyte development takes place in distinct differentiation steps that are characterized by the

specific structure of the BCR (B-Cell Antigen Receptor), which is composed of a surface Immunoglobulin, comprising two heavy chains and two light chains that are covalently linked by disulfide bridges, and the associated molecules CD79A and CD79B, which contain cytoplasmic ITAMs (immunoreceptor tyrosine activation motifs) for signal transduction. Depending on the differentiation stage, BCR ligation may lead to proliferation or further differentiation. Transformation occurs at particular differentiation stages, with most types of B-cell lymphoma being derived from germinal center or postgerminal center B-lymphocytes. The high recombinogenic activity of B-lymphocytes is important for generating antigenic specificity. It is also a predisposing factor for tumorigenic DNA recombinations. Specifically in the pathogenesis of B-cell chronic lymphocytic leukemia (CLL), there is involvement of the Immunoglobulin V(D)J Recombinase. In the *igH* gene at 14q32, there is a consensus site that is mimicked by heptamer–nonamer sequences at 11q13, 18q21, and 8q24. These are the genomic locations of the growth factor and survival genes *bcl-1*, *bcl-2*, and *c-myc* [Haluska et al. 1987].

Growth factors play important roles in transformation. Interleukins -2, -6, and -7 are proliferative factors for malignant lymphocytes and plasma cells. Interleukin-10 is a vital factor for the differentiation and survival of germinal center B-lymphocytes and is also a negative prognostic factor in non-Hodgkin lymphoma. TIMP-1 expression regulates IL-10 levels in B-cells and mediates specific B-cell differentiation steps. The TIMP-1 expression in B-lymphocyte non-Hodgkin lymphoma correlates closely with IL-10 expression and with high histologic grade.

16.1.1 Diffuse Large B-cell Lymphoma

Diffuse large B-cell lymphoma is the most common of the non-Hodgkin lymphomata, accounting for up to 30% of newly diagnosed cases. It is an aggressive, fast growing lymphoma, which can arise in the lymph nodes or outside of the lymphatic system, in the gastrointestinal tract, testes, thyroid, skin, breast, bone, or brain. Often, an early sign of diffuse large B-cell lymphoma is a painless or even painful rapid swelling in the neck, armpit, or groin caused by enlarged lymph nodes. Other symptoms include night sweats, unexplained fevers, and weight loss.

Molecular pathogenesis. Approximately 40% of diffuse large B-cell lymphomata are associated with chromosomal translocations that dysregulate the expression of *bcl-6* (*znf-51*, *laz-3*) {3q27} by juxtaposing heterologous promoters to the *bcl-6* coding domain,

- The t(3;6)(q27;p21) translocation fuses the *histone H4/m* gene to exons 3–9 of *bcl-6*. The translocation may cause the inappropriate expression of BCL-6 during the cell cycle, leading to the development of lymphoma.
- The t(3;7)(q27;p12) translocations replace the 5′ regulatory region of *bcl-6* with the 5′ regulatory region of *ikaros*. This leads to dysregulated expression of *bcl-6* throughout B-cell differentiation.
- A t(3;13)(q27;q14) translocation fuses *lcp1* (*L-plastin*, *cp64*, *plastin-2*) with *bcl-6* in chimeric transcripts. As a consequence of the translocation, the 5′ regulatory region of each gene is exchanged, which may create both *lcp1-bcl-6* and reciprocal *bcl-6-lcp1* fusion transcripts. LCP-1 is involved in Actin cytoskeleton organization.
- In t(3;16)(q27;p11) translocation, *interleukin-21 receptor* (*il21r*) is the fusion partner for *bcl-6*. As a result of this translocation, the promoter region of *il21r* is substituted for the regulatory sequence of *bcl-6* in the same transcriptional orientation.

The *bcl-6* proto-oncogene encodes a 79 kD protein that contains six COOH-terminal zinc finger motifs and an NH_2-terminal poxvirus and zinc finger (POZ) domain [Ye et al. 1993]. BCL-6 can repress transcription from promoters linked to its DNA target sequence, and this activity is dependent on the presence of an intact NH_2-terminal half of the protein. This part of the BCL-6 molecule contains an autonomous transrepressor domain and two noncontiguous regions, including the POZ motif [Chang et al. 1996]. The coactivator P300 binds and acetylates BCL-6. Acetylation disrupts the ability of BCL-6 to recruit Histone Deacetylases, thereby hindering its capacity to repress transcription. BCL-6 is acetylated under physiologic conditions in normal germinal center B-cells [Bereshchenko et al. 2002]. The BCL-6 protein is predominantly expressed in the B-lymphocyte lineage, where it is present in mature B-cells. The expression is topographically restricted to germinal centers, including all centroblasts and centrocytes. BCL-6 necessary for germinal center formation. Because diffuse large

B-cell lymphomata derive from germinal center B cells, deregulated BCL-6 expression may contribute to lymphomagenesis by preventing postgerminal center differentiation.

TNF-α induces proliferation in B-lymphoma cells. An autocrine loop may occur in diffuse large B-cell lymphoma with the expression of TNF and the TNFR-1.

Aberrations of micro-RNAs can be associated with lymphomata. In diffuse large cell lymphoma, *miR-155* is often amplified.

Mediastinal large B-cell lymphoma. Mediastinal large B-cell lymphoma is a subtype of diffuse large B-cell lymphoma that characteristically presents as localized tumors in young female patients. This form of B-cell lymphoma clinically resembles the nodular sclerosis type of classical Hodgkin lymphoma. Mediastinal large B-cell lymphoma has low expression levels of signaling molecules associated with the BCR, a profile resembling that of Reed-Sternberg cells of classical Hodgkin lymphoma. Like classical Hodgkin lymphoma, Mediastinal large B-cell lymphoma also has high levels of expression of the Interleukin-13 Receptor and its downstream effectors JAK-2 (Janus Kinase-2) and STAT-1 (Signal Transducer and Activator of Transcription-1), TNF family members, and TRAF-1 (TNF Receptor-Associated Factor-1). In almost all cases of mediastinal large B-cell lymphoma, c-REL is localized to the nucleus, consistent with activation of the NF-κB pathway. There are shared signaling and survival pathways between mediastinal large B-cell lymphoma and classical Hodgkin lymphoma [Savage et al. 2003].

16.1.2 Follicular Lymphoma

B-lymphocytes arise from the bone marrow, they mature and migrate to various areas of the body. Normal follicular B-lymphocytes reside in the germinal center of lymph nodes. Follicular lymphomata exhibit a follicular or nodular pattern of growth, which is reminiscent of the germinal centers. However, they often form outside the lymph nodes. Follicular lymphoma is among the most frequent hematologic malignancies. Although most follicular lymphomata are advanced at the time of diagnosis, the median survival is 8–12 years.

Molecular pathogenesis. The antiapoptotic regulator BCL-2 is not normally expressed in lymph follicles. Its presence in transformed B-lymphocytes interferes with their normal apoptosis. Most follicular lymphomata are characterized by the t(14;18) translocation, involving the *bcl-2* locus and the heavy chain *immunoglobulin* segments. A major breakpoint cluster is located within the 3′ noncoding region and a minor breakpoint cluster within the 3′ flanking region of *bcl-2*. A common reciprocal translocation is t(14;18)(q32;q21), which results in the dysregulated expression of *bcl-2* {18q21.3} [Bakhshi et al. 1985; Ngan et al. 1988]. A cAMP response element (CRE site) in the promoter of the translocated *bcl-2* allele functions as a positive regulatory module in these t(14;18) lymphomata. CREB binds to the CRE site in the *bcl-2* 5′ flanking region of the translocated allele. In contrast, access to this CRE site is blocked in the normal *bcl-2* allele. The translocation also brings an *immunoglobulin heavy chain enhancer* close to *bcl-2*, which may contribute to the high expression levels.

16.1.3 Mantle Cell Lymphoma

Mantle cell lymphoma (centrocytic lymphoma, diffuse follicular small-cleaved cell lymphoma, mantle zone lymphoma, intermediate lymphocytic lymphoma) is a lymphoproliferative disorder derived from a subset of naïve pregerminal center cells that are localized in primary follicles or in the mantle region of secondary follicles. B-lymphocytes travel from the bone marrow to reside in primary lymphoid follicles and in the mantle zones of secondary follicles in the lymph nodes and spleen. The cancerous cells are arrested at this stage of development and do not differentiate. Mantle cell lymphoma is constituted by atypical small lymphoid cells in wide mantles around benign germinal centers. It typically arises as a mixture of small lymphoid cells. Three histologic forms comprise

– Mantle zone architecture
– Nodular or diffuse architecture
– Blastic or blastoid architecture

Mantle cell lymphoma can occur from the late thirties to old age, but is more common above the age of 50. It is three times more common in men than in women.

Molecular pathogenesis. The transforming events in mantle cell lymphoma are specific chromosome translocations.

– Most cases of mantle cell lymphoma are associated with the chromosome translocation t(11;14)(q13;q32), which involves the *immunoglobulin heavy chain* gene on chromosome 14 and the *bcl-1* locus on chromosome 11 [Tsujimoto et al. 1984]. The molecular consequence is the overexpression of Cyclin D_1. Cyclin D_1 plays a key role in cell cycle regulation, specifically for the progression of cells from G_1 to S phase. The overexpression of Cyclin D_1 by translocation is a characteristic signature of many mantle cell lymphomata.
– A limited number of mantle cell lymphomata display dual t(11;14) and chromosome 8q24 *c-myc* gene rearrangements. They have an extremely aggressive course with a very poor prognosis.

16.1.4 MALT Lymphoma

Malt lymphomata (extranodal marginal zone B-cell lymphomata of MALT type) are solid tumors that originate from the transformation of immune cells that are recruited to secretory tissue, such as the gastrointestinal tract, the salivary glands, the lungs, and the thyroid gland. MALT lymphomata most commonly arise in the stomach and their growth depends on the continued presence of *Helicobacter pylori*. In general, the growth of MALT lymphoma cells is driven by antigenic stimulation.

Molecular pathogenesis. Chromosome translocations that lead to MALT lymphoma activate antiapoptotic pathways associated with BCL-10 or cIAP.
– *bcl-10* is involved in the translocation t(1;14)(p22;q32) in MALT lymphomata. This translocation brings the intact coding region of *bcl-10* from 1p22 into the vicinity of *immunoglobulin* genes on 4q32. By this translocation, *bcl-10* may be exposed to somatic hypermutation (point mutations that physiologically occur in the variable regions of *immunoglobulin* genes during B-lymphocyte differentiation). *bcl-10* mutations truncating the CARD region lead to a loss of apoptosis and to constitutive IKK (I-κB Kinase) activation that causes the activation of NF-κB. Truncations distal to the CARD region cause a loss of apoptotic function, but retain the capability of NF-κB activation. Due to the antiapoptotic role of NF-κB, this may confer an added survival advantage.
– Lymphomata of the intestinal mucosa frequently are characterized by translocations involving *malt1*. The cIAP2 gene *ciap2* (*api2*) {11q22–q23} is a target gene at the breakpoint of the translocation t(11;18)(q21;q21) that arises in about 50% of marginal cell lymphomata of the mucosa associated lymphoid tissue. The chimeric product of cIAP2 and MALT1 is predictive of a poor response to eradication therapy It may also enhance the resistance to apoptosis. The chimeric protein containing the NH_2-terminal portion of cIAP-2 and the COOH-terminal domain of MALT1 (Para-Caspase) mediates constitutive IKK activation and downstream NF-κB activation. Furthermore, CARMA-1 and BCL-10 activate NF-κB through MALT1. Mutations of cIAP2 occur in low-grade MALT lymphoma.

16.1.5 Burkitt Lymphoma

Burkitt lymphoma [Burkitt 1958] is a high-grade B-cell neoplasm with a large growth fraction. It exists in an endemic African form and a nonendemic sporadic form. The African form most often involves the maxilla or mandible. The affliction of abdominal organs, such as the kidneys or ovaries, is less common. In contrast, the sporadic form usually affects abdominal organs, with the most common involvement of the distal ileum, cecum, or mesentery. An early symptom is typically abdominal swelling that starts in the bowels. Burkitt lymphoma is most common in children with higher incidence in males, the mean patient age in Africa being 7 years and outside Africa being 11 years. The nonendemic form of Burkitt lymphoma has a high incidence among carriers of HIV.

Molecular pathogenesis. Burkitt lymphoma is caused by a monoclonal proliferation of B-lymphocytes that have a uniform appearance and produce a diffuse pattern of tissue involvement. Two pathogenetic pathways, related to EBV infection and MYC activation, lead to this form of lymphoma.

EBV infection is causally associated with nearly all Burkitt lymphomata in Africa, but is only linked to 15% or fewer sporadic cases worldwide. EBV infects more than 90% of humans and persists in most hosts for life without causing disease. EBV preferentially enters into B-lymphocytes. Although the virus uses its growth-promoting ability in the early stages of infection, it persists in resting memory B-lymphocytes rather than in proliferating cells. In Burkitt lymphoma, every tumor cell carries the virus in a latent infection but the number of normal cells infected is very low. The viral oncoprotein LMP-1 may play a critical role in the immortalization of B-lymphocytes. Polyclonal B-lymphocyte expansion therefore

proceeds unchecked, probably enlarging the population of cells susceptible to chromosome translocations that activate MYC. Point mutations of *rb2* (*p130*) can occur in EBV-positive Burkitt lymphomata.

MYC {8q24.12–q24.13} plays important roles in the pathogenesis of Burkitt lymphoma, in part, through the induction of *hmgA1* gene expression. Chromosomal translocations, t(8,2), t(8,14), or t(8,22), that dysregulate *c-myc* by juxtaposing it with one of the three *ig* loci are typical primary events in Burkitt lymphoma. The type of Immunoglobulin produced by this B-cell tumor correlates with the type of translocation. Lymphomata with the 8;2 translocation produce predominantly κ light chains {2p}, those with the 8;22 translocation produce λ light chains {22q}, and those with the 8;14 translocation produce Immunoglobulins with both types of light chain, because the breakpoint {14q32} is located at the *immunoglobulin* heavy chains.

Many Burkitt lymphomata carry point mutation in *p53*. Inactivation of the *cdkn2A* gene by promoter methylation or homozygous deletion is possible. It may lead to defects in the $P14^{ARF}$–|MDM2–|P53, and $P16^{INK4a}$–| Cyclin D/CDK4–| RB pathways. This suggests that the disruption of both the RB and P53 tumor suppressor pathways is critical for the development of Burkitt lymphoma. Alterations of other genes, including *bax, p73*, or *bcl-6*, may provide further growth stimulation and apoptosis protection.

16.1.6 Myeloma

Multiple myeloma is a neoplasm of terminally differentiated B-lymphocytes (plasma cells), which arises from germinal center B-cells. This form of lymphoma is usually preceded by premalignant monoclonal gammopathy of undetermined significance (MGUS). Multiple myeloma occurs at multiple intramedullary sites, causes osteolytic bone lesions, and may have increasing tumor mass. Dissemination to the pleura and skin is possible. When involving the blood, the disease is called plasma cell leukemia (Figure 16.1.6.A).

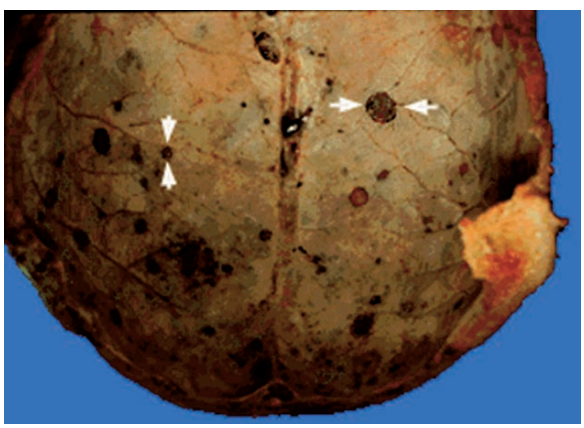


Figure 16.1.6.A. Multiple myeloma. This is a view of the skull from above, after the scalp has been removed. Note the "punched out" circular, hemorrhagic lesions (some indicated by arrows). These are the tumor deposits in the bones of the skull. Multiple myeloma is a malignant tumor of plasma cell origin that is typified by multiple round deposits in various bones and soft tissues of the body. These deposits are typically soft, gelatinous tan–white masses that are sharply demarcated. Most frequently affected is the spine, followed by the skull, the pelvis, and the chest bones. Characteristic of multiple myeloma is the presence of an abnormal, monoclonal antibody peak on serum electrophoresis. In some instances, the plasma cells do not make complete Immunoglobulin molecules. In such cases, the light chains made can be excreted in measurable quantities in the urine as Bence-Jones protein. X-ray of affected bone shows characteristic sharply defined "punched out" lesions that are purely lytic. Symptoms are caused by the local effects of tumor or by the effects of the paraprotein synthesis. They include bone pain and pathological fractures. In addition, the replacement of the marrow by the tumor can result in anemia, and the reduction in white blood cell formation can result in an increase in the frequency and severity of infections. The paraprotein may result in hyperviscosity symptoms that manifest as lethargy and abnormal bleeding. The paraprotein effects also include symptoms of renal damage due to cast formation in the tubules. Amyloid deposition due to the light chains is a frequent complication of multiple myeloma. The lytic lesions are likely caused by osteoclast activation through cytokines liberated by the plasma cells. These include IL-1 and TGF-β. IL-6 may be produced primarily by stromal cells, activated by the plasma cells. IL-6 levels are indicative of tumor progression. Multiple myeloma primarily affects individuals in the sixth and seventh decades of life. Males and females are approximately equally affected. The prognosis is dismal, with few, if any, survivors at 5 years. [Reproduced from http://pathweb.uchc.edu. With permission.]

Molecular pathogenesis. Numeric chromosomal abnormalities are present in virtually all multiple myelomata. Chromosome translocations frequently place oncogenes under the control of *immunoglobulin* enhancers. Primary chromosome translocations are mediated mainly by errors in *immunoglobulin* switch recombination, but sometimes by errors in somatic hypermutation. Although there is diversity in the translocation partners, most *immunoglobulins* translocate to just three groups of genes:

– *Cyclins* D_1 {1q13}, D_3 {6p21}, and possibly D_2 {12q13}
– Two genes on chromosome 4 {4p16}, *mmset* (*whsc1*, *nsd2*), and *fgfr3*
– The B-zip transcription factors *c-maf* {16q23} and *mafB* {20q11}

In myeloma, three categories of growth factors support the proliferation of malignant plasma cells.

– The IL-6 family cytokines, IL-10, and IFN-α (Interferon-α) activate the JAK/STAT and MAPK pathways.
– Growth factors that activate the Phosphatidylinositol 3-Kinase→PKB and MAPK pathways, including IGF-1, Hepatocyte Growth Factor, and members of the Epidermal Growth Factor family.
– BAFF (B-Cell-Activating Factor) and APRIL (Proliferation-Inducing Ligand) activate the NF-κB and PI 3-Kinase→PKB pathways. BAFF and APRIL bind to BAFFR and TACI and are major B-lymphocyte survival factors. These two growth factors may cooperate because they are localized together and with cytoplasmic transduction elements in Caveolin-linked membrane caveolae.

In addition to growth factor stimulation, the suppression of programmed cell death supports the expansion of myeloma cells. CD27 is a disulfide-linked, homodimeric receptor of the TNFR superfamily. It is induced upon lymphocyte activation, and when ligated by its counterreceptor CD70 induces programmed cell death through its cytoplasmic binding partner CD27BP (Siva) [Prasad et al. 1997]. In myeloma cells, CD27BP is unable to bind to CD27 consecutive to the engagement by CD70. Therefore, apoptosis may not be induced. During the progression of multiple myeloma, the expression of CD27 is lost. The loss of CD27 correlates with the loss of CD19 and is an indicator of a poor prognosis.

Disease progression. Activating mutations of oncogenes and secondary translocations contribute to tumor progression in multiple myeloma. The main paracrine growth factors of plasmablasts, IL-6 and IGF-1, activate the RAS→MAPK pathway. Consistently, activating mutations of *N-ras* or *K-ras2* distinguish multiple myeloma from monoclonal gammopathy of uncertain significance. Tumors may also have activating mutations of *fgfr3*, because it constitutively activates the MAPK pathway. Unbalanced chromosome structural changes are present in all plasma cell leukemias. Monoallelic loss of 13q sequences, specifically 13q14, is one of the most frequent abnormalities in multiple myeloma and a predictor of poor prognosis [Wada et al. 1999; Elnenaei et al. 2003]. This is likely to involve a tumor suppressor gene on 13q. Secondary translocations dysregulate *myc* and may contribute to disease progression. Tumor progression results in osteolytic bone lesions [Kuehl and Bergsagel 2002].

Paraneoplastic syndromes. The spectrum of neurologic complications of multiple myeloma ranges from direct compression (radiculopathy, spinal cord compression, base-of-the-skull tumor) to infiltrative damage (amyloid, peripheral neuropathies, numb chin syndrome of myeloma), to metabolic alterations (slowed mentation from hyperviscosity, hypercalcemia, or uremia), and to autoimmune or cytokine-mediated consequences (peripheral neuropathy). The most common presentations are compressive radiculopathy and peripheral neuropathies [Dispenzieri and Kyle 2005].

Primary plasma cell leukemia. Primary plasma cell leukemia is a rare plasma cell malignancy, which is related to multiple myeloma and is characterized by a poor prognosis. In contrast to multiple myeloma, primary plasma cell leukemia displays a high expression level of CD27. Upon ligation by CD70, CD27 inhibits apoptosis in these cells. This is associated with the activation of P38 and ERK-1/ERK-2. The ligation of CD27 leads to persistent DNA-binding activity of the transcription factor AP-1.

16.1.7 B-cell Acute Lymphocytic Leukemia

Acute lymphocytic leukemia (ALL, lymphoblastic leukemia) is a progressive malignant disease, characterized by the accumulation of large numbers of immature leukocytes. The malignant cells lose their ability to mature and differentiate. These cells are

present in the blood, the bone marrow, the lymph nodes, and the spleen. They multiply rapidly and replace the normal cells, resulting in bone marrow failure. The onset of symptoms is often abrupt and intense. They include poor healing of small injuries, bleeding events, petechiae, fatigue, recurrent minor infections, and bone pain. The patients become susceptible to fatigue because of anemia, to bleeding because of thrombocytopenia, and to infections because of a lack of functional lymphocytes. Most cases of ALL occur in children, adults who develop ALL are usually male and over 50 years. 85% of ALL cases are B-cell ALLs.

The stage of maturity of the leukemic B-cells ($CD19^+CD22^+$) determines the prognosis.

- Approximately 80% of ALL patients have the early precursor-B subtype (B1), which is the least mature. It also offers the best prognosis.
- Common B-cell ALL (B2) with expression of CD10 and precursor B-cell ALL (B3) with expression of surface IgM are intermediate forms.
- Mature B-cell ALL (B4) is akin to Burkitt non-Hodgkin lymphoma. It is therefore treated differently from other ALL cases.

Molecular pathogenesis. Transforming alterations in B-cell ALL affect MLL, E2A, or PEBP (Figure 16.1.7.A).

Homeobox transcription factors, encoded by *hox* genes, specify the differentiation of developing cells. The Histone Methyl Transferase MLL is an upstream regulator of *hox* gene expression through direct promoter binding and through Histone modification. *mll* (*all-1*, *trx*, *hrx*) {11q23} gene translocations are among the most common chromosomal abnormalities in B-lineage ALL. The resulting fusion proteins lack the MLL1 SET domain, which may have a dominant negative effect on wild-type MLL1.

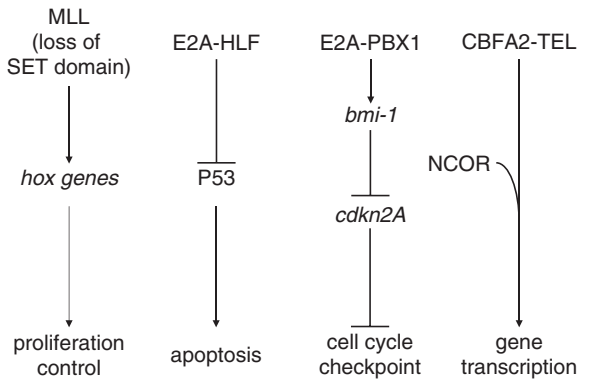


Figure 16.1.7.A. Molecular pathways to B-cell ALL. Genetic defects that initiate transformation to B-cell ALL affect four principal molecular pathways of growth control. The deregulation of any of these pathways can lead to excessive cell proliferation.

Chromosomal translocations involving t(4;11) in leukemic cells show clustering of the breakpoints in an area of 7–8 kb on chromosome 4, which contains heptamer and nonamer-like sequences, that are physiologically associated with the rearrangements of *immunoglobulin* genes. This suggests a direct involvement of the V(D)J Recombinase in the translocations that affect 11q23 [Gu et al. 1992]. Other translocations that affect MLL and lead to B-cell ALL include t(4;11), which generates the fusion protein MLL–AF4, t(9;11), which generates the fusion protein MLL–AF9, and t(11;19), which generates the fusion protein MLL-ENL.

In pro-B-cell ALL, the E2A–HLF fusion protein blocks P53-mediated apoptosis. This results in the prolonged survival of pro-B-cells, which may then acquire additional mutations that result in full transformation.

Loss-of-function alterations of $P16^{INK4a}$ commonly occur in lymphoid malignancies, but are consistently absent in pre-B-cell leukemias induced by the chimeric oncoprotein E2A-PBX1, which is created by t(1;19) chromosomal translocations. E2A-PBX1 enhances the expression of the lymphoid proto-oncogene *bmi1*. BMI-1 functions as a transcriptional repressor of the *cdkn2A* tumor suppressor locus that encodes $P16^{INK4a}$ and $P14^{ARF}$. This oncogenic pathway is likely to play a role in the pathogenesis of lymphoid leukemias through the downregulation of the *cdkn2A* gene.

The transcription factor PEBP2 (Core Binding Factor, CBF) is composed of α and β subunits. The α subunit binds to DNA via a Runt domain, while the β subunit increases the affinity of the α subunit for DNA without DNA binding by itself. *cbfa2* (*runx1*, *aml1*, *pebp2αB*) is one of several genes that encode the α subunit. The β subunit is encoded by *cbfb* (*pebp2B*). CBFA2 is one of the most frequent targets of chromosome translocations associated with leukemia. In pro-B-cell ALL, a translocation t(12;21) may arise, and place

cbfa2 {21q22} to *tel* (*etv6*) {12p13}, which encodes an ETS family transcription factor. This characteristic translocation involves a NH_2-terminal fusion, in which the RUNX1 moiety is virtually intact. The pointed oligomerization motif and a central repression domain in TEL recruit the nuclear corepressor (NCOR) complex. The TEL-CBFA2 gene rearrangement is the most common among all childhood cancers (20–25% of pediatric ALL).

Dissemination. ALL is a malignancy with the potential to infiltrate liver, spleen, and lymph nodes. The Chemokine Receptor CXCR4 is expressed on ALL cells and its ligand is abundant at sites associated with ALL-induced organ infiltration. This results in the chemotaxis of the leukemic cells from the bone marrow via the circulation to preferential sites of extramedullary organ infiltration, and it is associated with shorter disease-free survival. ALL may spread to the brain, spine, and testes (sanctuary disease sites). The brain is one of the first sites for relapsing leukemia.

16.1.8 B-cell Chronic Lymphocytic Leukemia

The vast majority of CLLs originate in the B-lymphocyte lineage. Men are twice as likely to develop CLL as women; however, the key risk factor is age, with over 75% of new cases being diagnosed in patients over the age of 50. B-cell CLL is closely related, and may be identical, to small lymphocytic lymphoma (SLL). Many newly diagnosed CLL patients have no clinical symptoms. Others may have fatigue, low-grade fever, night sweats, swollen lymph nodes, frequent infections, weight loss, or a loss of appetite.

B-cell CLL comprises two groups of diseases, characterized by slowly progressive indolent or aggressive disease course.

- The presence of somatic mutations in the *immunoglobulin* genes of CLL cells defines a group of patients with stable or slowly progressive disease.
- By contrast, the absence of *immunoglobulin* gene mutations in CLL cells defines a group of patients who have a progressive clinical course.

Molecular pathogenesis. A typical chromosomal translocation of B-cell CLL cells is t(11;14)(q13;q32). The breakpoint is in the joining segment of the *immunoglobulin heavy chain* locus on chromosome 14. The affected gene on 11q13 is *cyclin* D_1 (*bcl-1*, *ccnd1*) [Tsujimoto et al. 1984]. The translocation t(14;19)(q32;q13.1) recurs in the neoplastic cells of patients with CLL. It involves the rearrangement of the *ighA1* gene [McKeithan et al. 1987], placing it adjacent to *bcl-3*. In a fraction of CLLs, the 5′ flanking region of *bcl-2* is rearranged and preferentially linked to the *immunoglobulin light chain* genes on chromosome 2 or 22. The t(18;22) chromosome translocation dysregulates *bcl-2* gene expression by placing it into the *immunoglobulin light chain* locus [Adachi and Tsujimoto 1989]. This results in BCL-2 overexpression and resistance to apoptosis.

The loss of the micro-RNAs *miR-15* or *miR-16* is responsible for the indolent forms of CLL. In their absence, the levels of BCL-2 are elevated and the cells are protected from apoptosis. The micro-RNA signature of the tumor cells is predictive of the prognosis in CLL [Calin et al. 2004, 2005].

About 25% of B-cell CLLs are characterized by a chromosomal lesion involving 13q14. The deleted locus D13S25 on 13q14 likely lies close to a tumor suppressor locus whose inactivation contributes to the initiation or progression of low-grade B-cell malignancy. D13S25 lies distal to the *rb* tumor suppressor gene. Therefore, RB is unlikely to be responsible for this malignancy [Brown et al. 1993; Hawthorn et al. 1993].

The deregulation of the oncogene *tcl1* is a causal event in the pathogenesis of aggressive B-cell CLL. One genetic factor associated with poor prognosis of B-cell CLL is the deletion of chromosome 11q, which is associated with elevated expression of TCL-1 [Herling et al. 2006]. *tcl1* gene expression is regulated by *miR-29* and *miR-181*. The expression of these micro-RNAs is inversely correlated with the expression of TCL-1 in CLL [Pekarsky et al. 2006]. Only the aggressive group of B-cell CLL expresses ZAP-70.

Deletion of the *atm* locus {11q22.3} is associated with the absence of *immunoglobulin* gene mutations in CLL and with shortened survival in some patients [Staudt 2003].

Disease markers. B-cell CLL cells are not the neoplastic counterparts of normal resting B-lymphocytes. Similar to the peripheral blood B-lymphocytes, B-CLLs express CD19, CD20, CD21, CD24, CD40, CD44, CD45R, surface IgM, and surface IgD. However, unlike peripheral blood B-cells, B-cell CLL cells generally do not express C3b Complement Receptor, LFA-1 or CD22. In addition, B-CLLs

express the T-lymphocyte-associated antigen CD5 and various activation markers [Freedman 1990]. The activation marker CD23 is present on the surface of virtually all patients with classical B-cell CLL, but is not present in patients with mantle cell lymphoma.

Genetic predisposition. CLL is prone to familial clustering. The ionotropic receptor P2X7 (Purinergic Receptor P2X, P2RX7, Ligand-Gated Ion Channel 7) {12q24} is a ligand-gated cation channel that mediates the ATP-induced apoptotic death in hematopoietic cells. A single nucleotide polymorphism, E496A (A1513C) that results in a loss of function of P2X7 is present in the leukemic B-lymphocytes of patients with chronic lymphatic leukemia at a threefold higher frequency than in the healthy population. Whereas activation of P2X7 leads to lymphocyte apoptosis its reduced function has an antiapoptotic effect. The loss-of-function polymorphism of P2X7 may result in increased numbers of B-lymphocytes and thus contribute to the pathogenesis of CLL [Wiley et al. 2002]. In this condition, survival is significantly longer for patients who are heterozygous for the 1513C allele than for those with the 1513A/A genotype [Thunberg et al. 2002].

16.1.9 B-cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia (B-PLL) is a chronic progressive lymphoproliferative disorder that affects mature B-cells. It is a rare condition with a slight male predominance and a median age of 69. The patients often present with advanced stage disease. B-PLL is characterized by high white blood cell counts and splenomegaly without adenopathy, the bone marrow infiltration pattern is either diffuse or mixed. In the blood, there are elevated numbers of white blood cells with prolymphocytes representing more than 55% of the circulating lymphoid cells. Anemia and thrombocytopenia may exist.

Molecular pathogenesis. Deletions of the long arms of chromosomes 11 {11q23} and 13 {13q14} are the most frequent structural chromosome aberrations in various types of lymphoproliferative disorders, including B-PLL. In contrast to CLL, in B-PLL there is a preferential loss of RB1, suggesting that allelic loss of the *rb1* gene may play a role in the pathogenesis of this disease [Gardiner et al. 1997; Lens et al. 2000].

Molecular markers. B-PLL cells express the pan-B-cell markers CD19, CD20, and CD24. B-PLL cells are distinct from B-CLL cells in that they express high levels of surface Immunoglobulin and infrequently express CD5. The expression of CD22 is common, whereas CD23 is usually not expressed.

16.1.10 AIDS-Related Lymphoma

AIDS-related lymphomata often arise extranodally. They manifest as primary central nervous system lymphoma, gut lymphoma, or bone marrow lymphoma and have an aggressive clinical course. More than half of all cases are associated with EBV infection or HHV-8 infection.

AIDS-diffuse large cell lymphoma is EBV positive in 50–70% of all cases. The infection of B-lymphocytes with EBV is a preneoplastic event, which efficiently induces immortalization. The expression of the viral oncoprotein LMP-1 triggers several transforming signal transduction cascades. The afflicted cells express BCL-6, but not MUM1 or Syndecan-1, and represent germinal center centroblasts and early centrocytes.

Castleman disease (angiofollicular lymph node hyperplasia, angiomatous lymphoid, giant benign lymphoma) is a rare disorder with either unicentric or multicentric lymphadenopathy. The growths of the lymph node tissue are benign. Systemic signs may include fever, weight loss, nausea, and vomiting. Patients with multicentric disease may also have splenomegaly, edema, effusions, hepatomegaly, and occasionally a neuropathy [Bowne et al. 1999]. HHV-8-infected B-lymphocytes in AIDS may cause primary effusion lymphoma or plasmablastic lymphoma associated with multicentric Castleman disease (affecting plasmablasts that express IgM and the Igλ light chain). The cells express MUM-1 and Syndecan-1, but not BCL-6, and represent post-germinal center B-lymphocytes. Castleman tumors are benign lymphomata of variable location, but mostly intrathoracic. They are classified into:
– Common hyalin-vascular type (80–90%)
– More rare plasma cell type (10–20%)
– Intermediate type
There is a strong association of Castleman tumors with skin diseases (lichen planus, pemphigus vulgaris,

Kaposi sarcoma), neurologic diseases (POEMS syndrome, myasthenia gravis, arteritis temporalis, Guillain–Barré syndrome), and internal diseases (nephrotic syndrome, amyloidosis, plasmacytoma, rheumatoid arthritis, thrombotic thrombocytopenic purpura).

16.1.11 Genetic Predisposition to B-cell Lymphoma

Inherited disorders can increase the risk for leukemia. B-cell lymphomata occur with increased frequency in ataxia teleangiectasia (AT). Ataxia telangiectasia variant 1 is the designation applied to the Nijmegen breakage syndrome and ataxia telangiectasia variant 2 is the designation for the Berlin breakage syndrome. The clinical presentation is indistinguishable between variants 1 and 2, but they differ in complementation studies. t(11;14) translocations are characteristic of mantle cell lymphoma. A commonly deleted region of 11q22–q23 in this setting includes the *atm* locus. The deletion of one copy of *atm* may be accompanied by a point mutation in the remaining allele, which results in alterations of the protein. The 11q22–q23 deletion is associated with extensive lymph node involvement and poor survival. Biallelic *atm* mutations occur in mantle cell lymphomata that do not contain 11q deletions.

Patients with Down syndrome have a 20-fold greater risk of developing acute leukemia than the general population. About 2% of all cases of childhood leukemia occur in children who have Down syndrome. Leukemias and lymphomata in this condition may originate in any lymphoid or myeloid lineage. ALL in Down syndrome typically has a favorable prognosis. Primary lymphoma of the thyroid gland is rare. The histopathology of most low-grade thyroid lymphomata is of a MALT type. A typical feature of this type of lymphoma is a close lymphocyte–epithelium interaction. This form of lymphoma can arise in Down syndrome.

Wiskott–Aldrich syndrome (WAS) is a rare X-linked disorder caused by mutations in the *wasp* gene {6q21–q22}. WASP plays important roles in lymphoid development. Patients are prone to develop lymphoproliferative disorders of the B-lymphocyte lineage.

16.2 T-CELL AND NK-CELL NEOPLASMS

16.2.1 Anaplastic Large Cell Lymphoma

Anaplastic large cell lymphoma (ALCL) can occur at any time between childhood and old age, but is most common in children and young adults. It is about twice as frequent in men as in women. The lymphoma is usually composed of activated T-lymphocytes, although in some cases the constituting cell type is unclear and is referred to as a null-cell type. The first sign of the condition is often a painless swelling in the neck, armpit or groin, caused by enlarged lymph nodes. Multiple groups of lymph nodes may be affected. The lymphoma can also occur in the skin, and sometimes in the lungs, the liver, the bone marrow, or the bones.

Molecular pathogenesis. The t(2;5)(p23;q35) chromosomal translocation occurs in most anaplastic large cell lymphomata. This rearrangement fuses the *npm* gene {5q35} for Nucleophosmin to the protein tyrosine kinase gene *alk* {2p23}. In the resulting hybrid protein, the NH_2-terminus of the nucleolar phosphoprotein Nucleophosmin is linked to the catalytic domain of ALK (Anaplastic Lymphoma Kinase). ALK has sequence similarity to the Insulin Receptor subfamily of kinases. It is expressed in the small intestine, testis, and the brain, but not in normal lymphoid cells. The deregulated expression of the truncated ALK may contribute to the malignant transformation in these lymphomata [Morris et al. 1994].

Molecular markers. The transformed cells in anaplastic large cell lymphoma have an activated phenotype. They are characterized by the expression of the activation marker CD30 in more than 75% of cases. CD30 is a protein in the TNFR superfamily. Interleukin-9 (IL-9) is a cytokine that serves as a regulator of lymphoid and myeloid systems. It is expressed in large cell anaplastic lymphoma and may play a role as an autocrine growth factor.

The neoplastic cells typically express the helper T-lymphocyte surface marker profile $CD3^+CD4^+CD8^-$, whereas pan-T-cell antigens may be partially lost. These lymphoma cells are monoclonal according to their *T-cell antigen receptor* rearrangement. They are commonly characterized by defective expression of some components of the T-Cell Antigen Receptor (TCR) antigen. The transformed cells may lack TCRβ, CD3, or ZAP-70. The defective

TCRs contribute to the deregulation of signaling pathways that control activation and survival [Bonzheim et al. 2004].

16.2.2 Adult T-cell Leukemia

Two unique types of human retroviruses, HTLV types 1 and 2 are etiologically associated with leukemias [Ruscetti et al. 1977; Mier and Gallo 1980]. Human T-cell leukemia virus type 1 (HTLV-1), the first human retrovirus to be isolated and characterized, underlies adult T-cell leukemia and may be the causative agent of a relatively rare form of T-cell lymphoma that occurs mainly in Japan and the Caribbean Islands. HTLV-2 can cause hairy T-cell leukemia [Kalyanaraman et al. 1982]. HTLV-1 infects between 10 and 20 million people, of whom 2–3% develop adult T-lymphocyte leukemia or adult T-lymphocyte lymphoma.

The HTLV-1 oncoprotein TAX induces growth transformation and is critical for the pathogenesis of the HTLV-1-induced adult T-cell leukemia. It stimulates the cell cycle and transactivates the expression of growth-promoting cellular genes, such as *interleukin-13* (*il-13*). TAX activates the *il-13* promoter through an NF-AT binding P element and through a putative AP-1 site. IL-13 exerts proliferative and antiapoptotic functions. The IL-13 overexpression possibly leads to an autocrine stimulation of HTLV-1-infected cells [Waldele et al. 2004]. The spindle checkpoint protein MAD1 is targeted for degradation by the TAX protein of HTLV-1, which results in the accumulation of multinucleate aneuploid cells that are typical of adult T-cell leukemia.

16.2.3 T-cell Chronic Lymphocytic Leukemia

T-cell CLL [Brouet et al. 1975] comprises 2–5% of all CLL cases. It is likely that most cases of T-CLL are identical to large granular lymphocyte (LGL) leukemia. LGL leukemia is a chronic lymphoproliferative disorder with autoimmune features. T-cell CLL is typically monoclonal according to identical *tcrβ* (*T-cell antigen receptor β chain*) gene rearrangements [Foa et al. 1986].

An inversion of chromosome 14, due to breaks in q11.2 and q32.3, occurs in T-cell CLL. The proto-oncogene *tcl-1* (*tcl1a*) is situated at 14q32.3 and is activated by the resulting juxtaposition with the *tcrα* locus with inversion. TCL1 trimerizes and binds to the Plekstrin homology domain of PKB. This activates PKB and promotes cell survival and proliferation. TCL1 also stabilizes the mitochondrial transmembrane potential and enhances survival [Hecht et al. 1984; Croce et al. 1985; Laine et al. 2000].

T-cell prolymphocytic leukemia. T-cell prolymphocytic leukemia (T-PLL) is an uncommon chronic lymphoproliferative disorder characterized by lymphadenopathy, splenomegaly, and lymphocytosis. The disease has an aggressive clinical course with short survival.

T-PLL contains $CD3^+$ $CD4^+$ lymphocytes that display heterogeneous cytologic features. Cytogenetic anomalies in about 80% of T-PLLs comprise 14q11 and 8q rearrangements. The remaining cases usually carry Xq, 11q, 7q, or 6q anomalies. Additional chromosomal defects may lead to a complex karyotype. The *atm* gene {11q23} is often deleted in T-PLL, suggesting a tumor suppressor role for ATM in this disease. ATM is implicated in the translocation t(6;11)(q21;q23) in T-PLL [Wong et al. 1999].

Genetic predisposition. Patients with the autosomal recessive disorder ataxia teleangiectasia have a biallelic inactivation of the *atm* gene. As part of the resulting syndrome, these patients exhibit a predisposition to the development of lymphoid tumors. Adult patients with ataxia teleangiectasia are prone to the proliferation of phenotypically heterogeneous mature T-lymphocytes. This may lead to T-cell CLL [Saxon et al. 1979].

16.2.4 T-cell Acute Lymphocytic Leukemia

15% of all ALLs are T-cell ALLs. T-ALL (lymphoblastic lymphoma) is an aggressive cancer that preferentially affects children and adolescents. The most frequent type of childhood leukemia, it is common amongst adolescent males in the preteen-age and early teenage, but it also occurs in females and younger children. T-cell ALL may manifest as a mediastinal mass, cause hyperleukocytosis, and lead to hepatosplenomegaly.

T-cell ALL is defined by the expression of T-lymphocyte-associated antigens on the leukemic cells. They include CD2, CD3, CD5, and CD7. Of these, CD2 is associated with a more favorable prognosis. There are various forms of T-cell ALL ($CD3^+$):

– Pro-T-cell ALL (T1) express CD7, but are $CD3^-$ $CD5^-CD1^-CD10^-$
– Pre-T-cell ALL (T2) express CD2 or CD5

– Cortical T-cell ALL (T3) express CD1a
– Mature T-cell ALL (T4) are $CD3^+CD5^+CD1a^-$ $CD10^-$

Molecular pathogenesis. The major molecular subtypes of T-cell ALL comprise the misexpression of Notch pathway members, of the homeobox genes *hox11* or *hox11L2* or *hoxA*, of the genes for the helix–loop–helix transcription factors *tal1, tal2, lyl1,* or *myb*, or of the leucine zipper transcription factor gene *af10*. The transcriptional activation of these oncogenes in leukemic cells typically results from chromosomal rearrangements that place them next to highly active cis-acting transcriptional regulatory elements. Alternatively, the biallelic expression of oncogenic transcription factors in a significant fraction of T-ALLs may result from a loss of the upstream transcriptional mechanisms that normally downregulate the expression of these oncogenes during T-cell development [Ferrando et al. 2004]. The associated gene expression signatures are indicative of leukemic arrest at specific stages during normal thymocyte development, with LYL1 activity arresting at the pro-T-cell stage, HOX11 in early cortical thymocytes, and TAL1 in late cortical thymocytes [Ferrando et al. 2002]. In some forms of T-cell ALL, TGF-β-associated tumor suppressor pathways are inactivated.

Members of the Notch family, including Notch-1 (TAN1, Translocation-Associated Notch Homolog 1), are involved in mediating cell fate decisions during hematopoiesis. The Notch pathway is overactive in some forms of leukemia. Activating mutations of Notch-1, which involve the extracellular heterodimerization domain or the COOH-terminal PEST domain (proline-glutamate-serine-threonine), occur in 50% of T-ALLs. The heterodimerization domain is often affected by missense mutations, while mutations in the PEST domain are short insertions or deletions causing shifts in the reading frame [Weng et al. 2004]. Chromosome 7q34–q35, which contains the gene *tcrβ*, is a common site for translocations in T-cell neoplasms. In fewer than 1% of acute T-cell lymphoblastic leukemia cases, a translocation t(7;9)(q34;q34.3), the breakpoints occur within 100 bp of an intron in *notch-1*, resulting in the truncation of *notch-1* transcripts [Ellisen et al. 1991]. While Notch-1 is essential for T-lymphocyte lineage commitment, the continuous presence of its cleavage product Notch Intracellular Domain (NICD) maintains the T-lymphocytes in the immature, $CD4^+$ $CD8^+$ stage. The overexpression of a fragment similar to NICD occurs following the chromosome translocation t(7;9)(q34;q34.2).

The orphan homeobox gene *hox11* (*tlx-1*, *tcl-3*) is silent in normal mature T-lymphocytes. The gene is mutated in a subset of childhood T-cell ALLs. This may occur through the translocations t(10;14)(q24;q11) or t(7;10)(q35;q24), which bring the *hox11* gene into proximity of the *tcrβ* {7q35} or *tcrα/δ* {14q11} gene clusters and result in its transcriptional activation. The 14q11 breakpoint splits the *tcrα* locus in a region between the variable and constant genes. The leukemogenic process may result from the translocation of the Cα locus to *hox11* with the resulting deregulation of *hox11* transcription. A translocation t(10;14)(q24;q11) juxtaposes the *tcrδ* gene in chromosome 14q11 with a breakpoint that lies immediately centromeric of the *hox11* gene. This translocation is likely catalyzed by Recombinases normally involved in the generation of TCR diversity. The result is the deregulated expression of an unaltered homeobox gene in tumorigenesis. Overexpression of the *hox11* gene occurs in approximately 5–10% of childhood T-ALL cases and 30% of adult T-ALL cases. The prognosis is generally good.

HOX11L2 (HOX11-Like 2, TLX3) expression is elevated on the basis of a translocation t(5;14)(q35;q32). It is frequent in ALL with cortical T-lymphocyte immunophenotype and in children aged between 6 and 9 years. The *hox11l2* gene is transcriptionally activated as a result of the translocation, probably under the influence of CTIP2 transcriptional regulation elements. The prognosis is poor.

mll gene rearrangements are uncommon in T-cell ALL. The translocation t(11;19)(q23;p13) generates an in-frame fusion between the NH_2-terminus of MLL and the COOH-terminus of ENL (MLLT1, LTG19) [Tkachuk et al. 1992]. *mll* sequences telomeric to the breakpoint may be deleted, which precludes the formation of a reciprocal ENL–MLL fusion protein. The fusion of MLL with ENL creates a potent general transcriptional transactivator, which acts on the promoter of *hoxA7*, a potential target gene for the unaltered MLL protein [Schreiner et al. 1999]. This transactivation capability depends on the contributions of the Methyl Transferase homology region of MLL in combination with a protein–protein interaction domain on

the COOH-terminus of ENL. The relevant ENL domain recruits the polycomb protein and transcriptional repressor PC3. *hoxA9* and *hoxA7*, as well as the *hox* coregulators *meis1* and *pbx3* are among the target genes upregulated by MLL–ENL.

TAL1 (SCL, TCL5) is a transcription factor of the basic region helix–loop–helix family that is active in hematopoietic cells. Tumor-specific alterations of the *tal1* gene {1p32} occur in almost 25% of patients with T-cell ALL. They arise by distinct mechanisms. First, some T-ALL patients exhibit a 90 kb deletion of upstream sequences of one allele of the *tal1* locus. The site specificity of this deletion is mediated by aberrant activity of the Immunoglobulin Recombinase. Second, about 3% of T-ALL patients harbor a translocation t(1;14)(p34;q11), which transposes *tal1* from its normal location on chromosome 1 into the *tcrα/δ* (*T-cell receptor α/δ chain*) complex on chromosome 14. Third, a chromosomal rearrangement t(1;14)(p34;q11) brings the *sil* promoter into a relationship with the coding part of the *tal1* gene and is a mechanism for the generation of T-cell ALL.

tal2 is located 33 kb from the chromosome 9 breakpoint of t(7;9)(q34;q32), a recurring translocation specifically associated with T-ALL. The translocation juxtaposes *tal2* with sequences from the *tcrβ* (*T-cell antigen receptor β chain*) gene on chromosome 7. The TAL2 gene product contains a helix–loop–helix protein dimerization domain and a DNA-binding domain that are homologous to those encoded by the TAL1 and LYL1 proto-oncogenes.

lyl1 (*lymphoid leukemia 1*) {19p13.2–p13.1} is located at the breakpoint for a t(7;19) chromosomal translocation, which results in a truncation of LYL1. The point of crossover on chromosome 7 is immediately adjacent to a joining segment within the *tcrβ* gene, which implies that this translocation is a result of an error in *tcr* gene rearrangement [Cleary et al. 1988].

The *c-myb* oncogene encodes a transcription factor that is implicated in the switch between growth and differentiation of hematopoietic cells. The c-MYB protein consists of a NH_2-terminal DNA-binding domain, a central transactivation domain, and a COOH-terminal negative regulatory domain. The DNA-binding domain of c-MYB is composed of three repeats related to the helix–turn–helix motif (R1, R2, and R3). The R2 and R3 repeats are sufficient for sequence-specific DNA binding. The *myb* locus {6q22–6q24} is the breakpoint in translocations involved in T-lymphocyte acute lymphatic leukemia. Deletions of the long arm of chromosome 6 {6q-}, frequently occur in hematopoietic neoplasms, including ALLs. They are accompanied by high levels of *myb* mRNA levels despite the *myb* locus not being allelic.

The t(10;11)(p13–14;q14–21), associated with the generation of CALM-AF10, is a rare cause for acute lymphoid leukemias. It results in the fusion of the transcription factor AF10 (MLLT10) to the Clathrin assembly protein CALM [Dreyling et al. 1996]. The 109 kD protein AF10 contains an NH_2-terminal zinc finger region and a COOH-terminal leucine zipper. CALM-AF10 is specific for the γδ T-lymphocyte lineage. A large proportion of the *af10* or *af10-calm* transcripts encode truncated AF10 polypeptides, raising the possibility that these might act as dominant negative inhibitors of full length AF10 or related proteins.

The receptors for TGF-β and their associated signaling intermediates constitute an important tumor suppressor pathway. SMAD3 is a TGFβR-associated signal transduction molecule. Loss of the SMAD3 protein is a specific feature of pediatric T-cell ALL. A reduction in SMAD3 expression and the loss of the tumor suppressor $P27^{KIP1}$ work synergistically to promote T-cell leukemogenesis [Wolfraim et al. 2004].

16.2.5 Cutaneous T-cell Lymphoma

Sézary syndrome (Baccaredda–Sézary syndrome) [Sézary and Bouvrain 1938] is a rare form of erythrodermic cutaneous T-cell lymphoma with hematologic involvement and a poor prognosis. It becomes manifest through generalized exfoliative erythroderma, intense pruritus, and peripheral lymphadenopathy. Some patients also have alopecia and nail dystrophy. Facial infiltration may produce facies leonina. Chromosomal instability is common in Sézary syndrome [Mao et al. 2003] and clonal chromosome abnormalities are frequent. They include aberrations that affect chromosomes 1 and 17, rearrangement of chromosomes 10 and 14, and abnormality of 6q. Recurrent der(1)t(1;10)(p2;q2) and der(14)t(14;15) translocations are associated with Sézary syndrome. Chromosome imbalances affect loss of 1p, followed by losses of 10/10q, 17p, and 19, and gains of 17q and 18.

Mycosis fungoides (MF) [Alibert 1835] is the most common form of cutaneous T-cell lymphoma. It has a plethora of clinicopathological manifestations, in which the skin is variably affected by flat patches, thin plaques, or tumors. Mycosis fungoides may progress to the advanced form called Sézary syndrome. In this state, the entire skin is affected. There may also be patches, plaques, or tumors on the skin. Cancerous T-cells are present in the blood.

$CD30^+$ lymphoproliferative disorders of the skin represent a spectrum of primary cutaneous T-cell lymphomata. Lymphomatoid papulosis and anaplastic large-cell lymphoma share the expression of CD30 antigen as a common phenotypic hallmark, but differ in regard to their clinical and histologic features, as well as their biologic behavior. Primary cutaneous $CD30^+$ large-cell anaplastic T-cell lymphoma (LCAL) is a high-grade malignant lymphoma. These diseases need to be differentiated diagnostically from reactive inflammatory disorders that can contain a large number of $CD30^+$ cells [Kempf 2006].

16.2.6 AIDS-Related T-cell Lymphoma

Angioimmunoblastic T-cell lymphoma is a type of peripheral T-cell lymphoma that is clinically characterized by high fever, night sweats, weight loss, skin rash, and generalized lymphadenopathy that sometimes has cutaneous involvement. It may lead to a positive Coombs test and polyclonal hypergammaglobulinemia. This malignancy usually occurs in adult patients aged 40–90 years (median around 65), who are more often male. It often afflicts AIDS patients. As angioimmunoblastic T-cell lymphoma progresses, hepatosplenomegaly and hemolytic anemia may develop. The skin is involved in approximately 40–50% of patients.

16.2.7 Natural Killer-cell Lymphoma

Natural Killer (NK)-cell Lymphoma (cinonasal angiocentric T-cell/NK-cell lymphoma, CD56 lymphoma) comprises a group of aggressive lymphomata that have marked propensity to occur in the nose and paranasal sinuses. Blastic NK-cell lymphoma expresses CD4 in most cases and is related with skin tropism. Rarely, $CD4^-$ blastic NK-cell lymphomata arise with primary presentation in the skin, subsequent infiltration of the bone marrow, and aggressive behavior. Extranodal lymphomata expressing CD56 (N-CAM) are rare, are characterized by a high incidence of cutaneous involvement with a very aggressive clinical course, and frequently occur in the nasal or nasopharyngeal region. This manifestation is an EBV-associated lymphoma that arises in the nasal area and aggressively invades the surrounding tissues. It frequently occurs in the context of immunosuppression [O'Leary and Kennedy 1995; Chan et al. 1997; Ott et al. 1997; Garcia-Cosio et al. 2003].

Molecular and cellular pathology. The neoplastic NK-cells are pleomorphic, angiocentric growth is common, the expression of TIA-1 and Granzyme B is frequent. Aberrant promoter CpG methylation occurs frequently in NK-cell lymphomata. Primary tumors show the frequent methylation of *p73*, *p16*, *hmlh1*, *rarβ*, and *p15* [Siu et al 2003].

16.3 HODGKIN LYMPHOMA

Swollen but painless lymph nodes are the most common sign of Hodgkin lymphoma, often occurring in the neck (Figure 16.3.A). The lymph nodes of the chest may also be affected. Moderate splenomegaly occurs in about 30% of patients. The liver is enlarged in about 5% of cases due to liver involvement in the disease. Patients may also present with a cyclic high-grade fever, known as Pel-Epstein fever. Adverse prognostic factors include age over 45, stage IV disease, hemoglobin below 10.5 mg/dl, lymphocyte count below 600/μl, albumin below 4.0 mg/dl, and white blood count over 15,000/μl.

Hodgkin disease [Hodgkin 1832] may be initiated by EBV. The virus is localized to the malignant cells and is clonal. EBV has a strong propensity to transform germinal center B-lymphocytes, the likely cell lineage of origin for Hodgkin lymphoma. The *immunoglobulin* variable genes carry somatic mutations in Reed-Sternberg cells, a hallmark of germinal center B-cells and their descendants [Irsch et al. 1998]. There is evidence that cell fusion does not play a role in the generation of Hodgkin–Reed-Sternberg cells. According to histopathology, classical Hodgkin lymphoma and lymphocyte predominant Hodgkin lymphoma are distinguished. While the tumor cells in the lymphocyte predominant type of the disease resemble mutating and antigen-selected germinal center B-lymphocytes, Hodgkin–Reed-Sternberg cells in classical Hodgkin lymphoma originate from preapoptotic germinal center B-cells.

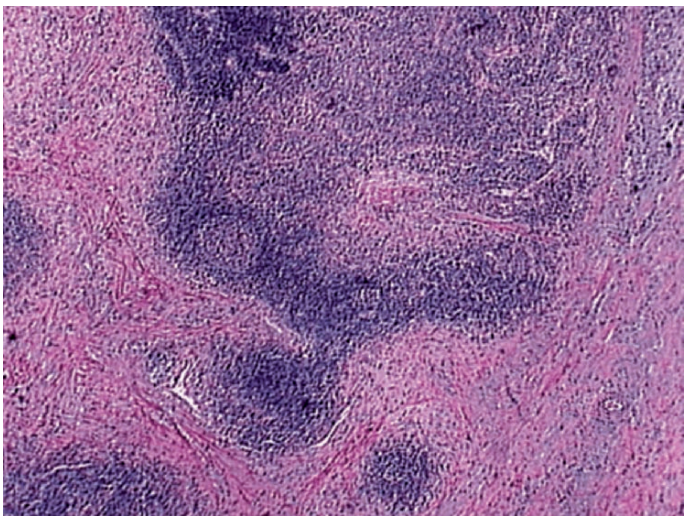


Figure 16.3.A. Hodgkin disease nodular sclerosis. The picture shows a very low power view of nodular sclerosing Hodgkin disease. Typical of this condition is the presence of large bundles of collagenous connective tissue, which separate the node into nodules. In this particular field, the pink staining fibrous, swirling material is Collagen. In the nodular sclerosing form of Hodgkin disease, there is sclerosis in broad bands of birefringent Collagen. Reed-Sternberg cells are classic binucleate cells with prominent eosinophilic macronucleoli (owl's eye cells). Lacunar cells are present with nuclei containing lacy chromatin, small- to medium-sized nucleoli, and polylobation. The cytoplasm may be clear or palely eosinophilic. The tumors cells express CD30 (KI-1) and CD15 (LEU M-1), but not CD45 (LCA). The growth is associated with a marked inflammatory reaction driven by cytokines that are secreted by the neoplastic cells, including IL-5 (attracts eosinophils), IL-4, TNF-α, GM-CSF, and TNF-β (associated with fibrosis). The disease manifests in enlarged, firm, fleshy lymph nodes with irregular nodules separated by bands of firmer, fibrotic tissue. It spreads sequentially along nodal groups. The prognosis is very good. The average age of patient is 32 years. Nodular sclerosis is among the most common variants of Hodgkin disease. It is the only variant that is more common in women. It often presents in the mediastinum. [Reproduced from http://pathweb.uchc.edu. With permission.]

Molecular pathogenesis. Immunoglobulin production is impaired in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma in spite of functional clonal rearrangements. About 25% of Hodgkin lymphomata have mutations that render originally functional *immunoglobulin* gene rearrangements nonfunctional (crippling mutations). Germinal center B-lymphocytes acquiring such mutations are usually removed effectively by apoptosis, because they have lost the capacity to be positively selected by the expression of high affinity BCRs.

Notch-1 is a transcription factor that regulates B-lymphocyte versus T-lymphocyte fate decisions in precursor cells by suppressing B-cell development. It is activated in Hodgkin–Reed-Sternberg cells [Jundt et al. 2002].

Polycomb genes play a critical role in hematopoiesis. Expression of the polycomb genes *bmi-1* and *ezh2* is separated during normal B-lymphocyte development in germinal centers. In contrast, Hodgkin and Reed-Sternberg cells coexpress these genes. The abnormal expression of BMI-1 may contribute to Hodgkin lymphoma [Raaphorst et al. 2000].

Whereas IL-13 expression is uncommon in non-Hodgkin lymphoma, IL-13 and IL-13Rα1 are frequently expressed in Reed-Sternberg cells. Loss of IL-13 induces the inhibition of proliferation and onset of apoptosis in Hodgkin lymphoma cells. It is associated with the decreased activation of STAT6, an important mediator of IL-13 function. Moreover, STAT6 is often activated in Reed-Sternberg cells, implying that IL-13 is an important growth factor in Hodgkin disease [Skinnider et al. 2002].

Molecular markers. Reed-Sternberg cells have large size, abundant amphophilic cytoplasm, two mirror image nuclei (owl eyes) each with an eosinophilic nucleolus and a thick nuclear membrane. The Hodgkin–Reed-Sternberg cells display a characteristic immunophenotype, with expression of CD15 and CD30, but only rare expression of CD20. The B-lymphocyte markers CD19, CD22, and surface Ig are often not expressed, whereas lineage marker expression for other cells, such as TARC, CD83, and

Fascin, can exist. The transcription factor NF-κB is constitutively active in these cells.

16.4 MYELODYSPLASTIC SYNDROMES

16.4.1 Acute Myelogenous Leukemia

Acute myelogenous leukemia (AML, acute myeloblastic leukemia, acute granulocytic leukemia, acute nonlymphocytic leukemia) is a fast growing cancer of the blood and bone marrow. It consists of blast cells that do not develop and cannot function normally in host immunity. The clinical symptoms of AML are caused by low numbers of healthy blood cells and high numbers of leukemia cells. They include fever, frequent infections, anemia, fatigue, shortness of breath, easy bleeding or bruising, petechiae, and pain in the bones or joints.

Acquired chromosome aberrations are present in the marrow of most patients with AML. Cytogenetically, AML is a very heterogeneous disease with over 160 structural chromosome abnormalities. AML can be divided into subclasses, M0–M7, on the basis of morphology, immunophenotype, and histochemistry (French–American–British classification). The subtypes correspond roughly to the maturation stages of myeloid, erythroid, and megakaryocytic development.

– M0 (undifferentiated AML)
– M1 (myeloblastic, without maturation), blasts with little maturation, low myeloperoxidase activity
– M2 (myeloblastic, with maturation), some maturation including the presence of promyelocytes or more mature neutrophils
– M3 (promyelocytic), or acute promyelocytic leukemia (APL), presence of many abnormal promyelocytes
– M4 (myelomonocytic), some monocytic differentiation
– M4eo (myelomonocytic together with bone marrow eosinophilia)
– M5 monoblastic (M5a) or monocytic (M5b)
– M6 (erythroleukemia), neoplastic proliferation of erythroblastic cells with atypical erythroblasts and myeloblasts in the peripheral blood; the acute form is Di Guglielmo syndrome
– M7 (megakaryoblastic)

The World Health Organization (WHO) classification of AML entails

– AML with characteristic genetic abnormalities, including AML with t(8;21), inv(16), or t(15;17) (which causes APL). These patients generally have a high rate of remission and a better prognosis compared to other types of AML.
– AML with multilineage dysplasia is based on prior myelodysplastic syndrome or a myeloproliferative diseases that transforms into AML. Afflicted are mostly elderly patients, the prognosis is poor.
– Therapy-related AML and myelodysplastic syndrome comprise patients who develop AML or myelodysplastic syndrome on the basis of prior chemotherapy or radiation. These leukemias may be characterized by specific chromosomal abnormalities, and often carry a poor prognosis
– AML, not otherwise categorized, includes subtypes of AML that do not fall into the above categories.
– Acute leukemias of ambiguous lineage (mixed phenotype, biphenotypic acute leukemia) are forms in which the leukemic cells cannot be classified as either myeloid or lymphoid cells, or both types of cells are present.

Molecular pathogenesis. Lineage commitment decisions of multipotent cells in hematopoiesis are made by specific activation patterns of certain transcription factors, which activate the expression of Growth Factor Receptors that allow these cells to proliferate and survive [Zhang et al. 1996]. Among the important transcription factors in this process are CBP and ETS family members, which act in part by inducing the expression of CSFR (Receptors for Colony-Stimulating Factors). Other transcription factors, such as CBF, support differentiation and limit cell expansion. A common feature in AML associated with balanced reciprocal translocations is the involvement of these transcription factors as fusion partners.

CBF (Core Binding Factor, Polyomavirus Enhancer Binding Protein 2, PEBP2) is a transcription factor complex composed of α and β subunits. Members of this class of transcription factors form heterodimers composed of 1 of 3 DNA-binding CBFα (RUNX, AML, PEBP2α) subunits and a common non-DNA-binding CBFβ (PEBP2β) subunit. The cellular CBF protein levels are crucial for the regulation of hematopoietic differentiation, because the Runt family of transcription factors (containing Runt domains, such as the RUNX proteins) associate with SMADs to transmit signals from TGF-β. CBFA2

(RUNX1, AML1, PEBP2αB) may act as a tumor suppressor. Biallelic somatic mutations of *cbfa2* occur in nearly 15% of AML cases. More than 10 different translocation fusion events, mostly occurring in myeloid leukemias, can involve *cbfa2*. They typically result in the formation of dominant inhibitors of the native CBF complex through the recruitment of the NCOR complex. Many translocations form protein fusions involving loss of the COOH-terminal transactivation domain of CBFA2. The translocation t(8;21)(q22;q22) is associated with some forms of AML [Erickson et al. 1992]. It fuses the DNA-binding domain of CBFA2 to the corepressor ETO (Eight–Twenty-One, CBFA2T1, AML1T1). The fusion protein CBFA2–ETO (AML1–ETO) immortalizes hematopoietic progenitor cells by repressing the transcription of *cdkn2A* (*p14^{ARF}*). The ETO oligomerization domain serves to recruit SIN3 and Histone Deacetylases. The E-Protein family includes the *e2A* gene products E12/E47, the *e2-2* gene products ITF-2A and ITF-2B, and the *heb* gene products HEB and HEBβ. CBFA2–ETO inhibits the activation of gene transcription by E-Proteins through stable interactions that preclude the recruitment of P300/CBP. These interactions are mediated by a conserved TAF4 homology domain in ETO, which binds to a 17 amino acid motif in P300/CBP and to the ETO target motif in E-Proteins. Thus, the basis for the CBFA2–ETO-mediated silencing of E-Protein function is an aberrant cofactor exchange mechanism that recruits NCOR instead of P300/CBP [Zhang et al. 2004]. The pericentric inversion inv(16)(p13q22) is a characteristic abnormality associated with AML, most commonly of the M4eo subtype that is characterized by eosinophilia. It causes the CBFβ–SMMHC (Smooth-Muscle Myosin Heavy Chain) fusion protein, which may act by sequestering CBFA2 on Actin filaments in the cytoplasm or by introducing a repression domain into CBFβ. *cbf* mutations are generally not sufficient to cause acute leukemia. It is likely that full transformation is generated by the cooperation between gene defects that confer a proliferative or survival advantage (class I mutations) and gene defects that impair hematopoietic differentiation (class II mutations). Rearrangements of the *cbf* genes are class II mutations [Speck and Gilliland 2002].

Translocations involving *cbp* (*crebbp*) can be causative for AML. The CBP–MOZ fusion protein is generated by a t(8;16)(p11;p13) chromosomal translocation in type M4/M5 AML. This form of AML displays monocytic differentiation, erythrophagocytosis by the leukemic cells, and a poor response to chemotherapy. The break points of this type of translocation cluster in *cbp* intron 2 and *moz* intron 16, close to repetitive elements. Additional genomic events, including deletions, duplications, and insertions in the breakpoint regions are common, indicating that the translocation does not originate in a simple end-to-end fusion. The transformation may be caused by mistargeting of the Histone Acetyl Transferase MOZ (MYST3, ZNF220), resulting in aberrant gene activation [Borrow et al. 1996].

The recurring translocation t(11;16)(q23;p13.3) mostly arises in cases of acute leukemia or myelodysplasia secondary to therapy with drugs that target DNA Topoisomerase II [Sobulo et al. 1997; Rowley et al. 1997]. This translocation fuses the *mll* gene to an exon of the *cbp* gene, producing chimeric proteins that contain the AT-hooks, the Methyl Transferase homology domain, and the transcriptional repression domain of MLL fused to the CREB-binding domain or to the bromodomain of CBP. Both fusion products retain the Histone Acetyl Transferase domain of CBP They may cause leukemia by promoting Histone acetylation of genomic regions targeted by the MLL AT-hooks, leading to transcriptional deregulation via aberrant chromatin organization.

A translocation t(10;16)(q22;p13), leading to MORF-CBP and CBP-MORF chimeras, is associated with childhood AML M5a [Panagopoulos et al. 2001]. The breaks are close to Alu elements in intron 16 of *morf* (*myst4*) and intron 2 of *cbp*. Duplications occur near the breakpoints. The MORF–CBP protein retains the zinc fingers, two nuclear localization signals, the Histone Acetyl Transferase domain, a portion of the acidic domain of MORF fused to the CBP protein downstream of codon 29. This includes the RARα-binding domain, the CREB-binding domain, the three cysteine/histidine-rich regions, the bromodomain, the HAT domain, and the glutamine-rich domains of CBP. In the reciprocal *cbp-morf*, part of the acidic domain and the COOH-terminal serine/methionine rich regions of MORF may be driven by the *cbp* promoter.

ets family proto-oncogenes may be activated by chromosome translocations that lead to AML. The ETS proteins (including ETS1, ETS2, ERG, ELK1, ELK2, ELG, ELF, and PEA3) are transcription factors that activate gene expression by binding to

purine-rich sequences. The DNA-binding activity of ETS1 is controlled by kinases and transcription factors. CBFA-2 (AML-1) regulates ETS1 by targeting its autoinhibitory module. *ets1* translocates from chromosome 11 to chromosome 4 in t(4;11)(q21;q23) in some case of AML.

The proto-oncogene product ERG {21q22.3} is a member of the ETS family. Like other ETS proteins, ERG is a sequence-specific DNA-binding protein. It is expressed at higher levels in early myeloid cells than in mature lymphoid cells. In myeloid leukemia with the t(16;21)(p11;q22) translocation, ERG is fused with TLS (FUS). The NH_2-terminal domain of TLS binds to RNA Polymerase II and this binding is retained by the TLS–ERG fusion protein. The *erg* gene is affected by the t(8;21)(q22;q22) translocation in patients with AML of the subtype M2 (AML-M2).

The proto-oncogene *mos* {8q11} is located at the breakpoint of a t(8;21) translocation associated with acute myeloblastic leukemia. This translocation places *ets2* into close proximity of *mos* [Sacchi et al. 1986]. MOS is a serine/threonine kinase that is expressed at high levels in germ cells and is a regulator of their maturation. The kinase is specifically expressed and functions during the G_2/M progression of oocytes. MOS activates MAPK. It also associates with Tubulin and may be involved in the microtubular reorganization that occurs during M phase. The inappropriate M phase activity of MOS during interphase may be responsible for its transforming capability.

Abnormalities in 7q or 12p are associated with AML. Recurrent translocations affecting *etv6* (*ets variant 6*, *tel*) {12p13}, t(7;12)(q36;p13) and t(7;12)(q32;p13), occur in AML cases in children below 19 months of age. They may arise in conjunction with trisomy 19. The prognosis is poor [Slater et al. 2001].

MLL is required for the proper maintenance of *hox* gene expression during development and hematopoiesis. MLL is an essential component of a chromatin remodeling complex that includes Histone Acetylases and Methyl Transferases. Translocations involving *mll* (*all-1*, *hrx*, *trx-1*) {11q23} are often reciprocal rearrangements and occur most commonly in secondary AML. The translocations result in the replacement of the COOH-terminal functional domains of MLL with those of a fusion partner, yielding a chimeric protein that endows hematopoietic progenitors with self-renewing and leukemogenic activity. The fusion partner contributes either direct transcriptional transactivation or oligomerization [Eguchi et al. 2005]. The immature phenotype of the resulting blasts implies that MLL plays roles at early stages of differentiation.

In some cases, a MLL–ELL fusion protein is generated by the translocation t(11;19)(q23;p13.1) [Thirman et al. 1994]. ELL (Elongation Factor RNA Polymerase II, Eleven Nineteen Lysine-Rich Leukemia Gene) is an elongation factor that can increase the catalytic rate of RNA Polymerase II transcription by suppressing the transient pausing of the Polymerase at multiple sites along the DNA. MLL–ELL increases the proliferation of myeloid cells.

ENL (MLLT1) is a very large protein involved in homeotic gene regulation. MLL–ENL expressing cells harbor the translocation t(11;19)(q23;p13) and proliferate as immature myeloid cells in the presence of Interleukin-3. This maturation block can be overcome by GM-CSF, which downregulates the expression of *c-myc*, resulting in the development of mature granulocytes and macrophages accompanied by growth arrest. Hence, the transforming ability of MLL–ENL may be exerted in part by *c-myc* activation [Tkachuk et al. 1992; Schreiner et al. 2001].

The fusion proteins MLL–FOXO3A, t(6;11)(q21;q23), and MLL–FOXO4, t(X;11)(q13;q23), enhance the self-renewal of myeloid progenitors and induce AMLs.

In the pathogenesis of AML, there may be cooperation between constitutively activated tyrosine kinases and transcription factor fusions. In this setting, the activated tyrosine kinase confers proliferation or antiapoptotic activity to the hematopoietic cells, while the transcription factor fusion impairs the normal differentiation pathways [Dash and Gilliland 2001]. In 30% of AML patients, FLT3 (STK1, FLK2) {13q12} mutation and aberrant signaling through this receptor occurs. The cytokine FLT3L (FMS-Like Tyrosine Kinase 3 Ligand) [Lyman et al. 1993] has multiple effects on the hematopoietic and immune systems. It is capable of stimulating the expansion and differentiation of hematopoietic progenitor and stem cells. Activating mutations of FLT3 are among the most common molecular abnormalities in AML. Internal tandem duplication mutations of *flt3* cause constitutive

activation of the encoded receptor through ligand-independent phosphorylation. Activation of the FLT3 receptor tyrosine kinase due to length mutations in the juxtamembrane domain arises in 20–25% of AML patients. This may be an insertion in the activation loop of FLT3 between the codons 840 and 841. Point mutations (D835Y, D835V, D835H, D835N, D835E) and deletions of the codons 835–836, which are located in the activation loop of the protein tyrosine kinase domain, occur in approximately 7% of all AML cases. The presence of *flt3* mutations is associated with a poor prognosis.

Gene silencing by aberrant promoter methylation of the tyrosine phosphatase *shp-1* (*ptpn6*, *hcp*, *sh-ptp1*, *ptp1C*) occurs in various leukemias and lymphomata. SHP-1 is expressed primarily in hematopoietic cells in a lineage specific manner from a promoter for exon 1b and usually functions as a negative regulator of signal transduction. [Oka et al. 2002]. In some forms of AML, the $CD34^+$ $CD117^+$ blasts express aberrantly spliced *ptpn6* mRNA. This is based on a conversion A7866→G, which represents the putative branch site in IVS3 of the *ptpn6* mRNA. The retention of intron 3 alters the N-SH2 domain [Beghini et al. 2000].

Myeloid malignancies can show a del(13)(q) or translocations affecting 13q [La Starza et al. 1998]. This may reflect a loss of *rb*.

Premalignant manifestations. Myelodysplastic syndromes (MDS, preleukemia) are disorders of bone marrow characterized by hypercellularity, dysplastic maturation, and an excess number of blasts. They may be accompanied by anemia, neutropenia, or thrombocytopenia. A high percentage of these cases progress to AML.

Secondary myelodysplastic syndrome (secondary acute leukemia) frequently evolves from severe aplastic anemia following immunosuppressive therapy.

Monosomy 7 syndrome of childhood is a rare from of myelodysplastic Syndrome that affects young children. It leads to anemia, thrombocytopenia, and leukocytosis. Within the marrow, there may be dysplastic changes and low numbers of megakaryocytes.

$5q^-$ syndrome is a rare myelodysplastic syndrome in adults, with a higher incidence in females. It is characterized by a prolonged clinical course, moderate to severe anemia, and normal to elevated leukocyte and platelet counts. In the marrow, there are clusters of abnormal megakaryocytes with round nuclei and an excess of myeloblasts.

Genetic predisposition. Various inherited genetic defects predispose to the development of AML.

- FPD/AML syndrome (familial platelet disorder with a propensity to develop AML) is an autosomal dominant condition that is characterized by moderate thrombocytopenia and impaired platelet function. It often leads to progressive pancytopenia and hematopoietic dysplasia with a markedly increased risk of developing AML. Inactivating mutations of *cbfa2* (*runx1*) in FDP/AML pedigrees are the basis of this rare leukemia predisposition syndrome (Table 16.4.1.A). Subtle changes in the CBF component CBFA2 can significantly affect the timing of stem cell emergence and the number of committed progenitors. Haploinsufficiency of this gene can contribute to inherited leukemia syndromes.
- Shwachman-Diamond syndrome is an inherited preleukemic disorder that is caused by a faulty bone marrow microenvironment. Shwachman–Diamond syndrome is characterized by growth retardation, exocrine pancreatic insufficiency, bone marrow dysfunction, and metaphyseal chondrodysplasia. It predisposes to myeloid leukemia.
- Some patients with congenital neutropenia (Kostmann syndrome) develop myelodysplastic

Table 16.4.1.A. runx1 mutations in leukemia and myelodysplasia

Mutation	Disease	DNA contract residue
W79C	AML (M0)	No
R80C	AML (M0)	Yes
K83N	AML (M0)	Yes
K83E	FDP/AML	Yes
R135G	AML (M0)	Yes
G138D	AML (M0)	No
R139Q	AML (M0)	Yes
R139Q	FDP/AML	Yes
R139Q	MDS	Yes
D171G	AML (M0)	Yes
D171Y	FDP/AML	Yes
R174Q	FDP/AML	Yes
R174G	AML (M0)	Yes
R177Q	AML (M0)	Yes
R177G	AML (M0)	Yes

AML (M0) = acute myeloid leukemia subclass M0; FDP/AML = familial platelet disorder with a propensity to develop AML; MDS = myelodysplastic syndrome

syndrome and AML. It is not clear whether the conventional treatment of the condition with G-CSF contributes to the propensity for myeloid transformation.
– Fanconi anemia is an autosomal recessive chromosomal instability disorder, which is characterized by congenital abnormalities, defective hematopoiesis, and a high risk of developing AML.
– AML in Down syndrome is usually of the FAB M7 subtype, which affects mainly the platelet-producing cells in the bone marrow. AML in these patients may be preceded by myelodysplastic syndrome. Transient abnormal myelopoiesis almost never arises in children who do not have Down syndrome. In transient abnormal myelopoiesis with a typical onset soon after birth, the blood and bone marrow are subject to changes that appear typical of leukemia. About 20–30% of these cases progress to leukemia, most frequently AML.

16.4.2 Promyelocytic Leukemia

In acute promyelocytic leukemia (APL), there is an abnormal accumulation of promyelocytes (immature granulocytes). The large number of promyelocytes in the bone marrow results in a reduced production of normal red blood cells and platelets. This leads to anemia and thrombocytopenia. Either leukopenia or leukocytosis may arise in the peripheral blood. APL is frequently associated with bleeding caused by disseminated intravascular coagulopathy. The bleeding can manifest itself as petechiae (bleeding spots in the skin), small ecchymoses (bruises), epistaxis (nose bleeds), bleeding in the mouth, hematuria (blood in the urine), and in women may cause menometrorrhagia (excessive irregular menstrual bleeding). The hemorrhagic diathesis may precede the diagnosis of leukemia.

APL represents 5–10% of AML cases. It afflicts predominantly young adults with a median age of approximately 40. The incidence is increased in Latin American countries.

Molecular pathogenesis. APL is a subtype of AML. It is uniquely associated with chromosomal translocations that disrupt the gene encoding the Retinoic Acid Receptor, RARα. In more than 99% of cases, this disruption results in the formation of a fusion of the *rarα* gene with the *pml* gene. In rare variants of APL, the *rarα* gene is fused to *plzf*, *npm*, or *numa1*.

PML belongs to a family of proteins harboring a distinctive C_3HC_4 zinc finger domain, termed the RING. The PML RING finger is located NH_2-terminally and is followed by two additional zinc fingers (B boxes) and an α-helical coiled-coil motif (collectively referred to as the RBCC domain). The RBCC domain mediates protein–protein interactions and is responsible for PML multimerization, localization in the PML-nuclear body (PML-NB), and heterodimerization with PML–RARα, but it does not confer DNA-binding capability. Multiple PML forms are generated through alternative splicing.

PML colocalizes with more than 30 proteins to the PML-nuclear body, a subnuclear macromolecular structure. The modification of PML by SUMO1 is essential for proper formation of the PML-nuclear body, in which PML can associate with corepressors such as NCOR (SMRT), SIN3, SKI/SNO, and Histone Deacetylases. This complex is important for the transcriptional repression mediated by the tumor suppressors MAD and RB. PML directly interacts with the Acetyl Transferase CBP and colocalizes with P300/CBP and P53 in the PML-nuclear body. HIPK2 (Homeodomain-Interacting Protein Kinase-2), which also colocalizes with PML into the PML-nuclear body, phosphorylates P53 at serine 46, resulting in P53 transcriptional activation and apoptosis.

The translocation t(15;17) is a reciprocal and balanced chromosomal translocations. The resulting two fusion genes encode PML–RARα and RARα–PML chimeric proteins, which are both expressed in the leukemic cells.
– Upon ligand binding, RARα transactivates target genes that are critical for the induction of terminal differentiation in myeloid hemopoietic cells. PML–RARα can inhibit this transcriptional function through the aberrant recruitment of corepressors and Histone Deacetylases, thus acting as a dominant negative RARα mutant.
– PML–RARα causes the delocalization of PML from the nuclear bodies and consecutively of all the other nuclear body components, even though many of these proteins do not interact directly with PML.
– PML is essential for multiple apoptotic pathways induced by DNA damage or other forms of stress. This proapoptotic activity can be antagonized by PML–RARα, lending the leukemic blasts a marked survival advantage. In cells that lack PML, the γ-radiation-induced acetylation of P53

is substantially impaired, implying that PML may regulate P53 transcriptional function by favoring its acetylation.
– PML is necessary for CD95 and TNF-α-induced apoptosis. The signaling molecule DAXX is activated upon CD95 or TNFα Receptor ligation. DAXX directly interacts with PML and its sequestration in the PML-nuclear body blocks the transcription suppressing activity but supports the apoptosis inducing activity of DAXX. Conversely, the expression of PML–RARα results in the delocalization of DAXX from the PML-nuclear body, in turn enhancing DAXX transcriptional repression [Salomoni and Pandolfi 2002].

Through these mechanisms, the PML–RARα fusion protein inactivates the differentiative pathway induced by RARα in conjunction with RXR and PML, leading to cell proliferation and inhibition of differentiation.

Progression. While the PML–RARα fusion initiates transformation, additional mutations may be required for full leukemogenesis. A candidate target gene for this mechanism is *flt3*, because it is mutated in approximately 40% of APL cases [Kelly et al. 2000].

16.4.3 Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (chronic granulocytic leukemia) is a clonal myeloproliferative disorder that arises in the hematopoietic stem cell compartment. It is characterized by a stepwise progression, driven by the accumulation of additional mutations. Chronic myelogenous leukemia is characterized by a chronic phase that can last for months or years and may have few or no symptoms. In the accelerated phase, fever, bone pain, and splenomegaly commonly arise.
– In the initial chronic phase, the myeloid compartment is expanded, but the cells retain their capacity to differentiate and function normally. Myeloid precursors proliferate in the bone marrow, peripheral blood, and tissues.
– Eventually, the chronic phase progresses to an accelerated phase, during which the leukemia cells grow more quickly. It may be reflected in 10–20% myeloblasts in the blood or bone marrow, more than 20% basophils in the blood or bone marrow, or platelet counts below 100,000/mm^3.
– The progression to blast crisis is characterized by a loss of differentiation capacity. This phase resembles acute leukemias, with rapid progression and short survival. It may be characterized by more than 20% myeloblasts or lymphoblasts in the blood or bone marrow, large clusters of blasts in the bone marrow, or the development of a chloroma [Burns 1811; King 1853] (granulocytic sarcoma, a solid focus of leukemia outside the bone marrow, the name chloroma is derived from a green tint that is caused by the high concentration of Myeloperoxidase).

CML accounts for 10–20% of leukemia cases. While the disease occurs in all age groups it afflicts mostly middle-aged adults. The incidence is 1–2 per 100,000, but exposure to radiation increases the risk.

The molecular signature of many chronic myelogenous leukemia cases is the *bcr-abl1* gene rearrangement. A t(9;22) translocation fuses the *c-abl* gene {9q24.1} to the *bcr* (*breakpoint cluster region*) locus {22q11.21}, resulting in the production of a chimeric oncoprotein. This generates a constitutively activated ABL protein tyrosine kinase in the cytoplasm of the affected cells [de Klein et al. 1982; Chissoe et al. 1995]. BCR-ABL1 transforms hematopoietic cells through the activation of multiple signaling pathways.

BCR-ABL autophosphorylates on tyrosine residues and attracts various adaptor proteins, setting up large signaling complexes that ultimately result in survival and growth signals. They are mediated predominantly through the PI 3-Kinase and STAT pathways [Griffin 2001]. BCR-ABL expression in leukemic cells exerts a potent effect against apoptosis by preventing the cytosolic accumulation of Cytochrome *c* and other preapoptotic mitochondrial perturbations, thereby inhibiting the activation of Caspase-3 and the execution of apoptosis. Further, the antiapoptotic effects of the BCR-ABL substrate PKB are required for transformation. The serine/threonine kinase PKB promotes the survival of hematopoietic cells by inducing the activity of mitochondrial RAF-1 in a RAS-independent, but PKC-dependent manner. By upregulating antiapoptotic members of the BCL-2 family, oncogenic tyrosine kinases protect form apoptosis. BCR-ABL can activate STAT5, which contributes to the overexpression of BCL-X_L. BCR-ABL also induces the phosphorylation of BAD.

c-ABL is a substrate for ATM and it contributes to DNA damage repair. The oncogenic tyrosine kinase BCR-ABL enhances the repair of DNA lesions,

especially homologous recombination repair of double strand breaks, through mediating the deregulated expression and phosphorylation of RAD51. Excessive RAD51 activity prolongs the activation of cell cycle checkpoints, specifically a G_2/M delay, providing more time for the repair of otherwise lethal lesions. BCR-ABL has three ways of increasing the activity of RAD51.

– BCR-ABL phosphorylates RAD51, leading to its activation.
– BCR-ABL may increase *rad51* gene expression through phosphorylation and activation of STAT5B, followed by initiation of transcription.
– BCR-ABL inhibits Caspase-3 and thus prevents it from degrading RAD51.

Paradoxically, affected cells have reduced repair fidelity and a tendency to accumulate more DNA damage, possibly because homologous end joining is not suitable for the repair of all DNA lesions (repair by nonhomologous end joining is diminished in BCR-ABL expressing cells). In addition, the antiapoptotic effect of the oncogenic tyrosine kinases allows cells with aberrantly repaired genomic defects to survive.

CBFA2 (AML-1, RUNX1, PEBP2αB) is essential for hematopoietic cell development in the fetal liver and its lineage specific differentiation in adults. In contrast, EVI-1 is barely expressed in normal hematopoietic cells. The chimeric *cbfa2-evi1* gene is generated by the t(3;21)(q26;q22) translocation and plays a pivotal role in some forms of chronic myelogenous leukemia and myelodysplastic syndrome. In CBFA2-EVI1, the NH_2-terminal half of CBFA2, including a Runt homology domain, is fused to the entire EVI-1 zinc finger protein. CBFA2-EVI1 leads to malignant transformation of hematopoietic stem cells through

– Exerting dominant negative effects over CBFA2-induced transcriptional activation; binding competition to specific DNA sequences and recruitment of Histone Deacetylase through a corepressor are underlying mechanisms.
– Interfering with TGF-β signaling and antagonizing the growth inhibitory effects of TGF-β; a zinc finger domain of EVI-1 associates with SMAD3, a TGF-β signal transducer, and represses its transcriptional activity by recruiting Histone Deacetylase.
– Blocking JNK activity and preventing stress-induced apoptosis; CBFA2-EVI1 associates with JNK through a zinc finger domain of EVI-1 and disturbs the association between JNK and its substrates.
– Enhancing AP-1 activity by activating the *c-fos* promoter, in a manner that depends on a zinc finger domain of EVI-1, and promoting cell proliferation [Mitani 2004].

Angiogenesis. Bone marrow angiogenesis occurs in chronic myeloproliferative disorders. This is reflected in the presence of the markers for endothelial cells CD34 and CD105. In comparison with the normal bone marrow, chronic myelogenous leukemia reveals a significant increase in microvascular density, which is functionally associated with elevated levels of angiogenic cytokines.

16.4.4 Hypereosinophilic Syndrome

Hypereosinophilic syndrome is a rare leukemia, in which abnormal PDGF Receptor activity contributes to the pathogenesis. Some cases of hypereosinophilic syndrome are caused by a complex chromosomal rearrangement involving an interstitial deletion on chromosome 4q12 and resulting in a fusion between *fip1l1* and *pdgfrα* [Cools et al. 2003]. The resulting FIP1L1–PDGFRα fusion protein is a constitutively activated tyrosine kinase that transforms hematopoietic cells. The syndrome is more common in men than women (ratio of 9:1) and occurs predominantly between the ages of 20 and 50.

16.4.5 Paraneoplastic Syndromes

Autoimmune paraneoplastic syndromes commonly arise in patients with myelodysplastic syndromes. As many as 10% of afflicted patients may experience various autoimmune syndromes. Clinical manifestations of such phenomena may include an acute systemic vasculitic syndrome, skin vasculitis, fever, arthritis, pulmonary infiltrates, peripheral polyneuropathy, inflammatory bowel disease, glomerulonephritis, and classical connective tissue disorders, such as relapsing polychondritis [Saif et al. 2002].

16.5 HISTIOCYTIC OR DENDRITIC CELL NEOPLASMS

Histiocytic sarcoma. The histiocytic sarcoma complex encompasses distinctive clinical entities.

– Histiocytic sarcoma is a histiocytic neoplasia that originates in a single site. This form is often

encountered on the extremities and has a good prognosis if removed early by surgical excision. When dissemination to distant sites beyond the local lymph nodes occurs, the disease is termed disseminated histiocytic sarcoma.
– Malignant histiocytosis is an aggressive, histiocytic neoplasm that simultaneously arises in multiple sites. In clinical practice, it is not always possible to differentiate the multicentric origin of malignant histiocytosis from widespread metastasis of disseminated histiocytic sarcoma.

Histiocytic sarcoma is a very rare disorder. The neoplastic cells may proliferate in organs throughout the body. They are large cells containing eosinophilic cytoplasm and pleomorphic nuclei with prominent nucleoli. The neoplastic cells are positive for Lysozymes, CD68, and the monocyte/macrophage marker CD163. The disease may be accompanied by erythrophagocytosis by neoplastic cells in the bone marrow [Mikami et al. 2004].

Dendritic cell neoplasms

– Langerhans cell tumors (Langerhans cell histiocytoses) are all defined by the presence of the Birbeck granule, a tennis racquet-shaped cytoplasmic organelle with unknown function. Langerhans cell histiocytoses comprise Histiocytosis X, eosinophilic granuloma, Hand-Schuller-Christian disease, Letterer-Siwe disease, and Hashimoto-Pritzker disease (congenital self-healing Langerhans cell histiocytosis). Allelic loss of chromosomes 9p. 22q, or 1p may be associated with Langerhans cell tumors.
– Interdigitating cell tumors are rare sarcomata that arise from the interdigitating reticulum cells of the lymph nodes. They are derived from nonlymphoid accessory cells of the T-cell areas in peripheral lymphoid tissue. They lack Birbeck granules and are important in antigen presentation to the T-lymphocytes. These tumors manifest with lymphadenopathy, and only rarely with associated systemic symptoms, including fever and weight loss. The tumors are located in the lymph nodes, intra-abdominal organs (pancreas and peripancreatic tissue), and tonsils.
– Follicular dendritic cell tumors do not express lysozyme or CD1a, but they are positive for CD21 and CD35. Most cases involve the lymph nodes of the neck, mediastinum, and axilla. Approximately 30% of the cases are located in extranodal sites, including the liver, the tonsils, and abdominal soft tissue.

REFERENCES

Adachi M, Tsujimoto Y. 1989. Juxtaposition of human bcl-2 and immunoglobulin lambda light chain gene in chronic lymphocytic leukemia is the result of a reciprocal chromosome translocation between chromosome 18 and 22. Oncogene 4:1073–1075.

Alibert JLM. 1835. Monographie des dermatoses, 2nd edn. Paris: Germer Balliere.

Beghini A, Ripamonti CB, Peterlongo P, Roversi G, Cairoli R, Morra E, Larizza L. 2000. RNA hyperediting and alternative splicing of hematopoietic cell phosphatase (PTPN6) gene in acute myeloid leukemia. Human Molecular Genetics 9:2297–2304.

Bereshchenko OR, Gu W, Dalla-Favera R. 2002. Acetylation inactivates the transcriptional repressor BCL6. Nature Genetics 32:606–613.

Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ. 1985. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J(H) on chromosome 14 and near a transcriptional unit on 18. Cell 41:899–906.

Bonzheim I, Geissinger E, Roth S, Zettl A, Marx A, Rosenwald A, Müller-Hermelink HK, Rüdiger T. 2004. Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. Blood 104:3358–3360.

Borrow J, Stanton VP Jr, Andresen JM, Becher R, Behm FG, Chaganti RSK, Civin CI, Disteche C, Dube I, Frischauf AM, Horsman D, Mitelman F, Volinia S, Watmore AE, Housman DE. 1996. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nature Genetics 14:33–41.

Brouet JC, Sasportes M, Flandrin G, Preud'Homme JL, Seligmann M. 1975. Chronic lymphocytic leukaemia of T-cell origin: immunological and clinical evaluation in eleven patients. The Lancet 8:890–893.

Brown AG, Ross FM, Dunne EM, Steel CM, Weir-Thompson EM. 1993. Evidence for a new tumour suppressor locus (DBM) in human B-cell neoplasia telomeric to the retinoblastoma gene. Nature Genetics 3:67–72.

Bowne WB, Lewis JJ, Filippa DA, Niesvizky R, Brooks AD, Burt ME, Brennan MF. 1999. The management of unicentric and multicentric Castleman's disease: a report of 16 cases and a review of the literature. Cancer 85:706–717.

Burkitt D. 1958. A sarcoma involving the jaws in African children. British Journal of Surgery 46:218–223.

Burns A. 1811. Observations of surgical anatomy in head and neck. London: Royce, p. 364.

Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. 2005. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. The New England Journal of Medicine 353:1793–1801.

Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, Croce CM. 2004. MicroRNA profiling reveals distinct signatures in B cell

chronic lymphocytic leukemias. Proceedings of the National Academy of Sciences USA 101:11755–11760.

Chan JK, Sin VC, Wong KF, Ng CS, Tsang WY, Chan CH, Cheung MM, Lau WH. 1997. Nonnasal lymphoma expressing the natural killer cell marker CD56: a clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. Blood 89:4501–4513.

Chang C-C, Ye BH, Chaganti RSK, Dalla-Favera R. 1996. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. Proceedings of the National Academy of Sciences USA 93:6947–6952.

Chissoe SL, Bodenteich A, Wang Y-F, Wang Y-P, Burian D, Clifton SW, Crabtree J, Freeman A, Iyer K, Jian L, Ma Y, McLaury H-J, Pan H-Q, Sarhan OH, Toth S, Wang Z, Zhang G, Heisterkamp N, Groffen J, Roe BA. 1995. Sequence and analysis of the human ABL gene, the BCR gene, and regions involved in the Philadelphia chromosomal translocation. Genomics 27:67–82.

Cleary ML, Mellentin JD, Spies J, Smith SD. 1988. Chromosomal translocation involving the β T cell receptor gene in acute leukemia. The Journal of Experimental Medicine 167:682–687.

Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, Cross NC, Tefferi A, Malone J, Alam R, Schrier SL, Schmid J, Rose M, Vandenberghe P, Verhoef G, Boogaerts M, Wlodarska I, Kantarjian H, Marynen P, Coutre SE, Stone R, Gilliland DG. 2003. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. New England Journal of Medicine 348:1201–1214.

Croce CM, Isobe M, Palumbo A, Puck J, Ming J, Tweardy D, Erikson J, Davis M, Rovera G. 1985. Gene for alpha-chain of human T-cell receptor: location on chromosome 14 region involved in T-cell neoplasms. Science 227:1044–1047.

Dash A, Gilliland DG. 2001. Molecular genetics of acute myeloid leukaemia. Best Practice & Research. Clinical Haematology 14:49–64.

de Klein A, Geurts van Kessel A, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR. 1982. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300:765–767.

Dispenzieri A, Kyle RA. 2005. Neurological aspects of multiple myeloma and related disorders. Best Practice and Research. Clinical Haematology. 18:673–688.

Dreyling MH, Martinez-Climent JA, Zheng M, Mao J, Rowley JD, Bohlander SK. 1996. The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. Proceedings of the National Academy of Sciences USA 93:4804–4809.

Eguchi M, Eguchi-Ishimae M, Greaves M. 2005. Molecular pathogenesis of MLL-associated leukemias. International Journal of Hematology 82:9–20.

Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, Sklar J. 1991. TAN-1, the human homolog of the Drosophila Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 66:649–661.

Elnenaei MO, Hamoudi RA, Swansbury J, Gruszka-Westwood AM, Brito-Babapulle V, Matutes E, Catovsky D. 2003. Delineation of the minimal region of loss at 13q14 in multiple myeloma. Genes, Chromosomes and Cancer 36:99–106.

Erickson P, Gao J, Chang K-S, Look T, Whisenant E, Raimondo S, Lasher R, Trujillo J, Rowley JD, Drabkin HA. 1992. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood 80:1825–1831.

Ferrando AA, Herblot S, Palomero T, Hansen M, Hoang T, Fox EA, Look AT. 2004. Biallelic transcriptional activation of oncogenic transcription factors in T-cell acute lymphoblastic leukemia. Blood 103:1909–1911.

Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, Behm FG, Pui CH, Downing JR, Gilliland DG, Lander ES, Golub TR, Look AT. 2002. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 1:75–87.

Foa R, Pelicci PG, Migone N, Lauria F, Pizzolo G, Flug F, Knowles DM 2nd, Dalla-Favera R. 1986. Analysis of T-cell receptor β chain (T β) gene rearrangements demonstrates the monoclonal nature of T-cell chronic lymphoproliferative disorders. Blood 67:247–250.

Freedman AS. 1990. Immunobiology of chronic lymphocytic leukemia. Hematology/Oncology Clinics of North America 4:405–29.

Garcia-Cosio M, Santon A, Mendez MC, Rivas C, Martin C, Bellas C. 2003. Nasopharyngeal/nasal type T/NK lymphomas: analysis of 14 cases and review of the literature. Tumori 89:278–284.

Gardiner AC, Corcoran MM, Oscier DG. 1997. Cytogenetic, fluorescence in situ hybridisation, and clinical evaluation of translocations with concomitant deletion at 13q14 in chronic lymphocytic leukaemia. Genes, Chromosomes and Cancer 20:73–81.

Griffin JD. 2001. Phosphatidyl inositol signaling by BCR/ABL: opportunities for drug development. Cancer Chemotherapy and Pharmacology 48:S11–S16.

Gu Y, Nakamura T, Alder H, Prasad R, Canaani O, Cimino G, Croce CM, Canaani E. 1992. The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. Cell 71:701–708.

Haluska FG, Tsujimoto Y, Croce CM. 1987. Mechanisms of chromosome translocation in B- and T-cell neoplasia. Trends in Genetics 3:11–15.

Hawthorn LA, Chapman R, Oscier D, Cowell JK. 1993. The consistent 13q14 translocation breakpoint seen in chronic B-cell leukaemia (BCLL) involves deletion of the D13S25 locus which lies distal to the retinoblastoma predisposition gene. Oncogene 8:1415–1419.

Hecht F, Morgan R, Hecht BK, Smith SD. 1984. Common region on chromosome 14 in T-cell leukemia and lymphoma. Science 226:1445–1447.

Herling M, Patel KA, Khalili J, Schlette E, Kobayashi R, Medeiros LJ, Jones D. 2006. TCL1 shows a regulated expression pattern in chronic lymphocytic leukemia that correlates with molecular subtypes and proliferative state. Leukemia 20:280–285.

Hodgkin T. 1832. On some morbid appearances of the absorbent glands and spleen. Medico-Chirurgical Transactions 17:68–114.

Irsch J, Nitsch S, Hansmann ML, Rajewsky K, Tesch H, Diehl V, Jox A, Kuppers R, Radbruch A. 1998. Isolation of viable Hodgkin and Reed-Sternberg cells from Hodgkin disease tissues. Proceedings of the National Academy of Sciences USA 95:10117–10122.

Jundt F, Anagnostopoulos I, Forster R, Mathas S, Stein H, Dorken B. 2002. Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. Blood 99:3398–3403.

Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. 1982. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571–573.

Kelly LM, Kutok JL, Williams IR, Boulton CL, Amaral SM, Curley DP, Ley TJ, Gilliland DG. 2000. PML/RAR-α and FLT3-ITD induce an APL-like disease in a mouse model. Proceedings of the National Academy of Sciences USA 99:8283–8288.

Kempf W. 2006. $CD30^+$ lymphoproliferative disorders: histopathology, differential diagnosis, new variants, and simulators. The Journal of Cutaneous Pathology 33(S1):58–70.

King A. 1853. A case of chloroma. Monthly Journal of Medicine 17:17.

Kuehl WM, Bergsagel PL. 2002. Multiple myeloma: evolving genetic events and host interactions. Nature Reviews Cancer 2:175–187.

Laine J, Kunstle G, Obata T, Sha M, Noguchi M. 2000. The protooncogene TCL1 is an Akt kinase coactivator. Molecular Cell 6:395–407.

La Starza R, Wlodarska I, Aventin A, Falzetti D, Crescenzi B, Martelli MF, Van den Berghe H, Mecucci C. 1998. Molecular delineation of 13q deletion boundaries in 20 patients with myeloid malignancies. Blood 91:231–237.

Lens D, Matutes E, Catovsky D, Coignet LJ. 2000. Frequent deletions at 11q23 and 13q14 in B cell prolymphocytic leukemia (B-PLL). Leukemia 14:427–430.

Lyman SD, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B, Hollingsworth LT, Picha KS, McKenna HJ, Splett RR, Fletcher FA, Maraskovsky E, Farrah T, Foxworthe D, Williams DE, Beckmann MP. 1993. Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell 75:1157–1167.

Mao X, Lillington DM, Czepulkowski B, Russell-Jones R, Young BD, Whittaker S. 2003. Molecular cytogenetic characterization of Sézary syndrome. Genes, Chromosomes and Cancer 36:250–260.

McKeithan TW, Rowley JD, Shows TB, Diaz MO. 1987. Cloning of the chromosome translocation breakpoint junction of the t(14;19) in chronic lymphocytic leukemia. Proceedings of the National Academy of Science 84:9257–9260.

Mier JW, Gallo RS. 1980. Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media. Proceedings of the National Academy of Sciences USA 77:6134–6138.

Mikami M, Sadahira Y, Suetsugu Y, Wada H, Sugihara T. 2004. Monocyte/Macrophage-specific marker $CD163^+$ histiocytic sarcoma: case report with clinical, morphologic, immunohistochemical, and molecular genetic studies. International Journal of Hematology 80:365–369.

Mitani K. 2004. Molecular mechanisms of leukemogenesis by AML1/EVI-1. Oncogene 23:4263–4269.

Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT. 1994. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263:1281–1284.

Ngan B-Y, Chen-Levy Z, Weiss LM, Warnke RA, Cleary ML. 1988. Expression in non-Hodgkin's lymphoma of the BCL-2 protein associated with the t(14;18) chromosomal translocation. New England Journal of Medicine 318:1638–1644.

Oka T, Ouchida M, Koyama M, Ogama Y, Takada S, Nakatani Y, Tanaka T, Yoshino T, Hayashi K, Ohara N, Kondo E, Takahashi K, Tsuchiyama J, Tanimoto M, Shimizu K, Akagi T. 2002. Gene silencing of the tyrosine phosphatase *SHP1* gene by aberrant methylation in leukemias/lymphomas. Cancer Research 62:6390–6394.

O'Leary G, Kennedy SM. 1995. Association of Epstein-Barr virus with sinonasal angiocentric T cell lymphoma. The Journal of Clinical Pathology 48:946–949.

Ott G, Kalla J, Ott MM, Müller-Hermelink HK. 1997. The Epstein-Barr virus in malignant non-Hodgkin's lymphoma of the upper aerodigestive tract. Diagnostic Molecular Pathology 6:134–139.

Panagopoulos I, Fioretos T, Isaksson M, Samuelsson U, Billstrom R, Strombeck B, Mitelman F, Johansson B. 2001. Fusion of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13). Human Molecular Genetics 10:395–404.

Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu C-G, Rassenti L, Calin GA, Hagan JP, Kipps T, Croce CM. 2006. Tcl1 expression in chronic lymphocytic leukemia is regulated by *miR-29* and *miR-181*. Cancer Research 66:11590–11593.

Prasad KVS, Ao Z, Yoon Y, Wu MX, Rizk M, Jacquot S, Schlossman SF. 1997. CD27, a member of the tumor necrosis factor receptor family, induces apoptosis and binds to Siva, a proapoptotic protein. Proceedings of the National Academy of Sciences USA 94:6346–6351.

Raaphorst FM, van Kemenade FJ, Blokzijl T, Fieret E, Hamer KM, Satijn DP, Otte AP, Meijer CJ. 2000. Coexpression of BMI-1 and EZH2 polycomb group genes in Reed-Sternberg cells of Hodgkin's disease. American Journal of Pathology 157:709–715.

Rowley JD, Reshmi S, Sobulo O, Musvee T, Anastasi J, Raimondi S, Schneider NR, Barredo JC, Cantu ES, Schlegelberger B, Behm F, Doggett NA, Borrow J, Zeleznik-Le N. 1997. All patients with the T(11;16)(q23;p13.3) that involves MLL and CBP have treatment-related hematologic disorders. Blood 90:535–541.

Ruscetti FW, Morgan DA, Gallo RC. 1977. Functional and morphologic characterization of human T cells continuously grown in vitro. Journal of Immunology 119:131–138.

Sacchi N, Watson DK, Geurts van Kessel AHM, Hagemeijer A, Kersey J, Drabkin HD, Patterson D, Papas TS. 1986. Hu-ets-1 and Hu-ets-2 genes are transposed in acute leukemias with (4;11) and (8;21) translocations. Science 231:379–382.

Saif MW, Hopkins JL, Gore SD. 2002. Autoimmune phenomena in patients with myelodysplastic syndromes and chronic myelomonocytic leukemia. Leukemia and Lymphoma 43:2083–2092.

Salomoni P, Pandolfi PP. 2002. The role of PML in tumor suppression. Cell 108:165–170.

Savage KJ, Monti S, Kutok JL, Cattoretti G, Neuberg D, De Leval L, Kurtin P, Dal Cin P, Ladd C, Feuerhake F, Aguiar RC, Li S, Salles G, Berger F, Jing W, Pinkus GS, Habermann T, Dalla-Favera R, Harris NL, Aster JC, Golub TR, Shipp MA. 2003. The molecular signature of mediastinal large B-cell lymphoma

differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood 102:3871–3879.

Saxon A, Stevens RH, Golde DW. 1979. Helper and suppressor T-lymphocyte leukemia in ataxia-telangiectasia. New England Journal of Medicine 300:700–704.

Schreiner S, Birke M, Garcia-Cuellar MP, Zilles O, Greil J, Slany RK. 2001. MLL-ENL causes a reversible and myc-dependent block of myelomonocytic cell differentiation. Cancer Research 61:6480–6486.

Schreiner SA, Garcia-Cuellar MP, Fey GH, Slany RK. 1999. The leukemogenic fusion of MLL with ENL creates a novel transcriptional transactivator. Leukemia 13:1525–1533.

Sézary A, Bouvrain Y. 1938. Erythrodérmie avec présence de cellules monstrueuses dans le derme et le sang circulant. Bulletin de la Société francaise de dermatologie et de syphiligraphie 45:254–260.

Siu LL, Chan JK, Wong KF, Choy C, Kwong YL. 2003. Aberrant promoter CpG methylation as a molecular marker for disease monitoring in natural killer cell lymphomas. British Journal of Haematology 122:70–77.

Skinnider BF, Kapp U, Mak TW. 2002. The role of interleukin 13 in classical Hodgkin lymphoma. Leukemia and Lymphoma 43:1203–1210.

Slater RM, von Drunen E, Kroes WG, Weghuis DO, van den Berg E, Smit EM, van der Does-van den Berg A, van Wering E, Hahlen K, Carroll AJ, Raimondi SC, Beverloo HB. 2001. t(7;12)(q36;p13) and t(7;12)(q32;p13) – translocations involving ETV6 in children 18 months of age or younger with myeloid disorders. Leukemia 15:915–920.

Sobulo OM, Borrow J, Tomek R, Reshmi S, Harden A, Schlegelberger B, Housman D, Doggett NA, Rowley JD, Zeleznik-Le NJ. 1997. MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). Proceedings of the National Academy of Sciences USA 94:8732–8737.

Speck NA, Gilliland DG. 2002. Core-binding factors in hematopoiesis and leukemia. Nature Reviews Cancer 2:502–513.

Staudt LM. 2003. Molecular diagnosis of the hematologic cancers. New England Journal of Medicine 348:1777–1785.

Thirman MJ, Levitan DA, Kobayashi H, Simon MC, Rowley JD. 1994. Cloning of ELL, a gene that fuses to MLL in a t(11;19)(q23;p13.1) in acute myeloid leukemia. Proceedings of the National Academy of Sciences USA 91:12110–12114.

Thunberg U, Tobin G, Johnson A, Soderberg O, Padyukov L, Hultdin M, Klareskog L, Enblad G, Sundstrom C, Roos G, Rosenquist R. 2002. Polymorphism in the P2X7 receptor gene and survival in chronic lymphocytic leukaemia. The Lancet 360:1935–1939.

Tkachuk DC, Kohler S, Cleary ML. 1992. Involvement of a homolog of Drosophila trithorax by 11q23 chromosomal translocations in acute leukemias. Cell 71:691–700.

Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell PC, Croce CM. 1985. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation. Nature 315:340–343.

Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC, Croce CM. 1984. Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. Science 224:1403–1406.

Wada M, Okamura T, Okada M, Teramura M, Masuda M, Motoji T, Mizoguchi H. 1999. Frequent chromosome arm 13q deletion in aggressive non-Hodgkin's lymphoma. Leukemia 13:792–798.

Waldele K, Schneider G, Ruckes T, Grassmann R. 2004. Interleukin-13 overexpression by tax transactivation: a potential autocrine stimulus in human T-cell leukemia virus-infected lymphocytes. Journal of Virology 78:6081–6090.

Weng AP, Ferrando AA, Lee W, Morris JP IV, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. 2004. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269–271.

Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C, Taper J, Gallo J, Manoharan A. 2002. A loss-of-function polymorphic mutation in the cytolytic P2X7 receptor gene and chronic lymphocyte leukaemia: a molecular study. The Lancet 359:1114–1119.

Wolfraim LA, Fernandez TM, Mamura M, Fuller WL, Kumar R, Cole DE, Byfield S, Felici A, Flanders KC, Walz TM, Roberts AB, Aplan PD, Balis FM, Letterio JJ. 2004. Loss of Smad3 in acute T-cell lymphoblastic leukemia. The New England Journal of Medicine 351:552–559.

Wong KF, Chan JK, Sin VC. 1999. T-cell prolymphocytic leukemia with a novel translocation (6;11)(q21;q23). Cancer Genetics and Cytogenetics 111:149–151.

Ye BH, Rao PH, Chaganti RSK, Dalla-Favera R. 1993. Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. Cancer Research 53:2732–2735.

Zhang DE, Hohaus S, Voso MT, Chen HM, Smith LT, Hetherington CJ, Tenen DG. 1996. Function of PU.1 (Spi-1), C/EBP, and AML1 in early myelopoiesis: regulation of multiple myeloid CSF receptor promoters. Current Topics in Microbiology and Immunology 211:137–147.

Zhang J, Kalkum M, Yamamura S, Chait BT, Roeder RG. 2004. E protein silencing by the leukemogenic AML1-ETO fusion protein. Science 305:1286–1289.

CHAPTER 17

MESENCHYMAL TUMORS

Sarcomata (Greek for fleshy growths) is derived from a variety of mesenchymal cells of the connective tissues. They can arise anywhere in the body and are frequently hidden deep in the limbs. Although sarcomata constitute about 1% of all adult cancers, they make up 15–20% of all children's cancers.

17.1 MYOSARCOMA

17.1.1 Rhabdomyosarcoma

Rhabdomyosarcomata occur early in life and are uniformly high-grade tumors. More than 50% of them develop in children under the age of 10, and they are slightly more frequent in males than in females. These tumors are the most common soft tissue sarcomata in childhood.

Molecular pathogenesis. Rhabdomyosarcomata are characterized by the presence of muscle-specific gene products, including Myogenin, MYO-D, Actin and Myosin, Myoglobin, Z-Band Protein, and Desmin. Two major histologic subtypes include embryonal rhabdomyosarcoma (ERMS, comprising spindle cell and botryoid variants), which accounts for more than two thirds of all cases, and alveolar rhabdomyosarcoma (ARMS). The most common sites of origin for the embryonal form are the head and neck, the retroperitoneum, and the genitourinary tract. The alveolar form has a predilection for the extremities.

– The embryonal form of rhabdomyosarcoma has a more spindled appearance, and tends to be less densely cellular and relatively rich in stroma. This cancer is characterized by loss of heterozygosity on the short arm of chromosome 11 {11p15.5} [Loh et al. 1992], which is referred to as the rhabdomyosarcoma chromosomal region (RMS1, RMSE1). This domain contains the *igf-2* gene that is uniformly overexpressed in this cancer. *igf-2* is normally imprinted from the maternal allele, however, in rhabdomyosarcoma the normally silent maternal allele becomes transcriptionally active (loss of imprinting). Besides the relaxation of imprinting, two or more copies of active *igf-2* alleles can arise by duplication of the active allele. IGF-2 acts as an autocrine growth factor for rhabdomyosarcoma cells, and its elevated expression may be an important step for tumor initiation or progression.
– Alveolar rhabdomyosarcoma frequently exhibits a more aggressive course. It is typically composed of small round cells with a dense appearance that may seem to line up along spaces reminiscent of pulmonary alveoli. Most cases of alveolar rhabdomyosarcoma are characterized by the presence of a translocation involving the long arm of chromosome 13 and either chromosome 2 or chromosome 1, which creates a transforming chimeric oncogene product, PAX3-FOXO1 in t(2;13)(q35;q14) [Douglass et al. 1987] or PAX7-FOXO1 in t(1;13)(p36;q14). PAX3 and PAX7 are highly related members of the paired-box transcription factor family, which contain a NH_2-terminal forkhead DNA-binding domain and a COOH-terminal transcription activation domain. The fusion of FOXO1 (FKHR) to PAX increases its transcriptional activity. The chimeric transcription factors activate PAX responsive genes with 10- to 100-fold higher potency than the wild type PAX proteins. Potentially important genes that may be activated by the PAX3-FOXO1 fusion protein include *c-met*, *igf-2*, and *igf-bp5*.

HH (Hedgehog) signaling promotes the expression of G_1/S Cyclins, including Cyclins D and E, and results in the growth of cells. SHH signaling also opposes epithelial cell cycle arrest by $P21^{CIP1/WAF1}$. rhabdomyosarcoma is associated with mutations that activate the proto-oncogene *smo* or that inactivate the tumor suppressor *ptc* [Hahn et al. 1998; Taipale et al. 2000]. Therefore, the excessive activation of HH signaling may lead to rhabdomyosarcomagenesis.

HGF-driven positive feedback loops, that sustain MET-induced oncogenic transformation, occur in mesenchymally derived tumors. Although MET is not expressed in adult skeletal muscles, a significant fraction of rhabdomyosarcomata that endogenously secrete HGF also express MET. This may form a positive feedback loop. Further, there is communication between MET signaling and the actions of the CDKN2A tumor suppressor (comprising $P16^{INK4a}$ and $P14^{ARF}$). MET activation and *cdkn2A* loss suppress myogenesis in an additive fashion, and synergism between CDKN2A inactivation and aberrant HGF signaling results in rhabdomyosarcomagenesis [Sharp et al. 2002].

Tumor progression. Nerve Growth Factor Receptor (NGFR, $P75^{NTR}$) {17q21-q22} is a 399 amino acid transmembrane glycoprotein that belongs to the TNFR family. $P75^{NTR}$ is consistently expressed in rhabdomyosarcomata.

The expression of highly sialylated, embryonic, less avidly adhesive forms of NCAM (PSA NCAM) on rhabdomyosarcomata is associated with increased metastatic potential.

The interactions of Collagen with Integrin $\alpha_2\beta_1$ are implicated in tumor metastasis. The expression of Integrin $\alpha_2\beta_1$ increases the metastatic potential of rhabdomyosarcoma cells.

Genetic predisposition. TSC-2 (Tuberin) acts as a tumor suppressor that negatively regulates Phosphatidylinositol 3-Kinase signaling downstream of PKB. Rhabdomyosarcomata are often associated with loss of heterozygosity of 16p13, the locus of the *tsc-2* gene. In tuberous sclerosis, cardiac rhabdomyomata may develop in utero.

Loss of $p57^{KIP2}$ (*cdkn1C*) underlies a fraction of cases of Beckwith–Wiedemann syndrome (BWS, Exomphalos–Macroglossia–Gigantism syndrome, EMG syndrome), which is characterized by organ overgrowth and predisposition to cancer. Rhabdomyosarcomata arise with increased frequency. $p57^{KIP2}$ is genomically imprinted, the maternal allele being preferentially expressed while the paternally inherited allele is methylated and transcriptionally repressed. A microdeletion involving the entire *lit1* gene {11p15} causes silencing of $p57^{KIP2}$ when inherited maternally, and results in BWS.

Mosaic variegated aneuploidy is a genetic defect in chromosome segregation, in which more than 25% of cells are aneuploid and childhood cancers, such as rhabdomyosarcoma, occur with increased frequency. It may be based on loss-of-function mutations (missense or truncations) in the repair gene *bub1B* {15q15}, which encodes BUBR1 (BUB Related 1), a key protein in the mitotic spindle checkpoint.

Basal cell nevus syndrome (Gorlin syndrome, Gorlin–Goltz syndrome) is an inherited multisystem disorder, in which multiple basal cell carcinomata are associated with various abnormalities. The condition predisposes to basal cell carcinoma, medulloblastoma, and rhabdomyosarcoma. The inheritance is autosomal dominant, with variable penetrance. The underlying genetic defect is a loss of the tumor suppressor *patched* (*ptc*) {9q22.3}.

An inherited predisposition to rhabdomyosarcoma exists in Costello syndrome (Faciocutaneoskeletal syndrome, FCS syndrome). The condition is caused by the germline mutation G12V of H-RAS.

17.1.2 Leiomyosarcoma

Leiomyoma is a benign neoplasm of smooth muscle tissue (Figure 17.1.2.A). It is common in the genitalia, skin, and gastrointestinal tract, but is rare in the upper aerodigestive tract. Leiomyomata are divided into solid leiomyoma (leiomyoma of deep soft tissue), vascular leiomyoma (angiomyoma, angioleiomyoma), and epithelioid leiomyoma (leiomyoblastoma). Leiomyoma does not normally progress to leiomyosarcoma, the cancerous tumor of smooth muscle.

Clinical forms. Uterine leiomyoma (uterine fibroid) is a common benign smooth muscle cell tumor of the myometrium, occurring in up to 30% of women over 35 years. Leiomyomata may lead to excessive menstrual bleeding (menorrhagia), often causing anemia, and may result in infertility. Leiomyoma

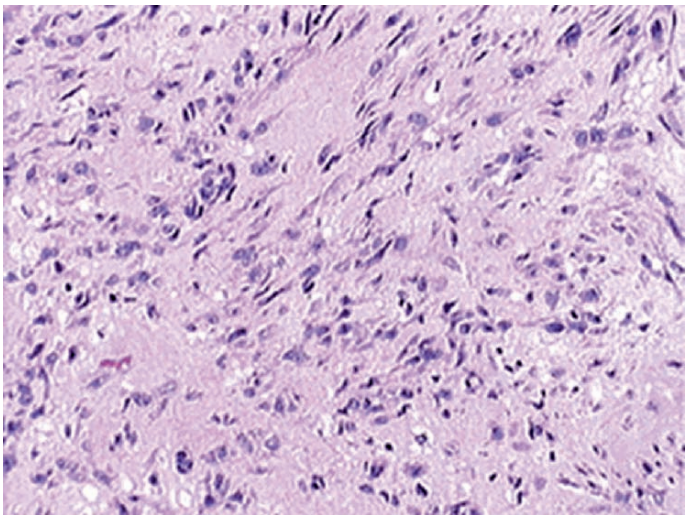


Figure 17.1.2.A. Leiomyoma. This low power view shows irregular bundles of cells. The nuclei are elongated. Some of the acellular material between the cells is collagen, a feature of aging. Leiomyoma is typically composed of spindle cells with blunt-ended elongate bland nuclei and thin elongated cytoplasm. The cells are arranged in interlacing bundles. There may be cytological atypia, necrosis, or hemorrhage. These tumors are solitary or multiple, well circumscribed growths. They may be submucosal, intramural, or subserosal with pedunculation. A variety of degenerative changes includes hemorrhagic, cystic, mucinous, fatty, myxoid, or fibrotic alterations. Leiomyomata are highly common neoplasms affecting 25% of reproductive age women. The vast majority of cases are asymptomatic. Symptoms, if present, include heavy, painful menses, pelvic fullness, infertility, spontaneous miscarriage, or urinary symptoms due to pressure on the bladder. The tumors are estrogen sensitive with occasional rapid growth during pregnancy and atrophy after menopause. [Reproduced from http://pathweb.uchc.edu.with permission.]

growth is dependent on ovarian steroids, specifically estrogen and progesterone. In this setting, the action of estrogen may be mediated in part by local growth factors, such as EGF and IGF-1, which are produced by the target cells. GAS6 and TYRO-7 signal transduction is aberrantly stimulated in uterine leiomyoma. The TYRO family receptor tyrosine kinases can immortalize cells, in part by preventing apoptosis. These receptors activate SRC family kinases and signal transduction pathways downstream of GRB-2. The ligand for TYRO, GAS6 (Growth Arrest Specific Gene 6), is able to induce cell cycle reentry and protect cells from apoptotic cell death.

Leiomyosarcoma of the uterus is a rare neoplasm with an aggressive growth pattern and high rate of recurrence. The most common sites of dissemination are lung, liver, and peritoneal cavity, with a low rate of lymph node involvement [Sandruck et al. 2004].

Three forms of cutaneous leiomyomata comprise piloleiomyomata, angioleiomyomata, and genital leiomyomata. Piloleiomyomata arise from the arrector pili muscle of the pilosebaceous unit. They may occur as single or multiple lesions. While multiple piloleiomyomata generally arise in the age range of 10–30 years, solitary piloleiomyomata usually appear later with a mean patient age of around 50 years at presentation. Angioleiomyomata originate from the tunica media of arteries and veins. They are typically solitary. The malignant form, primary leiomyosarcoma of a major peripheral artery is extremely rare [Blansfield et al. 2003]. Leiomyomata derived from the dartos muscle of the scrotum and the labia majora, as well as those derived from the erectile muscle of the nipple, are classified as genital leiomyomata. They are rare and typically arise as solitary lesions.

Primary leiomyosarcoma of the breast is very rare with an average age of affliction around 55 years. It is a slowly growing mass limited to the breast, however there is always a possibility of local recurrence or distant spread, which can occur many years after primary surgery [Hussien et al. 2001].

Leiomyoma is the most common benign esophageal tumor, although it accounts for less than 1% of all esophageal neoplasms. Patients are typically between the ages of 20 and 50 years. Most leiomyomata are solitary, but in about 5% of patients multiple esophageal leiomyosarcomata arise (leiomyomatosis). These tumors may lead to dysphagia and in rare cases to bleeding.

Most of the cancers that develop during states of immune deficiency are rare cancers associated with viral infection, often by EBV. Smooth muscle tumors in immunosuppressed individuals uniformly contain EBV, indicating a likely causal role for the virus in this condition. Specifically, EBV infection is strongly associated with leiomyomata and leiomyosarcomata in children with AIDS and in recipients of organ transplants. EBV can infect smooth muscle cells in these immunocompromised hosts. In contrast, EBV may not play a role in smooth muscle tumors of otherwise healthy children.

Genetic predisposition. Multiple cutaneous and uterine leiomyomatosis (Reed syndrome) is an autosomal dominant disorder caused by mutation in the gene encoding Fumarate Hydratase {1q42.1} [Alam et al. 2001; Tomlinson et al. 2002]. Mutations in the same gene cause Fumarase deficiency. Multiple small tumors composed of smooth muscle fibers develop in the skin. Malignant transformation is rare. The tumors are thought to arise from the erector pilorum muscles [Kloepfer et al. 1958; Wei et al. 2006].

Hereditary leiomyomatosis with renal cell cancer is caused by mutations in the gene encoding Fumarate Hydratase, an enzyme of the tricarboxylic acid cycle. The enzyme acts as a tumor suppressor in familial leiomyomata, and its activity is very low or absent in tumors from individuals with leiomyomatosis. The inheritance is autosomal dominant [Launonen et al. 2001].

The Carney triad comprises the association of gastric epithelioid leiomyosarcoma, functioning extraadrenal paraganglioma, and pulmonary chondroma [Carney et al. 1977].

Paraneoplastic syndromes. Recurrent hypoglycemia may result from the secretion of IGF-2 by a leiomyosarcoma. This is reflected in elevated plasma cortisol, but low Insulin and Growth Hormone levels during hypoglycemia. The concentrations of IGF-1 in the tumor and serum are low [Daughaday et al. 1988].

17.2 SOFT TISSUE SARCOMA

17.2.1 Liposarcoma

Liposarcoma is a malignant tumor that arises in fat cells within deep soft tissue, such as that inside the thigh or in the retroperitoneum (Figure 17.2.1.A). Liposarcomata are typically bulky tumors that tend to have multiple smaller satellites extending beyond the main confines of the growth. The prognosis varies depending on the site of origin, the tumor size, the depth, and the proximity to lymph nodes. Once grown to a very large size, a retroperitoneal liposarcoma may cause weight loss, emaciation, and abdominal pain. It may also compress a kidney or ureter, leading to kidney failure. Metastases are common. The 5-year survival rate for a high-grade liposarcoma is below 50%.

There are multiple histologic subtypes of liposarcoma, including well differentiated, dedifferentiated, myxoid, round cell, and pleomorphic. The histologic type is predictive of both the clinical course of the disease and the ultimate prognosis.

Molecular pathogenesis. The targeting of a conserved effector domain within RNA-binding proteins to DNA may play a role in tumor formation. The *fus* (*tls*, *translocated in liposarcoma*) gene [Rabbitts et al. 1993; Crozat et al. 1993] consists of 15 exons located within 11 kb of genomic DNA. *fus* exon 1 contains a 72 bp untranslated region and the translation initiation codon. The sequence upstream of the transcription start site contains no TATA boxes but does contain stretches of C and G, as well as several recognition sites for the transcription factors AP-2, GCF, and SP-1. FUS is a glycine-rich nuclear protein that associates with products of RNA Polymerase II transcription. CHOP is a nuclear protein that serves as a dominant negative inhibitor of the transcription factors C/EBP and LAP. The translocation t(12;16)(q13;p11) in malignant myxoid liposarcoma causes a fusion of the CHOP (DDIT3, GADD153) gene product with FUS (TLS). The result is a chimera, in which the RNA-binding domain of FUS is replaced by the DNA binding and leucine zipper dimerization domain of CHOP. The break in *fus* occurs in a specific ATGGTG hexamer, the break in intron 1 of *chop* is near an Alu sequence. The chromosomal translocation t(12;22;20)(q13;q12;q11) in myxoid liposarcoma creates a EWS–CHOP hybrid. The breakpoints are localized to intron 7 of *fus* and intron 7 of *ews*. The break in intron 7 of *ews* occurs close to an Alu sequence [Panagopoulos et al. 1997].

Primary liposarcoma cells can be induced to undergo terminal differentiation by activation of PPARγ, suggesting that the differentiation block in these cells may be due to insufficient induction of the PPAR pathway. RXR-specific ligands are

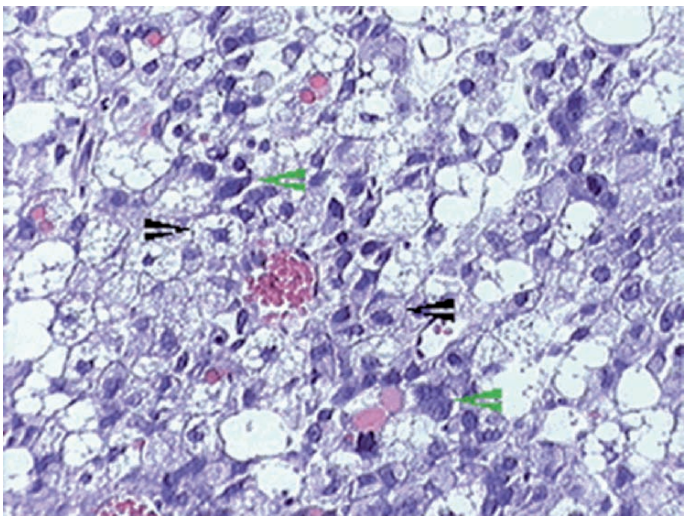


Figure 17.2.1.A. Liposarcoma. Shown is a medium power view of a liposarcoma. Notice that in contrast to adult fat, this tissue is intensely cellular. In addition, even at this power, many of these nuclei are unusually large in size, such as the ones under the green pointers. The proliferating cells in this type of lesion are lipoblasts (usually appearing as cells with a foamy or signet ring appearance), two examples of which are pointed by the black arrows. These cells are round, contain a central nucleus and some evidence of fat accumulation. Histologically, the most common type of liposarcoma is the myxoid type. The tumors have three components, including proliferating lipoblasts, a delicate capillary network (resembling chickenwire fence), and a myxoid matrix containing abundant ground substance and stellate myxomatous cells. Some liposarcomata are poorly differentiated with small round cells resembling a malignant lymphoma. The three most common locations are the thigh, the retroperitoneum, and the inguinal region. Liposarcomata tend to be large (5–10 cm in diameter) and manifest a lobulated appearance. Typically, at least some evidence of yellow color, due to the lipid material, is present. The neoplasms usually manifest as a slow growing, deep seated, poorly defined mass. Rare instances may be associated with pain or tenderness early in the disease. Liposarcoma is a tumor of adult life, with a peak incidence between 40 and 60 years of age. Approximately 55–60% of tumors occur in men. Retroperitoneal tumors occur somewhat more frequently in women. There is no evidence that any race or geographical area is more prone to develop liposarcomata. Five-year survival rates vary from 60% to 70%. [Reproduced from http://pathweb.uchc.edu. With permission.]

potent adipogenic agents in cells expressing the PPARγ/RXRα heterodimer, and simultaneous activation of PPARγ and RXR in liposarcoma cells results in an additive stimulation of differentiation, which is characterized by the accumulation of intracellular lipid, induction of adipocyte specific genes, and exit from the cell cycle.

lpsa (*liposarcoma oncogene*) is an oncogene that is activated in soft tissue tumors. It maps to 19p13.2-q13.3 and its transcripts have sizes of 3.5 and 8.5 kb [Gill et al. 1991].

17.2.2 Lipoma

Lipomata are common, benign lesions involving soft tissues. Lipomata have a soft consistency, are sometimes moveable, and are generally painless. They grow slowly and do not become cancerous. Lipomata grow most often superficially subcutaneously on the trunk (Figure 17.2.2.A). Lipomatosis represents a diffuse overgrowth of mature fat, affecting subcutaneous tissue, muscle or nerve. Lipoblastoma is a tumor of immature fat occurring in young children, and may contain a mixture of fat and nonadipose tissue.

Molecular pathogenesis. In lipomata, rearrangements of the *hmgA2* gene are common. *lhfp* (*lipoma HMGIC fusion partner*) is the fusion partner of *hmgA2* (*hmgIC*, *babl*, *lipo*) in lipoma with t(12;13). The expressed fusion transcript encodes the three DNA-binding domains of HMGA2, followed by 69 amino acids encoded by frame shifted *lhfp* sequences. LPP (Lipoma Preferred Partner) is fused with HMGA2 by a t(3;12) translocation in some forms of benign lipoma. In a subset of translocations associated with lipoma, the 3′ end of the *hmgA2* gene is deleted. Chimeric transcripts are formed, in which HMGA2 DNA-binding domains (AT hook motifs) are fused to either a LIM domain or an acidic transactivator domain.

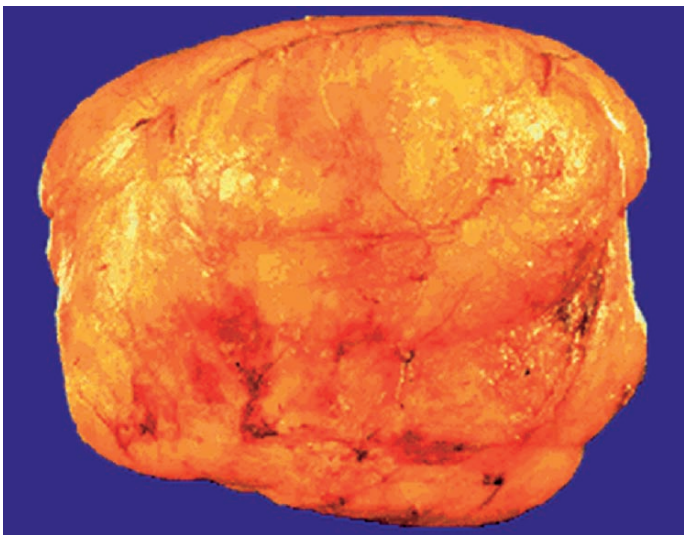


Figure 17.2.2.A. Lipoma. This is the external surface of a lipoma, a benign tumor of adipocyte origin. The bright yellow color due to the fat content is typical. Lipoma manifests as lobulated yellow mass surrounded by a thin capsule. These tumors may occur anywhere in the body, most often in the subcutaneous tissues. Lipomata are common growths of adult individuals that occur at all ages. They usually present as a painless, soft mass. Males and females are equally affected. [Reproduced from http://pathweb.uchc.edu. With permission.]

Deletions and structural rearrangements of the long arm of chromosome 13 {13q14} are frequently associated with benign and low malignant lipomatous tumors, including ordinary lipomata, spindle cell/pleomorphic lipomata, myxolipomata, angiomyxolipoma, and atypical lipomatous tumors.

Angiolipoma, myolipoma, and chondroid lipoma are rare lipomatous lesions.

- Intramuscular lipoma is a benign neoplasm that has a clear margin but an infiltrative growth pattern that has an effect on muscle bundles, although is never metastasizes. The infiltrating growth characteristic of intramuscular lipoma are modulated by type selective muscular degeneration and endomysial fatty growth as a result of atrophy [Mori et al. 2004].
- In angiomyolipomata, loss of heterozygosity of 16p13, which contains the *tsc-2* tumor suppressor gene, occurs in more than 50% of cases.

17.3 BONE CANCER

17.3.1 Osteosarcoma

Osteosarcoma (osteogenic sarcoma) is a malignant connective tissue tumor. Its neoplastic cells have osteoblastic differentiation and form tumoral bone. Osteosarcoma has a peak incidence in the second and third decade of life. A second peak in incidence occurs in the elderly, usually associated with an underlying bone pathology such as Paget disease, medullary infarct, or prior irradiation. There is a preference for the metaphyseal region of tubular long bones. About 50% of cases arise around the knee. For many patients, the initial symptom is recurring pain that may be worse at night. If the tumor is large, it can appear as a swelling. The affected bone is weakened and may suffer pathologic fracture with only minor trauma.

Histologic forms include chondroblastic, osteoblastic, fibroblastic, epitheloid, giant cell rich, small cell, and teleangiectatic osteosarcoma. Cortex-associated osteosarcoma may be paraosteal, periosteal, high-grade surface, or intracortical.

Molecular pathogenesis. Loss of heterozygosity affecting *rb1* (35%), *p53* (40%), and chromosome 18q (30%) occurs in osteosarcoma.

RB is essential for late osteoblast differentiation. It interacts with the osteoblast transcription factor CBFA1. The association of RB with CBFA1 (RUNX2, AML3) and osteoblast-specific gene promoter sequences results in the induction of osteoblast differentiation [Thomas et al. 2001]. This transactivating function is lost in certain RB mutants, and such loss-of-function mutations in the *rb* gene predispose to osteosarcoma. Alteration of RB1 in osteosarcoma is associated with reduced survival [Patino-Garcia et al. 2003]. There may be imprinting of *rb* in relation to osteosarcoma. The defective gene is more often transmitted from the father [Toguchida et al. 1989]. Survivors of the bilateral form of retinoblastoma (RB1) have an increased risk of osteosarcoma, while survivors of unilateral retinoblastoma show the same likelihood of developing osteosarcoma as the general

population. This suggests that sporadic osteosarcoma may be due to homozygosity, or hemizygosity for a mutation at the *rb1* locus {13}. P16, CDK4, and Cyclin D_1 act in the RB pathway. Their genes may be defective in osteosarcoma.

The chromosomal region 17p11.2 ~ p12 harbors many low copy repeats. Amplification of this region occurs in about 25% of high-grade osteosarcomata. The underlying mechanism may be repeated duplication by mitotic nonallelic homologous recombination. Candidate oncogenes in this area are *pmp22* and *cops3*. The overexpression of COPS3 is linked to P53 degradation and to the induction of genomic instability, which frequently occurs in high-grade osteosarcoma.

P53 plays a critical role in bone organogenesis and homeostasis by negatively regulating growth and suppressing transformation. *p53* mutations are present in about 40% of osteosarcoma cases. A similar effect may result from acquired gain-of-function mutations of MDM2. Mutation of *p53* is associated particularly with advanced patient age.

Genomic instability may lead to osteosarcoma, in particular if chromosome translocations form constitutively active receptor tyrosine kinases.

– *met* may be subject to gene translocations, such as *tpr-met* in osteosarcoma [Dean et al. 1987]. The TPR-MET fusion protein is constitutively active and potently oncogenic as a result of TPR leucine zipper interactions, which allow MET kinase dimerization, autophosphorylation, and activation.
– A constitutively active form of FGF Receptor 2 may be expressed in osteosarcoma secondary to a chromosomal rearrangement that fuses the NH_2-terminus of FGF Receptor 2 to an unknown protein, resulting in a constitutively active form.

Reflecting genomic instability, a substantial fraction of high-grade osteosarcomata is aneuploid [Kreicbergs et al. 1984; Hiddemann et al. 1987]. A wide range of karyotypic abnormalities may arise. Although areas of gene amplification, such as ring chromosomes, double minutes, and homogeneously staining regions, are fairly common in most osteosarcomata, the presence of ring chromosomes is frequently the sole cytogenetic alteration in parosteal osteosarcomata, which are low grade lesions.

Extension of life span. Survivin is present in the cytoplasm of about half of cases and in the nucleus in about half of cases with high-grade osteosarcoma. Its nuclear localization is correlated with prolonged survival, but its cytoplasmic localization does not correlate with patient outcome [Trieb et al. 2003].

Metastasis. While local osteosarcoma causes death in about 30% of cases, osteosarcoma that has spread is fatal for about 60% of patients. Metastasis to the lungs (Figure 17.3.1.A) depends on MCAM (MUC18) [McGary et al. 2003] and on CD44 [Weber et al. 2002]. CD44 signals through Ezrin, the expression of which in osteosarcoma cells is associated with metastasis formation [Khanna et al. 2001]. Ezrin expression provides an early survival advantage for cancer cells that reach

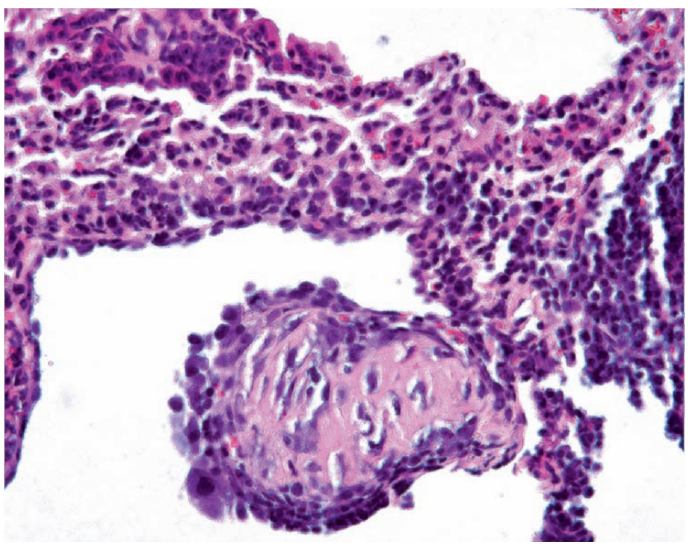


Figure 17.3.1.A. Osteosarcoma metastasis to the lung. Osteosarcomata have a tendency to disseminate to the lungs and the liver. The histologic picture shows a pulmonary metastasis of a murine osteosarcoma that arose spontaneously on a $p53^{+/-}$ genetic background. This secondary tumor is characterized by osteoid formation. The slide is stained with hematoxilin/eosin.

the lungs. This early metastatic survival is partially dependent on the activation of MAPK but not PKB [Khanna et al. 2004].

FOS, which is a component of the transcription factor AP-1, is frequently overexpressed in osteosarcomata [Van Beveren et al. 1983]. Sustained elevated levels of FOS require its phosphorylation by RSK-2 and lead to resistance to apoptosis. Furthermore, the overexpression of FOS results in accelerated S phase entry as a result of deregulated Cyclin/CDK2 activity. In dissemination, AP-1 contributes to inducing the expression of Interstitial Collagenase, which is important for the remodeling of the extracellular matrix.

Recurrence. Many osteosarcomata do not express Telomerase, but use the alternative pathway of telomere maintenance. Children with osteosarcoma are more likely to experience a recurrence of the cancer after treatment and less likely to survive if the cancer cells express Telomerase [Sanders et al. 2004].

Genetic predisposition. Paget disease of the bone (osteitis deformans) is a metabolic bone disease characterized by excessive bone resorption and formation due to activated osteoclasts. Approximately 1% of Paget patients develop osteosarcoma, which represents a substantial increase in risk over that of the general population. A form of Paget disease of the bone (PDB2) is linked to chromosome 18q. This may reflect a duplication involving bases 75–101 in exon 1 of the *tnfrsf11A* (*rank*) gene [Nellissery et al. 1998; Hughes et al. 2000]. The phenotype linked to chromosome 5q35 (PDB3) is caused by mutation in the *sqstm1* gene, the product of which is associated with the RANK signaling pathway. Other disease loci map to 6p (PDB1), 5q31 (PDB4), and 18q23.

The risk of osteogenic sarcoma is increased 500-fold in bilateral retinoblastoma patients. This is, in principle, unrelated to radiation treatment of the eye tumor, although osteosarcomata occur 1.2 years earlier inside than outside the radiation field. Rather, it is a direct effect of the genetic defect that underlies retinoblastoma. In addition, a radiation induced mutation of the intact *rb1* allele may be the cause of osteosarcomata occurring after a short delay [Abramson et al. 1976; Chauveinc et al. 2001].

OSLAM syndrome (osteosarcoma, limb anomalies, and erythroid macrocytosis with megaloblastic marrow) is an autosomal dominant tumor predisposition syndrome with impaired regulation of bone and bone marrow development [Mulvihill et al. 1977].

Li–Fraumeni syndrome [Li and Fraumeni 1969; Li and Fraumeni Jr. 1969] is a clinically and genetically heterogeneous inherited cancer syndrome with autosomal dominant inheritance. It is characterized by an early onset of tumors and multiple tumors within an individual. The most common types are soft tissue sarcomata and osteosarcomata, breast cancer, brain tumors, leukemia, and adrenocortical carcinoma. Li–Fraumeni syndrome is often caused by mutations in *p53*, and loss of P53 function typically occurs in osteosarcomata. Specifically, osteosarcoma is also a feature of Li–Fraumeni syndrome caused by mutations in the *chk2* gene. This is similar to sporadic osteosarcoma, which may be associated with mutations in *chk2*.

Osteosarcoma may arise in patients with osteogenesis imperfecta. However it is very rare and it is uncertain whether there is a pathogenetic link [Klenerman et al. 1967]. The tumor needs to be distinguished in differential diagnosis from hyperplastic callus formation, which is a common complication in osteogenesis imperfecta [Baker 1946].

The risk for osteosarcoma is increased in Mazabraud syndrome (intramuscular myxoma associated with fibrous dysplasia) [Mazabraud et al. 1967]. Myxomata occur as multiple masses in about 80% of patients. They appear in close proximity to the most severely affected bone. While sarcomatous transformation is uncommon in fibrous dysplasia, it is more frequent in Mazabraud syndrome. The underlying genetic defect may be an activating point mutation in *gnas1* {20q13.2},which encodes a $G\alpha_s$ subunit.

Osteosarcomata may be associated with progeria syndromes, such as Hutchinson-Gilford disease and premature aging syndrome Okamoto type.

17.3.2 Ewing Sarcoma

Ewing sarcoma (peripheral neuroectodermal tumor) [Ewing 1921] accounts for 10–15% of all primary bone tumors occurring between 10 and 20

years of age. The disease afflicts almost exclusively Caucasians and occurs more frequently in males. It can occur anywhere in the body, but most commonly in the pelvis and proximal long tubular bones. The femoral diaphyses are the most common sites, followed by the tibiae and the humeri. Ewing sarcoma is a small round cell tumor of neuroectodermal origin, but with limited neuronal differentiation. The Ewing tumor family includes classical Ewing sarcoma of bone and soft tissues, peripheral primitive neuroectodermal tumors (pPNET), Askin tumor, and other less frequent variants.

Molecular pathogenesis. The Ewing group of tumors is defined by the consistent presence of chromosomal translocations, resulting in gene fusions between *ews* and a member of the *ets* family, mainly *fli1* and *erg*. *ews* belongs to a family of genes that encode proteins that may serve as adapters between the RNA Polymerase II complex and RNA splicing factors. In Ewing sarcoma, chromosomal translocations lead to the formation of chimeric fusions between the *ews* gene and one of five *ets* transcription factor genes.

- Ewing sarcoma involves most often t(21;22)(q22;q12). The resultant EWS-ETS proteins promote oncogenesis in a dominant fashion and are necessary for continued cell cycle progression. EWS belongs to a family of gene products that may serve as adapters between the RNA Polymerase II complex and RNA splicing factors. The EWS-ETS fusions have biochemical characteristics of aberrant transcription factors and modulate the expression of a network of target genes. EWS-ETS proteins may also alter RNA processing.
- The *ews-fli1* fusion, resulting from the specific t(11;22)(q24;q12) translocation, occurs in 85% of Ewing sarcomata [Turc-Carel et al. 1984]. It generates an aberrant transcription factor that has various mitogenic properties and it plays a possible role as a deregulator of splicing. EWS-FLI can suppress *p21* gene expression. EWS-FLI1 downregulates, possibly through an indirect mechanism, the transcription of $p57^{KIP2}$. This facilitates cell cycle progression through G_1. The modulation of $p57^{KIP2}$ expression by EWS-FLI1 may be a fundamental step in Ewing tumorigenesis. The P53 pathway may modulate its oncogenicity. EWS has a NH_2-terminal nuclear localization domain and a glutamine rich, RNA-binding region at the COOH terminus.
- FEV is fused to EWS in a subset of Ewing tumors. *fev* encodes a 238 amino acid protein, which contains an ETS DNA-binding domain. The NH_2-terminal portion of FEV is only 42 amino acids long, suggesting that FEV is lacking important transcription regulatory domains. The COOH-terminal end of FEV is rich in alanine residues which may indicate that FEV is a transcription repressor. A t(2;22) chromosome translocation fuses the NH_2-terminal domain of EWS to the ETS DNA-binding domain of FEV [Peter et al. 1997].
- In Ewing tumors, EWS can also be fused to the ETS family members ERG, ETV1, or E1AF.

17.3.3 Other Bone Tumors

Giant cell tumor (osteoclastoma) is characterized by a massive destruction of bone near the epiphysis of a long bone, most commonly around the knee, distal end of the radius, and occasionally in the sacrum or pelvis. It causes pain and restricts movement. Whereas the tumor cells themselves may not be capable of bone destruction, they stimulate the formation of cells that function like osteoclasts and resorb bone. Giant cell tumors tend to be surrounded by new bone formation. These neoplasms account for 5–10% percent of all primary bone tumors and may be the most common bone tumors in adults aged 25–40 with a preponderance to afflict women. In rare instances, giant cell tumors metastasize to the lungs.

Osteoblastomata are mostly benign lesions distinct from osteosarcomata. Aggressive osteoblastoma arises predominantly in the spine or ribs. It is locally aggressive, but does not metastasize. It tends to recur near its original location.

17.4 CHONDROSARCOMA

Chondrosarcomata are rare cancers that originate in the cartilage. They can affect people of any age, however they are infrequent in individuals under the age of 20 years and are most common between the ages of 50 and 70 years. The incidence between males and females is equal. While any site of cartilage can transform, the most common sites are the pelvic and shoulder bones along with the superior regions of the arms and legs.

Chondrosarcoma is graded based on its growth rate. Grade 1 describes slow growing cancers, grades

2 and 3 are faster growing cancers. The forms of chondrosarcoma include dedifferentiated, mesenchymal, clear cell, and skeletal myxoid.

Molecular pathogenesis. Despite heterogeneity in the genetics of chondrosarcoma, certain chromosomal imbalances are recurrent. They include loss of chromosomes or chromosomal regions 1p36, 1p13~p22, 4, 5q13~q31, 6q22~qter, 9p22~pter, 10p, 10q24~qter, 11p13~pter, 11q25, 13q21~qter, 14q24~qter, 18p, 18q22~qter, and 22q13 and gain of 7p13~pter, 12q15~qter, 19, 20pter~q11, and 21q. Abnormalities of 9p and extra copies of chromosome 22 are prominent in central chondrosarcoma (arising centrally in bone) as compared to peripheral chondrosarcoma (arising within the cartilaginous cap of osteochondroma).

Extraskeletal myxoid chondrosarcoma is a rare soft tissue sarcoma of uncertain histogenetic origin. This group of tumors is characterized by recurrent chromosome translocations, resulting in fusions of the nuclear receptor NR4A3 {9q22} to various NH_2-terminal partners, often RNA-binding proteins. They include t(9;22)(q22;q12), t(9;17)(q22;q11–12), t(7;9;17)(q32;q22;q11), and t(9;15)(q22;q21). The gene for NR4A3 (Nuclear Receptor Subfamily 4 Group A, Member 3, NOR1, CSMF, CHN, MINOR) comprises eight exons and spans over 35 kb of genomic DNA. It encodes a member of the steroid/thyroid receptor gene superfamily. By alternative splicing, two variant transcripts can be generated. One variant has a different 5′ untranslated region and the other lacks COOH-terminal amino acid sequences corresponding to the putative ligand-binding domain [Ohkura et al. 1996]. *nr4A3* is subject to translocation in myxoid extraskeletal chondrosarcoma. The translocation t(9;22)(q22;q12) results in fusion of the NR4A3 and EWS genes, creating a chimeric EWS-NR4A3 oncoprotein. The chimeric EWS-NR4A3 fusion protein replaces the COOH-terminal RNA-binding domain of EWS with the entire NR4A3 protein, comprising a long NH_2-terminal domain, a central DNA-binding domain, and a COOH-terminal ligand binding and dimerization domain [Clark et al. 1996]. The translocation t(9;17)(q22;q11) results in fusion of the *nr4A3* and *rbp56* (*taf15*, *taf2N*) genes, creating a chimeric RBP56-NR4A3 protein.

In tumor progression, recurrent secondary abnormalities, including trisomy 1q, 7, 8, 12, and 19, and a deletion del(22)(q12–13), are common. A highly expressed gene is *chi3L1* (*chitinase-3 like*), which encodes the secreted glycoprotein YKL-40 (Cartilage Glycoprotein 39, GP39, Chondrocyte Protein YKL-40), implicated in various pathological conditions of extracellular matrix degradation as well as in cancer [Sjogren et al. 2003]. Microtubules in extraskeletal myxoid chondrosarcoma express MAP-2 and Class III β-Tubulin, and are similar to those expressed in neurons. This supports the possibility that neural/neuroendocrine differentiation occurs in a significant number of extraskeletal myxoid chondrosarcoma [Hisaoka et al. 2003].

The loss of tumor suppressor proteins is one of the hallmarks of chondrosarcomata. *p21* is important for the differentiation of cartilage. The expression of $P21^{CIP1/WAF1}$ is directly related to the level of chondrosarcoma differentiation. The *p16* (*cdkn2A*) gene is frequently deficient in chondrosarcoma. This may reflect deletions in the chromosome region 9p21 [Jagasia et al. 1996]. Functional inactivation of P53 by MDM2 expression seems to be the major cause of P53 accumulation. In contrast, overexpression, gene mutations, or loss of heterozygosity of the *p53* locus occur only in a minority of chondrosarcomata [Sandberg and Bridge 2003].

Genetic predisposition. Enchondromata are benign bone tumors that originate from cartilage and most frequently affect the hands. Enchondromata are usually in close proximity to, or in continuity with, growth plate cartilage and may result from an abnormal regulation of the proliferation and terminal differentiation of chondrocytes. In normal growth plates, differentiation of proliferative chondrocytes to postmitotic hypertrophic chondrocytes is regulated in part by a tightly coupled signaling relay that involves PTHrP (Parathyroid Hormone related Protein) and IHH (Indian Hedgehog). PTHrP delays the hypertrophic differentiation of proliferating chondrocytes, whereas IHH promotes chondrocyte proliferation. In some cases of enchondromatosis, a mutant PTHrP Receptor (R150C) may constitutively activate HH signaling and cause formation of the enchondromata [Hopyan et al. 2002].

– Spondyloenchondromatosis (spondyloenchondrodysplasia) may be a distinct entity, inherited in an autosomal recessive manner.

– The combination of enchondromatosis and multiple exostoses characterizes metchondromatosis.
– Ollier disease is constituted of a cluster of enchondromata (osteochondromatosis, dyschondroplasia). They may transform to chondrosarcomata.
– Maffucci syndrome is a combination of multiple enchondromata and angiomata. It is associated with an increased risk for chondrosarcoma [Sun et al. 1985].

Hereditary multiple exostoses (osteochondromata) are overgrowths of cartilage and bone at the end of the bone near the growth plate. Loss of heterozygosity for markers linked to *ext1* (*exostosin 1*) and *ext2* (*exostosin 2*) arises in chondrosarcomata originating in individuals with multiple exostoses as well as in sporadic chondrosarcomata.

Chondroblastoma is a benign neoplasm of the bones. It characteristically arises in the epiphysis of a long bone in young patients, most commonly at the age of 10–30 years. Men are afflicted about 2.5-fold more frequently than women. The symptoms include local pain, tenderness, swelling, and muscle wasting. Joint effusion occurs in approximately 30% of all patients. Malignant transformation is rare.

17.5 FIBROSARCOMA

Fibrosarcoma (fibroblastic sarcoma) is a rare malignant tumor derived from fibrous connective tissue. It is characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells. It can occur as a soft tissue mass or as a bone tumor. Fibrosarcomata typically originate at the ends of bones in the arms or legs, or in the head and neck area. They may then spread to surrounding soft tissues.
– Adult type fibrosarcoma (ATFS) is an aggressive malignancy of adults and older children that has a poor prognosis.
– An infantile form of fibrosarcoma, afflicting children below the age of 10 years, exists. It occurs on the extremities and axial regions. Unlike fibrosarcoma in adults, it has a very good prognosis, even if metastatic disease at present at the time of diagnosis.

Fibrosarcoma most frequently manifests as a tender mass. Pain or soreness, caused by suppressed nerves and muscles, can also constitute early symptoms.

Molecular pathogenesis. Fibrosarcomata may display aberrant tyrosine kinase signaling. RAS activation can contribute to transformation in fibrosarcoma [Brown et al. 1984, Kris et al. 1985]. It requires multiple downstream signaling pathways, including those dependent on RAF→MEK, RAC, and RHO-A [Gupta et al. 2000].

The overexpression of SP-1 plays a causal role in the malignant transformation of fibroblasts. SP-1 is a ubiquitously expressed transcription factor that binds GC box (GGCGGG) and GT box (CACCC) motifs via its C_2H_2 zinc finger domain. Upregulation of SP-1 transactivating function is closely correlated with the expression of *vegf*, *upa*, *upar*, and *egfr* [Lou et al. 2005].

Tumor progression. RHAMM (Receptor for Hyaluronan Mediated Motility) is required for the cell locomotion of *ras* transformed fibrosarcoma cells. This is mediated through $P60^{SRC}$ [Hall et al. 1995].

plf (proliferin) gene expression can occur in progressive fibrosarcoma, where it contributes to tumor angiogenesis. This may reflect the reactivation of an extraembryonic genetic program that has evolved to support fetal growth [Jackson et al. 1994; Toft et al. 2001].

Genetic predisposition. Congenital fibrosarcoma is a pediatric spindle cell malignancy that occurs in patients of 2 years or younger. It contains two specific cytogenetic abnormalities, trisomy of chromosome 11 and a t(12;15)(p13;q25) translocation. The t(12;15) rearrangement creates a transcriptionally active chimeric oncoprotein. It fuses the ETV6 (TEL) protein on chromosome 12p13 with NTRK3 (Neurotrophin-3 Receptor, TRKC) on chromosome 15q25 [Knezevich and McFadden et al. 1998]. The fusion protein tyrosine kinase ETV6-NTRK3 requires both RAS→ERK1/2 and PI3-Kinase→PKB signaling for fibroblast transformation [Tognon et al. 2001]. Congenital fibrosarcomata express Smooth Muscle Actin, Desmin, S-100, and CD34. They recur locally in 40–50% of cases but metastasize in only 10% of cases. The survival rates exceed 90%.

Bone dysplasia with medullary fibrosarcoma (BDMF, diaphyseal medullary stenosis with malignant fibrous histiocytoma) is characterized by the familial occurrence of multiple areas of necrosis in the diaphyses of the large tubular bones. It bears a high risk for medullary fibrosarcoma and resulting

death from metatases. A susceptibility locus maps to chromosome 9p22-p21.

17.6 ANGIOSARCOMA

The endothelium is one of the largest cellular compartments of the body and it has a high proliferative potential. Endothelial cells are normally resting, but changes in the net balance of angiogenic and antiangiogenic factors can shift them from almost complete quiescence into a phase of rapid growth.

Despite the endothelial potential to proliferate, angiosarcomata are among the rarest malignancies, containing anaplastic endothelial cells derived from blood or lymph vessels. They are aggressive, tend to recur locally, spread widely, have a high rate of lymph node and systemic metastases, and are frequently lethal. Angiosarcomata may occur in any region of the body, but are more common in skin and soft tissue. Approximately 50% arise in the head and neck. Angiosarcomata also can originate in the liver, breasts, spleen, bones, or heart.

Molecular pathogenesis. Angiogenesis is regulated by growth factors, which activate tyrosine kinase receptors leading to the activation of a number of intracellular signaling pathways. The expression of activated RAS (G12V) inhibits endothelial cell differentiation and induces a transformed phenotype with an increased rate of proliferation and a loss of contact inhibited growth [Rennel et al. 2003]. The PI 3-K pathway, but not the MEKK pathway, plays a major role in the regulation of RAS mediated tumor angiogenesis in angiosarcoma cells. PI 3-K regulates the production of the angiogenic mediators VEGF and MMPs. Increased VEGF expression is present in nearly 80% of angiosarcoma cases.

The *cdkn2A* (*p16*) locus is frequently inactivated in angiosarcoma of the liver, and this occurs independently of *p53* mutations [Weihrauch et al. 2002]. The most common somatic alteration is promotor methylation of the *cdkn2A* gene.

Clinical forms. Angiosarcomata of the liver (hemangioendothelial sarcomata) are frequently a consequence of occupational exposure to vinyl chloride [Creech and Johnson 1974], Thorotrast (contrast medium in X-ray diagnostics in the 1930s through 1940s), or arsenic. Vinyl chloride was first used commercially in the 1920s, but it was not until the 1930s that techniques were devised to polymerize vinyl chloride into stable forms of PVC. Vinyl chloride exposure can also occur when it is generated as a degradation product of chloroethylene solvents. After metabolic activation of vinyl chloride into chloroethylene oxide by Cytochrome P450 2E1 (CYP2E1), it exerts various genotoxic effects, including the induction of mutations and chromosomal aberrations. Among the mutagenic events caused, base pair substitutions are the most frequent [Bartsch et al. 1994] and are mediated by the metabolites 1,N^6-ethenoadenine, 3,N^4-ethenocytosine, N^2,3-ethenoguanine, and 1,N^2-ethenoguanine. The substitution mutations at A:T→T:A base pairs in codon 179 of *p53* and G:C→A:T transversions in codon 13 of *ras* are consistent with the procarcinogenic properties of the DNA adducts formed from vinyl chloride metabolites [Marion et al. 1991; Hollstein et al. 1994]. Abdominal pain, weakness, fatigue, and weight loss are the most prominent symptoms. Hepatosplenomegaly, ascites, and jaundice are the most common clinical signs. Liver function is typically only impaired in the final stages.

A hemangiopericytoma is a type of soft tissue sarcoma that originates in the pericytes in the walls of capillaries.

- Adult hemangiopericytoma occurs in elderly patients and is usually located in deep soft tissue, lower extremities, pelvis and retroperitoneum. It arises as a solitary well circumscribed tumor with a smooth surface. Areas of hemorrhage, necrosis, and cystic degeneration are often present. The tumor cells are small, ovoid to spindle shaped, with ill-defined cell boundaries. The spindle cells are each surrounded by a Reticulin sheath and are separated from the endothelial cells by a basement membrane. Actin and other myoid markers are absent, whereas Vimentin and CD34 are expressed.
- Infantile hemangiopericytoma is usually present at birth. It has a benign course. These tumors are mostly solitary lesions, located on the head and neck area, extremities or the trunk. The tumors contain polymorphic populations of cells consisting of spindle cells with myofibroblastic features and more primitive round cells (both are positive for Smooth Muscle Actin).

A benign tumor of the blood vessels is a hemangioma (Figure 17.6.A). These tumors are formed either during gestation or present as a birthmarks.

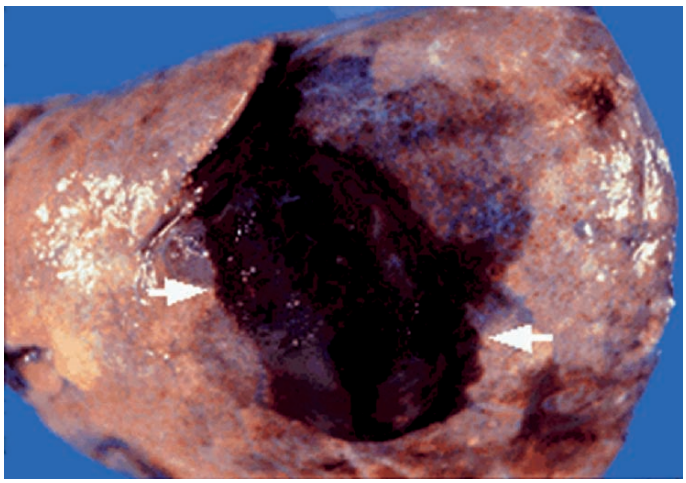


Figure 17.6.A. Hemangioma. View of the pleural surfaces of the lung. The arrows highlight a deep red brown region, which is a hemangioma. The remainder of the lung is pink and normal in appearance. Hemangiomata constitute a mass of blood vessels, which can be capillary, arterial, venous, or vessels with characteristics of both arteries and veins. Thrombosis and recanalization may be present. These tumors are congenital, they may be part of von Hippel-Lindau disease. [Reproduced from http://pathweb.uchc.edu. With permission.]

Approximately 80% of hemangiomata are located on the face and neck, with the next most prevalent location being the liver. It is estimated that 7% of healthy individuals bear hepatic hemangiomata. Females are afflicted 3–5 times more often than males, which may be due to a stimulating effect by female hormones. Hemangiomata of the adrenals, lungs, and liver may arise in von Hippel-Lindau disease.

There are three stages of development:

– In the proliferation stage (up to 12 months), the hemangioma grows quickly
– In the rest stage (between the age of 1 and 2 years), there is very little change in appearance
– in the involution stage, the hemangioma diminishes in size. Most of the lesions disappear by 10 years of age.

Lymphangiosarcoma is a rare malignant tumor, which occurs in long standing cases of primary or secondary lymphedema. It involves either the upper or the lower lymphedemateous extremities, being most common in upper extremities. The sarcoma first appears as a bruise mark, a purple discoloration, or a tender skin nodule, typically on the anterior surface. It progresses to an ulcer with crusting, and finally to an extensive necrosis involving the skin and subcataneous tissue. It metastasizes quickly.

17.7 KAPOSI SARCOMA

In 1872, the Hungarian dermatologist Moritz Kaposi published the case histories of five patients with idiopathic multiple pigmented sarcomata of the skin [Kaposi 1872]. There are four forms of Kaposi sarcoma:

– Classic (predominantly in elderly patients of Southern European, Arabic, or Jewish ancestry, median age 65 years, often an indolent disease that affects the extremities)
– Endemic (in some equatorial African countries, median age 40 years)
– Posttransplant (the incidence after renal transplant is increased about 150-fold compared to the population average)
– AIDS related (aggressive form, distribution of the lesions around the nose, mouth, and genitalia).

Immunosuppression, due to organ transplantation or old age, increases the risk for Kaposi sarcoma. The second most important risk factor for Kaposi sarcoma is HIV-1 infection, but not HIV-2 infection. In rare cases, Kaposi sarcoma can afflict the gastrointestinal tract [Zebrowska and Walsh 1991].

Etiology. The Human Herpesvirus 8 (HHV-8, Kaposi Associated Herpesvirus, KSHV) is a 165 kb double-stranded DNA virus that contributes essentially to the etiology of Kaposi sarcoma [Chang et al. 1994; Kedes et al. 1996; Gao et al. 1996]. The HHV-8 latent proteins have the ability to induce cell growth or to block apoptosis. Specifically, the viral gene product vGPCR (viral G-Protein Coupled Receptor, ORF74) is able to transform endothelial cells. Cells that express vGPCR are characterized by high PKB activity [Sodhi et al. 2004] and produce large amounts of VEGF and its receptor VEGFR-2 (KDR). This may initiate a positive feedback loop of antiapoptosis and cell cycle progression.

Molecular pathogenesis. Early Kaposi sarcoma lesions consist of a prominent inflammatory

component, blood vessels, and spindle-shaped cells. During tumor progression, the spindle cells proliferate and form the predominant cell type in advanced lesions. Individual nodules contain clonal populations of spindle cells. Kaposi sarcoma is a disseminated monoclonal cancer. The changes that permit the clonal outgrowth of spindle cells occur before the disease spreads [Rabkin et al. 1997]. The lineage origin of the spindle cells is unclear. They express markers for endothelial cells (CD31, CD34), lymphatic endothelial cells (VEGFR3, Podoplanin), smooth muscle cells, macrophages, and dendritic cells [Roth et al. 1992]. Spindle cells may be derived from pluripotent precursor cells.

The spindle cells and infiltrating inflammatory cells express high levels of IL-6, FGF-2, VEGF, TNF-α, and IFN-γ. IL-6, VEGF, and FGF-2 act as growth factors. IFN-γ induces endothelial cells to acquire the features of spindle cells. There is a strong association between the *il-6* promoter polymorphism -174G/C and susceptibility to Kaposi sarcoma in HIV-infected men. Homozygotes for the G allele, which is associated with elevated IL-6 expression, are overrepresented among patients with Kaposi sarcoma, whereas homozygotes for the C allele are underrepresented [Foster et al. 2000]. The viral gene product Kaposin B activates the $P38^{MAPK}$ signaling pathway leading to the stabilization of cytokine mRNAs [McCormick and Ganem 2005]. Tumor-infiltrating macrophages express CD169 (Sialoadhesin), which is an activation marker.

17.8 SARCOMATOID CARCINOMA

Sarcomatoid carcinomata are mixed tumors with highly variable histologic appearance that resembles both carcinomata and sarcomata. They are rare tumors that can occur in kidney, bladder, intestines, or prostate, and include mixed Müllerian tumors of the uterus. The carcinomatous and sarcomatous cells of the tumor are derived from a common clonal ancestor [Mayal et al. 1994; Thompson et al. 1996]. These tumors are likely of epithelial stem cell origin because their metastases have either carcinomatous or mixed appearance.

– Sarcomatoid carcinoma of the small intestine has a poor prognosis. It is distinguishable from primary and metastatic gastrointestinal stromal tumors by a lack of expression of CD117 (c-KIT) and CD34. In contrast to leiomyosarcoma, sarcomatoid carcinoma is generally negative for muscle-specific Actin and Desmin.
– Osteonectin is a multimodular protein component of the extracellular matrix that is implicated in tissue remodeling, occurring in neoplastic and nonneoplastic conditions. It is frequently expressed by tumor cells in pulmonary sarcomatoid carcinoma.

17.9 MYXOMA

Myxoma is a soft tumor composed of connective and mucoid tissue. It is the most common primary tumor of the heart. Cardiac myxomata are usually located in either the left or the right atrium, with about 86% being located in the left atrium. In contrast to cardiac lipomata or rhabdomyomata, myxomata are typically pedunculated, with a stalk that is attached to the interatrial septum. The most common location for attachment of the stalk is the fossa ovalis region (Figure 17.9.A).

Symptoms associated with cardiac myxomata are typically due to the mass of the tumor obstructing the normal flow of blood through the chambers of the heart. They include dyspnea, syncope (loss of conciousness), weight loss, and sudden death. Paraneoplastic symptoms may be caused by the release of IL-6 (Interleukin-6) from the tumor, which constitutes a higher risk of autoimmunity, embolism, and brain metastasis [Jourdan et al. 1990; Wada et al. 1993].

Molecular pathogenesis. Intracardiac myxoma is a solitary manifestation of mutations in the *pkaR1A* gene {17q23-q24}, which encodes a regulatory subunit of PKA (Protein Kinase A). Carney complex is a multiple neoplasia syndrome characterized by spotty skin pigmentation, cardiac and other myxomata, endocrine tumors, and psammomatous melanotic schwannomata [Carney et al. 1986]. Type 1 of Carney myxoma–endocrine complex (CNC1), linked to 17q, is caused by mutations in the *pkar1α* gene [Kirschner et al. 2000]. Type 2 (CNC2) is the designation for the form that is linked to chromosome 2.

A trismus-pseudocamptodactyly syndrome (Carney complex variant associated with distal arthrogryposis) may be based on a G2094A transition in exon 16 of the *myh8* (*myosin heavy chain 8*) gene {17p13.1}, resulting in a R674Q substitution. The arginine residue at position 674 localizes to the Actin-binding domain of the perinatal myosin head and is close to the ATP-binding site. The syndrome may lead to cardiac myxomata [Chaudron et al. 1992; Veugelers et al. 2004; Stratakis et al. 2004].

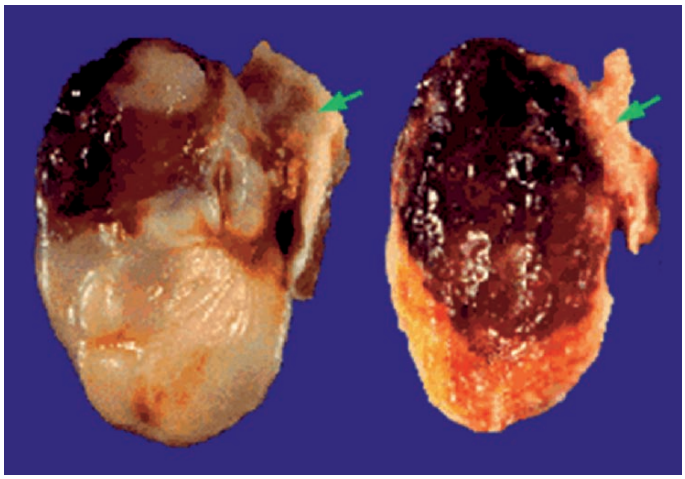


Figure 17.9.A. Myxoma. This is a bisected myxoma, measuring 4 × 2 × 2 cm, showing the surface of the tumor on the left and the cut surface on the right. Note the resected portion of the attached atrial wall (arrows), and the broad pedicle of the tumor. Shown is the typical smooth glistening surface and the variegated cut surface with hemorrhage, possibly due to surgical trauma. This benign neoplasm may clinically mimic mitral stenosis when it arises from the left atrium. Myxoma occurs in adolescence and all higher age groups. About 90% of myxomata arise in the atria with a 4:1 ratio of left/right involvement. The remainder can occur anywhere, in rare cases including the heart valves. Multiple tumors are rare. Papillary myxomata may present as emboli to a limb or a viscus. Some cases present with the paraneoplastic effects of Interleukin-6 secreted by the tumor, which include fever and malaise. [Reproduced from http://pathweb.uchc.edu. With permission.]

In Mazabraud syndrome (intramuscular myxoma associated with fibrous dysplasia) [Mazabraud et al. 1967], myxomata occur as multiple masses in about 80% of patients. They appear in close proximity to the most severely affected bone. The underlying genetic defect may be an activating point mutation in *gnas1* {20q13.2},which encodes a $G\alpha_s$ subunit.

REFERENCES

Abramson DH, Ellsworth RM, Zimmerman LE. 1976. Monocular cancer in retinoblastoma survivors. Transactions of the American Academy of Ophthalmology and Otolaryngology 81:454–457.

Alam NA, Bevan S, Churchman M, Barclay E, Barker K, Jaeger EE, Nelson HM, Healy E, Pembroke AC, Friedmann PS, Dalziel K, Calonje E, Anderson J, August PJ, Davies MG, Felix R, Munro CS, Murdoch M, Rendall J, Kennedy S, Leigh IM, Kelsell DP, Tomlinson IP, Houlston RS. 2001. Localization of a gene (MCUL1) for multiple cutaneous leiomyomata and uterine fibroids to chromosome 1q42.3-q43. American Journal of Human Genetics 68:1264–1269.

Baker SL. 1946. Hyperplastic callus simulating sarcoma in two cases of fragilitas ossium. The Journal of Pathology and Bacteriology 58:609.

Bartsch H, Barbin A, Marion MJ, Nair J, Gu Y. 1994. Formation, detection and role in carcinogenesis of ethenobases in DNA. Drug Metabolism Reviews 26:349–371.

Blansfield JA, Chung H, Sullivan TR Jr, Pezzi CM. 2003. Leiomyosarcoma of the major peripheral arteries: case report and review of the literature. Annals of Vascular Surgery 17:565–570.

Brown R, Marshall CJ, Pennie SG, Hall A. 1984. Mechanism of activation of an N-ras gene in the human fibrosarcoma cell line HT1080. EMBO Journal 3:1321–1326.

Carney JA, Headington JT, Su WPD. 1986. Cutaneous myxomas: a major component of the complex of myxomas, spotty pigmentation, and endocrine overactivity. Archives of Dermatology 122:790–798.

Carney JA, Sheps SG, Go VLW, Gordon H. 1977. The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. The New England Journal of Medicine 296:1517–1518.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994. Identification of herpes-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869.

Chaudron JM, Jacques JM, Heller FR, Cheron P, Luwaert R. 1992. The myxoma syndrome: an unusual entity: a family study. European Heart Journal 13:569–573.

Chauveinc L, Mosseri V, Quintana E, Desjardins L, Schlienger P, Doz F, Dutrillaux B. 2001. Osteosarcoma following retinoblastoma: age at onset and latency period. Ophthalmic Genetics 22:77–88.

Clark J, Benjamin H, Gill S, Sidhar S, Goodwin G, Crew J, Gusterson BA, Shipley J, Cooper CS. 1996. Fusion of the EWS gene to CHN, a member of the steroid/thyroid receptor gene superfamily, in a human myxoid chondrosarcoma. Oncogene 12:229–235.

Creech JL, Johnson MN. 1974. Angiosarcoma of liver in the manufacture of polyvinyl chloride. Journal of Occupational Medicine 16:150–151.

Crozat A, Aman P, Mandahl N, Ron D. 1993. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640–644.

Daughaday WH, Emanuele MA, Brooks MH, Barbato AL, Kapadia M, Rotwein P. 1988. Synthesis and secretion of insulin-like growth factor II by a leiomyosarcoma with associated hypoglycemia. New England Journal of Medicine 319:1434–1440.

Dean M, Park M, Vande Woude GF. 1987. Characterization of the rearranged TPR-MET oncogene breakpoint. Molecular and Cellular Biology 7:921–924.

Douglass EC, Valentine M, Etcubanas E, Parham D, Webber BL, Houghton PJ, Green AA. 1987. A specific chromosomal abnormality in rhabdomyosarcoma. Cytogenetics and Cell Genetics 45:148–155.

Ewing J. 1921. Diffuse endothelioma of bone. Proceedings of the New York Pathology Society 21:17–24.

Foster CB, Lehrnbecher T, Samuels S, Stein S, Mol F, Metcalf JA, Wyvill K, Steinberg SM, Kovacs J, Blauvelt A, Yarchoan R, Chanock SJ. 2000. An IL6 promoter polymorphism is associ-

ated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 96:2562–2567.

Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo CR, Saah A, Phair J, Detels R, Chang Y, Moore PS. 1996. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nature Medicine 2:925–928.

Gill S, Stratton MR, Patterson H, Spurr NK, Fisher C, Gusterson BA, Cooper CS. 1991. Detection of transforming genes by transfection of DNA from primary soft-tissue tumours. Oncogene 6:1651–1656.

Gupta S, Plattner R, Der CJ, Stanbridge EJ. 2000. Dissection of Ras-dependent signaling pathways controlling aggressive tumor growth of human fibrosarcoma cells: evidence for a potential novel pathway. Molecular and Cellular Biology 20:9294–9306.

Hahn H, Wojnowski L, Zimmer AM, Hall J, Miller G, Zimmer A. 1998. Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. Nature Medicine 4:619–622.

Hall CL, Yang B, Yang X, Zhang S, Turley M, Samuel S, Lange LA, Wang C, Curpen GD, Savani RC, Greenberg AH, Turley EA. 1995. Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for H-ras transformation. Cell 82:19–26.

Hiddemann W, Roessner A, Wormann B, Mellin W, Klockenkemper B, Bosing T, Buchner T, Grundmann E. 1987. Tumor heterogeneity in osteosarcoma as identified by flow cytometry. Cancer 59:324–328.

Hisaoka M, Okamoto S, Koyama S, Ishida T, Imamura T, Kanda H, Kameya T, Meis-Kindblom JM, Kindblom LG, Hashimoto H. 2003. Microtubule-associated protein-2 and class III β-tubulin are expressed in extraskeletal myxoid chondrosarcoma. Modern Pathology 16:453–459.

Hollstein M, Marion M-J, Lehman T, Welsh J, Harris CC, Martel-Planche G, Kusters I, Montesano R. 1994. p53 mutations at A:T base pairs in angiosarcomas of vinyl chloride-exposed factory workers. Carcinogenesis 15:1–3.

Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, Bell RS, Juppner H, Andrulis IL, Wunder JS, Alman BA. 2002. A mutant PTH/PTHrP type I receptor in enchondromatosis. Nature Genetics 30:306–310.

Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RGH, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. 2000. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nature Genetics 24:45–48.

Hussien M, Sivananthan S, Anderson N, Shiels A, Tracey N, Odling-Smee GW. 2001. Primary leiomyosarcoma of the breast: diagnosis, management and outcome. A report of a new case and review of literature. Breast 10:530–534.

Jackson D, Volpert OV, Bouck N, Linzer DI. 1994. Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. Science 266:1581–1584.

Jagasia AA, Block JA, Diaz MO, Nobori T, Gitelis S, Inerot SE, Iyer AP. 1996. Partial deletions of the CDKN2 and MTS2 putative tumor suppressor genes in a myxoid chondrosarcoma. Cancer Letters 105:77–90.

Jourdan M, Bataille R, Seguin J, Zhang XG, Chaptal PA, Klein B. 1990. Constitutive production of interleukin-6 and immunologic features in cardiac myxomas. Arthritis and Rheumatism 33:398–402.

Kaposi M. 1872. Idiopatisches multiples Pigmentsarcom der Haut. Archiv für Dermatologie und Syphillis 4:265–273.

Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. 1996. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nature Medicine 2:918–924.

Khanna C, Khan J, Nguyen P, Prehn J, Caylor J, Yeung C, Trepel J, Meltzer P, Helman L. 2001. Metastasis-associated differences in gene expression in a murine model of osteosarcoma. Cancer Research 61:3750–3759.

Khanna C, Wan X, Bose S, Cassaday R, Olomu O, Mendoza A, Yeung C, Gorlick R, Hewitt SM, Helman LJ. 2004. The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. Nature Medicine 10:182–186.

Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA. 2000. Mutations of the gene encoding the protein kinase A type I-α regulatory subunit in patients with the Carney complex. Nature Genetics 26:89–92.

Klenerman L, Ockenden BG, Townsend AC. 1967. Osteosarcoma occurring in osteogenesis imperfecta. Report of two cases. The Journal of Bone and Joint Surgery. British Volume 49:314–323.

Kloepfer HW, Krafchuk J, Derbes V, Burks J. 1958. Hereditary multiple leiomyoma of the skin. American Journal of Human Genetics 10:48–52.

Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PH. 1998. A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nature Genetics 18:184–187.

Kreicbergs A, Silvfersward C, Tribukait B. 1984. Flow DNA analysis of primary bone tumors: Relationship between cellular DNA content and histopathologic classification. Cancer 53:129–136.

Kris RM, Avivi A, Bar-Eli M, Alon Y, Carmi P, Schlessinger J, Raz A. 1985. Expression of ki-ras oncogene in tumor cell variants exhibiting different metastatic capabilities. International Journal of Cancer 35:227–230.

Launonen V, Vierimaa O, Kiuru M, Isola J, Roth S, Pukkala E, Sistonen P, Herva R, Aaltonen LA. 2001. Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proceedings of the National Academy of Sciences USA 98:3387–3392.

Li FP, Fraumeni JF. 1969. Rhabdomyosarcoma in children: an epidemiologic study and identification of a familial cancer syndrome. The Journal of the National Cancer Institute 43:1364–1373.

Li FP, Fraumeni JF Jr. 1969. Soft-tissue sarcomas, breast cancer, and other neoplasms: a familial syndrome? Annals of Internal Medicine 71:747–752.

Loh WE Jr, Scrable HJ, Livanos E, Arboleda MJ, Cavenee WK, Oshimura M, Weissman BE. 1992. Human chromosome 11 contains two different growth suppressor genes for embryonal rhabdomyosarcoma. Proceedings of the National Academy of Sciences USA 89:1755–1759.

Lou Z, O'Reilly S, Liang H, Maher VM, Sleight SD, McCormick JJ. 2005. Down-regulation of overexpressed sp1 protein in human fibrosarcoma cell lines inhibits tumor formation. Cancer Research 65:1007–1017.

Marion M-J, Froment O, Trépo C. 1991. Activation of Ki-ras gene by point mutation in human liver angiosarcoma associated with vinyl chloride exposure. Molecular Carcinogenesis 4:450–454.

Mayal F, Rutty K, Campbell F, Goddard H. 1994. p53 immunostaining suggests that carcinosarcomas are monoclonal. Histopathology 24:211–214.

Mazabraud A, Semat P, Roze R. 1967. A propos de l'association de fibromyxomes des tissues mous a la dysplasie fibreuse des os. Presse Médicale 75:2223–2228.

McCormick C, Ganem D. 2005. The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. Science 307:739–741.

McGary EC, Heimberger A, Mills L, Weber K, Thomas GW, Shtivelband M, Lev DC, Bar-Eli M. 2003. A fully human antimelanoma cellular adhesion molecule/MUC18 antibody inhibits spontaneous pulmonary metastasis of osteosarcoma cells in vivo. Clinical Cancer Research 9:6560–6566.

Mori K, Chano T, Matsumoto K, Ishizawa M, Matsusue Y, Okabe H. 2004. Type-selective muscular degeneration promotes infiltrative growth of intramuscular lipoma. BMC Musculoskeletal Disorders 5:20.

Mulvihill JJ, Gralnick HR, Whang-Peng J, Leventhal BG. 1977. Multiple childhood osteosarcomas in an American Indian family with erythroid macrocytosis and skeletal anomalies. Cancer 40:3115–3122.

Nellissery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ, Hansen MF. 1998. Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget disease of bone. American Journal of Human Genetics 63:817–824.

Ohkura N, Ito M, Tsukada T, Sasaki K, Yamaguchi K, Miki K. 1996. Structure, mapping and expression of a human NOR-1 gene, the third member of the Nur77/NGFI-B family. Biochimica et Biophysica Acta 1308:205–214.

Panagopoulos I, Lassen C, Isaksson M, Mitelman F, Mandahl N, Aman P. 1997. Characteristic sequence motifs at the breakpoints of the hybrid genes FUS/CHOP, EWS/CHOP and FUS/ERG in myxoid liposarcoma and acute myeloid leukemia. Oncogene 15:1357–1362.

Patino-Garcia A, Pineiro ES, Diez MZ, Iturriagagoitia LG, Klussmann FA, Ariznabarreta LS. 2003. Genetic and epigenetic alterations of the cell cycle regulators and tumor suppressor genes in pediatric osteosarcomas. Journal of Pediatric Hematology and Oncology 25:362–367.

Peter M, Couturier J, Pacquement H, Michon J, Thomas G, Magdelenat H, Delattre O. 1997. A new member of the ETS family fused to EWS in Ewing tumors. Oncogene 14:1159–1164.

Rabbitts TH, Forster A, Larson R, Nathan P. 1993. Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. Nature Genetics 4:175–180.

Rabkin CS, Janz S, Lash A, Coleman AE, Musaba E, Liotta L, Biggar RJ, Zhuang Z. 1997. Monoclonal origin of multicentric Kaposi's sarcoma lesions. The New England Journal of Medicine 336:988–993.

Rennel E, Cross MJ, Klint P, Bai X, Arbiser JL, Gerwins P. 2003. Regulation of endothelial cell differentiation and transformation by H-Ras. Experimental Cell Research 291:189–200.

Roth WK, Brandstetter H, Sturtzl M. 1992. Cellular and molecular features of HIV-associated Kaposi's sarcoma. AIDS 6:895–913.

Sandberg AA, Bridge JA. 2003. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: chondrosarcoma and other cartilaginous neoplasms. Cancer Genetics and Cytogenetics 143:1–31.

Sanders RP, Drissi R, Billups CA, Daw NC, Valentine MB, Dome JS. 2004. Telomerase expression predicts unfavorable outcome in osteosarcoma. The Journal of Clinical Oncology 22:3790–3797.

Sandruck J, Escobar P, Lurain J, Fishman D. 2004. Uterine leiomyosarcoma metastatic to the sphenoid sinus: a case report and review of the literature. Gynecologic Oncology 92: 701–704.

Sharp R, Recio JA, Jhappan C, Otsuka T, Liu S, Yu Y, Liu W, Anver M, Navid F, Helman LJ, DePinho RA, Merlino G. 2002. Synergism between INK4a/ARF inactivation and aberrant HGF/SF signaling in rhabdomyosarcomagenesis. Nature Medicine 8:1276–1280.

Sjogren H, Meis-Kindblom JM, Orndal C, Bergh P, Ptaszynski K, Aman P, Kindblom LG, Stenman G. 2003. Studies on the molecular pathogenesis of extraskeletal myxoid chondrosarcoma-cytogenetic, molecular genetic, and cDNA microarray analyses. American Journal of Pathology 162:781–92.

Sodhi A, Montaner S, Gomez-Roman JJ, Li Y, Sausville EA, Sawai ET, Gutkind JS. 2004. Akt plays a central role in sarcomagenesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. Proceedings of the National Academy of Sciences USA 101:4821–4826.

Stratakis CA, Bertherat J, Carney JA. 2004. Mutation of perinatal myosin heavy chain. The New England Journal of Medicine 351:2556.

Sun T-C, Swee RG, Shives TC, Unni KK. 1985. Chondrosarcoma in Maffucci's syndrome. The Journal of Bone and Joint Surgery 67A:1214–1219.

Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, Milenkovic L, Scott MP, Beachy PA. 2000. Effects of oncogenic mutations in smoothened and patched can be reversed by cyclopamine. Nature 406:1005–1009.

Thomas DM, Carty SA, Piscopo DM, Lee JS, Wang WF, Forrester WC, Hinds PW. 2001. The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. Molecular Cell 8:303–316.

Thompson L, Chang B, Barsky SH. 1996. Monoclonal origin of malignant mixed tumors (carcinosarcomas): evidence for a divergent histogenesis. American Journal of Surgical Pathology 20:277–285.

Toft DJ, Rosenberg SB, Bergers G, Volpert O, Linzer DI. 2001. Reactivation of proliferin gene expression is associated with increased angiogenesis in a cell culture model of fibrosarcoma tumor progression. Proceedings of the National Academy of Sciences USA 98:13055–13059.

Tognon C, Garnett M, Kenward E, Kay R, Morrison K, Sorensen PH. 2001. The chimeric protein tyrosine kinase ETV6-NTRK3 requires both Ras-Erk1/2 and PI3-kinase-Akt signaling for fibroblast transformation. Cancer Research 61:8909–8916.

Toguchida J, Ishizaki K, Sasaki MS, Nakamura Y, Ikenaga M, Kato M, Sugimot M, Kotoura Y, Yamamuro T. 1989. Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 338:156–158.

Tomlinson IPM, Alam NA, Rowan AJ, Barclay E, Jaeger EEM, Kelsell D, Leigh I, Gorman P, Lamlum H, Rahman S, Roylance RR, Olpin S, Bevan S, Barker K, Hearle N, Houlston RS, Kiuru M, Lehtonen R, Karhu A, Vilkki S, Laiho P, Eklund C, Vierimaa O, Aittomäki K, Hietala M, Sistonen P, Paetau A, Salovaara R, Herva R, Launonen V, Aaltonen LA. 2002.

Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nature Genetics 30:406–410.

Trieb K, Lehner R, Stulnig T, Sulzbacher I, Shroyer KR. 2003. Survivin expression in human osteosarcoma is a marker for survival. European Journal of Surgical Oncology 29: 379–382.

Turc-Carel C, Philip I, Berger M-P, Philip T, Lenoir GM. 1984. Chromosome study of Ewing's sarcoma (ES) cell lines: consistency of a reciprocal translocation t(11;22)(q24;q12). Cancer Genetics and Cytogenetics 12:1–19.

Van Beveren C, van Straaten F, Curran T, Muller R, Verma IM. 1983. Analysis of FBJ-MuSV provirus and c-fos (mouse) gene reveals that viral and cellular fos gene products have different carboxy termini. Cell 32:1241–1255.

Veugelers M, Bressan M, McDermott DA, Weremowicz S, Morton CC, Mabry CC, Lefaivre J-F, Zunamon A, Destree A, Chaudron J-M, Basson CT. 2004. Mutation of perinatal myosin heavy chain associated with a Carney complex variant. The New England Journal of Medicine 351:460–469.

Wada A, Kanda T, Hayashi R, Imai S, Suzuki T, Murata K. 1993. Cardiac myxoma metastasized to the brain: potential role of endogenous interleukin-6. Cardiology 83:208–211.

Weber GF, Bronson RT, Ilagan J, Cantor H, Schmits R, Mak TW. 2002. Absence of the CD44 gene prevents sarcoma metastasis. Cancer Research 62:2281–2286.

Wei M-H, Toure O, Glenn GM, Pithukpakorn M, Neckers L, Stolle C, Choyke P, Grubb R, Middelton L, Turner ML, Walther MM, Merino MJ, Zbar B, Linehan WM, Toro JR. 2006. Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. Journal of Medical Genetics 43:18–27.

Weihrauch M, Markwarth A, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. 2002. Abnormalities of the ARF-p53 pathway in primary angiosarcomas of the liver. Human Pathology 33:884–892.

Zebrowska G, Walsh NM. 1991. Human immunodeficiency virus-related Kaposi's sarcoma of the appendix and acute appendicitis. Report of a case and review of the literature. Archives of Pathology and Laboratory Medicine 115:1157–1160.

CHAPTER 18

NEUROLOGIC TUMORS

18.1 MEDULLOBLASTOMA

Medulloblastoma [Bailey and Cushing 1925] is the most common malignant tumor in childhood, accounting for 10–20% of primary central nervous system neoplasms and approximately 40% of all posterior fossa tumors. It arises mostly in the fourth ventricle, between the brain stem and the cerebellum. Medulloblastomata are derived from neurons of the central nervous system. They have the tendency to spread to other sites in the nervous system and, infrequently, to other organs. Common symptoms are caused by cerebellar damage (unsteadiness, dysmetria), hydrocephalus (headache, vomiting), or leptomeningeal dissemination (severe weakness, neck stiffness).

Risk group stratification is based on age, extent of postoperative residual disease, and the metastasis stage (M stage, derived from the Chang system with M0 = no gross subarachnoid or hematogenous metastasis, M1 = microscopic detection of tumor cells in the cerebrospinal fluid, M2 = gross nodular seeding in the cerebellum, the cerebral subarachnoid space, or in the third or fourth ventricles, M3 = gross nodular seeding in the spinal subarachnoid space, and M4 = extraneuraxial metastasis).

– Average risk disease: patients older than 3 years, who are at stage M0 with less than 1.5 cm^2 of residual tumor postoperatively (5-year survival rate 78%).
– Poor risk disease: patients older than 3 years, who are at stage M1–M4 or have more than 1.5 cm^2 of residual tumor postoperatively (5-year survival rate 30–55%).
– Infants: patients younger than 3 years, having the worst prognosis regardless of M stage and extent of postoperative residual disease (5-year survival rate 30%).

Medulloblastomata span a histologic spectrum that comprises overtly malignant anaplastic cell lesions characterized by increased nuclear size, marked cytological anaplasia, and increased mitotic and apoptotic rates.

– Desmoplastic medulloblastoma more commonly afflicts adults and is typically located in the cerebellar hemisphere. This form contains a dense Reticulin network, where cells are arranged in a bimodal pattern with areas of high and low cellularity.
– Medullomyoblastoma contains striated and smooth muscle cells. Some cells may display neuronal or glial differentiation.
– Melanotic medulloblastoma is a very rare form that is formed by small undifferentiated cells containing Melanin.
– Large-cell medulloblastoma has large vesicular nuclei with prominent nucleoli. The cells express high levels of Synaptophysin. The prognosis is poor.

Molecular pathogenesis. Multiple genetic defects can underlie this cancer (Figure 18.1.A)

– Many of the genes overexpressed in metastatic medulloblastoma are associated with signal transduction from the Platelet-Derived Growth Factor Receptor (PDGFR). PDGFRA and PDGFRB are often overexpressed [Smits et al. 1996]. PDGFRA increases the phosphorylation of MAPKK1 and MAPKK2, as well as their downstream kinases

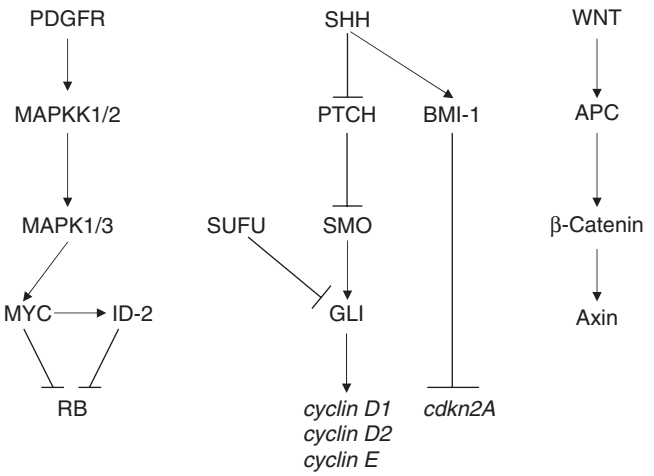


Figure 18.1.A. Molecular pathways to medulloblastoma. Loss of control over growth factor pathways associated with PDGF, SHH, or WNT may induce transformation to medulloblastoma. The defects may arise at various levels within these pathways.

MAPK1 and MAPK3 [MacDonald et al. 2001]. This induces a transforming pathway.

– MYC proteins circumvent the block on cell cycle progression imposed by the RB pathway. In medulloblastomata, *N-myc* is overexpressed, but not typically amplified. Neuroblastoma cells carrying high levels of N-MYC overexpress ID-2 and constitutively bypass the cell cycle checkpoint imposed by RB. The ID proteins (Inhibitor of DNA Binding, Inhibitor of Differentiation) act as dominant negative regulators of transcription factors within the basic helix-loop-helix family, because ID proteins contain a helix-loop-helix dimerization domain but lack a basic DNA-binding domain. The ID family comprises four members, ID-1, ID-2, ID-3, and ID-4. ID-1 is expressed at high levels in medulloblastoma, but not in normal cerebellum or lower grade astrocytoma [Lyden et al. 1999].
– The cells of origin for medulloblastoma derive from the external granular layer of the cerebellum. The proliferation of precursor neurons in this layer is controlled by SHH and its receptor PTCH. SHH-associated signaling regulates the levels of *cyclin* D_1, *cyclin* D_2, and *cyclin E* mRNA transcripts and proteins. Also, BMI-1 is rapidly induced by SHH and plays a crucial role in the clonal expansion of cerebellar granule cell precursors. SHH is dysregulated [Goodrich et al. 1997], or its receptor PTCH is mutated [Raffel et al. 1997] in distinct subsets of sporadic medulloblastomata. Linked overexpression of BMI-1 and PTCH occurs in a substantial fraction of primary medulloblastomata [Leung et al. 2004]. Although GLI is activated as a downstream target of SHH, it is rarely mutated in medulloblastoma [Erez et al. 2002]. SUFU (Suppressor of Fused) {10q24–q25} is a component of the SHH signaling pathway. Medulloblastomata with truncating mutations in SUFU may be of the desmoplastic subtype [Taylor et al. 2002]. Desmoplastic tumors, which make up about 20–30% of medulloblastomata, have a more nodular architecture than classical medulloblastomata and may have a better prognosis.
– β-Catenin can function in the decision of neural precursor cells to proliferate or differentiate during neuronal development. The genes of the pathway WNT→APC→β-Catenin are associated with the risk for developing medulloblastoma. Mutations of *apc* or *β-catenin*, but not both, occur in this tumor [Huang et al. 2000]. While mutations of *apc* are rare, a hot spot region of *β-catenin* (*ctnnb1*) mutations is associated with a subset of medulloblastomata. Point mutations and deletions in *axin-1* may also arise.

TRK-C (NTRK3, Neurotrophin-3 Receptor) is preferentially expressed in the hippocampus, cerebral cortex, and the granular cell layer of the cerebellum. Medulloblastomata express Neurotrophin-3 and its receptor TRK-C. The level of TRK-C is highly variable. Patients with tumors expressing high levels of *trk-C* mRNA have significantly longer intervals without disease progression than those with low levels and a more favorable overall survival [Segal et al. 1994].

Genetic predisposition. Medulloblastoma can occur as part of Turcot syndrome. This syndrome is a rare, autosomal recessive disorder, characterized by the development of primary neuroepithelial tumors of the central nervous system and numerous adenomatous colorectal polyps. Patients with Turcot

syndrome, who develop medulloblastomata, harbor germline *apc* mutations [Mori et al. 1994].

Somatic mutations in the *ptch2* gene {1p32} underlie basal cell carcinoma and medulloblastoma, both of which are features of the nevoid basal cell carcinoma syndrome (Gorlin–Goltz syndrome) [Evans et al. 1991]. Basal cell carcinomata and jaw cysts occur in more than 90% of patients by the age of 40 years. Additional complications include ovarian calcification or fibroma (24%), medulloblastoma (5%), cardiac fibroma (3%), cleft palate (5%), and ophthalmic abnormalities such as squint or cataract (26%) [Evans et al. 1993].

Medulloblastoma may arise on the basis of Nijmegen breakage syndrome, an inherited chromosome instability disorder. Afflicted patients are characterized by an adverse response to radiation treatment.

18.2 ASTROCYTOMA

Glia is sustentacular tissue that surrounds and supports neurons in the central nervous system. Glial cells retain their proliferative capacity throughout life. Consistently, most neurologic tumors are of glial lineage origin. Gliomata comprise astrocytomata, ependymomata, and oligodendrogliomata.

Astrocytomata are tumors of astrocytes or their glia-restricted precursor cells (Figure 18.2.A). They may arise in the cerebral hemispheres, in the posterior fossa, in the optic nerve, and rarely in the spinal cord. In almost 50% of cases, the initial clinical manifestation of an astrocytoma is a focal or generalized seizure. 60–75% of patients have recurrent seizures in the course of the illness. The symptoms of increased intracranial pressure, including headaches and vomiting, usually present late in the disease. In children, the tumor is usually located in the cerebellum, and therefore causes gait instability and unilateral ataxia.

A grading system is used to categorize astrocytomata:
- Grade 1: pilocytic astrocytoma (primarily a pediatric tumor with median age of diagnosis at 12 years) is associated with a loss of *nf1*.
- Grade 2: diffuse astrocytoma.
- Grade 3: anaplastic (malignant) astrocytoma. Over 60% of higher grade astrocytomata have loss of heterozygosity on 17p, including the *p53*

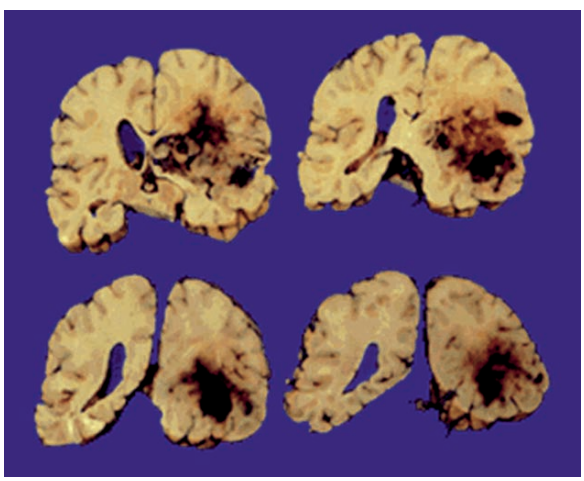


Figure 18.2.A. Glioblastoma. Multiple coronal sections of brain show a large hemorrhagic tumor in the right parietal and occipital lobes with marked mass effect. These finding are characteristic of glioblastoma multiforme, the most common glioma and also the most malignant. Glioblastoma multiforme has a multiform or variable appearance with evidence of old and recent hemorrhage, necrosis, and areas of firm tissue. It usually comprises a mass lesion involving a focal area, although it may cross the corpus callosum to the other hemisphere or be multifocal. Glioblastomata account for 50% of all gliomata and arise after the age of 50 years in most patients. Younger patients tend to have a better prognosis than the elderly. The clinical appearance of the glioblastoma is typical for brain tumors in general with a slowly progressive neurological deficit of a focal nature, that is, a slowly progressive hemiparesis. The average life span after diagnosis is 6 months to 1 year. There are several mutations in proto-oncogenes and tumor suppressor genes. Some appear as a part of several hereditary syndromes, such as neurofibromatosis or Turcot syndrome. Changes or loss of chromosome 17 and inactivation of p53 play a role. [Reproduced from http://pathweb.uchc.edu. With permission.]

locus, and the retained *p53* allele is mutated in the majority of cases.
– Grade 4: glioblastoma multiforme (most common form of astrocytoma). In malignant glioblastoma multiforme, Vimentin, Fibronectin, Tenascin-C, Collagen type IV α1 chain, Phospholipase A2 Receptor, Laminin α4 chain, Keratin-18, Desmoplakin, and Tropomodulin are overexpressed.

Molecular pathogenesis. Based on clinical and pathogenetic characteristics, grade IV astrocytoma can be divided into primary and secondary glioblastoma multiforme. Although these forms are generated by distinct genetic defects, the affected pathways in both forms of glioblastoma multiforme are identical, including P53, RB, and receptor tyrosine kinases.

Primary glioblastoma multiforme (grade IV astrocytoma) occurs as a consequence of
– Dysregulation of P53 function by amplification of *mdm2*.
– Disruption of the RB and P53 pathways through loss of *cdkn2A*. Deletions of this locus, which encodes $P16^{INK4a}$ and $P14^{ARF}$, are much more frequent in primary than in secondary glioblastoma [Biernat et al. 1997].
– Enhanced growth factor signaling through amplification or mutation of *egfr* [Libermann et al. 1985]. The expression of the mutant EGFR vIII in glioblastoma activates two pathways, the GRB→SOS→RAS→ ERK pathway and the PI 3-K→PKB→mTOR pathway, both of which converge in the activation of $P70^{Ribosomal\ S6\ Kinase}$ [Choe et al. 2003].
– EGFR signals through PKC. Malignant gliomata express higher levels of PKCα and lower levels of PKCδ than low-grade astrocytomata. PKCα induces enhanced proliferation and reduced expression of Glial Fibrillary Acetic Protein (GFAP), conversely PKCδ suppresses proliferation and induces the expression of GFAP. GFAP is a marker of astrocyte differentiation.
– Loss of *pten* [Wang et al. 1997]. Loss of the long arm of chromosome 10, which contains *pten*, is the most common genetic alteration associated with glioblastoma multiforme, affecting about 70% of all cases. This enhances the activity of the antiapoptotic PKB pathway. It also deprives the tumors of a potent angiogenesis suppressor.

In secondary glioblastoma multiforme, loss of *p53* and activation of the growth factor receptor→ tyrosine kinase→RAS signaling pathway initiate tumor formation.
– Secondary glioblastomata generally have no wild-type P53 due to loss of one allele and mutation of the retained allele. They lose their functional RB1 pathway in a similar manner, which contributes to the progression of tumor development [Zhu and Parada 2002].
– PDGF and PDGFR are often coexpressed on astrocytomata.
– The RAS pathway is upregulated not by *ras* mutations, but through the upregulation of upstream signaling or through the loss of function of NF1.
– During the progression of secondary glioblastoma multiforme, *cdk4* may be amplified.

Chromosome instability. ROS is a receptor tyrosine kinase. The fusion protein FIG–ROS may cause glioblastoma. A microdeletion on 6q21 results in the fusion of *fig*, a gene coding for a protein associated with the Golgi apparatus, to the kinase domain of the proto-oncogene *c-ros*. FIG acts to localize the tyrosine kinase portion of the plasma membrane protein ROS to the Golgi apparatus, where it exerts its transforming potential [Charest et al. 2003].

DNA maintenance and repair. Aberrant Histone acetylation, caused by the disruption of Histone Acetyl Transferase or Histone Deacetylase activity, may be associated with the development of cancer. Two closely related Histone Acetyl Transferase, CBP, and P300, are altered in some tumors by either mutation or translocation. Loss of heterozygosity of *p300* arises in 80% of glioblastomata.

While *mgmt* (*methylguanine DNA methyl transferase*) {10q26} is rarely mutated in cancer, low levels of the MGMT protein are associated with glioblastoma. This may reflect reduced transcription due to altered gene methylation.

Tumor progression. Two forms of NEGF-1, of sizes 15 and 18 kD, differentially promote glioblastoma migration and proliferation [Lu et al. 2005]. ALK (Anaplastic Lymphoma Kinase) is a tyrosine kinase receptor for NEGF-1. ALK is overexpressed in glioblastoma and its associated signaling is a rate-limiting factor in the growth of glioblastoma multiforme [Powers et al. 2002]. NEGF-1 (Pleiotrophin, PTN, HBGF-8, OSF-1) may also ligate the receptor RPTPζ to induce cell migration. Both NEGF-1 and RPTPζ are overexpressed in glioblastomata and in astrocytomata, suggesting the existence of an autocrine loop for dissemination [Ulbricht et al. 2003].

Life span extension. Malignant tumors overcome replicative senescence. Normal astrocytes express $P57^{KIP2}$. In contrast, this protein is absent from astrocytomata, although the gene does not contain mutations. *p57KIP2* gene expression is silenced in these cancers.

ID-1 is expressed at high levels in glioblastoma multiforme, but not in normal cerebellum or lower grade astrocytomata [Lyden et al. 1999].

Transformed precursor cells, which resemble stem cells, represent the fraction of tumor cells that drive growth. Similar to normal neural stem cells, these tumor-initiating precursors in the brain comprise a small fraction of glioblastoma cells. They belong to a $CD133^{+}$ pool that displays self-renewal, are multipotent, and generate a large number of progeny. These cells can perpetuate glioblastomata. BMPs (Bone Morphogenetic Proteins), specifically the CD133 ligand BMP-4, elicit the acquisition of an astroglial fate in the adult brain stem cell niche. BMP-4 supports the differentiation of the transformed precursor cells and suppresses glioblastoma growth [Piccirillo and Vescovi 2006].

Genetic predisposition. Subependymal giant cell astrocytomata, occurring as part of the tuberous sclerosis complex (TSC), display a loss of Tuberin expression due to germline mutations in the *tsc2* gene. Tuberin functions as an inactivator of the small G-Protein RAP1B (RAP1-GAP) and loss of its expression could lead to increased RAP1B activity with ensuing augmentation of $P21^{RAS}$-mediated signals.

Turcot syndrome is characterized clinically by the concurrence of a primary brain tumor and multiple colorectal adenomata. Patients with glioblastoma multiforme and colorectal tumors have replication errors characteristic of hereditary nonpolyposis colorectal cancer. They are caused germline mutations in the mismatch repair genes *mlh1* or *pms2* [Hamilton et al. 1995].

18.3 EPENDYMOMA

Ependymomata [Bailey 1924] are derived from ependymal cells. Intracranial ependymomata form intraventricular masses with frequent extension into the subarachnoid space. Spinal ependymomata present as intramedullary masses arising from the central canal, as myxopapillary tumors, or as metastases from an intracranial origin. Ependymomata account for 60% of primary neoplasms in the spinal cord and filum terminale [Slooff 1964].

The World Health Organization classification scheme includes
- Grade I, myxopapillary ependymoma (occurring almost exclusively in the region of the cauda equina) and subependymoma (uncommon benign lesion).
- Grade II, ependymoma (with cellular, papillary, and clear cell variants).
- Grade III, anaplastic ependymoma.

Subependymomata [Scheinker 1945] arise from the bipotential subependymal cells, which normally differentiate into either ependymal cells or astrocytes. They tend to be slow growing, noninvasive, and located in the ventricular system, the septum pellucidum, the cerebral aqueduct, or the proximal spinal cord. Ependymoblastomata are primitive neuroectodermal tumors (PNETs) and are distinct from ependymomata. Oligodendrogliomata are tumors of oligodendrocytes.

Molecular pathogenesis. A high frequency of aberrant 5′ CpG island methylation occurs of the *mgmt*, *gstp1*, $p14^{ARF}$, *thbs1*, *timp3*, and *tp73* genes. These aberrations are likely to arise early in the carcinogenesis process, because they are already present in the low-grade forms. However, the hypermethylation of the promoters for *dapk*, *thbs1*, and *tp73* may be associated with the genesis of anaplastic forms [Alonso et al. 2003].

Chromosome breaks may be features of ependymomata. Consistent genetic defects associated with these tumors include a loss of loci on chromosome 22, a recurring breakpoint at chromosome 11q13, and abnormal karyotypes that involve chromosomes 6 or 16. Chromosome 17 may be rearranged or lost.

Genetic predisposition. Familial clustering of ependymomata can occur. It is often associated with allelic loss of chromosome 22 [Gilchrist and Savard 1989; Yokota et al. 2003]. This suggests the presence of an ependymoma tumor suppressor gene in the region 22pter–22q11.2 of chromosome 22.

18.4 MENINGIOMA

Meningiomata are generally slowly growing benign tumors of the leptomeninges, derived from the meningothelial (arachnoidal) cap cells. They are usually solitary lobulated tumors attached to the dura (Figure 18.4.A). Meningiomata are probably the most common primary tumors of the central

nervous system. Exposure to ionizing radiation is a predisposing factor.

– Meningiomata are graded as malignancy grade I.
– Atypical meningiomata and certain rare histological variants (clear cell, chordoid, papillary, and rhabdoid) are malignancy grade II.
– Anaplastic meningiomata, displaying a more aggressive behavior, being associated with a high risk of local recurrence, and having a less favorable prognosis, are grade III.

Intraspinal clear cell meningiomata are rare morphological variants of meningioma. They usually have aggressive behavior with local recurrence in slightly more than half of all patients [Liu et al. 2005].

Molecular pathology. Consistent chromosomal abnormalities occurring in meningiomata include the loss of one copy of chromosome 22. This affects the tumor suppressor gene *nf2* (*merlin, schwannomin*). Other members of the protein 4.1 superfamily, which beside NF2 contains Protein 4.IB (DAL-1) and Protein 4.1R, may also be linked to meningioma initiation. The genetic alterations in atypical meningiomata are complex and involve losses on 1p, 6q, 10, 14q and 18q, as well as gains on multiple chromosomes. Anaplastic meningiomata show even more complex genetic alterations, including frequent alteration of the *cdkn2A*, *cdkn2B*, and $p14^{ARF}$ tumor suppressor genes at 9p21, as well as gene amplification on 17q23.

A balanced translocation t(4;22) can arise in meningioma. It disrupts the open reading frame of the gene *mn1* (*mgcr1*) {22q12.3–qter}, leading to a lack of MN1 expression. If this translocation is present in the germline it can be associated with multiple meningiomata [Lekanne Deprez et al. 1995].

Tumor progression. Meningiomata generally expand and displace, but do not invade adjacent brain or spinal chord. Invasion of the dura and skull does occur and may elicit an osteoblastic reaction, but it has no significance for malignancy grading. When brain invasion takes place it is associated with a greater likelihood of recurrence. Mutations in *pten* are not involved in the formation of low-grade meningioma, but may contribute to malignant progression in a fraction of anaplastic meningiomata [Peters et al. 1998].

Genetic predisposition. The vast majority of meningiomata are sporadic. Familial occurrence of meningioma is rare.

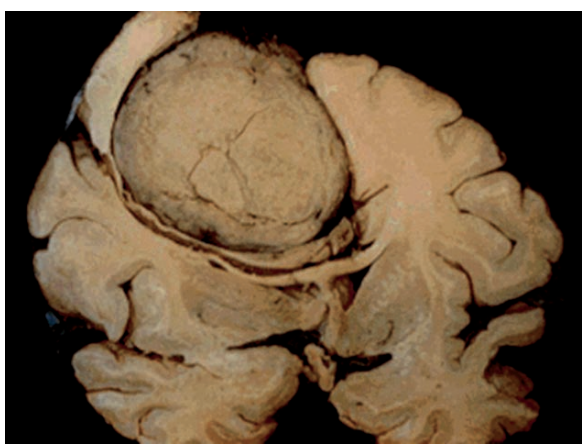


Figure 18.4.A. Meningioma. Shown is a coronal section through the brain at the level of the lenticular nuclei. A large mass between the hemispheres markedly distorts the brain. The mass is separable from the brain parenchyma. This is characteristic of a meningioma, which arises from the arachnoid cells. Meningiomata have a smooth bosselated external surface, which is usually pink–tan in color. These tumors may invade the dura, but normally do not invade the brain or show necrosis. The brain is compressed beneath the tumor. Meningiomata occasionally arise at sites of trauma or irradiation or in some hereditary tumor diatheses, such as neurofibromatosis. Patients usually have the slow onset of focal neurological signs, occasionally with seizures. Meningiomata comprise 15–20% of all intracranial tumors. They are more common in women than men, probably related to their tendency to express Estrogen Receptors and Progesterone Receptors. They are the only truly benign tumors of the central nervous system in that they usually do not recur after complete surgical removal. [Reproduced from http://pathweb.uchc.edu. With permission.]

In familial and sporadic meningiomata, a deletion in the fifth intron of the *pdgfβ* (*sis*) gene may occur. The intact *pdgfβ* gene has an Alu sequence in this region, which includes two perfect 130 nucleotide-repeated sequences, separated by five base pairs. The deleted allele in a fraction of meningioma cases misses one copy of the 130 base pairs repeat and the intervening five bp [Bolger et al. 1985].

Patients with neurofibromatosis type 2 are predisposed to a variety of central nervous system tumors, including vestibular schwannomata and meningiomata [Cogen et al. 1991]. Somatic mutations in the gene on chromosome 22q12 that encodes NF-2 (Merlin) also arise in tumor tissues of a subset of patients with meningiomata without other features of neurofibromatosis 2.

18.5 NEUROFIBROMA

Schwannomata are benign tumors derived from transformed Schwann cells. Their cell composition is relatively homogeneous. The disruption of *nf-1* in the Schwann cell lineage initiates neurofibroma formation. Neurofibromata are heterogenous tumors of peripheral nerves that include Schwann cells, neuronal processes, perineural cells, fibroblasts, and infiltrating mast cells [Greggio 1911]. The inciting factor for the mast cell infiltration is KIT Ligand, which is hypersecreted from NF-1-deficient Schwann cell populations. These mast cells contribute to tumorigenesis [Riccardi 1981; Yang et al. 2003].

The two forms of neurofibroma are dermal and plexiform. Dermal neurofibromata rarely progress to malignancy, whereas plexiform neurofibromata originate in less mature cells within the spinal or cranial nerves and undergo full transformation in about 5% of all cases. Neurofibromata may give rise to malignant peripheral nerve sheath tumors (MPNSTs). Genetic alterations in progression pathways from neurofibromata to malignant peripheral nerve sheath tumors include *p53* mutations, defects in *ink4a*, and loss of *kip1* [Zhu and Parada 2002]. $P75^{NTR}$ is consistently expressed in malignant peripheral nerve sheath tumors.

Genetic predisposition. Neurofibromatosis 1 (peripheral neurofibromatosis, von Recklinghausen disease) is an autosomal dominant familial cancer syndrome, in which patients develop multiple benign and malignant tumors of the central and peripheral nervous system. Consistent features of this disorder are café au lait spots and fibromatous skin tumors. A *nf1* (*neurofibromin*) microdeletion is the most frequent underlying mutation [Riva et al. 2000]. The loss of both *nf1* alleles is causative for the fibromata, myeloid leukemias (especially juvenile myelomonocytic myeloid leukemia, JMML), and pheochromocytomata associated with the syndrome. It also leads to the development of numerous neurofibromata in the Schwann cells of peripheral nerves, which become malignant peripheral nerve sheath tumors in 3–15% of cases.

Neurofibromatosis 2 (central neurofibromatosis) is characterized by bilateral vestibular schwannomata, with predisposition to spinal and cranial schwannomata, meningiomata, and astrocytomata. It is caused by loss-of-function mutations in the gene *nf2* (*merlin*, *schwannomin*), which has 17 exons and encodes a protein of 595 amino acids. It contains an NH_2-terminal domain, a α-helical domain, and a COOH-terminal domain that lacks the Actin-binding domain present in other ERM proteins. NF2 regulates cytoskeleton-mediated processes and operates in signaling pathways that link them to proliferation.

The malignant transformation potential of solitary neurofibroma, when not associated with a syndrome, is minimal. However, up to 12% of patients with neurofibromatosis will develop cancer, usually neurofibrosarcoma or malignant neurilemoma (malignant schwannoma). They result from transformations of long-term neurofibromata of the skin of the trunk or extremities. Oral lesions in neurofibromatosis very seldom transform into sarcoma but may become large enough to interfere with normal function.

Some patients with neurofibromatosis have manifestations of Noonan syndrome (an autosomal dominant dysmorphic syndrome characterized by hypertelorism, a downward eyeslant, and low set posteriorly rotated ears) [Allanson et al. 1985; Opitz and Weaver 1985]. Mutations in the *nf1* gene, which is the site of mutations causing classic neurofibromatosis type 1, are associated with neurofibromatosis–Noonan syndrome.

18.6 RETINOBLASTOMA

Retinoblastoma is an embryonic malignant neoplasm of retinal origin. It is a childhood tumor with an incidence of about 1 in 25,000. Retinoblastoma

accounts for 11% of cancers developing in the first year of life, and 3% of cancers in children under the age of 15 years. The tumors are generally confined to the eye. Their extension to surrounding soft tissues, the optic nerve, the brain, or the meninges leads to significant worsening of the prognosis. Approximately 60% of cases are sporadic, and 40% are hereditary. Tumors in patients with germline mutations have 85–95% penetrance, tend to occur at an earlier age than sporadic cases, and arise bilaterally with high frequency. Down syndrome may be associated with an increased occurrence of retinoblastoma. Spontaneous regression occurs in some cases of retinoblastoma.

Retinomata are benign lesions that may arise in the retina of retinoblastoma patients. The distinctive characteristics of these lesions include a translucent retinal mass protruding into the vitreous, calcification in 75%, and retinal pigment epithelial migration and proliferation in 60%. These tumors represent either spontaneous regression of retinoblastomata or benign manifestations of alterations in the *rb* gene, such as loss of function of both *rb* alleles in differentiated cells [Gallie and Phillips 1982].

Molecular pathogenesis. Germline mutations of the tumor suppressor gene *rb1* are associated with multiple neoplasms, primarily retinoblastoma, various sarcomata, and melanoma. Mutations leading to retinoblastoma are spread throughout all 27 exons of the *rb1* gene. They include insertions, deletions, point mutations (often generating stop codons), and promoter methylation.

Amplification of *N-myc* may occur in retinoblastoma. The N-MYC proto-oncogene product is a nuclear protein that forms heterodimers with MAX through a conserved helix-loop-helix zipper domain. The MYC/MAX complex transactivates a number of target genes that are important for cell cycle progression and transformation.

Secreted molecules contribute to the determination of stem cell fate. At least two members of the TGF-β family are important for the differentiation of neural crest stem cells. TGF-β signaling often induces cell cycle arrest. In general, tumors acquire TGF-β resistance at advanced stages. Whereas retinal cells bear receptors for TGF-β, retinoblastoma cells lack these receptors.

Invasion. In normal retina and in retinoblastoma, N-Cadherin is associated with α- and β-Catenin, but not with E-Cadherin or P-Cadherin. Retinoblastoma cells, in contrast with normal retina, express an *N*-Cadherin/Catenin complex that is irregularly distributed and weakly linked to the cytoskeleton. In retinoblastoma, this complex acts as an invasion promoter [Van Aken et al. 2002].

18.7 PITUITARY ADENOMA

Pituitary adenomata frequently secrete hormones. They also cause deficits in the visual field through pressure on the optic nerves.

- For prolactinomata, which produce excess of Prolactin, oligomenorrhea or amenorrhea, reduced fertility, loss of libido, erectile dysfunction, and galactorrhea are typical.
- Somatotroph adenomata produce excess in Growth Hormone (GH) and cause acromegaly.
- Corticotroph adenomata lead to Cushing syndrome.
- Thyrotrophin (TSH) secreting pituitary adenomata are characterized by hyperthyroidism without TSH suppression.

Molecular pathogenesis. A significant subset of pituitary adenomata has a constitutively activated α subunit of the stimulatory heterotrimeric G_s-Protein, which is involved in hormone receptor signaling. This aberrant activation leads to increased cyclic adenosine monophosphate, bypassing the normal requirement for trophic hormones. This induces mitogenic, tumor-promoting signals. Mutations in the gene coding for the α subunit of the G-protein G_s are the most frequent molecular events in the development of somatotroph adenomata in adults [Landis et al. 1989; Clementi et al. 1990].

A PKCα point mutation at position 294 of the V3 region is expressed in a subpopulation of pituitary adenomata, characterized by their invasive phenotype. In response to stimulation, this mutant PKCα translocates mainly to the perinuclear region and supports dysregulated growth.

Trisomy of chromosome 12 is a nonrandom chromosomal change in pituitary adenomata, particularly prolactinomata. It leads to overexpression of the high mobility group gene *hmgA2* {12q14–15}, suggesting a critical role for HMG-A2 in the generation of Prolactin-secreting pituitary adenomata [Finelli et al. 2002].

Genetic predisposition. Familial cancer syndromes associated with pituitary adenoma formation include:
- Multiple endocrine neoplasia type 1, an autosomal dominant condition associated principally with tumors of the parathyroid, pancreas and, in approximately 45% of patients, the pituitary.
- Carney complex, a rare autosomal dominant condition characterized by myxomata of the heart, skin, and breast, spotty skin pigmentation, Schwannomata, ovarian cysts, adrenal, testicular, and thyroid tumors, and in 5–20% of patients, pituitary adenomata.
- Isolated familial acromegaly, a condition in which individuals tend to develop acromegaly or gigantism at a relatively young age.

18.8 RHABDOID TUMOR

Rhabdoid tumors are a highly malignant group of neoplasms [Parham et al. 1994]. These tumors occur in young children, but not in adults, at a mean age of 3.5 years with a range of 2–13 years. Males and females are equally at risk. The location can be supratentorial, intraventricular, or infratentorial. The symptoms depend on the location of the tumor and the age of the child. They include seizures, raised intracranial pressure to hydrocephalus, and enlarged head size or fontanelles.

Molecular pathogenesis. A characteristic cytogenetic abnormality is the deletion of chromosome 22q11.2, which contains *snf5* [Versteege et al. 1998]. Most malignant rhabdoid tumors display biallelic inactivating mutations of the *snf5* gene, reflective of its nature as a tumor suppressor [Reincke et al. 2003]. The *snf5* (*ini1*, *integrase interactor 1*, *smarcB1*) gene encodes a protein component of the SWI/SNF chromatin remodeling complex. Two forms of SNF5, that differ by the variable inclusion of nine amino acids, potentially are produced through differential RNA splicing. Either form induces a dramatic change in morphology, growth suppression, and cell cycle arrest in rhabdoid tumor cells. Senescence associated proteins are upregulated, while the levels of proteins implicated in cell cycle progression are downregulated.

The tumor cells express Vimentin, Cytokeratin, and Epithelial Membrane Antigen. Neural markers are variably present.

18.9 NEUROENDOCRINE TUMORS

Neuroendocrine tumors (gastro-entero-pancreatic or neuroendocrine tumors, GEP-NETs) are cancers of the interface between the endocrine (hormonal) system and the nervous system. Endocrine tumors of the gastroenteropancreatic axis consist of cells that are capable of amine precursor uptake and decarboxylation and therefore are named APUDomata [Pearse 1968]. The peptides secreted from gut endocrine tissues and nerve cells include Gastrin, Cholecystokinin, Vasoactive Intestinal Polypeptide (VIP), Substance P, Somatostatin (SRIF), Enkephalins, Neurotensin, and Thyrotropin-Releasing Hormone (TRH).

18.9.1 Neuroblastoma

Neuroblastoma is the most common extracranial solid cancer in infancy and childhood. It is a neuroendocrine tumor that can arise from any neural crest element of the sympathetic nervous system. Neuroblastomata originate in the adrenal medulla or in paraspinal sites, where sympathetic nervous system tissue is present. The mean age for diagnosis in sporadic cases is 30 months and in rare familial cases is 9 months. Neuroblastoma is among the rare malignancies that may be subject to spontaneous regression from an undifferentiated state to a completely benign cellular appearance.

Molecular pathogenesis. N-MYC {2p24.1} is amplified in most neuroblastoma cells, which contain either homogeneous staining regions or double minutes (the karyologic manifestations of amplified genes) [Kohl et al. 1983]. *N-myc* amplification is most frequent in patients over 1 year and correlates with poor outcome. ID proteins coordinate proliferation and differentiation. ID-2 is an effector of N-MYC in neuroblastomata. It acts as a dominant antagonist of basic helix-loop-helix transcription factors and proteins of the RB family. ID-2 is recruited by MYC oncoproteins to bypass the tumor suppressor function of RB. *myc* amplification is often absent in localized neuroblastomata.

A gain of chromosome 17q is a frequent chromosomal anomaly in neuroblastoma. It correlates closely with the amplification of *N-myc* and is strongly associated with an unfavorable prognosis. Survivin is an IAP family member that protects cells from apoptosis. Its expression correlates with

more aggressive and unfavorable disease prognosis [Adida et al. 1998]. The gene maps to chromosome 17q25.

Loss of 1p36.2–36.3 is common in neuroblastoma. The *tp73* gene {1p36.3} maps to this frequently deleted region [Kaghad et al. 1997]. Furthermore, the expression of the variant form ΔNP73 in neuroblastomata is an adverse prognostic marker. In contrast to the full-length P73, which induces apoptosis, this NH_2-terminally truncated form inhibits the activity of P53 and of the full length P73.

Deletions of 11q14–23 occur in 15–25% of neuroblastoma cases and frequently characterize a subgroup of stage 4 neuroblastomata (tumors with distal dissemination) without *N-myc* amplification but with loss of chromosome 3p.

Brain tumors contain cells expressing the neuroectodermal stem cell markers CD133 (Prominin 1, PROM1) and Nestin, while lacking the expression of neural differentiation markers. The proliferation rate of these cells correlates with the clinical aggressiveness of the tumors. The gene for the intermediate filament Nestin is a transcriptional target for N-MYC and a mediator of N-MYC induced aggressiveness [Thomas et al. 2004].

The expression of the CDK Inhibitor $P27^{KIP1}$ is a prognostic indicator independently of the status of N-MYC.

Spontaneous regression occurs in neuroblastomata in conjunction with the overexpression of *H-ras* and the gene for the NGFR, *trkA*. During regression, dying cells have increased numbers of lysosomes, which is characteristic of autophagic degeneration [Kitanaka et al. 2002]. Terminal differentiation and reversal of the transformed phenotype of neuroblastoma cells may be induced by cyclic AMP.

Tumor progression and metastasis. Neuroblastomata metastasize predominantly to bone. A S120G substitution, which does not represent a common polymorphism, in the NME-1 metastasis suppressor gene product is associated with advanced neuroblastomata but not with limited stage neuroblastomata. Furthermore, the expression levels of Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ in the tumor vasculature correlate with the malignancy of neuroblastomata [Brooks et al. 1994; Erdreich-Epstein et al. 2000].

18.9.2 Multiple Endocrine Neoplasia

MEN1 (multiple endocrine neoplasia type 1, Wermer syndrome) [Wermer 1954] is an autosomal dominant disorder characterized by a high frequency of parathyroid hyperplasia, pancreatic endocrine tumors, and pituitary adenomata [Guo and Sawicki 2001]. The Zollinger–Ellison syndrome, comprising intractable peptic ulcer with pancreatic islet adenoma, is a facet of multiple endocrine adenomatosis. MEN1 may also lead to Cushing syndrome. The putative tumor suppressor Menin, the protein product of *men1* {11q13}, is mutated in MEN1 syndrome as well as in sporadic endocrine tumors. The *men1* gene spans 9 kb and consists of 10 exons, which encode a 610 amino acid protein. There are 28 putative phosphorylation sites, missense mutations in two of which produce disease. Menin's growth suppression is thought to be mediated, in part, through its interactions with transcription factors. Menin binds to JUN-D, but not to other members of the JUN or FOS families, and represses JUN-D-mediated transcriptional activation [Yazgan and Pfarr 2001].

MEN 1Burin is an incomplete variant of multiple endocrine neoplasia type 1. Compared to MEN 1, MEN 1Burin is characterized by a high prevalence of Prolactin-secreting pituitary adenomata, late onset of hyperparathyroidism and rare pancreatic involvement. The MEN 1Burin phenotype may be a consequence of the point mutations R460X or Y312X. Alternatively, it may be derived from a deletion of A at nucleotide 1,021 of codon 304, resulting in frameshift and downstream protein truncation at codon 320. No mutation within the coding sequence of *men1* was found in two families studied [Kong et al. 2001], suggesting that alterations of other genes can contribute to the condition.

MEN2A and MEN2B are allelic disorders. Multiple endocrine neoplasia type 2A (Sipple syndrome, pseudotumor cerebri, PTC syndrome), is an autosomal dominant syndrome of multiple endocrine neoplasms, including medullary thyroid carcinoma, pheochromocytoma, and parathyroid adenomata. There is evidence that MEN2A results from mutations in the *ret* oncogene. MEN2B (Wagenmann–Froboese syndrome, mucosal neuroma syndrome) is characterized by multiple true neuromata, pheochromocytoma, and medullary thyroid carcinoma. The neuromata occur as pedunculated nodules on the eyelid margins, lips, and

tongue. Enlarged nerves of the gastrointestinal tract (ganglioneuromatosis), often with megacolon, are features of MEN2B. Corneal nerve thickening (medullated corneal nerve fibers) and mucosal neuromata are comparable features. The patients sometimes have café au lait spots. A single point mutation in the catalytic core of the tyrosine kinase domain of *ret* is associated with both inherited and de novo MEN2B. In contrast to MEN2A, no parathyroid disease is associated with MEN2B.

18.9.3 Carcinoid Tumors

Carcinoid tumors [Langhans 1867; Lubarsch 1888] are neuroendocrine neoplasms that are derived from neuroectodermal cells of the neural crest (Figure 18.9.3.A). They comprise approximately 55% of all gastrointestinal endocrine tumors, with an overall incidence of 1.5 cases per 100,000. Although carcinoid tumors are located predominantly in the gastrointestinal tract they may arise in various organs of the body. More than 95% of these tumors originate in the appendix, the rectum, or the small intestine. The tumors grow slowly and metastasize to the regional lymph nodes, liver, and, less commonly, to bone. Carcinoid tumors may be grouped according to the embryologic site of origin [Williams and Sandler 1963].

- Foregut carcinoids affect the bronchus, stomach, first portion of the duodenum, and pancreas. They are argentaffin negative, have a low content of 5-hydroxytryptamine (serotonin), and often secrete the serotonin precursor 5-hydroxytryptophan, histamine, and a multitude of polypeptide hormones. Their functional manifestations include carcinoid syndrome (cutaneous flushing, bronchospasm, diarrhea, and right-sided valvular lesions) [Pernow and Waldenström 1954], gastrinoma syndrome, acromegaly, and Cushing disease. The expression of FOS is associated with ACTH secretion and the corticotroph phenotype in bronchial carcinoid tumors. The overexpression of P53 may distinguish atypical pulmonary carcinoid tumors from typical carcinoid tumors.
- Midgut carcinoid tumors derive from the second portion of the duodenum, the jejunum, the ileum, and the right colon. They are argentaffin positive, have a high 5-hydroxytryptamine content, rarely secrete 5-hydroxytryptophan, and often produce a number of other vasoactive compounds, such as kinins, prostaglandins, and Substance P. On rare occasions, these tumors produce ACTH. Various endocrine tumors have a high frequency of somatic deletions of the distal part of chromosome 11q, where the tumor suppressor gene *sdhD* (*succinate-ubiquinone oxidoreductase subunit D*) is located. In about half of midgut carcinoids, the constitutive missense mutations H50R and G12S, associated with loss of heterozygosity of the other allele, may occur [Kytola et al. 2002].
- Hindgut carcinoid tumors include those of the transverse colon, left colon, and rectum. They are argentaffin-negative, rarely secrete 5-hydroxytryptamine, 5-hydroxytryptophan, or other peptides. They may metastasize to bone.

In carcinoid tumor cells, IGF-1 stimulates Phosphatidylinositol 3-Kinase, $P70^{S6\ Kinase}$, and ERK-2. Through this mechanism, IGF-1 may act as an autocrine growth factor in these neuroendocrine tumor cells.

18.9.4 Gut Hormone-Secreting Tumors

Insulinoma. The prominent clinical symptom of insulinoma is hypoglycemia due to excess Insulin secretion. This leads to symptoms of both neuroglycopenia (ranging from diplopia and blurred vision, confusion, aberrant behavior, and amnesia to seizures and coma) and catecholamine response (sweating, weakness, hunger, tremor, nausea, sensations of warmth, anxiety, and palpitations). More than 90% of insulinomata are benign tumors. Malignant insulinoma occurs in a few patients, with metastasis to the periaortic or portal lymph nodes and to the liver.

Glucagonoma. The main features of the glucagonoma syndrome (4D syndrome for dermatosis, diarrhea, deep vein thromboses, depression) include necrolytic migratory erythema, painful glossitis, angular stomatitis, normochromic normocytic anemia, weight loss, mild diabetes mellitus, hypoaminoacidemia, deep vein thrombosis, and depression [McGavran et al. 1966; Sweet 1974]. Like other islet cell neoplasms, glucagonomata may secrete Insulin, ACTH, Pancreatic Polypeptide, Parathyroid Hormone, Gastrin, Serotonin, Vasocative Intestinal Peptide, or Melanocyte-Stimulating Hormone. Among neuroendocrine neoplasms, the highest median serum Glucagon values occur in patients with the glucagonoma syndrome or with insulinomata, and the lowest

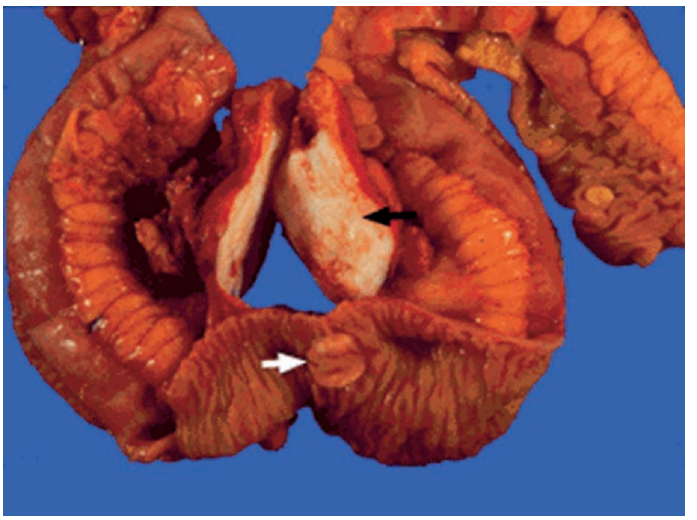


Figure 18.9.3.A. Carcinoid tumor. The small intestine has been opened disclosing a tumor (white arrow) that penetrates through the mucosa. The tumor is white with well-demarcated smooth margins, which are typical of carcinoid but do not distinguish it with assurance from other tumors of the small intestine. The adjoining mucosa is normal with delicate transverse folds. An enlarged lymph node of the mesentery has been transected (*black arrow*) disclosing metastatic tumor with white appearance typical of tumor as opposed to the tan appearance of normal lymphoid tissue. Carcinoids are slow-growing low-grade malignant tumors, most of which do not metastasize and are asymptomatic at death. The most common symptom is nonspecific abdominal pain, which has often been present for a number of years. Bleeding is rare, and obstruction can occur due to size of the tumor, desmoplastic thickening of the intestinal wall, intestinal infarction secondary to mesenteric vessel compression by tumor, or intussusception. Metastases first occur in regional nodes and then in the liver. While carcinoids can arise in the entire length of the gastrointestinal tract, 95% occur in the appendix, ileum, or rectum. Whereas the great majority of metastasizing carcinoids arise in the ileum, usually the distal ileum, appendiceal and rectal carcinoids rarely metastasize. Carcinoids can also arise from neuroendocrine cells of endodermal derivation, which are normally present in the stomach. The rare carcinoid tumors of the stomach are associated with pernicious anemia or prolonged exposure to H_2 blockers. These cause a multicentric neuroendocrine hyperplasia that eventually leads to tumors. The presumed mechanism is neuroendocrine cell proliferation stimulated by elevated Gastrin levels. The most striking presentation of these tumors is the carcinoid syndrome, caused by less than 1% of carcinoid tumors, and almost all are primary in the ileum. The presence of this syndrome indicates a release of polypeptide hormones or vasoactive amines into the general circulation and in most cases denotes bulky hepatic metastases. The two most common symptoms of the carcinoid syndrome are flushing and diarrhea, and these are almost always associated with elevated 5-hydroxyindoleacetic acid (5-HIAA). The peak incidence of carcinoids is the sixth decade. [Reproduced from http://pathweb.uchc.edu. With permission.]

median values occur in those with carcinoid syndrome or Zollinger–Ellison syndrome. A mild degree of hyperglucagonemia can commonly be associated with multifunctional neuroendocrine tumors, while the glucagonoma syndrome is less frequent [Wermers et al. 1996].

VIPoma. VIPomata (Vasoactive Intestinal Peptide tumors, watery diarrhea–hypokalemia–achlorhydria syndrome, WDHA syndrome) are noninsulin-secreting tumors of the pancreatic islets [Verner and Morrison 1958].

Somatostatinoma. Somatostatin inhibits various endocrine and exocrine secretory functions, including the majority of all gut hormones. Somatostatinoma [Ganda et al. 1977] causes diabetes, diarrhea or steatorrhea, gallbladder disease, hypochlorhydria, and weight loss.

18.9.5 Parathyroid Tumors

Sporadic parathyroid adenomata are benign tumors. They may be caused by overexpression of *cyclin* D_1 (*prad1*, *parathyroid adenomatosis 1*) in 20–40% of cases. Somatic mutation or deletion of the *men1* tumor suppressor occurs in about 15–20% of sporadic parathyroid adenomata. Low expression alleles of *vdr* (*vitamin D receptor*) are represented with elevated frequencies. The tumors of patients homozygous for the b, a, or T alleles have lower *vdr* and higher *pth* mRNA levels than those from patients with BB, AA, or tt genotypes, whereas those from heterozygotes have intermediate values. The lower *vdr* mRNA

levels associated with the b, a, and T alleles may affect the calcitriol (1,25-dihydroxycholecalciferol, 1,25-$(OH)_2D_3$)-mediated control of parathyroid function and thereby contribute to the development of sporadic primary hyperparathyroidism.

Parathyroid carcinomata may be causative for hyperparathyroidism. Sporadic parathyroid carcinomata frequently have inactivating *hrpt2* mutations that are likely to be of pathogenetic importance [Shattuck et al. 2003].

18.9.6 Other Neuroendocrine Tumors

In sporadic adrenocortical tumor, somatic mutations in the *pkar1α* gene, including a nonsense mutation A751G, a transition 1050T→C followed by a 22 base pair deletion, and a splicing mutation IVS9AS-1G-A, effect premature termination of the protein. These tumors may lead to Cushing syndrome [Bertherat et al. 2003].

Merkel cell cancer (Merkel cell carcinoma, trabecular cancer) is a rare and highly aggressive neuroendocrine cancer that originates on or just beneath the skin and in hair follicles. These tumors occur most often on the face, head, and neck, while 30% begin on the legs and 15% arise on the arms. The condition usually manifests as firm, painless nodules, which grow rapidly. From initial onset, Merkel cell cancer metastasizes quickly and spreads to the regional lymph nodes. The tumor also tends to invade underlying subcutaneous fat, fascia, and muscle. It can metastasize to the liver, lungs, brain, or bones. Merkel cell cancer is fatal in 30–50% of cases.

Additional tumors with neuroendocrine characteristics include pheochromocytoma, small cell lung cancer, and neuroblastoma.

REFERENCES

Adida C, Berrebi D, Peuchmaur M, Reyes-Mugica M, Altieri DC. 1998. Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. Lancet 351:882–883.

Allanson JE, Hall JG, Van Allen MI. 1985. Noonan phenotype associated with neurofibromatosis. American Journal of Medical Genetics 21:457–462.

Alonso ME, Bello MJ, Gonzalez-Gomez P, Arjona D, Lomas J, de Campos JM, Isla A, Sarasa JL, Rey JA. 2003. Aberrant promoter methylation of multiple genes in oligodendrogliomas and ependymomas. Cancer Genetics and Cytogenetics 144:134–142.

Bailey P. 1924. Tumors arising from ependymal cells. Archives of Neurology and Psychiatry 11:1–27.

Bailey P, Cushing H. 1925. A common type of midcerebellar glioma of childhood. Archives of Neurology and Psychiatry 14:192–224.

Bertherat J, Groussin L, Sandrini F, Matyakhina L, Bei T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C, Perlemoine K, Gicquel C, Bertagna X, Stratakis CA. 2003. Molecular and functional analysis of PRKAR1A and its locus (17q22–24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. Cancer Research 63:5308–5319.

Biernat W, Tohma Y, Yonekawa Y, Kleihues P, Ohgaki H. 1997. Alterations of cell cycle regulatory genes in primary (de novo) and secondary glioblastomas. Acta Neuropathologica (Berlin) 94:303–309.

Bolger GB, Stamberg J, Kirsch IR, Hollis GF, Schwarz DF, Thomas GH. 1985. Chromosomal translocation t(14;22) and oncogene (c-sis) variant in a pedigree with familial meningioma. The New England Journal of Medicine 312:564–567.

Brooks PC, Clark RA, Cheresh DA. 1994. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 264:569–571.

Charest A, Kheifets V, Park J, Lane K, McMahon K, Nutt CL, Housman D. 2003. Oncogenic targeting of an activated tyrosine kinase to the Golgi apparatus in a glioblastoma. Proceedings of the National Academy of Sciences USA 100:916–921.

Choe G, Horvath S, Cloughesy TF, Crosby K, Seligson D, Palotie A, Inge L, Smith BL, Sawyers CL, Mischel PS. 2003. Analysis of the phosphatidylinositol 3′-kinase pathway in glioblastoma patients *in vivo*. Cancer Research 63:2742–2746.

Clementi E, Malgaretti N, Meldolesi J, Taramelli R. 1990. A new constitutively activating mutation of the G_s protein α subunit-gsp oncogene is found in human pituitary tumours. Oncogene 5:1059–1061.

Cogen PH, Daneshvar L, Bowcock AM, Metzger AK, Cavalli-Sforza LL. 1991. Loss of heterozygosity for chromosome 22 DNA sequences in human meningioma. Cancer Genetics and Cytogenetics 53:271–277.

Erdreich-Epstein A, Shimada H, Groshen S, Liu M, Metelitsa LS, Kim KS, Stins MF, Seeger RC, Durden DL. 2000. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are expressed by endothelium of high-risk neuroblastoma and their inhibition is associated with increased endogenous ceramide. Cancer Research 60:712–721.

Erez A, Ilan T, Amariglio N, Muler I, Brok-Simoni F, Rechavi G, Izraeli S. 2002. GLI3 is not mutated commonly in sporadic medulloblastomas. Cancer 95:28–31.

Evans DGR, Farndon PA, Burnell LD, Rao Gattamaneni H, Birch JM. 1991. The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. The British Journal of Cancer 64:959–961.

Evans DGR, Ladusans EJ, Rimmer S, Burnell LD, Thakker N, Farndon PA. 1993. Complications of the naevoid basal cell carcinoma syndrome: results of a population based study. The Journal of Medical Genetics 30:460–464.

Finelli P, Pierantoni GM, Giardino D, Losa M, Rodeschini O, Fedele M, Valtorta E, Mortini P, Croce CM, Larizza L, Fusco A. 2002. The High Mobility Group A2 gene is amplified and overexpressed in human prolactinomas. Cancer Research 62:2398–2405.

Gallie BL, Phillips RA. 1982. Multiple manifestations of the retinoblastoma gene. Birth Defects Original Article Series 18:689–701.

Ganda OP, Weir GC, Soeldner JS, Legg MA, Chick WL, Patel YC, Ebeid AM, Gabbay KH, Reichlin S. 1977. "Somatostatinoma": a somatostatin-containing tumor of the endocrine pancreas. The New England Journal of Medicine 296:963–967.

Gilchrist DM, Savard ML. 1989. Ependymomas in two sisters and a maternal male cousin. American Journal of Human Genetics 45S:A22.

Goodrich LV, Milenkovic L, Higgins KM, Scott MP. 1997. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 277:1109–1113.

Greggio H. 1911. Les cellules granuleuses (Mastzellen) dans les tissus normaux et dans certaines maladies chirurgicales. Archivos de medicina experimental; trabajos del Instituto Nacional de Ciencias Medicas 23:323–375.

Guo SS, Sawicki MP. 2001. Molecular and genetic mechanisms of tumorigenesis in multiple endocrine neoplasia type-1. Molecular Endocrinology 15:1653–1664.

Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B, Burger PC, Wood PA, Taqi F, Booker SV, Petersen GM, Offerhaus GJA, Tersmette AC, Giardiello FM, Vogelstein B, Kinzler KW. 1995. The molecular basis of Turcot's syndrome. The New England Journal of Medicine 332:839–847.

Huang H, Mahler-Araujo BM, Sankila A, Chimelli L, Yonekawa Y, Kleihues P, Ohgaki H. 2000. APC mutations in sporadic medulloblastomas. American Journal of Pathology 156:433–437.

Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D. 1997. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90:809–819.

Kitanaka C, Kato K, Ijiri R, Sakurada K, Tomiyama A, Noguchi K, Nagashima Y, Nakagawara A, Momoi T, Toyoda Y, Kigasawa H, Nishi T, Shirouzu M, Yokoyama S, Tanaka Y, Kuchino Y. 2002. Increased Ras expression and caspase-independent neuroblastoma cell death: possible mechanism of spontaneous neuroblastoma regression. Journal of the National Cancer Institute 94:358–368.

Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, Alt FW. 1983. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 35:359–367.

Kong C, Ellard S, Johnston C, Farid NR. 2001. Multiple endocrine neoplasia type 1Burin from Mauritius: a novel MEN1 mutation. The Journal of Endocrinological Investigation 24:806–810.

Kytola S, Nord B, Elder EE, Carling T, Kjellman M, Cedermark B, Juhlin C, Hoog A, Isola J, Larsson C. 2002. Alterations of the SDHD gene locus in midgut carcinoids, Merkel cell carcinomas, pheochromocytomas, and abdominal paragangliomas. Genes, Chromosomes and Cancer 34:325–332.

Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L. 1989. GTPase inhibiting mutations activate the chain of G_s and stimulate adenylyl cyclase in human pituitary tumours. Nature 340:692–696.

Langhans T. 1867. über einen Drüsenpolyp im Ileum. Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin 38:559–560.

Lekanne Deprez RH, Riegman PHJ, Groen NA, Warringa UL, van Biezen NA, Molijn AC, Bootsma D, de Jong PJ, Menon AG, Kley NA, Seizinger BR, Zwarthoff EC. 1995. Cloning and characterization of MN1, a gene from chromosome 22q11, which is disrupted by a balanced translocation in a meningioma. Oncogene 10:1521–1528.

Leung C, Lingbeek M, Shakhova O, Liu J, Tanger E, Saremaslani P, Van Lohuizen M, Marino S. 2004. Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. Nature 428:337–341.

Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A, Schlessinger J. 1985. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 313:144–147.

Liu PI, Liu GC, Tsai KB, Lin CL, Hsu JS. 2005. Intraspinal clear-cell meningioma: case report and review of literature. Surgical Neurology 63:285–288.

Lu KV, Jong KA, Kim GY, Singh J, Dia EQ, Yoshimoto K, Wang MY, Cloughesy TF, Nelson SF, Mischel PS. 2005. Differential induction of glioblastoma migration and growth by two forms of pleiotrophin. The Journal of Biological Chemistry 280:26953–26964.

Lubarsch O. 1888. über den primären Krebs des Ileum nebst Bemerkungen über das gleichzeitige Vorkommen von Krebs und Tuberculose. Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin 111:280–317.

Lyden D, Young AC, Zagzag D, Yan W, Gerald W, O'Reilly R, Bader BL, Hynes RO, Zhuang Y, Manova K, Benezra R. 1999. Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumor xenografts. Nature 401:670–677.

MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, Packer RJ, Cogen P, Stephan DA. 2001. Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. Nature Genetics 29:143–152.

McGavran MH, Unger RH, Recant L, Polk HC, Kilo C, Levin ME. 1966. A glucagon-secreting alpha-cell carcinoma of the pancreas. The New England Journal of Medicine 274:1408–1413.

Mori T, Nagase H, Horii A, Miyoshi Y, Shimano T, Nakatsuru S, Aoki T, Arakawa H, Yanagisawa A, Ushio Y, Takano S, Ogawa M, Nakamura M, Shibuya M, Nishikawa R, Matsutani M, Hayashi Y, Takahashi H, Ikuta F, Nishihua T, Mori S, Nakamwa Y. 1994. Germ-line and somatic mutations of the APC gene in patients with Turcot syndrome and analysis of APC mutations in brain tumors. Genes, Chromosomes and Cancer 9:168–172.

Opitz JM, Weaver DD. 1985. The neurofibromatosis-Noonan syndrome. American Journal of Medical Genetics 21:477–490.

Parham DM, Weeks DA, Beckwith JB. 1994. The clinicopathologic spectrum of putative extrarenal rhabdoid tumors: an analysis of 42 cases studied with immunohistochemistry or electron microscopy. American Journal of Surgical Pathology 18:1010–1029.

Pearse AGE. 1968. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobronchial C cells and calcitonin. Proceedings of the Royal Society London, B Biological Science 170:71–80.

Pernow B, Waldenström J. 1954. Paroxysmal flushing and other symptoms caused by 5-hydroxytryptamine and histamine in patients with malignant tumors. The Lancet 2:951–953.

Peters N, Wellenreuther R, Rollbrocker B, Hayashi Y, Meyer-Puttlitz B, Duerr EM, Lenartz D, Marsh DJ, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A. 1998. Analysis of the PTEN gene in human meningiomas. Neuropathology and Applied Neurobiology 24:3–8.

Piccirillo SGM, Vescovi AL. 2006. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444:761–765.

Powers C, Aigner A, Stoica GE, McDonnell K, Wellstein A. 2002. Pleiotrophin signaling through anaplastic lymphoma kinase is rate-limiting for glioblastoma growth. The Journal of Biological Chemistry 277:14153–14158.

Raffel C, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, James CD. 1997. Sporadic medulloblastomas contain PTCH mutations. Cancer Research 57:842–845.

Reincke BS, Rosson GB, Oswald BW, Wright CF. 2003. INI1 expression induces cell cycle arrest and markers of senescence in malignant rhabdoid tumor cells. The Journal of Cell Physiology 194:303–313.

Riccardi VM. 1981. Cutaneous manifestation of neurofibromatosis: cellular interaction, pigmentation, and mast cells. In: Blandau R, Paul N, Dickman F (eds). Birth defects: original article series, Vol. 17. New York: Alan R. Lis Inc., pp. 129–145.

Riva P, Corrado L, Natacci F, Castorina P, Wu B-L, Schneider GH, Clementi M, Tenconi R, Korf BR, Larizza L. 2000. NF1 microdeletion syndrome: refined FISH characterization of sporadic and familial deletions with locus-specific probes. American Journal of Human Genetics 66:100–109.

Scheinker IM. 1945. Subependymoma: a newly recognized tumor of subependymal derivation. The Journal of Neurosurgery 2:232–240.

Segal RA, Goumnerova LC, Kwon YK, Stiles CD, Pomeroy SL. 1994. Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. Proceedings of the National Academy of Sciences USA 91:12867–12871.

Shattuck TM, Valimaki S, Obara T, Gaz RD, Clark OH, Shoback D, Wierman ME, Tojo K, Robbins CM, Carpten JD, Farnebo LO, Larsson C, Arnold A. 2003. Somatic and germ-line mutations of the HRPT2 gene in sporadic parathyroid carcinoma. The New England Journal of Medicine 349:1722–1729.

Slooff JL, Kernohan JW, MacCarty CS. 1964. Primary intramedullary tumors of the spinal cord and filum terminale. Philadelphia: W.B. Saunders.

Smits A, van Grieken D, Hartman M, Lendahl U, Funa K, Nister M. 1996. Coexpression of platelet-derived growth factor alpha and beta receptors on medulloblastomas and other primitive neuroectodermal tumors is consistent with an immature stem cell and neuronal derivation. Laboratory Investigations 74:188–198.

Sweet RD. 1974. A dermatosis specifically associated with a tumour of pancreatic α cells. British Journal of Dermatology 90:301–308.

Taylor MD, Liu L, Raffel C, Hui C, Mainprize TG, Zhang X, Agatep R, Chiappa S, Gao L, Lowrance A, Hao A, Goldstein AM, Stavrou T, Scherer SW, Dura WT, Wainwright B, Squire JA, Rutka JT, Hogg D. 2002. Mutations in SUFU predispose to medulloblastoma. Nature Genetics 31:306–310.

Thomas SK, Messam CA, Spengler BA, Biedler JL, Ross RA. 2004. Nestin is a potential mediator of malignancy in human neuroblastoma cells. The Journal of Biological Chemistry 279:27994–27999.

Ulbricht U, Brockmann MA, Aigner A, Eckerich C, Muller S, Fillbrandt R, Westphal M, Lamszus K. 2003. Expression and function of the receptor protein tyrosine phosphatase ζ and its ligand pleiotrophin in human astrocytomas. The Journal of Neuropathology and Experimental Neurology 62:1265–1275.

Van Aken EH, Papeleu P, De Potter P, Bruyneel E, Philippe J, Seregard S, Kvanta A, De Laey JJ, Mareel MM. 2002. Structure and function of the N-cadherin/catenin complex in retinoblastoma. Investigative Ophthalmology and Visual Science 43:595–602.

Verner JV, Morrison AB. 1958. Islet cell tumor and a syndrome of refractory watery diarrhea and hypokalemia. American Journal of Medicine 25:374–380.

Versteege I, Sevenet N, Lange J, Rousseau-Merck M-F, Ambros P, Handgretinger R, Aurias A, Delattre O. 1998. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature 394:203–206.

Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R. 1997. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Research 57:4183–4186.

Wermer P. 1954. Genetic aspects of adenomatosis of endocrine glands. American Journal of Medicine 16:363–371.

Wermers RA, Fatourechi V, Kvols LK. 1996. Clinical spectrum of hyperglucagonemia associated with malignant neuroendocrine tumors. Mayo Clinic Proceedings 71:1030–1038.

Williams ED, Sandler M. 1963. The classification of carcinoid tumors. The Lancet 1:238–239.

Yang FC, Ingram DA, Chen S, Hingtgen CM, Ratner N, Monk KR, Clegg T, White H, Mead L, Wenning MJ, Williams DA, Kapur R, Atkinson SJ, Clapp DW. 2003. Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for Nf1 $^{+/-}$ mast cells. The Journal of Clinical Investigation 112: 1851–1861.

Yazgan O, Pfarr CM. 2001. Differential binding of the Menin tumor suppressor protein to JunD isoforms. Cancer Research 61:916–920.

Yokota T, Tachizawa T, Fukino K, Teramoto A, Kouno J, Matsumoto K, Emi M. 2003. A family with spinal anaplastic ependymoma: evidence of loss of chromosome 22q in tumor. The Journal of Human Genetics 48:598–602.

Zhu Y, Parada LF. 2002. The molecular and genetic basis of neurological tumours. Nature Reviews Cancer 2:616–626.

CHAPTER 19

TUMORS OF SEROUS CAVITIES

19.1 MESOTHELIOMA

Mesotheliomata may be pleural, peritoneal, or pericardial. Malignant pleural mesothelioma is an aggressive neoplasm of the serosal lining of the pleural cavity, which arises from undifferentiated mesothelial cells representing the adult remnants of the surface celomic mesoderm. Mesothelial cells may give rise to both the lining epithelial cells (which express Keratin) and the paucicellular submesothelial layer. The etiology of mesothelioma is primarily associated with environmental factors, particularly exposure to asbestos and smoking. The latency period can be 10–20 years.

Subtypes of mesothelioma are:
– Epithelioid (the most common)
– Sarcomatous (a much more aggressive form)
– Biphasic or mixed (a combination of both of the other cell types)

Molecular pathogenesis. The loss of tumor suppressors plays a substantial role in mesothelioma development. Acquired genetic targets in malignant pleural mesothelioma include the 9p21 locus, which contains $p16^{INK4a}$ and $p14^{ARF}$, and the 22q11-q13.1 locus, which contains *nf-2*. Inactivation of the *cdkn2* gene is an essential step in the formation of malignant mesotheliomata [Kratzke et al. 1995]. *rassf1* is often silenced in malignant pleural mesothelioma.

Although malignant mesothelioma cells express normal levels of CD95 and the TRAIL Receptors DR4 and DR5, they are resistant to apoptosis mediated by CD95L or TRAIL. These cells constitutively express FLIP, which prevents programmed cell death [Rippo et al. 2004].

The Peroxiredoxins (PRX, Thioredoxin Peroxidases) are a family of six peroxidases (PRX I-VI) of about 25 kD that can reduce H_2O_2 using an electron from Thioredoxin or other substrates. They are located in the cytosol and play roles in the cell signaling system. Peroxiredoxin is greatly overexpressed in malignant mesothelioma [Kinnula et al. 2002].

Invasion. The Trypsin-like serine protease Matriptase (Suppressor of Tumorigenicity-14, ST-14, MTSP-1) is overexpressed in malignant epitheloid pleural mesothelioma. This protease is implicated in mesothelioma invasion and metastasis [Hoang et al. 2004].

Matrix Metalloproteinases, in particular the Gelatinases MMP-2 and MMP-9, play a significant role in tumor invasion and angiogenesis. While MMP-9 activities are not prognostic in malignant mesothelioma, MMP-2 may play an important role in metastasis [Edwards et al. 2003].

Genetic predisposition. Cases of familial aggregation of mesothelioma incidence suggest that genetic predisposition is an etiologic component [Roushdy-Hammady et al. 2001].

19.2 SYNOVIAL SARCOMA

Synovial sarcomata are high grade soft tissue neoplasms that usually develop in adolescents and young adults, are more common in males than in females, and have no racial predilection. Synovial sarcomata comprise approximately 5% of soft tissue sarcomata that occur primarily in adolescents and young adults. They typically develop in the deep soft tissues of the extremities. The head and neck region constitutes the

second most common site of involvement for synovial sarcoma, accounting for up to 10% of all cases. Other sites include the lungs and the kidneys. Primary synovial sarcomata of the heart are extremely rare and aggressive tumors.

Molecular pathogenesis. The chromosome translocation t(X;18)(p11.2;q11.2) is highly characteristic of synovial sarcomata. This specific translocation fuses the proximal portion of the *syt* (*synovial sarcoma translocation*) gene {18q11.2} to the distal portion of one of several duplicated *ssx* (*sarcoma, synovial, X breakpoint*) genes {Xp11.2}. The fusion partners are most frequently *ssx1* and *ssx2* [Clark et al. 1994], with *syt-ssx1* translocations outnumbering *syt-ssx2* translocations by threefold. The *syt-ssx1* fusion is associated with more aggressive tumor growth and poor outcome. Synovial sarcomata with *syt-sst1* fusions express ERBB2 and their histologic appearance is characterized by a dimorphic spindle and epithelioid cell appearance. *ssx1* and *ssx2* encode closely related proteins of 188 amino acids. Their NH_2-terminal portions exhibit homology to the Krüppel associated box, a transcriptional repressor domain characteristic of Krüppel type zinc finger proteins.

REFERENCES

Clark J, Rocques PJ, Crew AJ, Gill S, Shipley J, Chan AM-L, Gusterson BA, Cooper CS. 1994. Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. Nature Genetics 7:502–508.

Edwards JG, McLaren J, Jones JL, Waller DA, O'Byrne KJ. 2003. Matrix metalloproteinases 2 and 9 (gelatinases A and B) expression in malignant mesothelioma and benign pleura. The British Journal of Cancer 88:1553–1559.

Hoang CD, D'Cunha J, Kratzke MG, Casmey CE, Frizelle SP, Maddaus MA, Kratzke RA. 2004. Gene expression profiling identifies matriptase overexpression in malignant mesothelioma. Chest 125:1843–1852.

Kinnula VL, Lehtonen S, Sormunen R, Kaarteenaho-Wiik R, Kang SW, Rhee SG, Soini Y. 2002. Overexpression of peroxiredoxins I, II, III, V, and VI in malignant mesothelioma. The Journal of Pathology 196:316–323.

Kratzke RA, Otterson GA, Lincoln CE, Ewing S, Oie H, Geradts J, Kaye FJ. 1995. Immunohistochemical analysis of the $p16^{INK4}$ cyclin-dependent kinase inhibitor in malignant mesothelioma. Journal of the National Cancer Institute 87:1870–1875.

Rippo MR, Moretti S, Vescovi S, Tomasetti M, Orecchia S, Amici G, Catalano A, Procopio A. 2004. FLIP overexpression inhibits death receptor-induced apoptosis in malignant mesothelial cells. Oncogene 23:7753–7760.

Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. 2001. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. The Lancet 357:444–445.

CHAPTER 20

EMBRYONIC TUMORS

Germ cell tumors have the following subtypes and frequencies:
– Seminomata 40%
– Embryonal tumors 25%
– Teratocarcinomata 25%, teratomata 5%
– Choriocarcinoma 1%
– Others 5%

Male germ cell tumors result from the transformation of premeiotic or early meiotic germ cells and exhibit embryonal like differentiation of the three germinal layers. They are the most common malignant neoplasms of males aged 15–35 years and a major cause of cancer-induced deaths in this age group. In females, germ cell tumors account for 30% of all ovarian tumors, but only 1–3% of ovarian cancers in North America. In younger women, germ cell lesions are more common, accounting for 60% of ovarian tumors arising under the age of 21. Worldwide, the highest incidence for germ cell tumors is in Scandinavia.

Germ cell tumors may develop extragonadally. Mediastinal germ cell tumors are rare growths that predominantly affect young males. They may represent isolated metastases from inapparent gonadal primary sites, potentially as a consequence of the abnormal migration of germ cells during embryogenesis.

20.1 SEMINOMA

Seminomata are tumors that occur during the continued differentiation along germ cell lines (Figure 20.1.A). About 70% of classic seminomata are confined to the testes, but about 25% metastasize to the lymph nodes. These testicular primary tumors are usually homogenous and large. The incidence of spermatocytic seminomata peaks in the sixth decade of life. It often occurs bilaterally, is indolent, and rarely metastasizes.

A somatic mutation of the co-SMAD gene *smad4* leads to the loss of SMAD4 protein function in seminomata. This mutational inactivation may affect the activity of several members of the TGF-β superfamily, such as TGF-β, Activin, Inhibin, and BMP.

The placenta is rich in the enzyme 3-β Steroid Sulfatase {Xp22.32}. The testis also has potent Steroid Sulfatase activity, which may play a role in gonadal steroid regulation. Placental Steroid Sulfatase deficiency leads to low estriol levels in the urine and plasma, delay in the onset of labor, and increased risk of stillbirth. In postnatal life, this deficiency causes X-linked ichtyosis. It can be a cause of cryptorchidism. This manifests an increased risk for testicular tumors, such as seminomata [Traupe and Happle 1983; Lykkesfeldt et al. 1983].

20.2 EMBRYONAL CARCINOMA

Embryonal carcinoma is a primitive tumor derived from totipotent germ cells. Risk factors include a history of undescended testis, testicular dysgenesis, infertility, previous testicular germ cell tumor, and a family history of the disease.

Embryonal carcinoma is associated with a characteristic series of abnormalities in the Retinoblastoma pathway, including the upregulation of Cyclin D_2 and P27 and downregulation of RB1 and the Cyclin-Dependent Kinase Inhibitors P16, P18, P14, and P21.

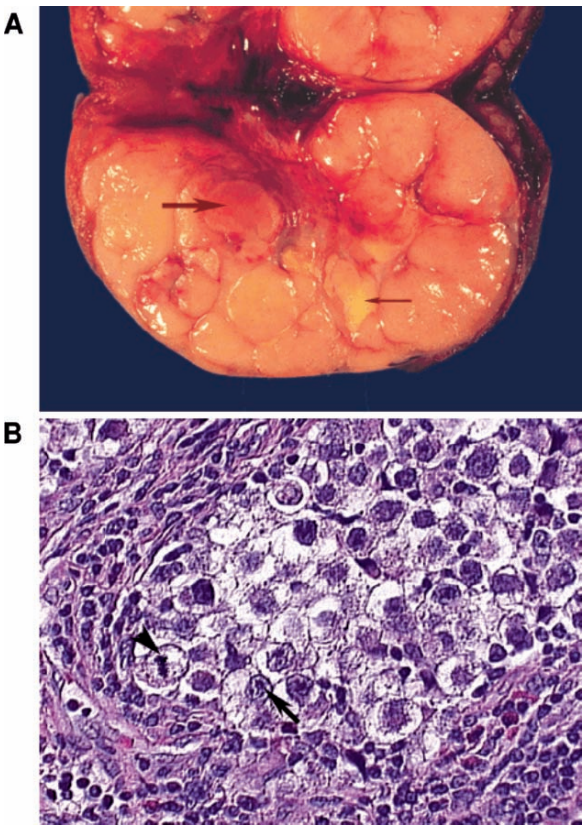


Figure 20.1.A. Seminoma. (A) The tumor replaces most of the testis proper except for a thin peripheral rim. The tumor has a lobulated cut surface and is rather homogeneous and fleshy. Areas of dusky coloration (*large arrow*) and flat yellow coloration (*small arrow*) represent areas of necrosis. (B) High magnification of seminoma cells showing distinct cell membranes, relatively clear cytoplasm, and central nuclei with prominent nucleoli (*arrow*). A mitotic figure is present (*arrowhead*). A typical seminoma is composed of monotonous sheets of uniform round cells with rather clear cytoplasm and centrally located round nuclei with prominent nucleoli. The stroma frequently contains lymphocytes. Granulomatous inflammatory foci can be present. Necrosis and hemorrhage are inconspicuous in small tumors and more notable in larger tumors. Seminomata are germ cell neoplasms that lead to unilateral, nonpainful testicular enlargement. They occur most frequently in the fourth and fifth decade of life. [Reproduced from http://pathweb.uchc.edu. With permission.]

20.3 TERATOMA

A teratoma is a tumor that derives from pluripotent germ cells. Teratomata usually start from cells in the testes in men, the ovaries in women, and in the sacrum in children. They are complex growths, having various cellular or organoid components reminiscent of normal derivatives from several germ layers. Teratomata often contain well differentiated cells, which can result in tissues growing in a teratoma that are different from the surrounding tissue. Some teratomata may contain a mixture of respiratory epithelium, hair follicle, fat tissue, or mature nervous tissue.

Teratomata are divided into three categories:
– Mature (benign)
– Immature (malignant)
– Monodermal or highly specialized

Clinical manifestations. High frequencies of loss of heterozygosity and allelic imbalance at several loci reflect the importance of tumor suppressor gene inactivation in developing testicular germ cell tumors. In these cancers, i(12p) (isochromosome for the short arm of chromosome 12), monosomy 12, and deletions in 12q occur frequently, implying that loss of genetic material on 12q is involved in the development of these growths [Rodriguez et al. 1992; Murty et al. 1992]. Loss of 3p or 11p alleles is associated with about 40–50% of testicular cancers [Lothe et al. 1989; Smith and Rukstalis 1995]. Afflicted genes in seminomatous, but not in nonseminomatous lesions include *mgf* {12q22} and *kit* {4q12}. MGF (Steel Factor) is a ligand for KIT, and both play key roles in the embryonal and postnatal developments of germ cells [Murty et al. 1994]. Cyclin D_2 may be overexpressed in germ cell tumors and contribute to pathogenesis.

Ovarian teratomata originate in a failure of extrusion of the second polar body or the refusion of it with the ovum. Ovarian teratomata manifest as dermoid cysts (mature teratomata), which are generally benign cystic tumors comprised predominantly of ectodermal elements. However, endodermal and mesodermal elements also may be included. These cysts are often filled with hair, skin, teeth, bones, neural tissue, and sebaceous material. Immature teratomata of the ovary have a malignant potential in line with the amount of neuroblastic tissue present.

Extragonadal manifestations of teratoma include pineal teratoma [Wakai et al. 1980], paraventricular germinoma [Schimke 1983], and presacral teratoma [Ashcraft and Holder 1974].

Endocrine factors. β-Human Chorionic Gonadotropin (bHCG) is a glycoprotein that may be secreted by syncytiotrophoblast cells within germinal cell tumors. It is elevated in 5–10% of patients with seminomata and may correlate with metastatic

disease. Placenta-like Alkaline Phosphatase (PAP) levels can be elevated in patients with seminoma and may increase with the tumor burden. Lactate Dehydrogenase (LDH) is a less specific marker for germ cell tumors, but its levels may correlate with overall tumor burden. Yolk sac elements secrete α-Fetoprotein (AFP). Therefore, elevated levels of AFP rule out the diagnosis of pure seminoma.

Genetic predisposition. Predisposing factors for the development of testicular teratomata include:
– Klinefelter syndrome (XXY genotype) may predispose to testicular teratoma or seminoma [Raghavan et al. 1980].
– Risk factors include cryptorchidism, carcinoma in situ, and a preceding contralateral testicular germ cell neoplasm.
– Testicular teratomata are part of the spectrum of cancers that arise in Li–Fraumeni syndrome based on mutations of *p53*.
– Familial testicular cancer [Patel et al. 1990].

Familial clustering of ovarian teratomata occurs [Plattner and Oxorn 1973; Simon et al. 1985].

Presacral teratoma is a manifestation of Currarino syndrome [Currarino et al. 1981]. It is caused by malformation at the caudal end of the developing notochord. The Currarino triad involves the association of partial sacral agenesis with intact first sacral vertebra (sickle-shaped sacrum), a presacral mass, and anorectal malformation. The specific sacral anomaly is distinct to this syndrome. At least some cases of the Currarino syndrome are caused by mutation in the homeobox gene *hlxB9* [Belloni et al. 2000].

20.4 CHORIOCARCINOMA

Choriocarcinoma occurs at stages of differentiation into extraembryonic structures. Choriocarcinoma is an advanced form of gestational trophoblastic disease, which initially manifests as a hydatidiform mole and then an invasive mole [Fisher et al. 1988]. In the course of pregnancy, fetal trophoblast cells invade the uterine mucosa without causing rejection by the decidual leukocytes. In choriocarcinoma, trophoblast-derived tumor cells invade the uterine mucosa. In normal pregnancy CD95L (FASL), which is expressed by the trophoblast cells, appears to contribute to the immune privilege of the pregnant uterus. Choriocarcinoma cells express biologically active CD95L, which may contribute to the control of antitumor immune responses by inducing apoptosis in activated, CD95 bearing leukocytes [Hammer et al. 2002].

20.5 GONADOBLASTOMA

Gonadoblastoma [Scully 1953] is a rare benign tumor that has the potential for malignant transformation. The gonads that transform to gonadoblastoma are nearly always located intraabdominally. Phenotypically, 80% of patients with gonadoblastoma are females and 20% are males.

An increased risk for gonadoblastoma exists in phenotypic females with dysgenetic gonads and the presence of Y chromosomal material. The risk is increased in complete androgen insensitivity/male pseudohermaphroditism with the karyotype 46,XY and in mixed gonadal dysgenesis (45,X/46,XY). Turner syndrome (45,XO) is associated with an elevated incidence of gonadoblastoma. Patients with Turner syndrome typically present with short stature, a short webbed neck, widely spaced nipples, sparse pubic and axillary hair distribution, and infantile genitalia. Their skin is thick from lymphedema, and many nevi are present.

Frasier syndrome (a variant of Denys–Drash syndrome) is caused by germline mutations in *wt1*. Patients have streak gonads, pseudohermaphroditism, and renal failure. Gonadoblastoma arising from the streak gonads is frequent. No mutant WT1 protein is produced by the mutations causing Frasier syndrome. Instead, the mutation results in an altered ratio of the two splice forms of the protein, which are characterized by the presence or absence of the three amino acids KTS. In Denys–Drash syndrome, the tumor risk is much greater than in Frasier syndrome. The dominant negative mutant allele is defective and loss of the other allele predisposes to tumor formation. In contrast, Frasier patients have one normal copy of *wt1* and 1 that can only produce the shorter form. Allele loss would thus lead to cells that cannot produce the +KTS form of WT1, but still have large amounts of the –KTS form [Klamt et al. 1998].

WAGR syndrome is a rare genetic disease, in which affected children are predisposed to develop Wilms tumor, aniridia, genitourinary anomalies (specifically gonadoblastomata), and mental retardation. The syndrome is caused by a mutation on

chromosome 11p13. Among the deleted genes in this region are the ocular development gene pax6 and the Wilms tumor gene *wt1*. The abnormalities in *wt1* may be causative for the genitourinary anomalies.

20.6 YOLK SAC TUMOR

Yolk sac tumor occurs at a stage of differentiation into extraembryonic structures. Yolk sac tumors are ovarian germ cell neoplasms, almost all of which arise in women under the age of 30 years. They typically cause a sudden onset of pain. The serum levels of AFP are virtually always elevated. Spread occurs to the peritoneum and regional lymph nodes in 30–70% of cases.

20.7 DESMOPLASTIC SMALL ROUND CELL TUMOR

Desmoplastic small round cell tumor is an aggressive and rare tumor that primarily occurs in the form of multiple masses in the abdominal serosa. Other areas affected include the lymph nodes, the diaphragm, and the pelvis. Symptoms include abdominal pain, gastrointestinal obstruction, ascites, and cachexia. The most common target sites for metatases are the liver, the lungs, and the bones. Desmoplastic small round cell tumor has a predilection for young males [Gerald et al. 1991].

Desmoplastic small round cell tumor is associated with the recurrent chromosomal translocation t(11;22)(p13;q12) [Gerald et al. 1995]. EWS-WT1 is a chimeric transcription factor resulting from fusion of the NH_2-terminal domain of the Ewing sarcoma gene product EWS to the three COOH-terminal zinc fingers of the Wilms tumor suppressor WT1. The expression of EWS-WT1 leads to the induction of growth associated genes, including the *β chain* of the *interleukin-2/15 receptor* (*IL-2/15Rβ*) in desmoplastic small round cell tumor. This tumor is characterized by an abundance of reactive stroma surrounding islets of tumor cells, indicative of paracrine signals that contribute to tumor cell proliferation. The high levels of IL-2/15Rβ within the tumor cells, along with the expression of IL-2 and IL-15 by the abundant hyperplastic endothelial cells within the reactive stroma, suggest that the transcriptional induction of a Cytokine Receptor by a tumor-associated translocation product enables a proliferative response of epithelial cancer cells to ligands secreted by the surrounding stroma [Wong et al. 2002].

20.8 CHORDOMA

Chordomata are rare malignant tumors of the embryonic backbone, derived from notochordal remnants. They occur along the length of the spinal axis, predominantly in the spheno-occipital, vertebral, and sacrococcygeal regions. They are characterized by slow growth, local destruction of bone, extension into adjacent soft tissues, and in some cases distant metastatic spread. There is a modest overall male predominance of chordomata (1.7:1), which is more striking (approximately 3:1) among patients with sacral chordomata. The age distribution at diagnosis is unimodal, with a median of 59 years [McMaster et al. 2001].

Genetic predisposition. Chordomata can arise in patients with the tuberous sclerosis complex (TSC), an autosomal dominant syndrome characterized by hamartomata in multiple organs, epilepsy, mental retardation, and behavioral problems. Tuberous sclerosis is caused by germline mutation in either the *tsc1* or the *tsc2* tumor suppressor genes.

Loci for familial chordoma map to chromosome 7q33 [Kelley et al. 2001] and chromosome 1p36 [Miozzo et al. 2000].

20.9 HAMARTOMA

The term hamartoma refers to a mass composed of the tissues that normally constitute the organ in which it occurs. However, the tissue elements are poorly organized. Hamartomata are dysontogenetic tumors. They grow along with, and at the same rate as, the organ from whose tissue they are made, and, unlike cancerous tumors, only rarely invade or substantially compress surrounding structures.

Clinical presentation. The most common hamartomata occur in the lungs. Pulmonary hamartoma is the most common benign lung tumor arising in 0.25% of general population. It accounts for 6–8% of all solitary pulmonary lesions. They affect mostly women older than 35 years. The patients are usually asymptomatic. About 15% of the pulmonary hamartomata are calcified. Hamartomata are

composed of variable amounts of adipose, glandular, and fibrous tissues.

Hypothalamic hamartoma typically generates progressive clinical symptoms. It most often causes gelastic seizures, can affect vision (the growths are frequently adjacent to the optic nerve), can underlay rage disorders of hypothalamic origin, and may induce early onset of puberty.

Other locations include colonic, Brunner gland, splenic, and renal hamartomata.

Genetic predisposition. Hamartoma syndromes are frequently based on mutations of the *pten* gene. The PTEN hamartoma-tumor syndromes are characterized by an increased risk of breast, thyroid, and endometrial cancers. Hyperplastic–dysplastic changes in the prostate, skin, and colon are characteristic of these syndromes. Over 100 germline *pten* mutations are known in afflicted patients. In the PTEN hamartoma-tumor syndrome Cowden disease, multiple hamartomata are located on the skin and in about 66% of cases hamartomata of the thyroid gland exist.

Tuberous sclerosis (TSC) is a disorder with autosomal dominant inheritance, characterized by the development of hamartomata in various tissues, including brain, skin, kidneys, and heart. Tuberous sclerosis is further characterized by epilepsy, mental retardation, and behavioral problems. TSC1 is caused by a defect on chromosome 9q24, while TSC2 is caused by a defect on chromosome 16p13.3 [Povey et al. 1994]. The *tsc1* (*hamartin*) gene [van Slegtenhorst et al. 1997] and the *tsc2* (*tuberin*) gene are tumor suppressor genes. Both gene products are negative regulators of the TOR signaling pathway. Tuberin, serving as a substrate of PKB and AMPK, mediates TOR activity by coordinating inputs from growth factors and energy availability in the control of cell growth, proliferation, and survival. Inactivation of the wild type alleles of the affected genes occurs in the hamartomata.

Isolated hemihyperplasia is an abnormality of cell proliferation leading to asymmetric overgrowth of one or more regions of the body. It is associated with an increased risk for embryonal cancers in childhood. A fraction of the patients have hypomethylation of the *kcnq1ot1* (*lit1*) locus on chromosome 11p15.5. This may be associated with somatic mosaicism and uniparental disomy [Martin et al. 2005; Shuman et al. 2006]. Although isolated hemihyperplasia is a distinct clinical entity, it can also occur as a feature of overgrowth syndromes, including Beckwith-Wiedemann syndrome, neurofibromatosis, PTEN hamartoma-tumor syndrome (Proteus syndrome), and Klippel–Trenaunay–Weber syndrome [Shuman et al. 2006].

REFERENCES

Ashcraft KW, Holder TM. 1974. Hereditary presacral teratoma. The Journal of Pediatric Surgery 9:691–697.

Belloni E, Martucciello G, Verderio D, Ponti E, Seri M, Jasonni V, Torre M, Ferrari M, Tsui LC, Scherer SW. 2000. Involvement of the HLXB9 homeobox gene in Currarino syndrome. The American Journal of Human Genetics 66:312–319.

Currarino G, Coln D, Votteler T. 1981. Triad of anorectal, sacral, and presacral anomalies. American Journal of Roentgenology 137:395–398.

Fisher RA, Lawler SD, Povey S, Bagshawe KD. 1988. Genetically homozygous choriocarcinoma following pregnancy with hydatidiform mole. The British Journal of Cancer 58:788–792.

Gerald WL, Miller HK, Battifora H, Miettinen M, Silva EG, Rosai J. 1991. Intra-abdominal desmoplastic small round-cell tumor. Report of 19 cases of a distinctive type of high-grade polyphenotypic malignancy affecting young individuals. American Journal of Surgical Pathology 15:499–513.

Gerald WL, Rosai J, Ladanyi M. 1995. Characterization of the genomic breakpoint and chimeric transcripts in the EWS-WT1 gene fusion of desmoplastic small round cell tumor. Proceedings of the National Academy of Sciences USA 92:1028–1032.

Hammer A, Hartmann M, Sedlmayr P, Walcher W, Kohnen G, Dohr G. 2002. Expression of functional Fas ligand in choriocarcinoma. American Journal for Reproductive Immunology 48:226–234.

Kelley MJ, Korczak JF, Sheridan E, Yang X, Goldstein AM, Parry DM. 2001. Familial chordoma, a tumor of notochordal remnants, is linked to chromosome 7q33. American Journal of Human Genetics 69:454–460.

Klamt B, Koziell A, Poulat F, Wieacker P, Scambler P, Berta P, Gessler M. 1998. Frasier syndrome is caused by defective alternative splicing of WT1 leading to an altered ratio of WT1 +/–KTS splice isoforms. Human Molecular Genetics 7:709–714.

Lothe RA, Fossa SD, Stenwig AE, Nakamura Y, White R, Borresen A-L, Brogger A. 1989. Loss of 3p or 11p alleles is associated with testicular cancer tumors. Genomics 5:134–138.

Lykkesfeldt G, Hoyer H, Lykkesfeldt AE, Skakkebaek NE. 1983. Steroid sulphatase deficiency associated with testis cancer. The Lancet 2:1456.

Martin RA, Grange DK, Zehnbauer B, DeBaun MR. 2005. LIT1 and H19 methylation defects in isolated hemihyperplasia. American Journal of Medical Genetics 134A:129–131.

McMaster ML, Goldstein AM, Bromley CM, Ishibe N, Parry DM. 2001. Chordoma: incidence and survival patterns in the United States, 1973–1995. Cancer Causes and Control 12:1–11.

Miozzo M, Dalpra L, Riva P, Volonta M, Macciardi F, Pericotti S, Tibiletti MG, Cerati M, Rohde K, Larizza L, Fuhrman Conti AM. 2000. A tumor suppressor locus in familial and sporadic chordoma maps to 1p36. International Journal of Cancer 87:68–72.

Murty VV, Houldsworth J, Baldwin S, Reuter V, Hunziker W, Besmer P, Bosl G, Chaganti RS. 1992. Allelic deletions in the long arm of chromosome 12 identify sites of candidate tumor suppressor genes in male germ cell tumors. Proceedings of the National Academy of Sciences USA 89:11006–11010.

Murty VVVS, Bosi G, Le Blanc-Straceski J, Kucherlapati R, Chaganti RSK. 1994. Molecular mapping of 12q22 deletions in male germ cell tumors. Cytogenetics and Cellular Genetics 67:271–272.

Patel SR, Kvols LK, Richardson RL. 1990. Familial testicular cancer: report of six cases and review of the literature. Mayo Clinic Proceedings 65:804–808.

Plattner G, Oxorn H. 1973. Familial incidence of ovarian dermoid cysts. Canadian Medical Association Journal 108:892–893.

Povey S, Burley MW, Attwood J, Benham F, Hunt D, Jeremiah SJ, Franklin D, Gillett G, Malas S, Robson EB, Tippett P, Edwards JH, Kwiatkowski DJ, Super M, Mueller R, Fryer A, Clarke A, Webb D, Osborne J. 1994. Two loci for tuberous sclerosis: one on 9q34 and one on 16p13. Annals of Human Genetics 58:107–127.

Raghavan D, Jelihovsky T, Fox RM. 1980. Father-son testicular malignancy: does genetic anticipation occur? Cancer 45:1005–1009.

Rodriguez E, Mathew S, Mukherjee AB, Reuter VE, Bosl GJ, Chaganti RSK. 1992. Analysis of chromosome 12 aneuploidy in interphase cells from human male germ cell tumors by fluorescence in situ hybridization. Genes, Chromosomes and Cancer 5:21–29.

Schimke RN. 1983. Familial extragonadal germ cell tumors. American Journal for Medical Genetics 15:175–176.

Scully RE. 1953. Gonadoblastoma; a gonadal tumor related to the dysgerminoma (seminoma) and capable of sex-hormone production. Cancer 6:455–463.

Smith RC, Rukstalis DB. 1995. Frequent loss of heterozygosity at 11p loci in testicular cancer. The Journal of Urology 153:1684–1687.

Shuman C, Smith AC, Steele L, Ray PN, Clericuzio C, Zackai E, Parisi MA, Meadows AT, Kelly T, Tichauer D, Squire JA, Sadowski P, Weksburg R. 2006. Constitutional UPD for chromosome 11p15 in individuals with isolated hemihyperplasia is associated with high tumor risk and occurs following assisted reproductive technologies. American Journal of Medical Genetics 140A:1497–1503.

Simon A, Ohel G, Neri A, Schenker JG. 1985. Familial occurrence of mature ovarian teratomata. Obstetric Gynecology 66:278–279.

Traupe H, Happle R. 1983. Clinical spectrum of steroid sulfatase deficiency: X-linked recessive ichthyosis, birth complications, and cryptorchidism. European Journal of Pediatrics 140:19–21.

van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, Burley M, Jeremiah S, Woodward K, Nahmias J, Fox M, Ekong R, Osborne J, Wolfe J, Povey S, Snell RG, Cheadle JP, Jones AC, Tachataki M, Ravine D, Sampson JR, Reeve MP, Richardson P, Wilmer F, Munro C, Hawkins TL, Sepp T, Ali JB, Ward S, Green AJ, Yates JR, Kwiatkowska J, Henske EP, Short MP, Haines JH, Jozwiak S, Kwiatkowski DJ. 1997. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science 277:805–808.

Wakai S, Segawa H, Kitahara S, Asano T, Sano K, Ogihara R, Tomita S. 1980. Teratoma in the pineal region in two brothers: case reports. Journal of Neurosurgery 53:239–243.

Wong JC, Lee SB, Bell MD, Reynolds PA, Fiore E, Stamenkovic I, Truong V, Oliner JD, Gerald WL, Haber DA. 2002. Induction of the interleukin-2/15 receptor beta-chain by the EWS-WT1 translocation product. Oncogene 21:2009–2019.

INDEX

B