REVIEW

Epigenetics and human disease: translating basic biology into clinical applications

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ABSTRACT

Epigenetics refers to the study of heritable changes in gene expression that occur without a change in DNA sequence. Research has shown that epigenetic mechanisms provide an "extra" layer of transcriptional control that regulates how genes are expressed. These mechanisms are critical components in the normal development and growth of cells. Epigenetic abnormalities have been found to be causative factors in cancer, genetic disorders and pediatric syndromes as well as contributing factors in autoimmune diseases and aging. In this review, we examine the basic principles of epigenetic mechanisms and their contribution to human health as well as the clinical consequences of epigenetic errors. In addition, we address the use of epigenetic pathways in new approaches to diagnosis and targeted treatments across the clinical spectrum.

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The field of genetics has become an integral part of modern medicine in the 50 years since Watson and Crick described their structural model of DNA. In this time, significant advances have been made in identifying specific mutations associated with such human diseases as Huntington's chorea and cystic fibrosis. Further advances, including the Human Genome Project, are providing new opportunities to develop better diagnostic tools and targeted gene therapies.

A new field that will also have an enormous impact on medicine is epigenetics. It is the study of heritable changes in gene function that do not change the DNA sequence but, rather, provide an "extra" layer of transcriptional control that regulates how genes are expressed.[1] This rapidly evolving field offers exciting new opportunities for the diagnosis and treatment of complex clinical disorders. In this review, we will provide clinicians with an understanding of the principles of epigenetic mechanisms and how these principles relate to human health and disease. We will also address the use of epigenetic pathways in new approaches to molecular diagnosis and novel targeted treatments across the clinical spectrum.

Basic principles of epigenetics: DNA methylation and histone modifications

The human genome contains 23 000 genes that must be expressed in specific cells at precise times. Cells manage gene expression by wrapping DNA around clusters (octamers) of globular histone proteins to form nucleosomes (Fig. 1A).[2] These nucleosomes of DNA and histones are organized into chromatin. Changes to the structure of chromatin influence gene expression: genes are inactivated (switched off) when the chromatin is condensed (silent), and they are expressed (switched on) when chromatin is open (active) (Fig. 1B).[3] These dynamic chromatin states are controlled by reversible epigenetic patterns of DNA methylation and histone modifications.[4] Enzymes involved in this process include DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone acetylases, histone methyltransferases and the methyl-binding domain protein MECP2. Alterations in these normal epigenetic patterns can deregulate patterns of gene expression, which results in profound and diverse clinical outcomes.

The loss of normal DNA methylation patterns is the best understood epigenetic cause of disease, based on the initial studies during the 1980s that focused on X chromosome inactivation,[5] genomic imprinting[6] and cancer.[4] DNA methylation involves the addition of a methyl group to cytosines within CpG (cytosine/guanine) pairs[7,8] (Fig. 1A). Typically, unmethylated clusters of CpG pairs are located in tissue-specific genes and in essential "housekeeping" genes, which are involved in routine maintenance roles and are expressed in most tissues. These clusters, or CpG "islands," are targets for proteins that bind to unmethylated CpGs and initiate gene transcription. In contrast, methylated CpGs are generally associated with silent DNA, can block methylation-sensitive proteins and can be easily mutated. DNA methylation patterns are established and maintained by DNMTs, enzymes that are essential for proper gene expression patterns.[9] In animal experiments, the removal of genes that encode DNMTs is lethal; in humans, overexpression of these enzymes has been linked to a variety of cancers.

In addition to DNA methylation, changes to histone proteins orchestrate DNA organization and gene expression.[2] Histone-modifying enzymes are recruited to ensure that a recep-

tive DNA region is either accessible for transcription or that DNA is targeted for silencing[10–12] (Fig. 1B). Active regions of chromatin have unmethylated DNA and have high levels of acetylated histones, whereas inactive regions of chromatin contain methylated DNA and deacetylated histones.[2] Thus, an epigenetic "tag" is placed on targeted DNA, marking it with a special status that specifically activates or silences genes. These reversible modifications ensure that specific genes can

be expressed or silenced depending on specific developmental or biochemical cues, such as changes in hormone levels, dietary components or drug exposures.[11,13]

Nature or nurture ... or both?

DNA methylation patterns fluctuate in response to changes in diet, inherited genetic polymorphisms and exposures to envi-
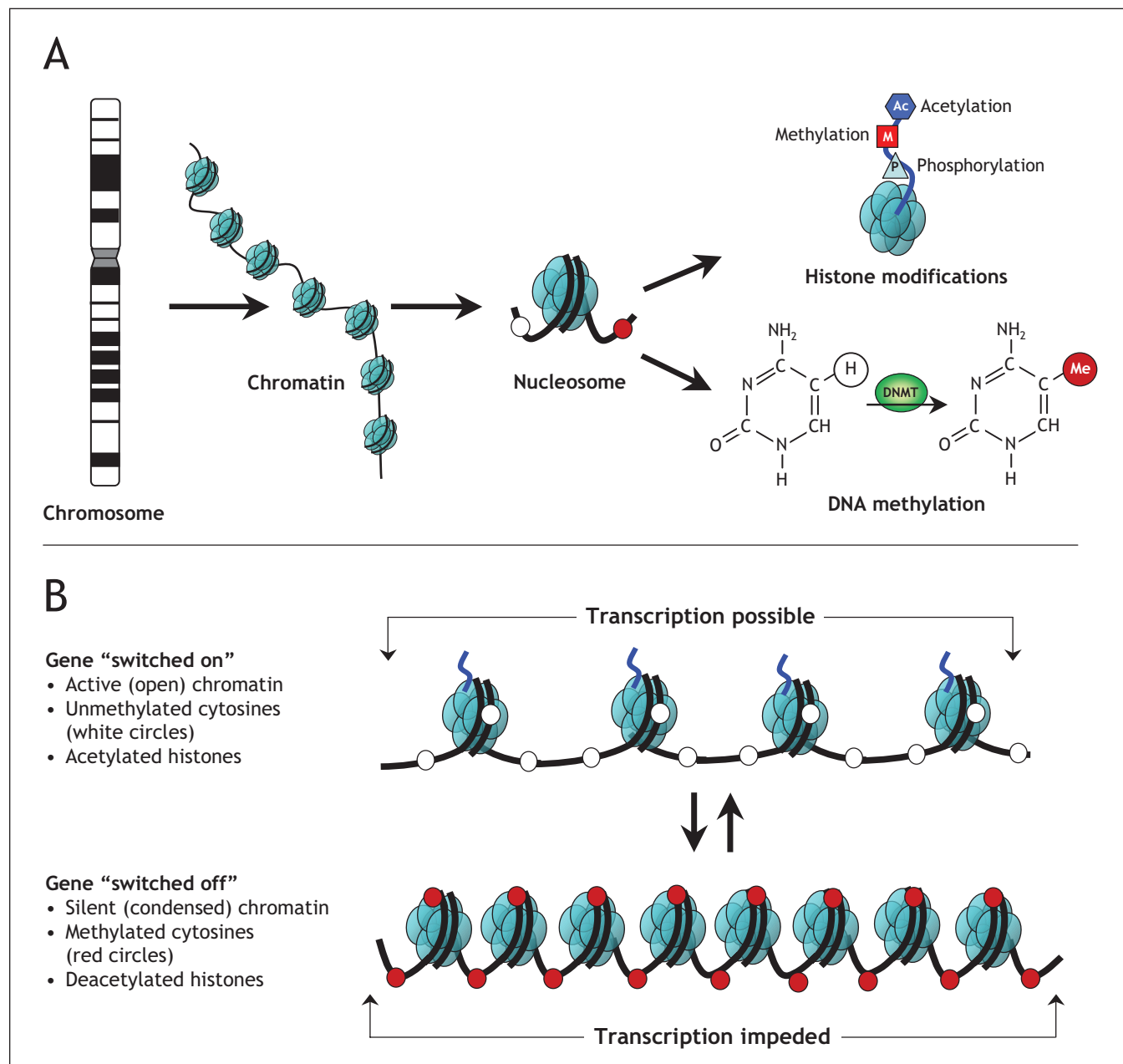

Fig 1: (A) Schematic of epigenetic modifications. Strands of DNA are wrapped around histone octamers, forming nucleosomes. These nucleosomes are organized into chromatin, the building block of a chromosome. Reversible and site-specific histone modifications occur at multiple sites through acetylation, methylation and phosphorylation. DNA methylation occurs at 5-position of cytosine residues in a reaction catalyzed by DNA methyltransferases (DNMTs). Together, these modifications provide a unique epigenetic signature that regulates chromatin organization and gene expression. (B) Schematic of the reversible changes in chromatin organization that influence gene expression: genes are expressed (switched on) when the chromatin is open (active), and they are inactivated (switched off) when the chromatin is condensed (silent).[3] White circles = unmethylated cytosines; red circles = methylated cytosines.

ronmental chemicals.[14,15] Methyl groups are acquired through the diet and are donated to DNA through the folate and methionine pathways.[16] Changes in DNA methylation may occur as a result of low dietary levels of folate, methionine or selenium, which can have profound clinical consequences such as neural tube defects, cancer and atherosclerosis.[17–21] Such imbalances in dietary nutrients can lead to hypomethylation (which contributes to improper gene expression) and genetic instability (chromosome rearrangements).[21] For example, hyperhomocysteinemia and global hypomethylation have been observed in vitro in atherosclerosis models, which supports an emerging view that alterations in global methylation patterns are characteristic of early stages of this disease.[19] In advanced stages of atherosclerosis, hyperproliferation may further contribute to DNA hypomethylation and altered gene expression.[22]

Environmental agents such as metals (e.g., arsenic) and aromatic hydrocarbons (e.g., benzopyrene) can also destabilize the genome or modify cellular metabolism, or both.[22,23] These environmental contaminants are found in occupational chemicals, fossil fuel emissions, contaminated drinking water and cigarette smoke.[14] People's sensitivity to diet or to environmental toxins may vary owing to pre-existing genetic variants that can challenge methyl metabolism and predispose a person to epigenetic change. Some reports have linked a common polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene to altered DNA methylation patterns in response to diet, alcohol consumption and hormone replacement and an apparent increased incidence of breast and colorectal cancer in certain populations. For example, the common *MTHFR* 677CT polymorphism has been shown to increase the risk of breast cancer up to 3-fold in premenopausal women.[24,25] Other studies suggest that the 677TT genotype confers a 40% decreased risk of breast cancer, particularly in postmenopausal women using hormone replacement therapy, by limiting the availability of nucleic acid precursors required by hyperproliferating cells.[26] Such examples underscore the complex interplay between epigenetics, the environment (nurture) and genetic individuality (nature) that potentially increase the risk of epigenetic disease.

Clinical consequences of epigenetic errors

Epigenetic mechanisms regulate DNA accessibility throughout a person's lifetime. Immediately following fertilization, the paternal genome undergoes rapid DNA demethylation and histone modifications.[27] The maternal genome is demethylated gradually, and eventually a new wave of embryonic methylation is initiated that establishes the blueprint for the tissues of the developing embryo. As a result, each cell has its own epigenetic pattern that must be carefully maintained to regulate proper gene expression. Perturbations in these carefully arranged patterns of DNA methylation and histone modifications can lead to congenital disorders and multisystem pediatric syndromes or predispose people to acquired disease states such as sporadic cancers and neurodegenerative disorders (Box 1).

Box 1: Normal cellular functions regulated in part by epigenetic mechanisms and molecular abnormalities caused by epigenetic errors

Normal functions

Correct organization of chromatin	Controls active and inactive states of embryonic and somatic cells
Specific DNA methylation and histone modifications	Controls gene- and tissue-specific epigenetic patterns
Silencing repetitive elements	Ensures that chromatin order and proper gene expression patterns are maintained
Genomic imprinting	Is essential for development
X chromosome inactivation	Balances gene expression between males and females

Abnormalities

DNA hypermethylation	Results in chromatin condensation and silencing of tumour suppressor and other genes
DNA hypomethylation	Activates oncogenes, results in chromosomal instability, activates transposons
Mutations at methylated cytosines	Results in inappropriate gene expression
Imprinting defects	Results in loss of parental identity

Genomic imprinting and imprinting disorders

Genomic imprinting allows genes to "remember" whether they were inherited from the mother or the father so that only the maternally or paternally inherited allele is expressed. Imprinting is regulated by DNA methylation and histone modifications[6] and is important in the context of a variety of developmental and pediatric disorders[54–58] (Table 1).

Prader–Willi, Angelman and Beckwith–Weidemann syndromes best characterize congenital imprinting disorders.[54,55] Prader–Willi and Angelman syndromes are caused by genetic and epigenetic errors to the same part of chromosome 15; errors inherited from the father result in Prader–Willi syndrome, and those inherited from the mother, Angelman syndrome.[54] Beckwith–Wiedemann syndrome is caused by genetic or epigenetic mutations resulting in loss of imprinting on chromosome 11.[55]

Besides gene-specific imprinting effects, global imprinting changes can occur in embryos that completely lack one parental genome (Table 1). For example, spontaneous activation of oocytes in situ leads to ovarian teratomas that lack a paternal genome.[64] In contrast, complete hydatidiform moles have been found that lack a maternal genome and an embryo and exhibit hyperproliferation of trophectoderm tissues, with the potential of forming choriocarcinoma.[65] The recessive disorder "familial biparental complete hydatidiform mole" also leads to recurrent development of moles when maternal-specific imprints fail to be established during oogenesis.[67] Interestingly, imprinting effects that appear to target trophoblast cells have been recently implicated as a cause of preeclampsia.[68]

Assisted reproductive technology

Recent evidence suggests that the manipulation of embryos for the purposes of assisted reproduction or cloning may impose inherent risks to normal development. For example, assisted reproductive technologies (ARTs) have been linked to an increased risk of intrauterine growth retardation (odds ratio [OR] 1.59, 95% confidence interval [CI] 1.20–2.11), premature birth (< 33 weeks' gestation, OR 2.99, 95% CI 1.54–5.80; < 37 weeks' gestation, OR 1.93, 95% CI 1.36–2.74), low birth weight (< 1500 g, OR 3.78, 95% CI 4.29–5.75) and prenatal death (OR 2.40, 95% CI 1.59–3.63).[69,70] Furthermore, an apparent association with ARTs was recently found in registries of children with Angelman syndrome and Beckwith–Wiedemann syndrome.[71] The prevalence of ART use was about 4–9 times greater among children with Beckwith–Wiedemann syndrome than among children in the general population (OR ≥ 3.2, 95% CI 1.4–7.3). Molecular analyses of patients with Angelman syndrome and Beckwith–Wiedemann syndrome conceived by in vitro fertilization or intracytoplasmic sperm injection revealed a loss of maternal-specific DNA methylation at imprinting centres, which indicates that the errors were epigenetic in nature. Although individually rare, as a group, epigenetic errors may impose significant risk for people conceived by ART. Similarly, in vitro culture in animal models has been found to lead to reduced viability and growth, developmental abnormalities, behavioural changes and loss of imprinting.[72,73] Such reports suggest that technologies involving the manipulation of cultured embryos may be the "tip of the iceberg" for a wider spectrum of epigenetic alterations.[74] Further study is required to determine whether epigenetic changes give rise to other imprinting disorders (or to cancer), and why such viability and growth defects appear to occur at increased frequency in the ART population.

Table 1: Associations between epigenetic modifications and human diseases and conditions

Disease/condition	Gene	Biological process	Disease/condition	Gene	Biological process
Cancer			**Neurologic**		
Bladder	Multiple genes	Hypermethylation[20]	Schizophrenia	RELN	Hypermethylation[46,47]
Brain (glioma)	RASSF1A	Hypermethylation[28,29]	Bipolar disorder	11p?	Unknown[48]
Brain (glioblast)	MGMT	Hypermethylation[30]	Memory formation	Multiple genes	Hypo-, hypermethylation[49]
Breast	BRCA1	Hypermethylation[31]	**Lupus**	Retroviral DNA	Hypomethylation[50]
Breast	Multiple genes	Hypermethylation[32,33]	**Cardiovascular**		
Cervix	p16	Hypermethylation[34]	Atherosclerosis	Multiple genes	Hypo-, hypermethylation[19,51]
Colon	Multiple genes	Hypermethylation[20]	Homocysteinemia	Multiple genes	Hypomethylation[52]
Colorectal	L1 repeats	Hypomethylation[35]	Vascular endothelium	eNOS	Hypomethylation[53]
Esophagus	CDH1	Hypermethylation[20]	**Imprinting and pediatric syndromes**		
Head/neck	p16, MGMT	Hypermethylation[20]	PWS or AS	15q11-q13	Imprinting[54]
Kidney	TIMP-3	Hypermethylation[20]	BWS	11p15	Imprinting[55]
Leukemia	p15	Hypermethylation[20]	SRS	Chromosome 7	Imprinting[56]
Liver	Multiple genes	Hypermethylation[36]	UPD14	14q23-q32	Imprinting[57]
Lung	p16, p73	Hypermethylation[20]	PHP, AHO, MAS	20q13.2	Imprinting[58]
Lymphoma	DAPK	Hypermethylation[20]	Rett syndrome	MECP2	Mutation[59]
Myeloma	DAPK	Hypermethylation[37]	ICF syndrome	DNMT3B	Mutation[60]
Ovary	BRCA1	Hypermethylation[38]	ATRX	ATRX	Chromatin structure[61]
Ovary	Sat2	Hypomethylation[39]	FraX	Triplet repeat	Silencing[62]
Pancreas	APC	Hypermethylation[20]	FSHD	3.3 kb repeat	Chromatin structure[63]
Pancreas	Multiple genes	Hypomethylation[40]	**Reproductive**		
Prostate	BRCA2	Hypermethylation[20,41]	Ovarian teratoma	No paternal genome	Imprinting[64]
Rhabdomyosarcoma	PAX3	Hypermethylation[42]	CHM	No maternal genome	Imprinting[65]
Stomach	Cyclin D2	Hypomethylation[43]	BiCHM	Maternal genome	Imprinting[65]
Thymus	POMC	Hypomethylation[44]	**Aging**	Chromatin	Hypo-, hypermethylation[66]
Urothelial	Satellite DNA	Hypomethylation[45]			
Uterus	hMLH1	Hypermethylation[20]			

Note: PWS = Prader-Willi syndrome; AS = Angelman syndrome; BWS = Beckwith-Weidemann syndrome; SRS = Silver-Russell syndrome; UPD14 = uniparental disomy 14; PHP = pseudohypoparathyroidism; AHO = Albright hereditary osteodystrophy; MAS = McCune-Albright syndrome; ICF = immunodeficiency, centromeric instability and facial anomalies; ATRX = α-thalassemia/mental retardation syndrome, X-linked; FraX = Fragile X syndrome; FSHD = facioscapulohumeral muscular dystrophy; CHM = complete hydatidiform mole, BiCHM = familial biparental CHM.

In addition, epigenetic abnormalities, including imprinting defects, are likely responsible for the substantial embryonic losses (up to 98%), placental hyperplasia and respiratory abnormalities observed in animal cloning models.[75] This may be because, in cloning experiments, the nucleus of a somatic cell must be reprogrammed to the epigenetic state of an embryonic nucleus; genes expressed specifically in somatic cells must be silenced, while those required for embryonic development must be activated. The presence of epigenetic defects in the majority of cloned embryos indicates that the donated nucleus is not efficiently reprogrammed to the epigenetic state of the embryonic nucleus for which it is substituting.[72,76] In contrast, a recent report showed that imprinting may be preserved in human embryonic stem cells, which suggests that these cells may be better candidates for therapeutic cloning.[77] A thorough understanding of epigenetic mechanisms and errors is therefore required before ARTs can reach their true potential in assisted reproduction, animal cloning or the engineering of replacement tissue transplants in cases of Parkinson's and other diseases.

Cancer and epigenetic therapies

Cancer is a multistep process in which genetic and epigenetic errors accumulate and transform a normal cell into an invasive or metastatic tumour cell. Altered DNA methylation patterns change the expression of cancer-associated genes (Table 1). DNA hypomethylation activates oncogenes and initiates chromosome instability,[78–80] whereas DNA hypermethylation initiates silencing of tumour suppressor genes. The incidence of hypermethylation, particularly in sporadic cancers, varies with respect to the gene involved and the tumour type in which the event occurs. For example, $p16^{INK4A}$ promoter hypermethylation occurs in varying degrees (9%–49%) in as many as 15 cancer types; in contrast, *BRCA1* hypermethylation is primarily associated with 10%–20% of sporadic breast and ovarian cancers.[20] These epigenetic changes can be used in the molecular diagnosis of a variety of cancers.[81]

To date, epigenetic therapies are few in number, but several are currently being studied in clinical trials or have been approved for specific cancer types.[1,82,83] Nucleoside analogues such as azacitidine are incorporated into replicating DNA, inhibit methylation and reactivate previously silenced genes.[84] Azacitidine has been effective in phase I clinical trials in treating myelodysplastic syndrome and leukemias characterized by gene hypermethylation. For example, 54% of patients with chronic myelogenous leukemia resistant to imatinib exhibited a complete or partial hematologic response, and 46% had a major or minor cytogenetic response to 5-aza-2′-deoxycytidine.[85] The antisense oligonucleotide MG98 that downregulates DNMT1 is showing promising results in phase I clinical trials[86] and in targeting solid tumours and renal cell cancer (www.methylgene.com/content.asp?node=14 [accessed 2005 Dec 22]). Molecular analysis of biopsies of head and neck cancer following MG98 treatment revealed demethylation of 2 methylated tumour suppressor genes and methylation of an oncogene. Similarly, small molecules such as valproic acid that downregulate HDACs are being used to induce growth arrest and tumour cell death. Combination epigenetic therapies (demethylating agents plus HDAC inhibitors) or epigenetic therapy followed by conventional chemotherapy (or immunotherapy) may be more effective since they reactivate silenced genes, including tumour suppressor genes, resensitize drug-resistant cells to standard therapies and act synergistically to kill cancer cells.[1,82,87] The key challenge for the future will be to limit toxic effects in normal cells and ensure that these novel drug effects reach critical target genes in tumour cells.

Aging

Both increases and decreases in DNA methylation are associated with the aging process, and evidence is accumulating that age-dependent methylation changes are involved in the development of neurologic disorders, autoimmunity and cancer in elderly people.[88] Methylation changes that occur in an age-related manner may include the inactivation of cancer-related genes. In some tissues, levels of methylated cytosines decrease in aging cells, and this demethylation may promote chromosomal instability and rearrangements, which increases the risk of neoplasia.[88] In other tissues, such as the intestinal crypts, increased global hypermethylation may be the predisposing event that accounts for the increased risk of colon cancer with advancing age.[89]

Immunity and related disorders

The activation of the immune response involves stepwise epigenetic changes, which allow individual cells to mount a specific immune response that can be maintained over multiple cell generations.[90,91] For example, shifts in both acetylation and methylation are required to coordinate DNA accessibility and permit recombination, thereby allowing cells to mount an immune response against a specific antigen.[92] Recent reports suggest that loss of epigenetic control over this complex process contributes to autoimmune disease. Abnormal DNA methylation has been observed in patients with lupus whose T cells exhibit decreased extracellular signal-regulated kinase pathway signalling, decreased methyltransferase activity and hypomethylated DNA.[93] Disregulation of this pathway apparently leads to overexpression of methylation-sensitive genes such as the leukocyte function-associated factor (*LFA1*), which causes lupus-like autoimmunity.[93,94] Interestingly, *LFA1* expression is also required for the development of arthritis, which raises the possibility that altered DNA methylation patterns may contribute to other diseases displaying idiopathic autoimmunity.[95]

Neuropsychiatric disorders

Recent reports have begun to address the role of epigenetic errors in the causation of complex adult psychiatric, autistic and neurodegenerative disorders (Table 1).[96] Several reports have associated schizophrenia and mood disorders with DNA rearrangements that include the DNMT genes.[96] *DNMT1* is selectively overexpressed in gamma-aminobutyric acid

(GABA)-ergic interneurons of schizophrenic brains,[97] whereas hypermethylation has been shown to repress expression of Reelin (a protein required for normal neurotransmission, memory formation and synaptic plasticity) in brain tissue from patients with schizophrenia and patients with bipolar illness and psychosis.[98] In addition, the HDAC inhibitor valproic acid has been shown to prevent Reelin promoter hypermethylation in a mouse model of schizophrenia.[99] A role for aberrant methylation mediated by folate levels has been suggested as a factor in Alzheimer's disease; however, there is contradictory evidence regarding hypomethylation and overexpression of the presenilin 1 gene that is involved in synaptic plasticity, long-term memory and neuronal survival.[100,101] As well, some preliminary evidence supports a model that incorporates both genetic and epigenetic contributions in the causation of autism.[102] Autism has been linked to the region on chromosome 15 that is responsible for Prader–Willi syndrome and Angelman syndrome. Findings at autopsy of brain tissue from patients with autism have revealed deficiency in *MECP2* expression that appears to account for reduced expression of several relevant genes.[103] These results suggest that *MECP2* deficiency plays a role in chromosome organization in the developing brain in autism, Rett syndrome and several other neurodevelopmental disorders.[104] There may be a role for epigenetics in the diagnosis and treatment of complex neuropsychiatric disorders in the future.

Pediatric syndromes

In addition to epigenetic alterations, specific mutations affecting components of the epigenetic pathway have been identified that are responsible for several syndromes: *DNMT3B* in the ICF (immunodeficiency, centromeric instability and facial anomalies) syndrome,[60] *MECP2* in Rett syndrome,[105] *ATRX* in ATR-X syndrome (α-thalassemia/mental retardation syndrome, X linked)[61] and DNA repeats in facioscapulohumeral muscular dystrophy[63] (Table 1). In Rett syndrome, for example, *MECP2* encodes a protein that binds to methylated DNA; mutations in this protein cause abnormal gene expression patterns within the first year of life. Girls with Rett syndrome display reduced brain growth, loss of developmental milestones and profound mental disabilities.[105] Similarly, the ATR-X syndrome also includes severe developmental deficiencies due to loss of ATRX, a protein involved in maintaining the condensed, inactive state of DNA.[61] Together, this constellation of clinical pediatric syndromes is associated with alterations in genes and chromosomal regions necessary for proper neurologic and physical development.

The road ahead

Our increased knowledge of epigenetic mechanisms over the last 10 years is beginning to be translated into new approaches to molecular diagnosis and targeted treatments across the clinical spectrum. With the Human Genome Project completed, the Human Epigenome Project has been proposed and will generate genome-wide methylation maps.[106]

By examining both healthy and diseased tissues, specific genomic regions will be identified that are involved in development, tissue-specific expression, environmental susceptibility and pathogenesis. Use of these epigenetic maps will lead to epigenetic therapies for complex disorders across the clinical spectrum.

Our understanding of the role of epigenetic abnormalities in disease processes is still in its infancy. The primary goals over the next decade will include improving our understanding of the interplay between epigenetic mechanisms, gene expression and the environment, and moving from animal models to clinical trials of novel epigenetic therapies directed at a wide variety of diseases. By decreasing the risk of epigenetic instability that leads to disease and by correcting epigenetic abnormalities that predispose to diseases later in life, the promise for epigenetic therapies as an essential treatment option will be fulfilled.

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