

Epigenetics and Human Disease

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Although the term epigenetics has been around for more than 50 years, it is perhaps not surprising that there have been several revisions to its definition. Originally used to describe the cascade of changes within an organism associated with growth and development that are directed by both the organism's genome and its surrounding environment, the term now refers to potentially heritable information that is not encoded by DNA sequence variation, but can change how the DNA is interpreted. Information can be modified by internal and external environmental cues and the resultant changes can be faithfully passed on to daughter and progenitor cells through both mitosis and meiosis. This definition is important from the perspective of understanding the contribution of epigenetic disturbances to human disease. Several epigenetic disturbances involving single genes are now recognised to be responsible for a range of disorders associated with abnormalities of growth, neurodevelopmental delay and childhood onset cancers. Epigenetic disturbances within somatic cells may be stably transmitted into daughter cells, a phenomenon that is now recognised as an important mechanism in the progression to common adult-onset diseases. Increasingly, there is evidence that epigenetic disturbances contribute to the development of complex non-Mendelian diseases such as diabetes mellitus, asthma, epilepsy and neuropsychiatric disorders such as autism, bipolar disorder and schizophrenia. A common theme to disease epigenetics is loss of phenotypic responsiveness, or the ability of cells to adapt to internal or external environmental cues. As well as offering many new insights into the pathogenesis of human disease, the recent characterisation of an increasing number of epigenetic mechanisms is fuelling the development of epigenetic therapies, some of which are already in clinical use.

Until recently, few would have considered the possibility that some heritable information may not be encoded by DNA. The fast growing field of epigenetics has quickly changed all this and, at the same time, the recent surge of epigenetic discovery is offering new insights into the etiology of many human diseases. Contemporary use of the term epigenetic refers to potentially heritable changes in gene expression that are not encoded within a DNA sequence and which may also be reversed. Recognition that these changes to gene expression may be transmitted through meiosis as well as mitosis challenges the long-standing notion that the genetic inheritance does not include any 'memory' of an organism's past experiences. Persistent epigenetic changes may also occur in post-mitotic cells, in which case the changes may be regarded as non-heritable. The emerging discipline of epigenetics is challenging many long-standing dogmas and also opening up important new insights into how genes interact with the environment to maintain health. It is also contributing to increasing recognition of the important contribution of epigenetic dysregulation to human disease.

Epigenetic Mechanisms

Epigenetic regulation is mediated principally by chemical modifications to DNA and histones. The nucleoside cytosine, particularly in the context of guanine being an immediately downstream neighbour (referred to as a CpG dinucleotide) may be epigenetically modified by the addition of a methyl (-CH₃) group. Histone proteins, around which DNA coils to form chromatin, are also subject to post-translational biochemical modifications. These DNA and histone changes occur in the context of higher-order changes to the looping and packaging of chromatin, with chromatin remodelling playing a major regulatory role in gene expression. The net effect of these changes is to regulate access of the transcription machinery to specific genomic sequences. Inaccessible genes are silent whereas accessible genes are transcribed. Beyond the inheritance of DNA, it is now appreciated that epigenetic changes to both DNA and histones may persist through cell division into daughter cells. This fundamental recent insight, together with increasing evidence that aberrant epigenetic signals may be therapeutically modified, provides the scientific basis and impetus for an emerging new era of medical discovery.

An understanding of the range of epigenetic mechanisms that influence gene expression, most notably regulation of the rate of RNA transcription from the underlying genomic sequence, which includes regulation of repressive heterochromatin by microRNA and other non-coding regulatory RNAs, is key to appreciating the importance of epigenetics. Almost certainly there are more epigenetic mechanisms to be discovered, including the processes that control the higher-level organisation of chromatin within the nucleus. Epigenetic changes can be regarded as analogous to the turning of a garden water tap, which has a dynamic range from fully open to completely off, with the rate and duration of flow ideally being matched to a garden's specific water requirements. Just like a garden tap, which is likely to be mostly turned off during winter months, epigenetic regulation of gene expression can be changed in response to environmental cues. But the amount of available RNA is not just dependent on the rate of transcription; the pace of RNA destruction is also important. RNA degradation is an actively regulated process that can also be influenced by several mechanisms including siRNA interference, which is activated when RNA molecules occur as double-stranded pairs that prompt silencing of the corresponding gene.

Epigenetic Regulation and Health

The concept of a genome having an accompanying epigenome, which regulates the activity of individual genomic sequences within the genome, offers new insights into how organisms adapt to environmental change and the important role of epigenetics in health maintenance. Epigenetic changes allow an organism to respond to a diversity of external environmental stresses, which may

include nutritional, chemical and physical challenges (1). Epigenetically-driven phenotypic diversity, which may also be referred to as developmental plasticity (2), has the potential to extend the environmental range within which an organism can thrive. Conversely, maladaptive epigenetic changes may limit the responsiveness of an organism to its surrounding environment, with an accompanying increased vulnerability to disease. At a more fundamental level, it is now appreciated that epigenetic modifications are a central component of fetal development and many epigenetic settings are established before conception. In fact, most epigenetic marks are erased from primordial germ cells from the time of their arrival in the embryonic gonad, but then subsequently reintroduced at different stages of spermatocyte and oocyte development in patterns that are gender-specific. The totipotency of sperm and ova, which are the only cells capable of generating an entirely new competent organism, is maintained by active transcriptional repression of cascading programs of somatic differentiation (3). This repression is maintained by a range of mechanisms including histone-mediated epigenetic repression. Similarly, a range of controlling epigenetic mechanisms contribute to the activity and fate of progenitor stem cells (4).

Imprinting

In recent years, details have emerged about epigenetic variation in germ line cells that persists in any resultant zygotes and which is set by the gender of the transmitting parent. Parent-of-origin defined epigenetic allelic differences within the developing embryo, which results in differential expression of parental alleles during development and, in many instances, into adulthood, is referred to as 'genomic imprinting' (5). Programmed epigenetic changes to the regulation of gene expression from fertilisation onwards prompt the emergence of different tissue types, which ultimately contribute to the integrity of a mature organism. These programmed changes are guided by an intracellular memory of the parental origin of imprinted alleles and are driven by active processes that involve methylation of CpG repeat sequences, histone deacetylation, microRNAs, polycomb proteins, SWI/SNF protein complexes and non-coding anti-sense RNAs, all of which play a part in the imprinting mechanisms of different genes (6).

Epigenetic Dysregulation and Disease

Since the epigenome affects gene activity, it is perhaps not surprising that there is a strong link between epigenetic errors and human disease. An epimutation or disease-associated epigenetic disturbance may be wholly responsible for the occurrence of a disease. However, epigenetic disturbances, particularly in cancer, may include both localised hypermethylation, which results in silencing of growth regulating genes (7), and global hypomethylation, which leads to activation of growth promoting genes and genes required for metastatic spread of cancer cells (8). It is now appreciated that global hypomethylation may be triggered by a range of demethylating carcinogens (9,10). Included among these is cigarette smoke, which has recently been shown to stimulate the demethylation of a prometastatic oncogene in lung cancer cells (11). Low birth weight, a marker of adverse intrauterine circumstances, is now recognised to be associated

with a range of disease outcomes in later life, including coronary heart disease, hypertension, type 2 diabetes, and osteoporosis (12,13). A rat model of uteroplacental insufficiency, which in humans is a common cause of intrauterine growth retardation, is associated with later adult onset of the typical pancreatic cell secretory defects and insulin resistance changes of type 2 diabetes (14). Genome-wide DNA hypomethylation and other widespread epigenetic changes in the liver have been identified in the model (15). These findings have been further extended recently by observations that dietary protein restriction induces gene-specific epigenetic changes that alter hepatic mRNA expression (16). Encouragingly, the same group has also observed that these epigenetic responses to impaired prenatal nutrition are prevented by folic acid supplementation.

Disorders Arising from Constitutional Dysregulation of Imprinted Genes

Several childhood-onset developmental disorders arise from epimutations that disrupt imprinting-mediated regulation of the genes involved. These include the well-studied examples of Angelman syndrome and Prader-Willi syndrome, both of which may be caused by genomic deletions involving an identical region of chromosome 15, but with the clinical phenotype determined by whether the deletion has occurred on the maternally- or paternally-derived chromosome. This is an example of a sequence-driven genetic factor prompting the occurrence of an epimutation in a parent-of-origin specified pattern. Both disorders, however, may also arise from epimutations in the absence of an accompanying underlying genomic defect. Similarly, Beckwith-Wiedemann syndrome, which is a somatic overgrowth syndrome associated with an 800-fold increased risk of embryonal tumours (17), is caused by a number of different categories of epigenetic and genetic disruptions to the normal regulation of two neighbouring imprinted subdomains on 11p15 (18). Included among the epigenetic lesions is loss of maternal imprinting of *IGF2*, which leads to a double dose of this potent growth factor receptor, or loss of methylation of *LIT1* leading to silencing of p57^{KIP2}, which is a growth-repressing cyclin-dependent kinase inhibitor.

GNAS1 is another imprinted locus that has an unusually high level of transcriptional and regulatory complexity, which includes maternal, paternal and biallelic expression, some of which is tissue specific, as well as a noncoding antisense transcript. *GNAS1* gives rise to alternatively spliced isoforms, including *GS α* , which couples a large variety of hormone and neurotransmitter receptors to the activation of adenylate cyclase and the regulation of intracellular levels of the second messenger cAMP (19). As *GS α* is the only protein with this activity in most tissues, it has a role in a wide range of homeostatic mechanisms, growth and differentiation pathways, and endocrine functions, including reproduction. It has been hypothesised that *GS α* imprinting in proximal renal tubules is important for maintaining maternal serum calcium homeostasis during pregnancy, helping to regulate the demand on maternal calcium during fetal bone development (20). However, even if eventually proven to be correct, the theory does not explain why *GNAS1* has such a high level of additional imprinting complexity.

Insights from Monogenic Disorders Involving Components of the Epigenetic Apparatus

It is interesting to consider the effects of monogenic disorders involving genes that disrupt core components of epigenetic regulation, including DNA methylation, methyl CpG binding proteins and histone modifications that regulate repressive chromatin remodelling activities. Mutations that inactivate the *MECP2* gene, which encodes methyl CpG-binding protein 2, one of a small family of proteins that mediate 'cross-talk' between DNA methylation and histone modifications (21), are associated with seemingly normal prenatal growth and development, which progresses into an early childhood phase of severe neurodevelopmental arrest with an associated decline in brain growth. Despite MeCP2's role as a transducer of epigenetic signals associated with DNA methylation, the biological consequences of MeCP2 deficiency appear to be restricted to specific populations of post-mitotic neurones during a particular window of central nervous system development. Mutations in the *ATRX* gene, which encodes a helicase involved in chromatin remodelling, lead to severe defects in psychomotor development, as well as urogenital and erythroid cell maturational defects that resemble α -thalassemia (22). Again, this is mostly a neurodevelopmental phenotype. By contrast, mutations in the DNA methyltransferase gene *DNMT3B*, which encodes a DNA methyltransferase that enzymically mediates *de novo* DNA methylation, prompt a milder neurodevelopmental defect associated with some physical dysmorphisms. However, among those with *DNMT2B* mutations, death occurs during childhood as a result of infections arising from a severe immunodeficiency (23). It is notable among many of these constitutional disorders arising from mutations in genes encoding seemingly key components of the epigenetic machinery that many organ systems continue to function normally, which suggests the presence of redundancies of epigenetic function that are yet to be characterised.

Disorders Arising from Somatic Cell Epigenetic Dysregulation

Imprinted gene dysregulation may arise in somatic cells, prompted by either epigenetic or genetic mutations. As many imprinted genes are oppositely acting growth factors, it is perhaps not surprising that epigenetic disturbances are ubiquitous in cancer (24) and several epigenetic disturbances in a malignant cell may contribute to the cascade of pathogenic steps that have progressed the cell towards tumourigenesis (see Showcase on Research article by Hinshelwood and Clark). Suffice to say here that a number of tumour suppressor genes are inactivated by epimutations (25) and that imprinted oncogenes are commonly overexpressed in cancer cells as a result of loss of imprinting (26). Epigenetic mechanisms are now also recognised in the pathogenesis of the autoimmune disease systemic lupus erythematosus. In addition to a number of genetic factors known to predispose to lupus, ultraviolet light and a number of drugs may also trigger the disorder. Procainamide and hydralazine are two well-known trigger drugs, both of

which prompt altered gene expression in T lymphocytes by inhibiting DNA methylation as a result of reduced expression of *DNMT1*, which encodes DNA methyltransferase 1. In the same way, T cells from lupus patients have decreased total genomic methylated cytosine content associated with decreased *DNMT1* expression (27). Similarly, bearing in mind its lupus-triggering effect, ultraviolet light also inhibits DNA methylation (28).

Epigenetic Responses to the Environment

The potency of epigenetic responses to the environment is powerfully illustrated by amphibian gonadal sex determination, which is defined by the ambient temperature during organogenesis instead of the genetic mechanisms used by mammals (29). In terms of human health, relatively recent epidemiological evidence that early life influences can alter later disease risk (30) has spawned the 'developmental origins of health and disease' (DOHaD) paradigm, which is bringing new insights into the pathogenesis of disease. The important observation of increased risk of coronary heart disease, stroke, hypertension, type 2 diabetes and osteoporosis associated with intrauterine growth retardation has led to the hypothesis that a range of disorders may have their origins in a mismatch between the environment during early development and the environment encountered in adulthood (31). Although DOHaD is now a burgeoning research area in both basic and clinical sciences, the concept of epigenetically driven developmental plasticity seems likely to be relevant to all stages of life, although with a progressive diminution of epigenetic plasticity associated with developmental maturation and the aging process.

Long-term Sequelae of Epigenetic Responses to the Fetal Environment

Prenatal environmental exposures to an increasing range of nutritional, chemical and physical factors are now known to induce long-term changes to epigenetic programming. Animal studies are now providing evidence that nutritional status at the earliest stages of development may cause persistent epigenetic changes (32). Striking evidence of the important role of diet in the maintenance of epigenetic integrity comes from several elegant studies of the *Agouti variable yellow* (*A^{vy}*) mouse, which have shown that maternal nutrition during pregnancy can alter the epigenetic programming within offspring (33).

Environmentally induced gene expression changes are mediated by a range of mechanisms including mutations within the promoter regions of genes, as well as epigenetic changes at critical genomic regions (34). The genomic targets of environmental triggers of epigenetic dysfunction contain CpG islands that may be either normally methylated, unmethylated or differentially methylated. An example of the potentially life-long consequences of epigenetic changes induced by an altered early fetal environment is the increased risk of birth defects among children conceived by assisted reproductive technology (ART). A well-constructed and statistically powerful evaluation of the rate of congenital malformations in an Australian population has revealed a doubling of the rate

of congenital malformations among children conceived by ART (35). Potent evidence that at least some of these congenital malformations have an epigenetic basis comes from recognition that the incidences of Beckwith-Wiedemann syndrome (BWS) and Angelman syndrome among ART-conceived children are higher than their rates of occurrence in the background population – a large Victorian case-control study has identified a nine-fold increase in the risk of BWS associated with ART conceptions (36). Epimutations, which are normally found in a minority of cases of BWS and Angelman syndrome, are detected in a very high proportion of the ART-conceived cases and virtually all involve loss of methylation of the maternal allele. Although these findings point to an urgent need to define the details of the mechanism underlying the occurrence of these aberrant epigenetic lesions among ART-conceived children, a recent survey of the incidence of identifiable imprinting disorders in children conceived by ART suggests that the absolute risk of imprinting disorders in these children is less than 1% (37).

Epigenetic Responses to the Infant Environment

In addition to epigenetic changes induced by undernutrition (and also toxins), there is now growing evidence that psychosocial exposure early in life may also prompt epigenetic reprogramming with an associated legacy of altered behaviour later in life (38). Evidence of epigenetic responsiveness to psychosocial stimuli has important implications. As well as revealing a biological mechanism for the effects of nurture on nature, it also demonstrates that the social behaviour of one subject may affect the epigenetic programming in another. It also introduces the possibility that epigenetic re-programming arising from exposure to chemical and environmental pollutants may have persistent behavioural effects (39).

Epigenetics and Common Diseases – Interaction Between the Epigenome, Genome and the Environment

There is increasing evidence that the field of epigenetics will provide new insights into the biological basis of complex non-Mendelian diseases. An important feature of epigenetic change is that stability may be partial, sometime referred to as metastability, which can be relatively long-lived but ultimately transient in nature (40). A hallmark of complex non-Mendelian disease is discordance among monozygotic twins, which strongly suggests the influence of factors other than inherited gene variants. Although a number of important risk factors, such as smoking and cancer and high fat dietary intake in relation to coronary artery disease, have been identified over the past 50 years, they, in fact, explain only a small proportion of the discordance observed among monozygotic twins. Recognition now that the epigenome is a dynamic link between an organism's genome and its environment offers a powerful new opportunity to identify factors contributing to the high discordance of complex diseases among monozygotic twins. A useful hint towards the likely significance of epigenetic-based causality for complex diseases comes from a study of identical twins of different ages, which suggests a greater

discordance in epigenetic marks such as the amount and patterning of DNA methylation in older twin pairs compared with younger twin pairs (41). As parent-of-origin specific linkage, which is strongly suggestive of the involvement of imprinted genes, has been identified in a number of complex non-Mendelian disorders, including autism, bipolar disorder, epilepsy, type 1 diabetes and schizophrenia, it seems that epigenetic disturbances are contributing significantly to the occurrence of these human disorders. Carolyn Ptak and Art Petronis, in a recent review of epigenetics and complex disease (40), succinctly summarise the likely place of epigenetics in complex disease: "*The epigenetic model of complex disease can be imagined as a chain of aberrant epigenetic events that begins with a pre-epimutation, a primary epigenetic problem that takes place during the maturation of the germline; the pre-epimutation increases the risk for the disease but is not necessarily sufficient to cause the disease. The misregulation can be tolerated to some extent, and age of disease onset may depend on the effect of tissue differentiation, stochastic factors, hormones, and probably to some external environmental factors such as nutrition, infections, medications and additions. It may take decades to reach a critical threshold beyond which the genome, cell, or tissue can no longer function normally. Only some predisposed individuals will reach the threshold of epigenetic misregulation and acquire phenotypic changes that meet the diagnostic criteria for a clinical disorder. Severity of epigenetic misregulation may fluctuate over time, which in clinical terms is called remission and relapse. In some cases, 'aging' epimutations may slowly regress back to the norm.*"

Epigenetics and Aging

The time-dependent decline in responsiveness to the environment associated with aging comes with a cumulative epigenetic legacy that reflects a lifetime of environmental exposures. Many aged mammalian tissues have reduced levels of DNA methylation (42), and data from monozygotic twins provide corroborating evidence that epigenetic variants accumulate during aging independently of the genetic sequence (41). 'Aging epigenetics' is another nascent research field that promises exciting discoveries in the near future. Already, plans are afoot for the definition of age-specific DNA methylomes and histone modification maps that will help to define a 'young' versus an 'old' cell, and to characterise all the chromatin modifier enzymes involved in the process (43).

Epigenetic Therapies

As epigenetics yields new insights into etiological and pathogenic mechanisms, it is providing a scientific base for the development of new drugs that have the potential to target the primary epigenetic lesions responsible for a range of complex non-Mendelian diseases. As epigenetic factors such as DNA methylation and histone deacetylation are known to contribute to the malignant transformation of cells by silencing critical genes, most trials of epigenetic drugs to date have been assessments of their effects on cancer. Already, several anti-cancer drugs targeting components of the epigenetic machinery have moved from clinical trials

into clinical practice. At present, the targets for epigenetic drugs are DNA methyltransferase inhibitors and histone deacetylase inhibitors. The first treatments approved by the US Food and Drug Administration for the treatment of myelodysplastic syndromes were the DNMT inhibitors 5-azacytidine (azacitidine) and 5-aza-2'-deoxyazacytidine (decitabine). However, as many other molecules are also involved in epigenetic mechanisms in gene expression, there are other potential targets as well. Since epigenetic defects are thought to underlie a broad range of diseases, the scope of epigenetic therapy is likely to expand.

Conclusion

There is growing awareness of the important place of the epigenome, particularly serving as an interface between the dynamic environment and the inherited static genome. Like the environment, the epigenome is dynamic and changes induced by a range of environmental exposures may dictate both immediate and later responses of an organism to environmental change. The field of epigenetics is opening up many new opportunities to learn about how diseases arise from adverse interactions between the human genome and a range of environmental exposures. At the same time, opportunities are fast emerging that will enable us to understand how these interactions may be modulated to improve human health. Andrew Feinberg, in a recent review in *Nature* on the relevance of epigenetics to human disease, makes the point that as epigenetics is at the heart of phenotypic variation in health and disease, it is likely that increasing knowledge of the epigenome will bring major new opportunities to treat or prevent common human illness (18).

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