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Review

Epigenetic mechanisms in the context of complex diseases

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Abstract. Complex diseases arise from a combination of heritable and environmental factors. The contribution made by environmental factors may be mediated through epigenetics. Epigenetics is the study of changes in gene expression that occur without a change in DNA sequence and are meiotically or mitotically heritable. Such changes in gene expression are achieved through the methylation of DNA, the post-translational modifications of histone proteins, and RNA-based silencing. Epigenetics has been implicated in complex diseases such as cancer, schizophrenia, bipolar disorder, autism and systemic lupus erythematosus. The prevalence and severity of these diseases may be influenced by factors that affect the epigenotype, such as ageing, folate status, *in vitro* fertilization and our ancestors' lifestyles. Although our understanding of the role played by epigenetics in complex diseases remains in its infancy, it has already led to the development of novel diagnostic methods and treatments, which augurs well for its future health benefits.

Keywords. Epigenetics, DNA methylation, histone modifications, complex diseases, cancer, schizophrenia, autism, systemic lupus erythematosus.

Complex diseases

Complex diseases exhibit a heritable component but do not follow Mendel's laws of inheritance. Examples of complex diseases are cancer, schizophrenia, lupus and cardiovascular disease. These diseases are generally considered to derive from a combination of multiple heritable and environmental factors. The contribution made by environmental factors may be mediated through epigenetic changes (see Fig. 1).

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Epigenetics

Epigenetics is the study of changes in gene expression that occur without a change in DNA sequence and are mitotically, and sometimes meiotically, heritable. Epigenetic changes in gene expression may occur via changes in the folding of DNA to form chromatin and the architecture of that chromatin within the nucleus. A person's epigenotype is established during their development and is maintained to a degree throughout their lifetime. Systems that initiate and sustain the epigenetic state of DNA, and hence a person's epigenotype, include histone modifications, RNA-associated silencing and DNA methylation. These systems tend to interact and stabilize each other [1 – 3].

Histones are globular proteins, around which DNA is spooled to form chromatin. Histone 'tails' protrude

from their globular domains. These tails are post-translationally modified, and the modifications can include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation and glycosylation. The histone tail modifications act together to form a code, which is recognised by proteins that regulate chromatin structure and thus gene expression (reviewed in [4, 5]). For example, heterochromatin protein 1 (HP1) binds to the methylated lysine 9 of histone H3 (H3K9me). H3K9me, together with overall histone under-acetylation, is characteristic of silent genes. Conversely, the nucleosome remodelling factor, chromodomain helicase DNA binding protein 1 (CHD1), binds to the trimethylated lysine 4 of histone H3 (H3K4me3), and causes local loosening of chromatin. The combination of H3K4me3 and overall histone hyperacetylation is characteristic of active genes.

Non-coding RNAs (ncRNAs) can trigger gene silencing by regulating chromatin structure (reviewed in [6–8]). For example, the non-coding RNA, X-inactive-specific transcript (*Xist*), is involved in silencing the inactive X chromosome in females. More recently, short ncRNAs, including short interfering RNA (siRNA) and microRNA (miRNA) molecules have been identified. These molecules target homologous genes for silencing. The precursors for siRNAs are single-stranded RNA transcripts that fold into 'stem-loop' structures. The double-stranded 'stem' region is cleaved by the enzyme Dicer into siRNAs, which are 20–25 nucleotides long. In plants, these siRNAs silence homologous DNA regions through the recruitment of DNA methyltransferases and histone-modifying enzymes, although it is not yet clear whether this occurs in the same way in mammalian systems. Intriguingly, there has been a controversial finding recently that these short ncRNAs may also be able to activate some genes [9]. Piwi-interacting RNAs (piRNAs) are a newly discovered example of non-coding RNAs. These are 25–31 nucleotides long and found in the testes. They are probably involved in gene silencing, although their function is as yet unknown.

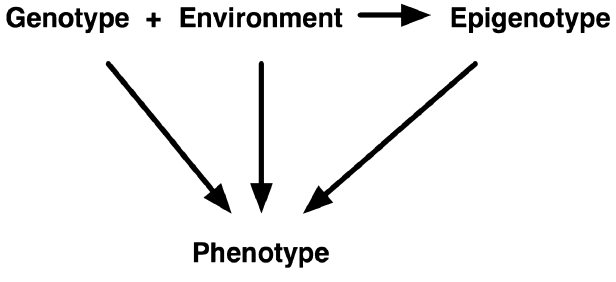

Figure 1. The phenotype of an organism is directly influenced by its genotype and its environment. In addition, these two factors indirectly influence phenotype via the epigenotype.

Epigenetic gene silencing can be achieved by DNA methylation. Methylation exists on about 80% of all CpG dinucleotides in mammalian DNA. Methylation is associated with the repression of genes and transposable elements, and with chromosomal stability. Methylation can inhibit gene expression by recruiting proteins, such as methyl CpG binding protein 2 (MeCP2), which in turn recruit transcriptional co-repressors. Methylation can also directly block binding of transcriptional activators such as myelocytomatosis viral oncogene homolog (Myc) (reviewed in [10, 11]).

Methyl groups are attached to CpG dinucleotides by DNA methyltransferases. This group of enzymes includes the *de novo* methyltransferases DNMT3a and -3b, which introduce methylation at previously unmethylated sites, and the maintenance methyltransferase, DNMT1, which copies pre-existing methylation patterns onto newly replicated DNA strands (reviewed in [10]).

DNA methyltransferases use the molecule *S*-adenosylmethionine (SAM) as their source of methyl groups. SAM is manufactured through the folate and methionine pathways, using methionine, choline, folic acid and vitamin B12 ingested in our diet [12]. DNA methyltransferases are inhibited by *S*-adenosylhomocysteine (SAH), which, together with homocysteine, is a by-product of DNA methylation. Studies of individuals with compromised folate pathways have found that genomic DNA methylation correlates positively with folate status and negatively with homocysteine status [13, 14]. In mice, an increase in folic acid intake can lead to increased DNA methylation of an allele of the agouti locus, causing gene silencing and a change in phenotype [15, 16]. Cancer, cardiovascular disease, schizophrenia and autism are complex diseases that have been linked to levels of folate and homocysteine. This raises the intriguing possibility that these diseases have an epigenetic component.

The involvement of epigenetics in complex diseases

Cancer

There is strong evidence for the role of epigenetics in cancer, although it is not always clear whether these changes are a cause or effect of the transformation process. In tumours with a well-defined progression, such as those occurring in colon cancer, aberrant DNA hypermethylation is detectable in a subset of precursor lesions. This suggests that hypermethylation directly contributes to cellular transformation, rather than being merely secondary to genetic alterations [17]. Changes in DNA methylation are thought to contribute to cell transformation in a variety of ways.

DNA hypomethylation is believed to initiate chromosome instability and activate oncogenes like related RAS viral oncogene homolog (*r-ras*), melanoma antigen 1 (*MAGE1*) and paired box gene 2 (*PAX2*) (reviewed in [18]). Conversely, DNA hypermethylation may initiate the silencing of tumour suppressor genes. Aberrant hypermethylation has been found in the promoters of genes involved in virtually all the steps of carcinogenesis, namely cell-cycle regulation, DNA repair, cell signalling, drug resistance and detoxification, apoptosis, differentiation, angiogenesis and metastasis (reviewed in [19]). Aberrant hypermethylation can encompass multiple neighbouring genes in large chromosomal regions [2].

The general pattern of histone modifications also appears to be disturbed in cancer. The genome-wide loss of acetylation at lysine 16 and trimethylation at lysine 20, both in histone H4, is present in multiple cancers, such as lymphomas, colorectal adenocarcinomas and squamous carcinoma. These changes appear in pre-cancerous cells and accumulate during the tumorigenic process [3]. Other patterns of histone modifications that are altered in cancer are the aberrant dimethylation of lysine 9 of histone H3 [2] and reduced levels of histone acetylation (reviewed in [20]).

Schizophrenia and bipolar disorder

There appears to be a degree of environmental input into the development of schizophrenia and bipolar disorder. The concordance for these diseases in monozygotic twins is roughly 50 % for schizophrenia [21] and 70 % for bipolar disorder [22]. At least in the case of schizophrenia, there seems to be a role for folate status in the development of the disease. Several lines of evidence indicate that folic acid deficiency and the polymorphism of genes related to folate metabolism are associated with schizophrenia (reviewed in [23]).

Many studies have shown that patients with schizophrenia have low circulating folate levels (reviewed in [21]). Serum folate concentrations in schizophrenia patients correlate inversely with the severity of their symptoms [24]. Risk factors for schizophrenia include not only low folate levels outright, but also famine, maternal malnutrition, late winter/early spring births, urban environments and low socioeconomic status, which may themselves be proxies for folate deficiency (reviewed in [25]). For example, the offspring of mothers affected by famines in the Netherlands [26] and China [27] were at an approximately twofold increased risk of risk of developing schizophrenia. The high incidence of schizophrenia in a Dutch cohort coincided with a 2.5 times higher incidence of neural tube defects, suggestive of low folate status [26].

5,10-Methylenetetrahydrofolate reductase (MTHFR) is an enzyme in the folate pathway. People who are homozygous for the common hypoactive variant (677C to T) of the MTHFR gene require a higher folate intake to generate the same amount of SAM [28] and have a 1.4-fold increased risk of schizophrenia [29].

Candidate genes that might account for the epigenetic sensitivity of schizophrenia and bipolar disorder include catechol-O-methyltransferase *(COMT)*, reelin *(RELN)* and glutamate decarboxylase *(GAD_{67})*.

COMT catalyses the first step in the degradation of neurotransmitters such as dopamine, epinephrine and norepinephrine. It is found in two forms in tissues: a soluble form (S-COMT) and a membrane-bound form (MB-COMT). A recent study of post-mortem brains has shown that the *MB-COMT* promoter is significantly more often hypomethylated and more active in the frontal lobe of schizophrenia and bipolar disorder patients relative to controls [23]. Hyperactivity of MB-COMT is associated with disturbances in attention, executive cognition and working memory. It has also been linked to an increased risk of schizophrenia, bipolar disorder and depression, although reports have been conflicting (reviewed in [23]). There is a positive correlation between hyperactive COMT and the hypermethylation of the *RELN* promoter [23].

A reduction in reelin and GAD_{67} in cortical structures of the brain is the most consistent finding of several studies on post-mortem brains from schizophrenia and bipolar disorder patients [30–32]. Reelin is an extracellular matrix protein thought to control the cell-cell interactions that are critical for cell positioning and neuronal migration during brain development, whereas GAD_{67} catalyses the production of the neurotransmitter gamma-aminobutyric acid (GABA). The gene encoding DNA methyltransferase 1 (*DNMT1*) is upregulated in the same GABA-ergic interneurons that downregulate *RELN* and *GAD_{67}* [30, 33]. It has been proposed that *DNMT1* overexpression is at least partly responsible for the downregulation of *RELN* in these neurons [32], since the *RELN* promoter is methylation-sensitive [34] and is hypermethylated in schizophrenia [35]. Chronic treatment of rodents with methionine (a dietary source of methyl groups) induces hypermethylation of the *RELN* promoter, downregulates *RELN* and *GAD_{67}* expression, and induces a psychotic-like state (as indicated by a decrease in pre-pulse inhibition of the startle reflex) [36]. Interestingly, an early study of patients with schizophrenia showed that the administration of methionine exacerbated the symptoms of the majority of patients [37].

Autism

RELN is a not only a candidate gene for schizophrenia but also for autism spectrum disorders (ASDs), which include autism and Asperger disorder. There are reduced levels of reelin messenger RNA (mRNA) and protein in the brains of autistic patients [38, 39].

Evidence for the involvement of epigenetics in ASDs remains slight. Children with autism have a lower ratio of SAM/SAH in their plasma, possibly causing an impaired methylation capacity [40]. Additionally, autism has been observed in conjunction with an incidence of prenatal exposure to the histone deacetylase (HDAC) inhibitor, valproic acid [41].

Brain tissues from patients with autism have reduced expression of *MeCP2*, the gene encoding the methyl CpG binding protein [42, 43]. ASD is also commonly found in conjunction with mutated *MeCP2* (in Rett syndrome). In *Mecp2*-null mice, imprinted genes such as ubiquitin protein ligase E3A (*Ube3A*) show loss of normal expression in brain tissue. Abnormalities in the methylation of *UBE3A* are also found in the brains of autistic patients [42]. About 5% of patients with ASD have duplications of the imprinted region of chromosome 15q11-q13, in which *UBE3A* is located [44–47].

Anxiety and depression

Epigenetics may play a role in anxiety and depression. A recent study in mice has shown that after chronic treatment with the antidepressant imipramine, levels of histone deacetylase 5 (*Hdac5*) mRNA are down-regulated in the hippocampus. Overexpression of *Hdac5* antagonises imipramine's antidepressant action [48].

A study in rats has raised the possibility that epigenetics is involved in the behavioural programming of anxiety. In rats, the stress-response is altered by the extent to which, as a pup, they are licked and groomed by their mother. More grooming is associated with more stress-resistant adult offspring. This effect is mediated by demethylation of a key site in the glucocorticoid receptor (*GR*) promoter in the hippocampus [49]. The resulting increased expression of GR gives rise to lower levels of circulating glucocorticoids because the hippocampus plays a crucial role in the negative feedback control of the hormones' release (reviewed in [50]). Although the hypomethylation of the *GR* promoter persists into adulthood in these rats, there is no evidence, as yet, that this hypomethylation is mitotically heritable, meaning that this phenomenon cannot be labelled as truly epigenetic. It does, however, provide a mechanism by which events in early life can program later behaviour.

Lupus

Systemic lupus erythematosus (SLE) is characterised by the production of autoantibodies to multiple nuclear antigens, which initiates an autoimmune reaction. T cells are believed to drive the autoantibody response, and this has been proposed to result from a loss of DNA methylation, which may be secondary to the disruption of cell signalling pathways [51]. In the T cells of patients with active SLE, DNMT1 levels are reduced [51], and the genomes of these cells are globally hypomethylated [52]. Treatment of normal murine T cells with DNA methyltransferase inhibitors renders them auto-reactive, and the transfer of these cells into mice causes a lupus-like disease [53]. There is some evidence to suggest that, in humans, exposure to DNA methylation inhibitors can induce a similar disease [54].

Metabolic syndrome

Several epidemiological studies have linked metabolic syndrome (which includes type II diabetes, cardiovascular disease, hypertension and obesity) to maternal malnutrition and diabetes [55, 56]. More recently, epidemiological studies have linked paternal behaviour to the development of metabolic syndrome in their offspring. Such paternal behaviour includes smoking [57], chewing betel nut [58] and food intake [57, 59]. It has also been suggested that the risk of metabolic syndrome may be affected by grandparental nutrition. Survivors of a famine in Sweden had grandchildren who were less likely to die from diabetes and heart disease than controls [59]. Epigenetics provides a possible mechanism by which organ development and physiologic homeostasis could be developmentally programmed and inherited. As yet, this theory lacks a credible molecular basis.

Epigenetics can make simple diseases more complicated

Although the diseases discussed thus far have been complex diseases, epigenetics is also involved in simple diseases such as Angelman syndrome, Beckwith-Wiedemann syndrome and transient neonatal diabetes. An in-depth discussion of these locus-specific diseases is beyond the scope of this review, but it is worth noting that some simple diseases, previously thought to be solely due to genetic variation at individual genes, may sometimes occur through aberrant epigenetic marks at these genes. Examples are α-thalassemia [60], hereditary non-polyposis colorectal cancer [61, 62] and caudal duplication anomaly [63].

Factors influencing epigenotype

The prevalence and severity of diseases with an epigenetic component may be influenced by factors that affect the epigenotype. Considering that the epigenotype displays a relatively high degree of developmental, temporal and inter-individual variability, it is probably more susceptible to environmental influences than the genotype [64]. In a study of monozygotic twins, the twins with the most disparate patterns of DNA methylation and histone acetylation were those that were older and those with a history of non-shared environments [65]. This review has already discussed the influences of folic acid levels on the epigenotype. Other factors that may affect the epigenotype are ageing, *in vitro* fertilization and exposure to endocrine disruptors.

As cells age, there is a generalised decrease in DNA methylation [66, 67], in association with an increase in localized hypermethylation at promoters [68]. It is intriguing that these epigenetic changes mirror those associated with cancer, which also tends to appear during or after middle age. Several genes in human sperm exhibit age-related DNA methylation changes, raising the possibility of patterns of epigenetic inheritance that vary with paternal age [69].

Children arising from assisted reproductive technologies (ARTs), including *in vitro* fertilization and intracytoplasmic sperm injection, are at a higher risk of epigenetic disorders (reviewed in [70, 71]). For example, ART is associated with a 3–6-fold increased risk of the imprinted disorders Angelman syndrome and Beckwith-Wiedemann syndrome [17, 72–75]. These diseases could result from epigenetic changes produced by *in vitro* culture conditions. Experiments in mice have shown that changes in the culture medium can alter the methylation status and expression of imprinted genes, and affect the birth weight of offspring [76, 77]. On the other hand, the risk of epigenetic disorders in the offspring may be heightened by the existence of epigenetic disturbances present in their parents' gametes, which may be responsible for their parents' infertility.

Diseases in offspring may arise from their ancestors' exposures to endocrine disrupters. Vinclozin is a fungicide used in the wine industry and an endocrine disruptor. In studies of rats, transient exposure of pregnant rats to vinclozin was found to cause abnormalities in their offspring, including reproductive abnormalities, prostate disease, kidney disease, immune system abnormalities and tumour development. These abnormalities were observed in several generations of descendents of the mice originally exposed. The incidence of the disease states was high and consistent across generations; 85 % of all animals across four generations were affected. The diseased phenotype was primarily transmitted through the male germ-line [78, 79], and there was an associated change in the methylation of multiple genes in sperm [80]. The absence of normal Mendelian transmission of the disease states indicates that they are not due to a single genetic mutation. The authors propose that vinclozin causes epigenetic changes to DNA in the developing male embryos, and that this change is maintained and transmitted via the sperm to the next generation.

Diethylstilbestrol (DES) is another example of an endocrine disrupter and, like vinclozin, is suspected to induce trans-generational epigenetic changes. Between the 1940 s and the 1970 s it was used to reduce the risk of miscarriage. In humans and rodent models, maternal exposure to DES leads to abnormal reproductive tract development and uterine tumours in the offspring [81, 82]. These abnormalities are also evident in granddaughters of mice exposed to DES during pregnancy [83].

Diagnosis and treatment of diseases harnessing the epigenotype

Epigenetics can be important in the diagnosis of complex diseases. For example, cancer-specific methylation can be detected in sputum to detect lung cancer [84] and in pancreatic secretions to detect pancreatic cancer [85]. Patterns of methylation in cancerous tissue, such as in neuroblastoma, breast cancer and prostate cancer [86–88], can be linked with the patient's prognosis and response to chemotherapies.

Several epigenetic therapies are being studied in clinical trials or have been approved for specific cancers. Inhibitors of DNA methylation and histone deacetylases (HDACs) reactivate the expression of genes that have undergone epigenetic silencing, particularly if this silencing has occurred in a pathological situation. Normal cells are almost always considerably more resistant than tumour cells to these drugs [89]. DNA methylation inhibitors include 5-azacytidine, which has received FDA approval for the treatment of myelodysplasia (a pre-leukemic syndrome); 5-aza-2′-deoxycytidine, which is in phase III clinical trials; and epigallocatechin-3-gallate and antisense oligomers, which are in phase I clinical trials (reviewed in [90–92]). HDAC inhibitors induce many tumours to stop growing and differentiate [20]. Examples of HDAC inhibitors are phenylbutyric acid, suberoylanilide, depsipeptide and valproic acid, which are under investigation in clinical trials (reviewed in [90]).

It appears that epigenetic therapies may be beneficial in schizophrenia. The HDAC inhibitor, valproic acid, enhances the effectiveness of antipsychotics in schizophrenia and bipolar patients [93]. Methylfolate improves both clinical and social recovery in patients with schizophrenia [94].

HDAC inhibitors, such as sodium butyrate, may be useful in the treatment of anxiety and depression. In a mouse model, treatment with sodium butyrate outperformed the selective serotonin reuptake inhibitor, fluoxetine, at improving behavioural despair [95].

Concluding remarks

Our understanding of the role played by epigenetics in the context of complex diseases remains in its infancy. Although epidemiological studies continue to be useful, studies aiming to measure the contribution made by epigenetics to the aetiology of complex diseases are often confounded by genetic variation. Most advances in our knowledge of mammalian epigenetics have come from studying genetically identical mouse strains, in which phenotypic variation may be attributed to epigenetic differences between individuals. In humans, the analogous model is discordant monozygotic twins. Needless to say, these are relatively rare. Another challenge facing researchers is the difficultly of accessing relevant tissue samples, such as from the human brain. Despite this, epigenetic research on humans holds the potential to explain much of the influence of environment on phenotype. It will be interesting to discover whether we inherit not only our gene sequence from our ancestors, but also the effects of their lifestyles.

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