Epigenetics and its Medical Repercussions

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Inheritable traits are not only encoded in the sequence of DNA, but also determined by factors 'on top' of the DNA, the epigenetic information ('epi' is classical Greek for 'on top')1. Epigenetics is the study of those mechanisms that control gene expression which are independent of the DNA sequence itself.

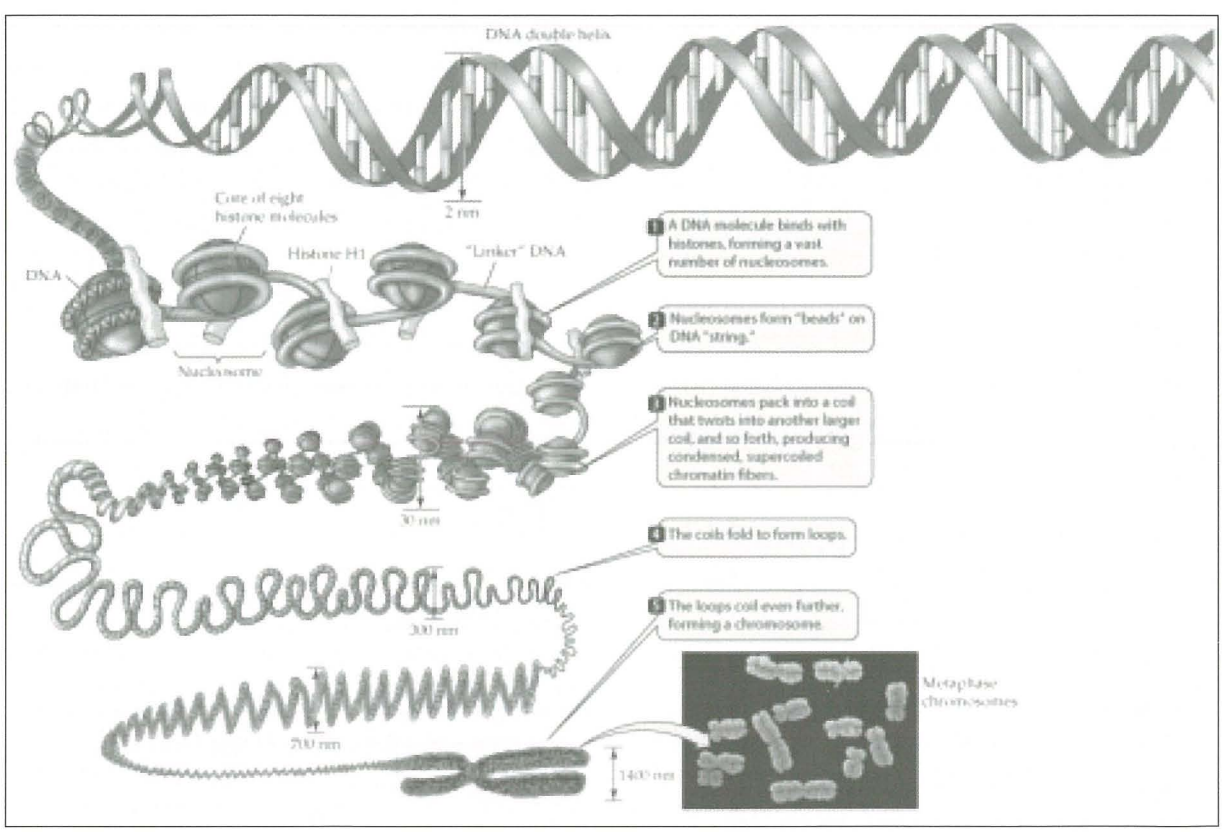
Molecularly (mechanistically), epigenetics can be defined as 'the sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome". DNA methylation and histone modification are the main epigenetic mechanisms in mammals2. Epigenetic factors are being found to play a role in disease (including cancer, atherosclerosis, autoimmunity, psychiatric diseases, diabetes mellitus and obesity), heredity, cell differentiation and cell development.

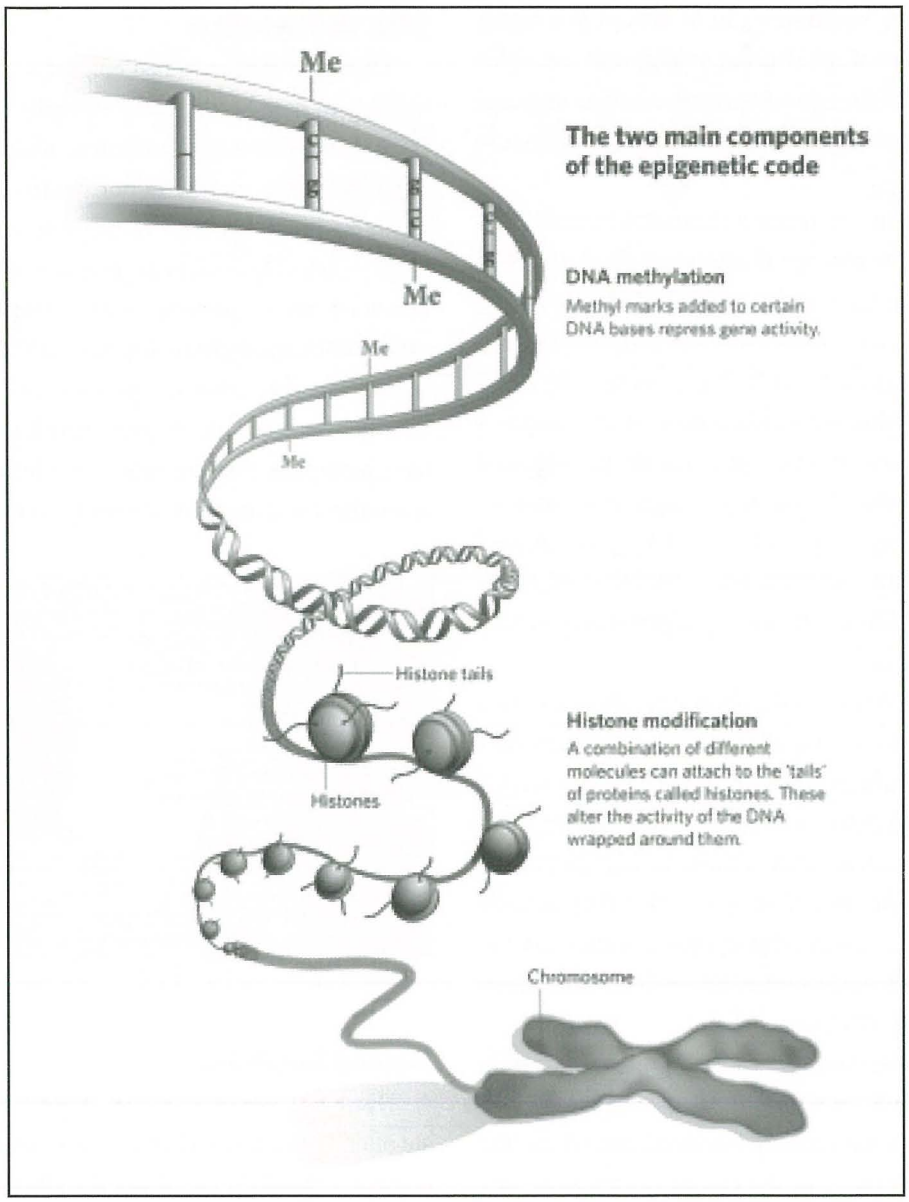
Epigenetic therapy is the use of drugs to correct epigenetic defects (epimutations). It is a new, realistic and rapidly developing area of pharmacology believed to treat several medical diseases.

Introduction

A eukaryotic chromosome contains a DNA molecule bound to proteins (histone type and non-histone type) in a complex called **chromatin**. During interphase, the DNA in chromatin is wound around cores of **histones** to form what are called **nucleosomes**. DNA folds over and over again, packing itself within the nucleus. During mitosis or meiosis, it folds even more[3].

The nucleosome is the basic structural unit of chromatin. Each nucleosome consists of about 146





bp (base pairs) of DNA wrapped around an octamer of core histone (including two each of histone H3, H4, H2B and H2A)[4]. Members of the linker histone H1/H5 family further stabilise the nucleosomal arrays. Initially, chromatin was thought to be an inert structure involved in packaging DNA into the confines of the nucleus, but today research is showing a more dynamic view of chromatin, particularly transcriptional activation and repression[5].

The DNA contains the instructions for building up all the parts of the body. But DNA is only half of the story. Both the DNA and histones are covered with chemical tags. This second layer of structure is called the **epigenome**. The epigenome shapes the physical structure of the genome. It tightly wraps inactive genes making them unreadable. It relaxes active genes, making them easily accessible. Different sets of genes are active in different cell types. The DNA code remains fixed for life, but the epigenome is flexible and can be modified. **Epigenetic tags** react to signals from the outside world, such as diet and stress. The epigenome adjusts specific genes in our 'genomic landscape' in response to our rapidly changing environment. Indeed, signals from the outside world can work through the epigenome to change a cell's gene expression[6].

Many, but not all, of the chromatin modifications and changes are reversible and, therefore, are unlikely to be propagated through the germ line. Intrinsic and external stimuli can bring about these transitory marks that impose changes to the chromatin template. In so doing, they regulate the access and/or processivity of the transcriptional machinery, which are needed to 'read' the underlying DNA template. Some modifications can however be stable through several cell divisions. This may establish 'epigenetic states' or a means of achieving 'cellular memory', which remain to be poorly understood.

Thus, chromatin 'signatures' can be viewed as a highly organised system of information storage that can index distinct regions of the genome accommodating a response to environmental signals that dictate gene expression programs.

The importance of having a chromatin template that can potentiate the genetic information is that it provides an extra layer to the readout of DNA. This might be a necessity, when one considers the size and complexity of multicellular organisms. In such organisms, a fertilised egg progresses through development: it starts with a single genome that becomes epigenetically programmed to generate a multitude of distinct 'epigenomes' in more than 200 different types of cells. This programmed variation constitutes what has been called the 'epigenetic code' that significantly extends the information potential of the genetic code[7].

Waddington described the phenotypic alterations that occur from cell to cell during the course of development in a multicellular organism as the 'epigenetic landscape'. All cells ranging from stem cells to fully differentiated cells, share identical DNA sequences but they differ remarkably in the 'profile of genes' that they actually express. With this knowledge, epigenetics later came to be defined as the 'nuclear inheritance which is not based on differences in DNA sequence'[8].

Many enzyme families are being discovered that modify chromatin (see below). And epigenetics can now be defined molecularly (mechanistically) as 'the sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome"[2]. While genetics is concerned with the information transmitted on the basis of gene sequence, epigenetics deals with the inheritance of information based on gene expression levels.

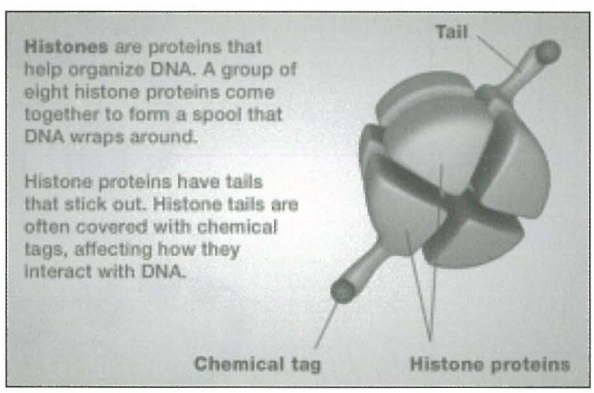
Thomas Jenuwein compares the eukaryotic genome to a "library containing instructions for living organisms to develop, where books represent chromosomes". As he says, "We know the sequence of all the letters in the books. But how these letters are organised into chapters is far less clear. Epigenetics has the promise of producing an index that will order the chapters and books of the genetic library.'

The Main Epigenetic Mechanisms

DNA methylation and **histone modification** are the main epigenetic modifications in mammals[9].

DNA Methylation

The main widely studied epigenetic modification in humans to date has been the **cytosine methylation of DNA**. This consists of the covalent addition of a methyl group from the methyl donor S-adenosylmethionine to the carbon-5 position of cytosine within the CpG dinucleotide. This enzymatic reaction occurs after DNA synthesis and is performed by a family of enzymes called **DNA methyltransferases (DNMTs)**. Research is showing that DNA methylation can be a clonally inherited mechanism of gene silencing. Genes which have promoters that are heavily methylated cannot be transcribed and are thus effectively silenced[10,11].



Histone Modification

Histone proteins have tails that stick out. Histone tails are often covered with chemical tags, affecting how they interact with DNA, modifying the chromatin structure and thus act as an epigenetic mechanism.

The histone tails can be acetylated, methylated, phosphorylated, poly-ADP ribosylated, ubiquitinated, and glycosylated. The combination of these modifications determines the histone-DNA interaction and the interaction of nonhistone proteins with chromatin through what has been called the **histone code**[12]. It is this histone code (i.e. the combination of histone modifications at a certain region of the chromatin), that determines the structure and function of the chromatin and hence gene expression. The histone code differs depending on the region of the chromatin, the cell type, the tissue type and the external conditions of a cell.

Histone acetylation is one of the best-studied histone modifications[13]. **Histone acetyltransferases (HATs)** catalyses this acetylation and uses acetyl-coenzyme A as a donor group. MORF, MOZ, MOF, TIP60 and HBO1

are all HATs. Histone acetylation occurs primarily at lysine residues of the histone H4 and H3, which are core histones. This alters the histone-DNA binding, (since the lysine loses a positive charge in the process) and also alters the binding codes of chromatin-interacting transcription factors. The level of histone acetylation depends on the balance between the action of HATs and **histone deacetylases (HDACs)**. There are four families of HDACs: class I (HDAC1,2,3, and 8), class II (HDAC4,5,6,7,9, and 10), class III (sirtuins 1-7), and class IV (HDAC11). 'Those of classes I, II, and IV have similar sequence and structure, but the sirtuins have a different structural homology and use a different catalytic mechanism that is dependent on NAD+ (nicotinamide adenine dinucleotide)'[9].

Another well-studied histone modification is **histone methylation**. It is linked to transcriptional activation and repression[13,14]. Histone tails can become methylated at several lysine and arginine residues. Among the lysine residues, the best studied are K4, -9, -27, -36, and -79 for H3 and K20 for H4. Lysine can be mono-, di-, and tri-methylated, whereas arginine can only be mono-methylated. Histone methylation is catalysed by a family of enzymes called **histone methyltransferases (HMTs)** and the methyl group can be removed by the a group of proteins called **histone demethylases (HDMs)**.

Medical Repercussions

Epigenetic aberrations (epimutations) are being found to play a role in several medical diseases. Indeed, epigenetics may be responsible in the development of cancer, atherosclerosis, autoimmunity, psychiatric disorders, diabetes mellitus and obesity. Also, epigenetics may be responsible for several hereditary disorders like disorders of genomic imprinting.

Several examples illustrate the involvement of DNA methylation in disease. In **Rett syndrome** there are mutations in the methyl-binding protein MeCP. In **lupus**, patients have severe degrees of DNA hypomethylation. Aberrant DNA methylation features in **fragile X mental retardation-1** (FMR). Here, there is DNA methylation of the fragile X mental retardation-1 (FMR) gene. **Atherosclerosis** is also characterised by aberrant patterns of DNA methylation; specifically protective cardiovascular genes are aberrantly hypermethylated. Patients with ICF (immunodeficiency, centromere instability, and facial anomalies) have mutations in a major DNA methyltransferase (DNMT3b). DNA methylation also features as an important player in **cancer** development.

Modifications in the histone code are also implicated in disease, especially in cancer. Acetylation of histone H4 is one of the best-studied alterations in the histone code in cancer. Histone H4 is found to be hypoacetylated in oesophageal squamous cell carcinoma, gastric cancer, testicular cancer, and acute promyelocytic leukemia (APL). Another histone modification that is observed is the tri-methylation of K20-H4. Tri-methylation of K20-H4 is enriched in differentiated cells, and increases with age; it is commonly reduced in cancer cells. Global aberrations of histone H3 modifications have also been observed in cancer. Low levels of acetylation at lysine 9 and 18 of histone H3 are associated with high recurrence of prostate cancer. H3 acetylation has been found to be reduced in human colon primary tumours and in several human colon cancer cell lines[2].

Cancer

Cancer encompasses a group of about 100 different and distinctive diseases[2]. A characteristic of these diseases is an abnormal growth of cells that generally lead to an uncontrolled proliferation. Research in recent decades have concentrated on identifying a wide variety of genomic changes, such as amplifications, translocations, deletions and point mutations. These genomic changes have been shown to be involved in this uncontrolled proliferation, and thus in the development of cancer. Also, analysis of these genomic alterations has led to the identification of **oncogenes** and **tumour-suppressor genes** involved in tumour development. An oncogene is a gene in which mutation, loss of function or other reduction of expression induces neoplasia[15]. A tumour-suppressor gene is a gene normally restraining a cell from excessive proliferation, by involvement of its product in the transduction of an anti-mitotic signal. Currently, research is showing that the occurrence of cancer is due not only to these genetic changes, but also to epigenetic changes, specifically to alterations of the histone code and DNA methylation involving such genes.

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DNA methylation was the first epigenetic alteration to be observed in cancer cells. Many cancer cells feature **hypermethylation of CpG islands at tumour suppressor genes**. This is responsible for the silencing of these genes. Another feature is that **global hypomethylation** leads to genome instability and **inappropriate activation of oncogenes and transposable elements**[16]. It also appears that genomic DNA methylation levels, which are maintained by **DNA methyltransferase enzymes** (DNMTs) are delicately balanced within cells; over-expression of DNMTs has been linked to cancer in humans[17,18].

Aberrant pattern of histone modifications at gene promoters is an important epigenetic route to cancer development. One such histone modification is the acetylation of lysine residues that is controlled by HATs and HDACs. Commonly, the existence of acetylated lysines within histone tails is associated with less-condensed chromatin and so a transcriptionally active gene status. If the lysines are deacetylated, heterochromatin forms and there is transcriptional gene silencing.

Studies are showing that there is a direct link between DNA methylation and histone modification. In fact, it is suggested that DNA methylation and histone methylation are tied together in a reinforcing loop. Upsetting this relationship will almost certainly have severe consequences on the epigenome and chromatin organisation.

Atherosclerosis

Coherent progress has been made in recent years to understand the epigenetic bases of cardiovascular disease (CVD) and atherosclerosis, the underlying cause of CVD. Yet, the field is still in its infancy in comparison with cancer studies.

Studies are showing 'epigenetic signatures' in cell types that are involved in CVD. Specifically, protective cardiovascular genes are being found to be aberrantly hypermethylated and so silenced. The description of such 'epigenetic signatures' are crucial for two reasons. First, environmental and nutritional factors represent an important part of the multifaceted aetiology of CVD. Second, the incidence of risk factors for atherosclerosis such as obesity and diabetes is increasing worldwide and this is not likely to be genetic; rather, it must reflect nongenetic mechanisms of gene expression regulated by environmental and nutritional factors. Epigenetics is providing conceptual and experimental tools to understand how these CVD risk factors act at the molecular level to change gene expression patterns. Furthermore, epigenetic mechanisms of gene regulation are conducive to a degree of flexibility and reversibility, and this would justify 'epigenomic therapies' to be developed in the future.

Autoimmunity

Interactions between environmental and genetic factors are being proposed to explain why autoimmunity afflicts certain individuals and not others. Genes and genetic loci predisposing to autoimmunity are being identified. Regional modification of chromatin structure through epigenetic mechanisms (including DNA methylation and histone modifications) are being proposed as to how the environment contributes to autoimmunity.

Epigenetic mechanisms are being implicated in the pathogenesis of human systemic lupus erythematosus (SLE)[20]. It is proposed that environmentally induced epigenetic changes contribute to the disease pathogenesis in those who are genetically predisposed. Such implication is based on the observation that identical changes in T cell DNA methylation and cellular function are found in patients with SLE. Also SLE-inducing drugs such as procainamide and hydralazine affect T cell DNA methylation.

Similar interactions between genetically determined susceptibility and environmental factors are also being implicated in other systemic autoimmune diseases such as rheumatoid arthritis and scleroderma, as well as in organ specific autoimmunity[21].

Disruption of epigenetic mechanisms that alter immune function are being proposed to explain the development of autoimmune conditions affecting the skin such as atopic dermatitis, psoriasis and some form of vitiligo. The skin is exposed to a wide variety of environmental agents (such as UV radiation) which bring about the disruption of epigenetic mechanisms.

The role of epigenetic mechanisms in autoimmune disease is only recently starting to become clear. Understanding these mechanisms, their effect on cellular function and the role of environmental factors is important in order to determine how to manage these often debilitating and fatal diseases.

Psychiatric Disorders

There is accumulating evidence to suggest that schizophrenia and bipolar disorders are due to epigenetic defects rather than genetic defects[22]. Specifically,

epimutations have been found in genes that code for neurotransmitters in certain parts of the brain. Epigenetic therapies are also being evaluated to treat such disorders. For example, valproic acid (which is a **histone deacetylase (HDAC) inhibitor**), increases the effectiveness of anti-psychotic medications in the treatment of schizophrenia and bipolar disorder.

Obesity

Bisphenol A (BPA) is a building block of polycarbonate plastics and expoy resins. Such items are used to produce consumer items that range from water bottles to dental sealants. Researchers have demonstrated that exposure of pregnant mice (specifically, viable yellow agouti mice) to bisphenol A (BPA), significantly reduced DNA methylation in these mice. This resulted in the birth of more mice that were doomed to become obese and to have higher incidence of diabetes and cancer (breast and prostate) as adults[23]. Moreover, maternal dietary supplementation, with either methyl donors like folic acid or the phytoestrogen genistein, was shown to off set the negative effects of BPA on the epigenome.

Such evidence is being taken to point to a connection between the increase in plastics in our environment and the rising incidence of obesity in humans. However, such an association will be demonstrated unequivocally when the expression and function of genes involved in human obesity are shown to be altered by BPA.

Table I. Classification of epigentic drugs with therapeutic potential

DNMT inhibitors	HDAC inhibitors
Nucleoside analogue inhibitors	*Hydroxamates*
	Trichostatin A
5 - Azacytidine (5-aza-CR)	Suberoylanilide hydroxamic
Decitabine (5 - aza - CdR)	acid (SAHA)
Zebularine	
	Cyclic tetrapeptides
Non-nucleoside analogue inhibitors	Depsipeptide
	Apicidin
Procainamide	
Procaine	*Aliphatic acids*
Epigallocatechin-3-gallate (EGCG)	Valproic acid
	Phenyl butrate
Antisense oligonucleotides	*Benzamides*
DNMTI ASO	MS - 275
	CI - 994
	Electrophilic ketones
	Trifluoromethyl ketones
	α- Ketaomides

DNMT, DNA methyltransfarase; HDAC, histone deacetylase

Disorders of Genomic Imprinting

Genomic imprinting is a phenomenon by which certain genes are expressed in a parent-of-origin-specific manner[24]. It is a non-Mendelian inheritance process. Imprinted genes are either expressed only from the allele inherited from the mother, or in other instances from the allele inherited from the father.

Genomic imprinting is an epigenetic process. It involves DNA methylation and histone modifications of the monoallelic gene. These epigenetic marks are established in the germline and are maintained throughout all somatic cells of an organism.

Prader-Willi syndrome (PWS) and **Angelman syndrome (AS)** were the first genomic imprinting disorders to be studied in humans. **Beckwith-Wiedemann syndrome**, **Psueodo-hypoparathyroidism**, and **Silver-Russell syndrome** soon expanded the list and introduced many intriguing questions about how epigenetic defects lead to the disease phenotype.

Importance Of Epigenetics In Clinical Setting

Understanding the epimutations associated with cancer development, in particular with respect to DNA changes, will lead to the development of new strategies for assessing cancer risk status, detecting tumours as early as possible and monitoring prognosis. One of the most promising approaches towards achieving these goals is the **detection of hypermethylated promoter region CpG islands**. Since DNA methylation epimutations are know to occur early on in carcinogenesis, such aberrations can be potential and good indicators of existing disease and even indicators of the risk of developing the disease in the future.

Epigenetic-based treatment strategies are also realistic and the first generation of epigenetics-based drugs have been approved by FDA. Indeed, such drugs have established that epigenetic modulation is a viable treatment option, not only for cancer, but also for a

Table II. Classification of epigenetic drugs according to potentail therapeutic uses and development phase

Drug	Use	Development phase
DNMT inhibitors		
5 - Azacytidine	MDS	FDA approved for clinic use
	Solid tumours	Phase II
	Leukaemia	Phase II
Decitabine	MDS	Phase II
	Leukaemia	Preclinical
Zebularine	Urinary bladder cancer	Preclinical
Procainamide	Prostate cancer	Preclinical
Procaine	Breast cancer	Preclinical
EGCG	Photocarcinogenesis	Preclinical
	Cancer of cervix	Preclinical
DNMTI ASO	Solid tumours	Phase I
HDAC Inhibitors:		
Trichostatin A	Breast cancer	Preclinical
	Ovarian cancer	Preclinical
SAHA	Solid tumors	Phase I/II
	Leukaemias	Phase I/II
Depsipeptide	Leukaemias	Phase I/II
	Melanoma	Preclinical
	Colon cancer	Preclinical
Apicidin	Leukaemia	Preclinical
Valproic Acid	Bipolar disorder	In routine use (exact mechanism unclear)
	Breast and ovarian cancer	Preclinical
Phenyl butyrate	MDS; Leukaemia	Phase I
MS - 275	Solid tumours	Phase I
CI - 994	Solid tumours	Phase I
Trifluoromethyl ketones	Cancer	Preclinical
a- Ketoaides	Cancer	Preclinical

MDS; Myelodysplastic syndrome; FDA, Food and Drug Adminstraion; DNMT, DNA methyltransferase; EGCG, epigallocatectin-3-gallate; ASO, antisense oligonucleotide; SAHA, suberoylanilide hydroxamic acid

growing list of diseases[25]. Since epigenetic changes are thought to underlie a wide range of diseases, the scope of epigenetic therapy is likely to expand[26]. The four epigenetic drugs available for clinical use in the U.S. include two DNA demethylating agents, **5-azacytidine** and **decitabine**, and two histone deacetylase (HDAC) inhibitors, **vorinostat** and **valproic acid. 5-azacytidine** and **decitabine** inhibit DNA methyltransferase (DNMTs) enzymes and reduce the overall levels of DNA methylation. **Vorinostat** and **valproic acid** block histone deacetylases (HDACs) which are enzymes that remove acetyl groups from histone tails. These epigenetic drugs have been approved mainly for the treatment of blood cancers, in particular **myelodysplastic syndromes** (MDS). At present, the targets for epigenetic drugs are DNMTs and HDACs, but it is worth mentioning that since many other

molecules are also involved in epigenetic mechanisms in gene expression, there are other potential targets as well.

Conclusion

New methods to characterise genome-wide epigenetic variations in humans are being developed, and it is being shown that many diseases are due to epigenetic alterations. Studies are also showing that such epimutations might even be trasgenerationally inherited, putting responsibilities on parents since their lifestyle might affect the health of their children and even their grandchildren. Indeed, epigenetics is showing that our lifestyle and environment can change the way our genes are expressed.

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