Epigenetics in human disease and prospects for epigenetic therapy

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Epigenetic mechanisms, which involve DNA and histone modifications, result in the heritable silencing of genes without a change in their coding sequence. The study of human disease has focused on genetic mechanisms, but disruption of the balance of epigenetic networks can cause several major pathologies, including cancer, syndromes involving chromosomal instabilities, and mental retardation. The development of new diagnostic tools might reveal other diseases that are caused by epigenetic alterations. Great potential lies in the development of 'epigenetic therapies' — several inhibitors of enzymes controlling epigenetic modifications, specifically DNA methyltransferases and histone deacetylases, have shown promising anti-tumorigenic effects for some malignancies.

The term 'epigenetics' defines all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. Three systems, including DNA methylation, RNA-associated silencing and histone modification, are used to initiate and sustain epigenetic silencing. Unravelling the relationships between these components has led to surprising and rapidly evolving new concepts, showing how they interact and stabilize each other (Fig. 1). Disruption of one or other of these interacting systems can lead to inappropriate expression or silencing of genes, resulting in 'epigenetic diseases'. Here we discuss potential causes of some of these diseases, and suggest how they might be treated in the future.

Methylation of the C^5 position of cytosine residues in DNA has long been recognized as an epigenetic silencing mechanism of fundamental importance[1,2]. The methylation of CpG sites within the human genome is maintained by a number of DNA methyltransferases (DNMTs) and has multifaceted roles for the silencing of transposable elements, for defence against viral sequences and for the transcriptional repression of certain genes. 5-Methylcytosine is highly mutagenic, causing C:G to T:A transitions and resulting in a strong suppression of the CpG methyl-acceptor site in human DNA (Box 1). CpG islands, which are regions of more than 500 base pairs in size and with a GC content greater than 55% (ref. 3), have been conserved during evolution because they are normally kept free of methylation. These stretches of DNA are located within the promoter regions of about 40% of mammalian genes and, when methylated, cause stable heritable transcriptional silencing. Aberrant *de novo* methylation of CpG islands is a hallmark of human cancers and is found early during carcinogenesis[4].

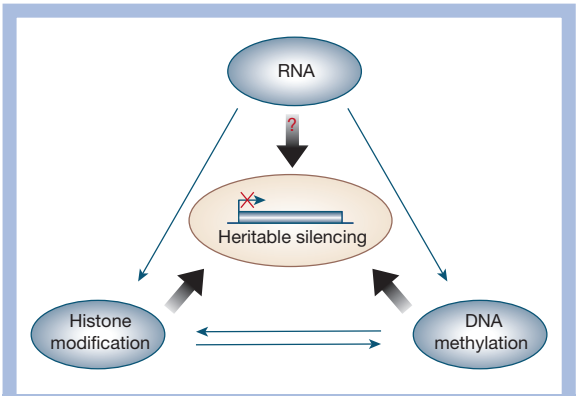
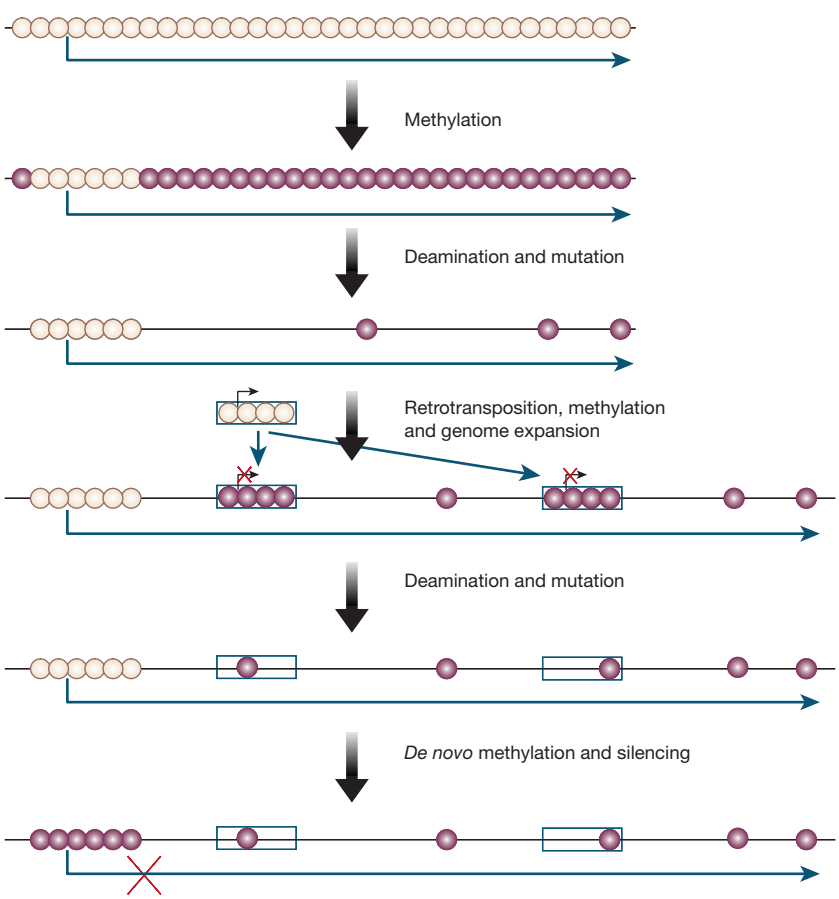


Figure 1 Interaction between RNA, histone modification and DNA methylation in heritable silencing. Histone deacetylation and other modifications, particularly the methylation of lysine 9 within histone H3 (H3-K9) residues located in the histone tails, cause chromatin condensation and block transcriptional initiation. Histone modification can also attract DNA methyltransferases to initiate cytosine methylation, which in turn can reinforce histone modification patterns conducive to silencing. Experiments in yeast and plants have clearly shown the involvement of RNA interference in the establishment of heterochromatic states and silencing. RNA triggering of heritable quiescence might therefore also be involved in higher organisms.

Histone modifications have also been defined as epigenetic modifiers. Post-translational modifications of histones, including acetylation and methylation of conserved lysine residues on the amino-terminal tail domains, have been studied closely over the past few years. Generally, the acetylation of histones marks active, transcriptionally competent regions, whereas hypoacetylated histones are found in transcriptionally inactive euchromatic or heterochromatic regions. Histone methylation can be a marker for both active and inactive regions of chromatin. Methylation of lysine 9 on the N terminus of histone H3 (H3-K9) is a hallmark of silent DNA and is globally distributed throughout heterochromatic regions such as centromeres and telomeres. It is also found on the inactive X chromosome and at silenced promoters[5]. In contrast, methylation of lysine 4 of histone H3 (H3-K4) denotes activity and is found predominantly at promoters of active genes[5]. Because lysine methylation can be monomeric, dimeric or trimeric, and histones may also be subject to other post-translational modifications such as phosphorylation[6], this enormous variation leads to a multiplicity of possible combinations of different modifications. This might constitute a 'histone code'[7], which can be read and interpreted by different cellular factors.

Links between histone modifications and DNA methylation have been found in plants and fungi (Fig. 1), where H3-K9 methylation is a prerequisite for DNA methylation[8–10]. DNA methylation can also trigger H3-K9 methylation[11–13], and this has also been shown in mammals,

Box 1
Evolution and methylation of CpG islands



Early genomes did not contain 5-methylcytosine, and CpG sites (cream circles in the figure) occurred as frequently as expected on a statistical basis. As genomes become methylated at CpG sites (pink circles), promoters of about half of human genes were somehow protected from this modification in the germ line and remained as CpG islands. Because of the well-known enhanced mutability of methylated cytosine, CpGs were converted to TpGs and depleted from the rest of the genome, particularly in the transcribed regions of genes in which most of the methylation occurs. About 40% of the human genome is made up of transposable elements (four cream circles in a group) or their relics, and the active elements are capable of inserting themselves both into genes and into heterochromatic regions. Methylation of the CpG sites within these transposed elements results in the silencing of their promoters at the same time as it does not hinder the transcription of the host gene. In this way, mammals are different from *Neurospora crassa*, in which methylation of cytosine residues in the transcribed regions blocks elongation by polymerase II. Organisms such as *Drosophila melanogaster*, which have very little cytosine methylation, show a higher degree of transposition of transposable elements[91]. The methylation of transposable elements such as Alus, LINEs and LTR sequences increases the rate of C to T transition mutations at these sites, so these sequences eventually become depleted of CpGs. The interaction between transposable elements and the cytosine methylation system results in genomic expansion and CpG depletion. Physiological methylation of CpG islands in the promoters of X-linked or imprinted genes, for example, leads to their long-term silencing. Pathological methylation can result in the silencing of tumour-suppressor or other cancer-relevant genes. Epigenetic therapy seeks to reverse these changes.

where H3-K9 methylation directs DNA methylation to pericentromeric heterochromatin[14]. Interactions between histone deacetylases (HDACs), histone methyltransferases and methylcytosine-binding proteins[15,16] lead to the recruitment of DNA methyltransferases[17], although it is not yet clear what initiates the recruitment of the different epigenetic modifiers to their specific target sequences.

The role of RNA in post-transcriptional silencing has attracted much interest. However, RNA, in the form of antisense transcripts, noncoding RNAs (such as *Xist*) or RNA interference (RNAi), can also lead to mitotically heritable transcriptional silencing by the formation of heterochromatin. In *Schizosaccharomyces pombe*, for example, the deletion of different components of the RNAi machinery results in an impairment of centromere function, a derepression of transgenes integrated at centromeres, and a loss of the characteristic H3-K9 methylation within the same region[18,19]. A similar connection between RNAi, DNA methylation and H3-K9 methylation has been demonstrated in *Arabidopsis thaliana*[20]. Although RNAi-directed silencing of heterochromatic regions has not yet been shown in mammals, the involvement of RNA in different silencing mechanisms has been described. For example, antisense RNAs are involved in the silencing of some mammalian imprinted genes[21] and in dosage compensation in mammals[22]. A recent report of a case of α-thalassaemia showed how antisense transcription could lead to DNA methylation and stable silencing of a globin gene[23]. RNA might therefore be a key trigger to direct histone modifications (for example, H3-K9 methylation) and DNA methylation to specific loci (for example, pericentromeric heterochromatin), thereby evoking heritable and stable silencing. The therapeutic activation of abnormally silenced genes thus requires drugs that can target the inherent stability of these multifaceted changes.

Epigenetic diseases

Multicellular organisms require mutually reinforcing mechanisms permitting heritable patterns of gene silencing. Mutations in genes that affect global epigenetic profiles can give rise to human diseases, which can be inherited or somatically acquired (Table 1). Interestingly, many of these epigenetic abnormalities result in chromosomal alterations and learning disabilities. For example, mutations in the *ATRX* gene result in consistent changes in the pattern of methylation of ribosomal DNA, Y-specific repeats and subtelomeric repeats. Fragile X syndrome results when a CGG repeat in the *FMR1* 5′ untranslated region expands and becomes methylated *de novo*, causing the gene to be silenced and creating a visible 'fragile' site on the X chromosome under certain conditions. On a more global level, the ICF (immunodeficiency, centromeric region instability and facial anomalies)

Table 1 **Epigenetic diseases**

Disease	Symptom	Aetiology	References
ATR-X syndrome	Intellectual disabilities, α-thalassaemia	Mutations in *ATRX* gene, hypomethylation of certain repeat and satellite sequences	82
Fragile X syndrome	Chromosome instability, intellectual disabilities	Expansion and methylation of CGG repeat in *FMR1* 5′ UTR, promoter methylation	83
ICF syndrome	Chromosome instability, immunodeficiency	*DNMT3b* mutations, DNA hypomethylation	84
Angelman's syndrome	Intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (maternal)	85
Prader–Willi syndrome	Obesity, intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (paternal)	86
BWS	Organ overgrowth	Deregulation of one or more imprinted genes at 11p15.5 (e.g. *IGF2*)	87
Rett syndrome	Intellectual disabilities	*MeCP2* mutations	25,26
α-Thalassaemia (one case)	Anaemia	Methylation of α2-globin CpG island, deletion of *HBA1* and *HBQ1*	23
Various cancers	Microsatellite instability	*De novo* methylation of *MLH1*	29
	Disruption of Rb, p53 pathway, uncontrolled proliferation	*De novo* methylation of various gene promoters	4
	Disruption of SWI–SNF chromatin remodelling complex	Mutations in *SNF5*, *BRG1*, *BRM*	36
	Overexpression of IGF2, silencing of *CDKN1C*	Loss of imprinting	88, 89
Leukaemia	Disturbed haematopoiesis	Chromosomal translocations involving HATs and HMTs	62
Rubinstein–Taybi syndrome	Intellectual disabilities	Mutation in CREB-binding protein (histone acetylation)	90
Coffin–Lowry syndrome	Intellectual disabilities	Mutation in *Rsk-2* (histone phosphorylation)	90

ATR-X syndrome, α-thalassaemia, mental retardation syndrome, X linked; BWS, Beckwith–Wiedemann syndrome; CREB, cAMP-response-element-binding protein; HAT, histone acetyltransferase; HMT, histone methyltransferase; ICF, immunodeficiency, centromeric region instability and facial anomalies syndrome; UTR, untranslated region.

syndrome is caused by mutations in the *DNMT3b* gene, which is an essential enzyme required for the establishment of DNA methylation patterns[24]. The fact that all of these diseases show gross chromosomal anomalies points to a central role for epigenetic mechanisms in chromosome architecture.

Several inherited syndromes are due to faulty genomic imprinting — defined as parent-specific, monoallelic expression of a gene — such as Angelman's syndrome, Prader–Willi syndrome and Beckwith–Wiedemann syndrome (BWS). In these conditions, an abnormal phenotype is established as a result of the absence of the paternal or maternal copy of an imprinted gene or because of deregulation of an imprinted gene.

For example, a cluster of imprinted genes at 11p15.5 is involved in the pathology of BWS, a syndrome characterized by organ overgrowth and association with embryonal tumours such as Wilms' tumour. Loss of methylation in imprinting control regions can cause a deregulation of imprinting and either biallelic expression (such as *IGF2*) or silencing (such as *CDKN1C*) of imprinted genes, which is found in most sporadic cases of BWS.

Particular interest has recently been generated by the finding that one of the most common forms of intellectual disability in young girls, namely Rett syndrome, is due to germline mutations of *MeCP2* (ref. 25). The MeCP2 protein binds to methylcytosine residues[26] and the disease pathogenesis might be linked to the derepression of genes normally suppressed by DNA methylation. No direct evidence has yet been found for this, because human cells with *MeCP2* mutations do not show global gene derepression[26]. However, MeCP2 might have a key role in the control of neuronal gene activity resulting in the pathology of Rett syndrome[27,28].

As mentioned above, a fascinating recent discovery was that the transcription of an antisense RNA led to gene silencing and to the methylation of a structurally normal α-globin gene in a patient with thalassaemia[23]. Other examples of inappropriate gene silencing might contribute to disease yet might be missed as part of conventional diagnosis. This case of thalassaemia might well be the tip of the iceberg and indicates that several other kinds of human disease might be the result of genomic rearrangements that cause epigenetic silencing.

Epigenetic changes can also have a major role in the development of human cancer. For example, a high percentage of patients with sporadic colorectal cancers with a microsatellite instability phenotype show methylation and silencing of the gene encoding MLH1 (ref. 29). Thus, epigenetic silencing can result directly in genetic instability. In some cases, promoter-associated methylation of *MLH1* is found not only in tumour but also in normal somatic tissues, including spermatozoa. These germline 'epimutations' predispose individuals carrying aberrant methylation patterns to multiple cancers[30,31]. Disruption of pathways that lead to cancer is often caused by the *de novo* methylation of the relevant gene's promoters[4]. Epigenetic silencing has been recognized as a third pathway satisfying Knudson's hypothesis that two hits are necessary for the silencing of tumour-suppressor genes[32].

Chromatin-modifying enzymes have also been associated with the aetiology of different haemopathologies. A characteristic of human leukaemias is the presence of various chromosomal translocations, leading to the expression of fusion proteins. Both histone acetyltransferases and histone methyltransferases can be part of such fusions and cause the upregulation of target genes[33]. In acute promyelocytic leukaemia, the oncogenic fusion protein PML–RARα (promyelocytic leukaemia–retinoic acid receptor-α) recruits an HDAC to repress genes essential for the differentiation of haematopoietic cells[34]. Similarly, in acute myeloid leukaemia, AML1–ETO fusions recruit the repressive N-CoR–Sin3–HDAC1 complex and inhibit myeloid development[35].

The importance of correct chromatin composition is further underlined by the roles of ATP-dependent chromatin remodelling complexes in disease. These are multisubunit complexes that are capable of moving and shifting nucleosomes, thereby regulating transcription. Several members of the highly conserved SWI–SNF complex have been implicated in cancer[36]. For example, a loss of SNF5 is observed in paediatric cancers, and the ATPase subunits BRM and BRG1 are mutated in a variety of cancer cell lines and primary tumours; this is associated with a poorer prognosis in patients with non-small-cell lung cancer[36].

Epigenetic drift

A distinguishing feature of epigenetic changes in comparison with genetic changes is that they tend to be acquired in a gradual rather than an abrupt process. For example, a generalized decrease in genomic 5-methylcytosine concentrations occurs as mammalian cells age[37,38], and this gradual loss of DNA methylation can result in aberrant gene activation. The decrease in 5-methylcytosine occurs at the same time as there is a localized hypermethylation of CpG islands at gene promoters[39]. The accumulation of methylated CpGs within the promoter might be acting as a 'rheostat' rather than a 'switch' for gene silencing[40]. These alterations are therefore targets for prevention strategies. It has already been shown that a lowering of DNA methylation or DNA methyl-binding proteins can decrease the number of intestinal polyps in cancer-prone mice[41]. Although the full extent of epigenetic changes is largely unknown, there are obvious advantages to designing strategies that can prevent the aberrant regulation of genes as a function of age.

Table 2 **Epigenetic drugs**

Target	Drug	Clinical trials
DNA methylation	5-Azacytidine	Phase I/II/III
	5-Aza-2′-deoxycytidine	Phase I/II/III
	FCDR	
	Zebularine	
	Procainamide	
	EGCG	Phase I
	Psammaplin A	
	Antisense oligomers	Phase I
Histone deacetylase	Many[55], including:	
	Phenylbutyric acid	Phase I/II
	SAHA	Phase I/II
	Depsipeptide	Phase I/II
	Valproic acid	Phase I/II

EGCG, epigallocatechin-3-gallate; FCDR, 5-fluoro-2′-deoxycytidine; SAHA, suberoylanilide hydroxamic acid.

Epigenetic therapy

The fact that many human diseases, including cancer, have an epigenetic aetiology has encouraged the development of a new therapeutic option that might be termed 'epigenetic therapy.' Many agents have been discovered that alter methylation patterns on DNA or the modification of histones, and several of these agents are currently being tested in clinical trials (Table 2).

Inhibitors of DNA methylation rapidly reactivate the expression of genes that have undergone epigenetic silencing, particularly if this silencing has occurred in a pathological situation. The prototype inhibitors, 5-azacytidine (5-aza-CR) and 5-aza-2′-deoxycytidine (5-aza-CdR), were initially developed as cytotoxic agents[42], but it was subsequently discovered that they are powerful inhibitors of DNA methylation and induce gene expression and differentiation in cultured cells[43,44]. Both nucleoside analogues are converted to the deoxynucleotide triphosphates and are then incorporated in place of cytosine into replicating DNA (Fig. 2). They are therefore active only in S-phase cells[43], where they serve as powerful mechanism-based inhibitors of DNA methylation[44]. DNA methyltransferases get trapped on DNA containing modified bases such as azacytosine, 5-fluorocytosine, pseudoisocytosine or zebularine, resulting in the formation of heritably demethylated DNA[44,45]. Covalent attachment of the various DNA methyltransferases to DNA might well be responsible for the cytotoxicities of these agents, particularly at high doses[46].

Initial clinical trials used increasingly cytotoxic doses of these agents, even though the induction of gene expression shows a bell-shaped response curve[44]. Exciting new clinical trials have shown that low doses of these agents might be efficacious in treating myeloid dysplastic syndrome and other leukaemias[47]. In addition, azanucleosides are being tested for the treatment of haemoglobinopathies[48], in which the aim is to cause the demethylation of the promoters of the fetal globin genes that have become silenced in the patient as part of normal development, leading to an increase in haemoglobin F to correct the anaemia that characterizes these diseases.

A disadvantage of the azanucleosides is their instability in aqueous solutions, but this might be overcome by the use of other analogues, such as zebularine or 5-fluoro-2′-deoxycytidine, which also inhibit DNA methylation after incorporation into DNA and might be orally active[49]. Procainamide, which is used to treat cardiac arrhythmias, is also an inhibitor of DNA methylation[50]. In addition, natural products derived from tea[51] and from sponges[52] have shown activity *in vitro*. Clinical trials with antisense oligonucleotides that target the DNA methyltransferases are also underway[53].

Epigenetic silencing is almost universally associated with histone deacetylation, which is catalysed by at least three classes of HDACs in human cells. The HDACs are partly redundant in function, and a growing series of small molecules has been designed to inhibit their activities either globally or more specifically (Table 2). HDAC inhibitors can induce differentiation, growth arrest and/or apoptosis in transformed cells in culture and in tumours. The driving hypothesis is that accumulation of acetylated proteins, particularly histones, results in the induction of genes and the upregulation of others that have become epigenetically silenced. In particular, the gene encoding p21, which is a cell-cycle kinase inhibitor, is commonly upregulated in tumour cells treated with these agents in the absence of p53 (ref. 54). This is important in terms of cancer therapy, because many cancers have no functional p53 and are therefore unable to arrest cells in a p53-dependent fashion. Different HDAC inhibitors are being used intravenously or orally in several phase I and II clinical trials[55], in which changes in histone acetylation have been documented. Because there are many different HDACs, it will be important in the future to design therapies that can target individual enzymes and thus increase the precision of this approach.

Coupling therapies

The links between histone modification and DNA methylation (see Fig. 1) have encouraged investigators to think about dual therapies

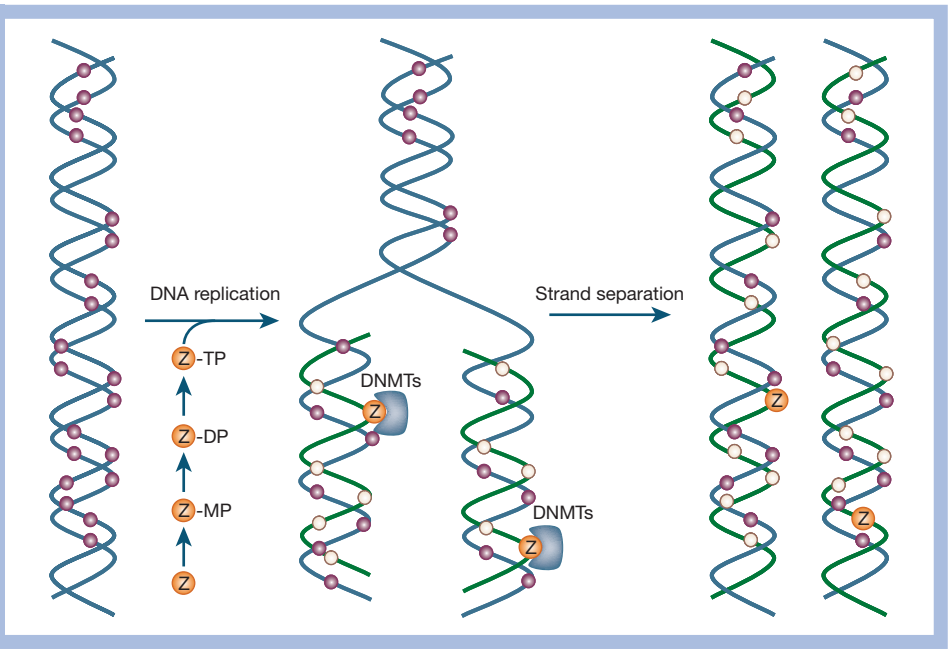
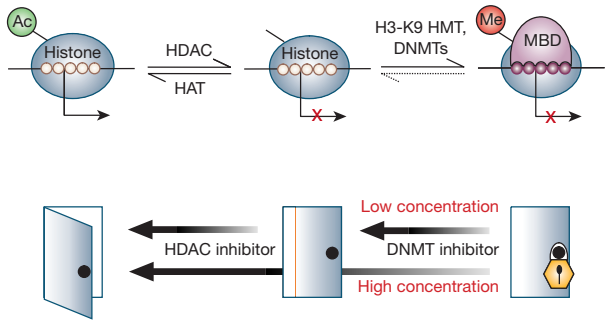


Figure 2 Mechanism of action of nucleoside analogue inhibitors. Deoxynucleoside analogues such as 5-aza-2′-deoxycytidine (depicted by Z) are converted into the triphosphate inside S-phase cells and are incorporated in place of cytosine into DNA. Ribonucleosides such as 5-azacytidine or zebularine are reduced at the diphosphate level by ribonucleotide reductase for incorporation (not shown). Once in DNA, the fraudulent bases form covalent bonds with DNA methyltransferases (DNMTs), resulting in the depletion of active enzymes and the demethylation of DNA. Pink circles, methylated CpG; cream circles, unmethylated CpG.

Box 2
Gene silencing and pharmacological reactivation



Genes containing CpG islands in their promoters (for example, the p16 tumour-suppressor gene) can be reversibly controlled (opened or shut) by altered levels of histone acetylation and the presence or absence of transcription factors at the promoter. Heritable silencing (locking) is achieved by multiple histone modification changes, including trimethylation of the H3-K9, binding of methylated DNA-binding proteins such as MeCP2, and *de novo* methylation of the CpG island[92]. The exact order in which these changes are acquired is not certain, although it seems likely that H3-K9 methylation precedes cytosine methylation[93]. Once these changes have occurred, they tend to reinforce each other and the gene becomes refractory to reactivation (upper panel). Demethylated, silenced genes can often be activated by histone deacetylase (HDAC) inhibitors, but these agents are largely ineffective with methylated promoters[58]. In contrast, DNA methylation inhibitors are highly effective not only at removing the cytosine methylation but also at rapidly reversing the chromatin structural changes[94,95], and not only in unlocking the gene but also in opening it for transcription. New clinical trials seek to take advantage of the synergy between low doses of DNA methylation inhibitors and HDAC inhibitors (lower panel). DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HMT, histone methyltransferase; MBD, methyl-CpG-binding domain protein.

combining DNA methylation inhibitors with HDAC inhibitors (Box 2). Jahangeer *et al.*[56] first showed that 5-aza-CR and butyrate (an HDAC inhibitor) acted synergistically to upregulate the β-adrenergic receptor in HeLa cells. Subsequently, Ginder *et al.*[57] showed that the two agents acted synergistically in anaemic chickens to increase the level of embryonic p-type globin messenger RNA in the haematopoietic cells. The synergy of demethylation and HDAC inhibitors was investigated in greater detail by Cameron *et al.*[58]. Suzuki *et al.*[59] and Yamashita *et al.*[60] have used the approach of treating cultured cells with 5-aza-CdR and trichostatin A to isolate new tumour-suppressor genes efficiently. In addition, a recent study has shown a strong synergy between 5-aza-CdR and phenyl butyrate (an HDAC inhibitor) in the prevention of murine lung cancer[61]. This is therefore an exciting time, as clinical trials are developed that can target these two epigenetic modifications in patients[62]. In addition to these approaches, it might be advantageous to sensitize cells by epigenetic therapy followed by treatment with chemotherapy[63], interferon[64] or immunotherapy[65], among others. In terms of cancer therapy, it will be essential to include both genetic (see reviews in this issue by Strausberg *et al.* (page 469) and Bell (page 453)) and epigenetic markers[66], which will permit an individually targeted therapy.

Potential pitfalls of epigenetic therapy

Despite the promise of epigenetic therapy, there are several concerns regarding the clinical applications of these agents. These relate mainly to the nonspecific activation of genes and transposable elements in normal cells, and also to potential mutagenicity and carcinogenicity. Unfortunately, few studies have examined the effects of azanucleosides on completely normal cells as opposed to cell lines. The drugs have profound effects on immortal lines[44], yet only 0.4% of 6,600 genes (compared with 1% in tumour cell lines) analysed were upregulated more than fourfold in normal human fibroblasts exposed to 5-aza-CdR[67]. Early studies showed that 5-aza-CR could activate a human X chromosome in a rodent somatic cell hybrid[68] but not in normal human cells[69]. These data suggest that DNA methylation is only one of the mechanisms enforcing silencing in normal cells (Fig. 1) and that they are therefore less sensitive to drug-induced gene activation. Imprinted genes can be activated by 5-aza-CdR[70], implying a need for caution. Azanucleosides have been shown to be mutagenic[71] and possibly carcinogenic in rats[72], and might be able to activate silenced oncogenes[73], although they can clearly act as cancer-prevention agents[41,61].

Azanucleosides have shown some promising results in clinical trials without much evidence of adverse effects. For example, treatment of 41 leukaemia patients with 5-aza-CdR showed only mild effects on global genomic demethylation, as measured by changes in Alu methylation[74]. Original methylation levels were regained within two weeks after therapy, and no development of a secondary malignancy was observed in follow-up studies. Furthermore, administration of a low dose of 5-aza-CdR induced cytogenetic remissions in a substantial number of patients with myelodysplastic syndrome with pre-existing chromosomal abnormalities[75]. No increase in chromosomal instability was observed after therapy, which argues against a strong effect of 5-aza-CdR on chromosomal integrity in patients.

The therapeutic mechanism of action of DNA methylation and HDAC inhibitors is by no means straightforward. Evidently, both classes of agents can activate genes — but is this the way that they work in patients? HDAC inhibitors and DNA methylation inhibitors are cytotoxic agents and induce cell-cycle arrest and apoptosis by upregulating p21 and/or p53 (refs 55, 76). Loss of genomic methylation causes p53-dependent apoptosis[77], and p53 represses DNMT1 (ref. 78), suggesting a feedback loop between the two proteins. In addition, the binding of proteins, including DNA methyltransferases, to the DNA of cells treated with azanucleosides can result in cytotoxicity[46,79]. These points make it imperative that surrogate endpoints be examined in patients to gain a better understanding of the mechanism of action.

Future perspectives

Epigenetic disorders give rise to several significant human diseases, and the race is on to find therapies that can reverse silencing. Sometimes it might be possible to reuse a wild-type embryonic gene in place of a mutated adult gene such as in thalassaemia or sickle-cell anaemia. Rett syndrome is caused by mutations in the *MeCP2* gene, which resides on the X chromosome. Because X-inactivation is mostly random, about 50% of the cells in these girls harbour a suppressed wild-type gene, so a valid target might be the reactivation of this good but unused copy. Because of the potential risks of epigenetic therapy, it is likely that trials to validate the approach will be based on patients with life-threatening diseases such as cancer. Here, it is important to remember that the target is an abnormally methylated CpG island and that these seem to be particularly sensitive to reactivation by DNA methylation inhibitors in cancer cells. In addition, because multiple genes become methylated in individual cancers[80], there is the possibility of hitting many targets with one drug. Finally, because methylation of CpG islands increases with age[39] and could therefore contribute to the development of chronic diseases in addition to cancer, there might be benefits in drugs and lifestyle changes that could bring about reversion, or slow gradual epigenetic silencing.

It is apparent that we are just at the beginning of understanding the substantial contributions of epigenetics to human disease, and there are probably many surprises ahead. For example, the finding

that loss of imprinting can be seen not only in normal colonic epithelium but also in the lymphocytes of colorectal patients was completely unexpected[81]. Elucidating the whole bandwidth of epigenetic mechanisms is an exciting challenge and will eventually lead to a clearer understanding of the development of human disease and direct therapeutic concepts into new directions.

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