

MiniReview

Epigenetic Changes in Cancer as Potential Targets for Prophylaxis and Maintenance Therapy

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Abstract: Epigenetic silencing of gene transcription by methylation of DNA or modification of histones is a key event in neoplastic initiation and progression. Alterations of the epigenome have been identified in virtually all types of cancer and involve multiple genes and molecular pathways. Recent studies have suggested that epigenetic gene inactivation may represent the first step in tumorigenesis, possibly by affecting the normal differentiation of stem cells and by predisposing these cells to additional oncogenic insults. The mechanisms that drive epigenetic silencing in pre-malignant cells are still unknown, but may reflect simple stochastic events that are beneficial to cancer precursor cells. It is now well established that epigenetically silenced genes may be reactivated pharmacologically. Some inhibitors of DNA methyltransferases (5-aza-cytidine and 5-aza-2'-deoxycytidine) or histone deacetylases (vorinostat) have been approved for clinical use by the US Food and Drug Administration and have reached clinical phase III trials elsewhere. The prospect that epigenetic alterations may play an essential role in renewing and maintaining the malignant clone has opened up new perspectives for the use of epigenetic therapy in cancer prevention and maintenance.

Disruption of cellular pathways that control the proliferation and death of cells is a fundamental event in the initiation and progression of cancer. The expression or function of proteins in these pathways may be altered by gene mutations or by epigenetic changes that alter gene expression without disrupting the nucleotide template. Like genetic changes, epigenetic alterations are somatically heritable and may lead to a clonal expansion of cells. Recent evidence suggests that early-stage cancer cells carry epigenetic modifications of growth- and differentiation-associated genes, which may predispose the cells to accumulation of additional changes in oncogenes and tumour suppressor genes and eventually lead to a full-blown cancer.

Changes in the epigenome involve several types of covalent modifications of DNA and associated proteins. The best-studied changes include modifications of the amino-terminal ends ('tails') of core histones (the proteins around which the DNA is wrapped), and methylation of cytosines in DNA (fig. 1). It is well established that hypermethylation of DNA and deacetylation of histones in promoter regions is associated with down-regulation of tumour suppressor genes in most cancers. In contrast to genetic alterations, gene silencing by epigenetic modifications is potentially

reversible. Treatment with drugs that target the epigenome, for example, by inhibiting DNA methyltransferases or histone deacetylases, can reactivate the transcription of silenced genes and restore normal cellular growth and differentiation.

Recently, the effects of DNA methyltransferase inhibitors and histone deacetylase inhibitors in advanced cancers have been investigated in many clinical trials. However, if epigenetic changes are involved in the earliest stages of cancer and contribute to maintaining the malignant clone after treatment, epigenetic therapy may also become a powerful strategy for prevention of cancer initiation and/or relapse.

Epigenetic regulation of gene expression

The transitory up- and down-regulation of gene transcription is directed mainly by the ephemeral activities of transcription factors. However, more permanent patterns of gene regulation are established by somatically heritable changes in the epigenome. Basically, two main structures may be altered by epigenetic modifications; cytosine bases of DNA, which can be either methylated or unmethylated, and histones, which attain various covalent modifications, such as methylation and acetylation, which are critical for the regulation of gene transcription. Together, these modifications result in a complex series of molecular events that cause re-modelling of the chromatin configuration and render genes either active or silent.

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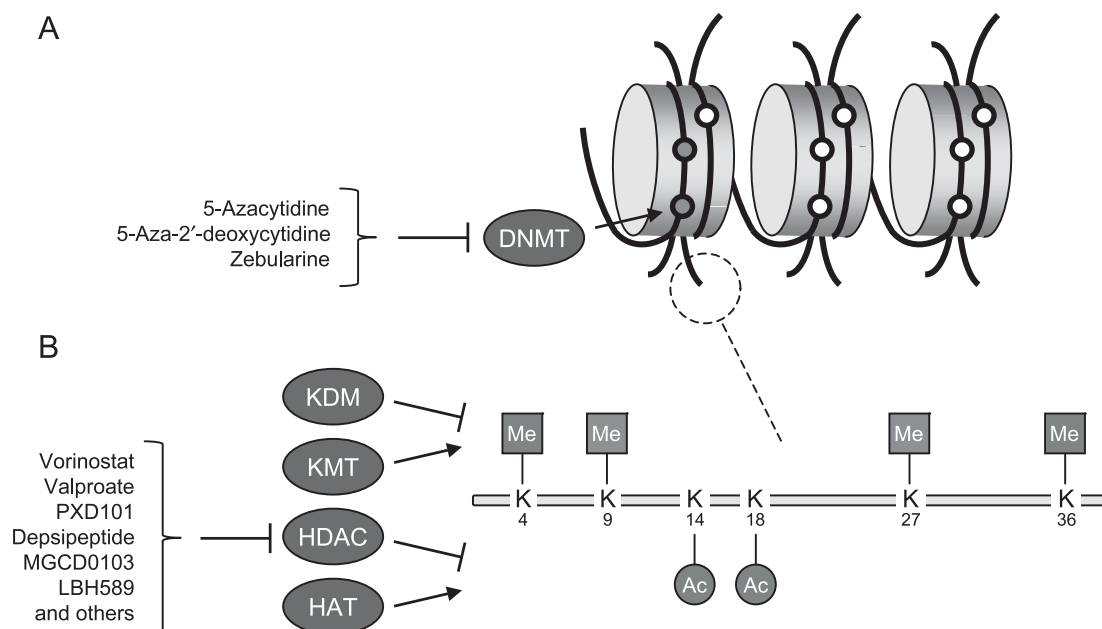


Fig. 1. Epigenetic regulation of gene transcription by covalent modifications of DNA and histones. (A) Regulation of gene transcription by DNA methylation. Gene silencing by methylation of cytosine bases is catalysed by DNA methyltransferase (DNMT). DNA methyltransferases are therapeutic targets of 5-azacytidine, 5-aza-2'-deoxycytidine and zebularine which can reactivate gene transcription. (B) Regulation of gene transcription by modification of the histone tails. Histone acetylation is mediated by histone acetyltransferases (HATs) and leads to an open chromatin structure at transcriptionally active promoters. Histone deacetylation is mediated by histone deacetylases (HDACs) and causes dense condensation of the nucleosomes at transcriptionally inactive promoters. Several histone deacetylase inhibitors are listed. Histone lysine methylation is catalysed by histone lysine methyltransferases (KMTs). Methyl groups can be removed by histone lysine demethylases (KDMs). Methylation of H3K27 and H3K9 is associated with gene silencing; H3K4 and H3K36 methylation is associated transcriptional activation.

Eukaryotic chromatin contains DNA and histones that are organized in the nucleosome, which consists of a histone octamer (two H2A/H2B dimers and an H3/H4 tetramer), around which ~146 bp of DNA is wrapped. Most chromatin exists as transcriptionally ineffectual *heterochromatin*, in which the nucleosomes are densely packaged to form a 'closed chromatin structure'. Conversely, an 'open chromatin structure' is characteristic of transcriptionally competent *euchromatin*, which has widely spaced nucleosomes and is accessible to the transcriptional machinery. Accordingly, euchromatic chromatin is flexible to meet the requests for individual proteins under particular cellular circumstances.

DNA methylation.

5-Methylcytosine is generated when a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM/Ado-Met), is added to the fifth carbon atom in the cytosine pyrimidine ring [1]. This process is catalysed by the enzymatic activity of DNA methyltransferases, which reacts only with cytosine bases of cytidine-phosphate-guanosine (CpG) dinucleotides in mammalian DNA. During DNA replication, the methylation pattern from the parental DNA strand is copied onto the newly synthesized DNA strand by the maintenance methyltransferase, DNMT 1. In embryonic stem (ES) cells and cancer cells, methylation of previously unmethylated DNA (so-called *de novo* methylation) is catalysed by the enzymes DNMT 3a or DNMT 3b [2]. The mecha-

nisms that initiate methylation of mammalian DNA are not yet clear, but recent discoveries suggest a link between histone modifications in ES cells and the DNA methylation patterns of mature cells (see below).

To inactivate transcription, methylation must occur at clusters of CpG sites ('CpG islands') in the promoter regions of genes. The current definition of a CpG island is a >0.5 kb long stretch of DNA with a G + C content greater than 55% and an observed CpG/expected CpG ratio greater than 0.65 [3]. Approximately, half of all genes harbour CpG islands in their promoters. The remainder of the human genome is mostly depleted of CpGs because of the spontaneous hydrolytic deamination of 5-methylcytosine to thymine (T). During evolution, mutations at CpG sites have caused a global reduction in the number of CpGs in the genome, and CpG sites in the coding regions of genes remain important mutational hot spots in human disease [4].

During normal embryonic development, cytosine methylation is crucial for establishing tissue-specific gene expression, for silencing imprinted genes and for X-chromosome inactivation. Methylation also protects against the transcription of parasitic elements that have become integrated in the genome [5].

Histone modifications.

The linkage between methylated cytosines and histone modifications has been described in some detail. Methylated

CpG islands attract a group of repressive proteins, the so-called methyl-CpG-binding-domain proteins, in a complex with histone deacetylases, which remove acetyl groups from lysine residues in the histone tails [6,7]. Deacetylated lysines are positively charged and react strongly with the negatively charged DNA. This leads to dense condensation of the nucleosomes at transcriptionally inactive promoters. Conversely, acetylation of the lysines neutralizes this charge, which generates the open chromatin structure at transcriptionally active promoters. Histone acetylation is mediated by histone acetyl transferases, which form a 'transcription activator complex' with transcription factors and co-activator proteins [8].

In addition to acetylation, a number of different covalent histone modifications have been identified that mark the transcriptional status of chromatin. Histone lysines may be unmethylated, or mono-, di- or trimethylated (-me1, -me2, -me3). The level of methylation at particular residues is important for the interaction with cofactors and is therefore essential for regulation of transcriptional activity. Repressive marks include di- and trimethylation of lysine 9 on histone H3 (H3K9me2/me3), trimethylation of lysine 27 on histone H3 (H3K27me3) and trimethylation of lysine 20 on histone H4 (H4K20me3). Conversely, activation is associated with histone H3 di- and trimethylation of lysine 4 (H3K4me2/me3) and trimethylation of lysines 36 and 79 (H3K36me3, H3K79me3) [9–12] (fig. 1). Methyl groups are provided to the histones by SAM/Ado-Met and the methylation reaction is catalysed by histone lysine methyltransferases [13]. Methyl groups can also be removed from histone lysine residues by the action of histone lysine demethylases [12,14].

Polycomb-mediated gene repression in cancer.

Recent investigations have generated the hypothesis that the epigenetic signatures of cancer cells may be predetermined already at the stem cell level and suggest the existence of early progenitor cells with cancer stem cell features. In ES cells, gene transcription is regulated by the so-called polycomb group (PcG) complexes, PRC1, PRC2/3 and PRC4 [15]. These protein complexes can reversibly repress genes that encode transcription factors necessary for differentiation. The PRC2 polycomb protein EZH2 is a histone lysine methyltransferase that catalyses the formation of H3K27me3, which is associated with gene silencing. Interestingly, EZH2 is an oncogene that is up-regulated in several types of cancer and associated with aggressive subtypes of melanoma, endometrial and breast cancer [16], and is a predictor of poor prognosis in metastatic prostate cancer [17] and mantle cell lymphoma [18].

It was recently shown that a significant overlap exists between gene promoters that are occupied by PcG complexes in ES cells and gene promoters that become methylated in cancer [19–21]. In contrast to the reversible gene silencing by histone H3K27me3 in ES cells, DNA hypermethylation in cancer cells confers deep transcriptional repression that can only be reversed by the use of DNA methyltransferase inhibitors. It has been suggested that an epigenetic switch

from PcG to DNA methylation-mediated gene silencing may be involved in the transformation of normal stem cells to cancer stem cells. Although it has been proposed that a direct link exists between H3K27me3 and DNA methylation [22], this has not been verified in other studies [23]. Furthermore, new observations indicate that histone H3K27me3 may be an independent mechanism of gene silencing in cancer [24].

Current knowledge and applications of epigenetic therapy

DNA methyltransferase inhibitors.

Some 30 years ago, it was discovered that analogues of cytidine, 5-aza-cytidine (5-aza-CR) and 5-aza-2'-deoxycytidine (5-aza-CdR), could induce differentiation of cultured mouse embryo cells to muscle cells [25]. It was demonstrated that genes involved in muscular differentiation were inactivated by DNA methylation in the mouse embryo cells, and that these genes could be reactivated by 5-aza-CR and 5-aza-CdR.

Within recent years, these drugs have been shown to be potent alternatives to conventional chemotherapy, particularly in the treatment of myelodysplastic syndrome and acute myeloid leukaemia (table 1). 5-Aza-CR and 5-aza-CdR differ from their normal counterparts (cytidine and deoxycytidine, respectively) by carrying a nitrogen atom in position 5 of the pyrimidine ring. Inside the cells, these drugs are converted into the corresponding nucleotides. 5-Aza-CdR is incorporated into DNA, while 5-aza-CR is preferentially integrated into RNA but also into DNA. Both drugs covalently bind DNA methyltransferase, which then becomes trapped and unable to catalyse the methylation of newly synthesized DNA strands [26]. Over subsequent cell divisions, methylation is gradually lost leading to cellular differentiation over time. Accordingly, the clinical responses are typically detected after three to four series of 5-aza-CR treatment [27], and it has been shown that DNA methylation gradually disappears in the tumour cells from responders [28]. Therefore, in contrast to conventional cancer chemotherapy, 5-aza-CR and 5-aza-CdR should not be administered at the maximum tolerable dose, which will kill the cells, but at smaller doses over a longer period of time in order to induce cellular differentiation [25,29]. In recent clinical trials, this schedule of drug administration has been used in advanced myelodysplastic syndrome and acute myeloid leukaemia [27,30]. It was recently demonstrated that treatment of myelodysplastic syndrome with 5-aza-CR as compared to conventional care regimens doubled the 2-year survival rate, (51% versus 26%, respectively; $P = 0.0001$) [31]. Both 5-aza-CR (VidazaTM) and 5-aza-CdR (DacogenTM) have been approved by the US Food and Drug Administration for use in the treatment of myelodysplastic syndrome [32].

Histone deacetylase inhibitors.

Histone deacetylase inhibitors are a class of molecules with diverse effects on the regulation of cell growth. First, they inhibit the deacetylation of histones and thus are directly involved in the regulation of gene transcription. Second,

Table 1.

Epigenetic drugs: functions and current applications.

Main function	Drug	Mechanism of action	Trade name	US FDA approved	Main clinical* application	Clinical trials*	Reference
DNMT inhibitors	5-Azacytidine	Binds DNMT when incorporated in RNA (and DNA)	Vidaza	Yes	AML/MDS many cancers in combination therapy	Phase III, maintenance and combination therapy	[31,42]
	5-Aza-2-deoxyeytidine	Binds DNMT when incorporated in DNA	Dacogen	Yes	AML/MDS many cancers in combination therapy	Phase III, maintenance and combination therapy	[28,30]
	S110: 5-aza-CpG	Oligonucleotide; no degradation by cytidine deaminase. Binds DNMT when incorporated in DNA	–	–	–	Pre-clinical	[47]
	Zebularine	For oral use – stable in water. Binds DNMT when incorporated in RNA (and DNA)	–	–	–	Pre-clinical	[48]
	RG108	Binds to the DNMT catalytic site	–	–	–	Pre-clinical	[51]
	MG98	Antisense to DNMT mRNA	–	–	Advanced solid tumours	Phase I-II	[53]
HDAC inhibition: class I	MGCD0103	Benzamide analogues HDACI	–	–	AML/MDS and lymphoma in combination therapy	Phase II	[33]
	MS275	Benzamide analogues HDACI	–	–	AML/MDS in combination therapy	Phase I/II	[33]
	Depsipeptide (Romidepsin)	Cyclic tetrapeptide HDACI	–	–	T-cell lymphoma	Phase II	[33]
HDAC inhibition: class I and II	Valproic acid	Short chain fatty acid HDACI	Depakene and others	Yes	AML/MDS in combination therapy	Phase I/II	[33]
	Butyric acid	Short chain fatty acid HDACI	–	–	EBV-associated lymphoma	Phase I	[33]
HDAC inhibition: class I, II and IV	Vorinostat (SAHA)	Hydroxamate HDACI	Zolinza	Yes	Cutaneous T-cell lymphoma	Phase III	[36]
	PXD101 (Belinostat)	Hydroxamate HDACI	–	–	Multiple myeloma and other cancers	Phase I-II	[33]
	LBH589	Hydroxamate HDACI	–	–	Lymphoma and other cancers	Phase I-II	[33]
		EZH2 depletion/H3K27me3↓	–	–	–	Pre-clinical	[57]
HDAC inhibition: class III	Niacinamide (Splitomicin)	SIRT1 inhibition may activate DNA-methylated genes	–	–	–	Pre-clinical	[39]
SAH hydrolase inhibitor	Deazane-planocin A (Dznep)	Inhibits the donation of methyl groups from SAM ⇒ H3K27me3↓	–	–	–	Pre-clinical	[56]
KDM inhibitor	Polyamine analogues	Inhibitor of LSD1 ⇒ H3K4me2↑, H3K9me2↓	–	–	–	Pre-clinical	[60]

DNMT, DNA methyltransferase; HDAC, histone deacetylase; HDACI, HDAC inhibitors; SAH, S-adenosylhomocysteine; SAM, S-adenosyl-L-methionine; KDM, histone lysine demethylase; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome.

*This and further information at *ClinicalTrials.gov*.

they indirectly modulate gene transcription by inhibiting the deacetylation of proteins, including transcription factors. Third, they may play a significant role in controlling cell division and differentiation in cancer, because they are capable of inducing G1 cell cycle arrest via induction of p21 in tumours that have defective p53 function [33]. Other important activities of histone deacetylase inhibitors relate to their ability to inhibit proliferation at the G2 cell cycle checkpoint and to up-regulate pro-apoptotic molecules.

At present, 18 different histone deacetylases are known, which have been assigned to four classes according to their homologues in yeast. Class I and II histone deacetylases mainly reside in the nucleus of cells and are involved in the deacetylation of histones and other proteins (e.g. p53) [33]. Class III histone deacetylases (sirtuins) constitute a specific class of histone deacetylases that are involved in the regulation of gene transcription in response to changes in cellular stress, including the levels of reactive oxygen species [34]. The specificity of histone deacetylase inhibitors to these different histone deacetylase classes varies; some are highly specific to one or two classes (e.g. depsipeptide and MS-275), while others [e.g. vorinostat (ZolinzaTM) and PXD101] cover the whole spectrum of histone deacetylases except for class III, which are inhibited by a specific group of drugs (e.g. niacinamide). In general, histone deacetylase inhibitors can be given with minimal toxicity and some drugs are available for oral administration, including vorinostat [33,35].

A large number of histone deacetylase inhibitors of different chemical classes are currently under investigation in clinical trials, alone or in combination with other anticancer therapeutics (table 1). While in most cancers, histone deacetylase inhibitors are most efficient in combination therapy, cutaneous T-cell lymphoma was successfully treated by vorinostat alone [36,37]. This drug has recently been approved by the US Food and Drug Administration for use in cutaneous T-cell lymphoma.

Combination epigenetic therapy.

It is well established from *in vitro* studies that histone deacetylase class I/II inhibitors given as single drugs do not turn on genes that are silenced by promoter hypermethylation. However, *in vitro* studies of the combination of histone deacetylase inhibitors and DNA methyltransferase inhibitors generally show strong synergy in up-regulating hypermethylated genes [26], and clinical studies using combination therapy of these drugs induced complete or partial responses in acute myeloid leukaemia patients [38]. In contrast to histone deacetylase class I/II inhibitors, histone deacetylase class III inhibitors given as single drugs may turn on methylated genes *in vitro* [39]; however, it remains to be shown if this is also the case *in vivo*. In *in vitro* studies of acute promyelocytic leukaemia, it was shown that the pathognomonic fusion proteins PML-RAR α and PLZF-RAR α recruit histone deacetylases to initiate their tumorigenic effect [40], and the combined use of histone deacetylase inhibitors and all-trans retinoic acid induced

differentiation of acute myeloid leukaemia blasts and haematological response in refractory acute myeloid leukaemia patients [41].

In vitro and clinical studies have also shown promising effects of combinations with other anticancer agents, such as chemotherapeutics. Conventional chemotherapy may be used *before* DNA methyltransferase inhibitors and histone deacetylase inhibitors to debulk solid tumours, which will allow subsequent induction of stem cell differentiation by the epigenetic therapy. Accordingly, it is investigated in clinical trials whether long-term, low-dose DNA methyltransferase inhibitor administration after chemotherapy may efficiently maintain patients in remission [42]. Alternatively, conventional chemotherapy administered *after* treatment with DNA methyltransferase inhibitors and histone deacetylase inhibitors may act via tumour suppressor proteins that are reactivated by the epigenetic therapy. Preclinical studies have also shown that histone deacetylase inhibitors enhance sensitivity to radiotherapy, and clinical trials are ongoing. Furthermore, in tumour cell lines, synergism has been shown with many of the novel targeted therapies, such as proteasome inhibitors [e.g. bortezomib (VelcadeTM)], kinase inhibitors and death receptor agonists [43–46].

New drugs for epigenetic therapy.

Within recent years, a number of attempts have been made to identify or design new drugs directed towards epigenetic targets. One major focus is to circumvent the inherent limitations of 5-aza-CR and 5-aza-CdR, including the fact that they are unstable in aqueous solution, have transient effects and are inactivated by cytidine deaminase to 5-azauridine [47]. 5-aza-CR is being developed for oral administration, and zebularine, another cytidine analogue that lacks the amino group on C-4 of the pyrimidine ring, is stable in aqueous solution and can be administered orally [48]. Unfortunately, this drug is degraded in the liver and, therefore, further development will be required [49]. Short oligonucleotide derivatives of 5-aza-CdR [e.g. 5-aza-CpG (S110)], seem to be resistant to degradation by cytidine deaminase. However, the stability of 5-aza-CpG in aqueous solution is not improved relative to the single nucleotide forms [47]. In general, DNA methylation seems to be re-established once DNA methyltransferase inhibitors are withdrawn; however, this may be overcome by using long-term maintenance therapy [50].

Other approaches to therapeutically down-regulate DNA methylation include the DNA methyltransferase 1 antisense compound MG98 as well as the small compound RG108, which binds to and inhibits the catalytic site of DNA methyltransferase 1. As RG108 is not incorporated into DNA, it is supposed to be less toxic to the cells than the azanucleotides; however, this compound has not yet entered clinical trials [51]. The antisense molecule MG98 showed promising results in a phase I clinical trial against renal cell carcinoma, but this result could not be confirmed in phase II [52]. Further investigations of MG98 are planned in combination with interferon [53].

Toxicity of epigenetic therapy.

Because epigenetic therapy is not specifically directed against cancer cells, a number of concerns have been raised regarding the toxicity of these drugs when administered to patients. Obviously, in theory, genes and parasitic elements that are normally silenced may be inappropriately expressed, and oncogenes may be turned on that could aggravate the malignant phenotype. These concerns are pertinent; however, because the growth rate of cancer cells is typically higher than that of normal cells and because DNA methyltransferase inhibitors are only active during cell division, they are more likely to reactivate transcription in cancer cells. Furthermore, in the majority of genes in normal cells, the bulk of DNA methylation is not at gene promoters, but within the coding regions of genes where it has no influence on gene transcription, and gene expression studies show that only a limited number of genes are up-regulated by DNA methyltransferase inhibitors in normal fibroblasts [54]. However, because global genomic hypomethylation may cause genomic instability, leading to chromosomal breaks, there is still a reason for concern. Because most experience with DNA methyltransferase inhibitors has been obtained during treatment of advanced cancers, little is known about their long-term side effects, including their carcinogenic potential.

The long-term effects of histone deacetylase inhibitors also are not well described. However, valproic acid, a potent histone deacetylase inhibitor which is given for years to patients suffering from epilepsy, showed no influence on the cancer incidence in a recent large population-based case-control study [55].

Potential new targets for epigenetic therapy.

Recently, it has become evident that histone methylation is also a reversible epigenetic modification that is essential for regulating gene transcription during normal cellular differentiation and in disease states. A considerable number of histone methyltransferases [13] and demethylases [12,14] have been discovered, and many of these enzymes are reported to be deregulated in cancer by deletions, amplifications and translocations, and have been suggested as tumour suppressors and oncogenes, respectively. Accordingly, these enzymes may be potential new targets for cancer therapy. For example, inhibition of the oncogenic histone lysine methyltransferase EZH2 may inhibit the formation of histone H3K27me₃, which is thought to play a role in gene silencing in cancer [24]. The S-adenosylhomocysteine hydrolase inhibitor 3-deazanaplanocin A (DZNep) was recently described as a specific inhibitor of EZH2-mediated H3K27me₃ *in vitro* [56]; however, it seems unlikely that this drug will not have side effects on other cellular methylation reactions. Recent studies have also suggested that histone deacetylase inhibitors may deplete EZH2 and down-regulate H3K27me₃ [24,57]; however, this also most likely occurs via an indirect mechanism, and hopefully, new and more specific EZH2 inhibitors may be developed for clinical use.

Two main types of histone demethylases have been discovered: the amino oxidase LSD1 (histone lysine demethylase 1) and the Jumonji (JmjC) domain proteins [12,14]. While LSD1 is capable of demethylating mono- and dimethylated lysines, the Jumonji enzymes also target trimethylated lysines. LSD1 demethylates both active and repressive methylation marks (H3K4me₂ and H3K9me₂) [58] as well as non-histone substrates (e.g. p53) [59]; however, small molecule inhibitors of LSD1 have been reported to up-regulate silenced tumour suppressors and may potentially be effective anticancer agents [60]. Some of the JmjC-domain histone enzymes demethylate specific methylation marks that may be associated with inappropriate gene transcription in cancer, such as H3K4me₃ (demethylated by JARID1), H3K9me₃ (demethylated by JMJD2) and H3K27me₃ (demethylated by UTX/JMJD3) [12]. Accordingly, these histone lysine demethylases may also be attractive new targets for therapy. However, at least in some cellular contexts, histone methyltransferases and demethylases interact in complex in transcriptional regulation at the same gene promoters [61], and a pharmacological targeting of individual molecules might be difficult. Nevertheless, the development of drugs that target the Jumonji demethylases is a high priority.

A potential role of epigenetic therapy in cancer prevention and maintenance.

Protection against the development and recurrence of cancer is a challenging task and new achievements in this field would have great socio-economical impact. If epigenetic alterations are involved in the abnormal clonal expansion of pre-cancerous cells, epigenetic therapy may potentially both reverse the early stages of cancer and maintain patients in remission. The most straightforward role for epigenetic therapy may be the latter, to ensure maintenance of remission after chemotherapy or surgery (e.g. used as so-called adjuvant therapy). If remaining cells with epigenetic changes constitute a source for cancer relapse, one approach to maintain remission would be continuous eradication of these cancer progenitors. This possibility is already being investigated in several clinical trials, including, for example, the ongoing Medical Research Council acute myeloid leukaemia16 (MRC-AML 16) clinical trial. In this study, one arm of the trial investigates whether older patients (≥60 years) with acute myeloid leukaemia or advanced myelodysplastic syndrome will benefit from maintenance treatment with 5-aza-CR after remission has been obtained by conventional chemotherapy (www.aml16.bham.ac.uk).

At the present time, the available drugs that target the epigenome may be too toxic for use in cancer prevention. However, with the rapid development of more specific drugs and with the discovery of new targets, new and milder forms of epigenetic therapy may be used in patients with cancer predisposition. Obvious candidates for such treatment are patients with recognized precancerous lesions such as patients with carcinoma *in situ* lesions and patients with chronic infections that predispose to cancer (e.g. HIV, HHV-6, HPV, Hepatitis B and Hepatitis C infection). Other groups of

individuals that may benefit from a cancer-preventive approach are members of cancer families. Although the primary inherited component in such families typically is a point mutation in a tumour suppressor gene or proto-oncogene [62], additional molecular hits are required for a cancer to fully develop, and at least some of these additional hits may be epigenetic alterations.

It will be interesting to learn from ongoing and future studies whether we can prevent cancer development and/or relapse by targeting the epigenome. Hopefully, in the years to come, more specific and non-toxic therapies with convenient dose schedules, such as oral administration, will provide new tools for cancer prevention and maintenance.

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