nature biotechnology

# Epigenetic modifications as therapeutic targets

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**Epigenetic modifications work in concert with genetic mechanisms to regulate transcriptional activity in normal tissues and are often dysregulated in disease. Although they are somatically heritable, modifications of DNA and histones are also reversible, making them good targets for therapeutic intervention. Epigenetic changes often precede disease pathology, making them valuable diagnostic indicators for disease risk or prognostic indicators for disease progression. Several inhibitors of histone deacetylation or DNA methylation are approved for hematological malignancies by the US Food and Drug Administration and have been in clinical use for several years. More recently, histone methylation and microRNA expression have gained attention as potential therapeutic targets. The presence of multiple epigenetic aberrations within malignant tissue and the abilities of cells to develop resistance suggest that epigenetic therapies are most beneficial when combined with other anticancer strategies, such as signal transduction inhibitors or cytotoxic treatments. A key challenge for future epigenetic therapies will be to develop inhibitors with specificity to particular regions of chromosomes, thereby potentially reducing side effects.**



Epigenetics encompasses the wide range of heritable changes in gene expression that do not result from an alteration in the DNA sequence itself. DNA methylation, the reversible post-translational modification of the range of histone variants, and nucleosome positioning collectively define the epigenetic landscape of a cell[1,2]. DNA methylation occurs when a methyl group is added to the 5′ position of the cytosine ring of CpG dinucleotides. Recently, methylation in embryonic stem cells was also suggested to occur at sites other than CpG dinucleotides, mainly on the cytosine of CHH or CHG trinucleotides (where H = A, C or T)[3]. In addition, it was recently shown that 5- methylcytosine can be converted into 5-hydroxymethylcytosine by members of the TET protein family[4], mainly in embryonic stem cells and Purkinje cells[5] . The biological relevance of these recently described types of methylation is an area of active investigation. Histones can be covalently modified after translation by the addition of methyl, acetyl, phosphoryl, ubiquityl or sumoyl groups. Whether the modification facilitates or inhibits transcription depends on the histone residue modified and the type of modification. The localization of nucleosomes within genomic regulatory regions has an important role in creating environments that either permit or prevent transcription. Nucleosomes consist of DNA wrapped around a core of two copies of each of the H2A, H2B, H3 and H4 histone proteins, thus linking DNA methylation and histone modifications. The presence of particular variants of core histone proteins, such as H3.3 and H2A.Z, at specific genomic loci influences the stability of nucleosome occupancy. Thus, multiple levels of epigenetic control account for appropriate orchestration of gene expression in healthy cells and dysregulated gene expression in disease.

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Published online 13 October 2010; doi:10.1038/nbt.1678

Here, we focus on recent examples in which epigenetic modifications have been used to evaluate disease risk, progression and clinical response. We aim to provide a broad overview of the accomplishments, remaining challenges and unrealized potential of epigenetic therapies in a range of diseases, with a particular emphasis on cancer.

## Epigenetic disease mechanisms and their clinical relevance

Epigenetic aberrations have been well established in cancer[6,7] and occur in several other diseases, including diabetes[8], lupus[9], asthma[10] and a variety of neurological disorders[7,11–13] (**Table 1** and references within). In cancer cells, a global loss of DNA methylation (hypomethylation), particularly in gene bodies and intergenic regions (including repetitive elements) leads to genomic instability. This global hypomethylation is accompanied by increased *de novo* methylation (hypermethylation) of many promoters of tumor suppressors and other genes that are contained within CpG islands. This results in stable gene silencing (**Fig. 1**). In addition to changes in DNA methylation, cancer cells are characterized by a global loss of histone H4 Lys16 (H4K16) acetylation and H4K20 trimethylation. There is also increased expression of BMI1, a component of the polycomb repressive complex (PRC)-1, and EZH2, a histone-methylating component of PRC2, which both inhibit gene expression[6,14]. Notably, recent evidence has shown that genes targeted by the PRC in embryonic stem cells are more likely than others to become methylated in cancer[15–17], suggesting that aberrant linkage between polycomb repression and the silencing of gene expression by DNA methylation may at least partly account for early changes seen during oncogenesis. Further understanding of the basis of this switch in epigenetic silencing mechanisms may provide new avenues to evaluate the tumorigenic potential of abnormal tissue.

Epigenetic modifications can be used to stratify disease subtypes, severity or treatment responsiveness[18] and to predict clinical outcomes[19,20]. H3 acetylation and H3K9 dimethylation can discriminate between cancerous and nonmalignant prostate tissue, and H3K4 trimethylation can predict the recurrence of prostate-specific antigen

**Table 1 Selected examples of epigenetic alterations associated with disease**

| Epigenetic aberration | Enzyme responsible | Disease | Epigenetic alteration | Comments | Reference |
|---|---|---|---|---|---|
| **DNA methylation** | DNMT1, DNMT3A, DNMT3B and DNMT3L | Rett syndrome | Inability to 'read' DNA methylation | *MECP2* mutation | 11–13 |
| | | Diabetes | Hypermethylation of *PPARGC1A* promoter | | 8 |
| | | Cancer | Global hypomethylation, hypermethylation of some CpG island promoters, including CIMP | | 6,7,11 |
| | | Systemic lupus erythematosus | Hypomethylation of CpG islands at specific promoter regions | Decreased DNMT1 and DNMT3B expression | 9 |
| | | ICF syndrome | Hypomethylation at specific sites | *DNMT3B* mutation | 11–13 |
| | | ATR-X syndrome | Hypomethylation of specific repeat and satellite sequences | *ATRX* mutation | 11,12 |
| **Histone acetylation** | HATs and HDACs | Rubinstein-Taybi syndrome | Hypoacetylation | Mutation in gene encoding CBP, a known HAT | 11–13 |
| | | Diabetes | Hyperacetylation at promoters of inflammatory genes | | 8 |
| | | Asthma | Hyperacetylation | Increased HAT activity and decreased HDAC activity | 10 |
| | | Cancer | H4K16 acetylation loss | Hypomethylation of DNA repetitive sequences | 6 |
| **Histone methylation** | HMTs and HDMs | Cancer | H4K20me3 loss | Hypomethylation of DNA repetitive sequences | 6 |
| | | Sotos syndrome | Decreased H4K20me3 and H3K36me3 | Loss of function of NSD1, a HMT | 113 |
| | | Huntington's disease | Increased H3K9me3 and possibly increased H3K27 trimethylation | Increased expression of the HMT ESET; enhanced PRC2 activity | 12 |
| **miRNA expression** | N/A | Cancer | Decreased miR-101 | Increased EZH2, H3K27 trimethylation | 74,87 |
| | | | Decreased miR-143 | Increased DNMT3A | 88 |
| | | | Decreased miR-29 | Increased DNMT3A and DNMT3B | 89 |
| | | | Increased miR-21 | Decreased PTEN | 96 |
| | | | Increased miR-155 | Lower survival rates | 95 |

ATR-X, alpha-thalassemia X-linked; CIMP, CpG island methylator phenotype; HAT, histone acetyltransferase; HDM, histone demethylase; HMT, histone methyltransferase; ICF, immunodeficiency, centromere instability and facial anomalies; me3, trimethylation.



accumulation after prostatectomy[21]. EZH2 expression is an independent prognostic marker that is correlated with the aggressiveness of prostate, breast and endometrial cancers[22]. Expression of the DNA repair gene O(6)-methylguanine-DNA methyltransferase (*MGMT*) antagonizes chemotherapy and radiation treatment[23]. Accordingly, silencing of *MGMT* by endogenous hypermethylation is correlated with positive treatment response. Furthermore, epigenetic alterations can precede tumor formation and are thus potential diagnostic indicators of disease risk[24]. For example, infection with *Helicobacter pylori* is associated with DNA hypermethylation of specific genes, which are often methylated in cancer[25]. Thus, reversal of epigenetic alterations that occur as a result of an acute illness may prevent progression to a more chronic disease state.

The growing development of technologies to analyze the epigenome has led to the emergence of pharmacoepigenomics, the use of epigenetic profiles to identify molecular pathways most sensitive to cancer drugs[26] as a means of prioritizing therapeutic strategies. In non–small-cell lung cancer, an unmethylated *IGFBP3* promoter indicates responsiveness to cisplatin-based chemotherapy[27]. A polymorphism in the gene encoding the CYP2C19*2 variant of a cytochrome P450 protein necessitates the use of higher doses of valproic acid (VPA) to achieve target plasma concentrations[28]. Furthermore, epigenetic changes can be monitored to measure treatment efficacy and disease progression. Methylation of *PITX2* can be used to predict outcomes of individuals with early-stage breast cancer after adjuvant tamoxifen therapy[29]. Patients with hypermethylation of the gene encoding p16 (*CDKN2A*) have lower recurrence rates of bladder cancer compared to patients with no hypermethylation after interleukin-2 treatment[30]. As epigenetic mechanisms determine which genes, and thus signaling pathways, can be activated, the presence of distinct modifications on specific genes and subsets of genes can aid at several steps in determining and monitoring optimal therapeutic approaches.

The reversibility of epigenetic modifications makes them more 'druggable' than attempts to target or correct defects in the gene sequence itself. Moreover, it is possible that cancer cells can become 'addicted' to the aberrant epigenetic landscape resulting from multiple epigenetic abnormalities[31], rendering them more sensitive than normal cells to epigenetic therapy though a mechanism similar to an inverted oncogene addiction. A classic example of oncogene addiction is mesenchymal-epithelial transition factor (MET), a tyrosine kinase that acts as a receptor for hepatocyte growth factor and controls tissue homeostasis in normal cells[32]. MET can be aberrantly activated in cancer by ligand-dependent mechanisms or by overexpression[32]. Although MET has roles in both normal and cancer cells, the latter are more sensitive to MET inhibition owing to their greater reliance on MET signaling[32]. Thus, cancer cells become dependent (and consequently addicted) to increased activity of a few highly important oncogenes. It is possible that cancer cells undergo a parallel process by which they become dependent on aberrant silencing or inactivation of a few crucial tumor suppressor genes. As it is well known that several tumor suppressor genes are silenced in cancer by epigenetic mechanisms[6], it is possible that cancer cells become addicted to their aberrant epigenetic landscape and consequently become more sensitive

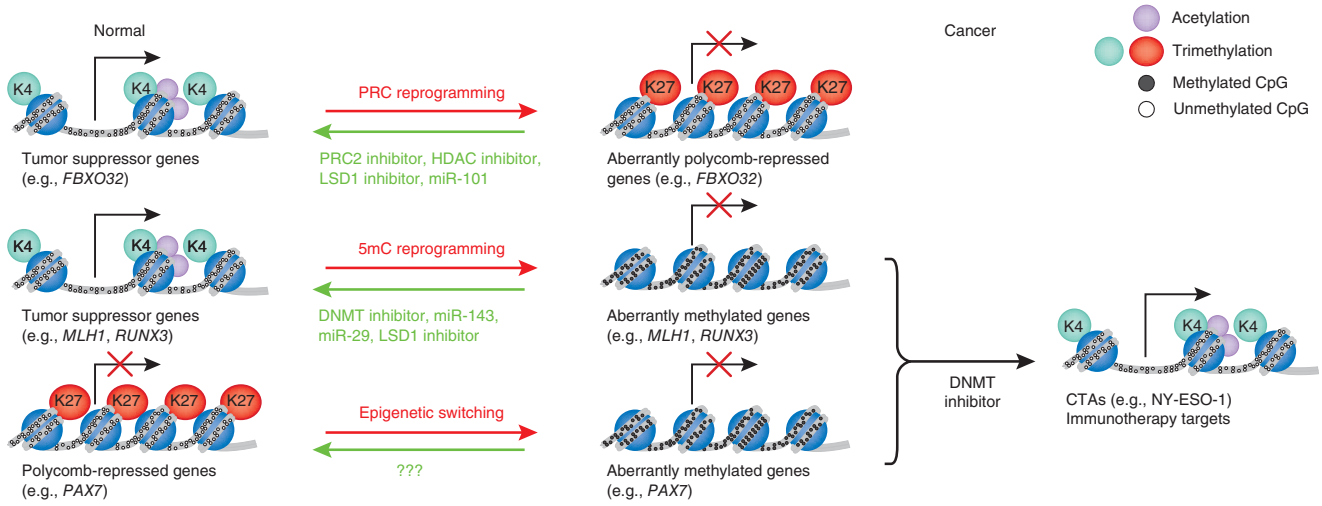


**Figure 1** Epigenetic aberrations of CpG island promoters in cancer cells and the epigenetic therapies that target them. Tumor suppressor genes (such as *FBXO32*, *MLH1* and *RUNX3*) are expressed in normal cells and become silenced in cancer cells. This can occur either by PRC reprogramming (as for *FBXO32*), where the polycomb group protein EZH2 catalyzes the methylation of H3K27, or by 5-methylcytosine (5mC) reprogramming (as for *MLH1* and *RUNX3*) owing to *de novo* DNA methylation by DNMT3A and DNMT3B. Polycomb-mediated repression can be targeted by inhibitors of PRC2, such as DZNep, and re-expression of these genes can be enhanced by HDAC and LSD1 inhibitors allowing acetylation of H3 and H4 and methylation of H3K4, respectively. Polycomb-mediated repression can also be reversed by inducing miR-101 expression, which inhibits the expression and function of EZH2. 5mC reprogramming can be reversed, mainly by DNMT inhibitors, but also by re-expression of miR-143 and miR-29, two miRNAs that target *de novo* DNMTs. LSD1 inhibitors may also reactivate tumor suppressor genes by inhibiting DNMT1 stabilization, leading to loss of DNA methylation maintenance. Genes that are polycomb-repressed in normal cells (such as *PAX7*) can undergo epigenetic switching by DNA methylation, thus losing their plasticity during transformation. It is not known whether treatment of cancer cells with DNMT inhibitors alone can reverse epigenetic switching to restore the polycomb-repressed state or whether it will reactivate this set of genes. Cancer-testis antigens (CTAs, such as NY-ESO-1) can become silenced by DNA methylation in cancer. Treatment with DNMT inhibitors can induce CTA expression, allowing the immune system to recognize and kill the cancer cells. Red arrows represent epigenetic alterations during transformation; green arrows represent reversion of these alterations by epigenetic therapy.



to epigenetic therapy than normal cells. There is some evidence that cancer cells are preferentially, affected by epigenetic therapies[33] .

We next consider progress and remaining challenges in manipulating DNA methylation and histone modifications for therapeutic purposes, including microRNAs (miRNAs), which can also affect gene expression without altering DNA sequence and regulate as well as be regulated by epigenetic mechanisms. What are the merits and limitations of therapeutic strategies that intervene at these distinct levels of regulation of the epigenetic landscape? Moreover, how might they be used together or in combination with nonepigenetic therapies to prevent disease and remission?

## DNA methylation

Cancer is characterized by global hypomethylation, with hypermethylation of a subset of gene promoters contained within CpG islands leading to gene silencing (**Fig. 1**)[6]. This hypermethylation has recently been described to extend past the boundaries of CpG islands into so-called DNA shores[34]. DNA (cytosine-5)-methyltransferase (DNMT)-3A and DNMT3B are responsible for *de novo* DNA methylation patterns, which are then copied to daughter cells during S phase by DNMT1. DNA methylation inhibitors have been well characterized and tested in clinical trials[35]. 5-Azacytidine (5-Aza-CR; Vidaza; azacitidine), a nucleoside analog that is incorporated into RNA and DNA, is approved to treat patients with high-risk myelodysplastic syndromes (MDS) and successful clinical results have recently been reported (**Tables 2** and **3**)[36]. 5-Aza-2-deoxycytidine (5-Aza-CdR; Dacogen; decitabine) is the deoxy derivative of 5-Aza-CR and is incorporated only into DNA. At low doses, both azanucleosides act by sequestering DNMT enzymes after incorporation into DNA, leading to global demethylation as cells divide. At higher doses, they induce cytotoxicity. Zebularine is a cytidine analog that acts similarly to 5-Aza-CR but has lower toxicity and greater stability and specificity[37]. Another drug for which promising preclinical data are available is S110, a decitabine derivative with better stability and activity than 5-Aza-CdR (**Fig. 2**)[38]. In addition to inhibiting DNMT activity, azanucleosides act through nonspecific mechanisms, which are likely to contribute to their clinical effectiveness.

Analysis of promoter DNA methylation can classify cancers[26,39,40], predict the progression of cancer[41,42] and direct therapy[43,44]. For example, DNA methylation of specific promoters may identify a subset of colorectal cancers that are responsive to 5-fluorouracil[43]. Furthermore, use of DNA methylation inhibitors to reverse the silencing of *MLH1* restores sensitivity to cisplatin[45]. This suggests that combining DNA methylation inhibitors with conventional chemotherapy drugs increases therapeutic efficacy. Successful conventional chemotherapy depends on activation of proapoptotic genes that respond to cytotoxic agents, leading to cell death. DNA methylation of these proapoptotic genes can prevent cell death, which in turn confers resistance to chemotherapy. Thus, reactivation of epigenetically silenced apoptotic genes should increase the efficacy of chemotherapy. For example, *APAF1* is silenced in metastatic melanoma cells, and treatment with 5-Aza-CdR restores expression and chemosensivity[44]. Conversely, methylation-induced silencing of DNA repair genes can be detrimental (by leading to microsatellite instability[46]) or beneficial (by preventing the repair of genes targeted by chemotherapy, causing cells to undergo apoptosis rather than repair[47]). Methylation-induced silencing of cancer-testis antigens, such as NY-ESO-1, can protect cancer cells from being recognized by T cells. Treating cancer cells with demethylating agents can induce the expression of these antigens, allowing recognition and killing by engineered cytotoxic

**Table 2 Selected clinical trials of epigenetic cancer therapies with published findings**

| Epigenetic target | Agent | Phase of study | Disease | Findings | Number of subjects | Reference |
|---|---|---|---|---|---|---|
| **DNMT inhibitor alone** | | | | | | |
| DNMTs | 5-Aza-CR | 2/3 | MDS and AML | Complete remission in 10–17% and hematological improvement in 23–36% | 309 | 114 |
| | | 3 | MDS | Better overall survival than with conventional care (24.5 vs. 15 months) | 358 | 36 |
| | 5-Aza-CdR | 2 | MDS and CMML | Anti-MDS and anti-CMML activities with a safe toxicity profile; 34% of patients achieved complete response and 73% had objective response | 95 | 115 |
| **HDAC inhibitor alone** | | | | | | |
| HDAC | Phenylbutyrate | 1 | MDS and AML | Well tolerated; no patients achieved complete or partial remission, although four achieved hematological improvement | 27 | 116 |
| | Vorinostat (SAHA) | 1 | Relapsed or refractory AML, CLL, MDS, ALL and CML | Seven of 31 AML patients showed hematological improvement, including two complete responses and two complete responses with incomplete blood count recovery | 41 | 117 |
| | | 1 | Advanced solid and hematologic malignancies | One complete response (diffuse large B-cell lymphoma), three partial responses (cutaneous T-cell lymphoma) | 73 | 118 |
| **Combination therapy** | | | | | | |
| DNMTs and HDAC | 5-Aza-CR and VPA | 1 | Advanced solid cancers | Combination is safe; 25% of patients showed stable disease (median, 6 months) | 55 | 110 |
| | 5-Aza-CR and phenylbutyrate | 1 | Refractory solid tumors | Combination is safe; no clinical benefit | 27 | 112 |
| HDAC | Vorinostat and doxorubicin | 1 | Solid tumors | Two of 24 showed partial responses (breast and prostate cancer) and two stable disease for more than 8 months (melanoma) | 32 | 119 |
| | Vorinostat plus carboplatin and paclitaxel | 1 | Advanced non–small-cell lung cancer | Better response ratio (34% versus 12.5%), progression-free survival (6 versus 4.1 months) and overall survival (13 versus 9.7 months) than with placebo plus carboplatin and paclitaxel | 94 | 106 |

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; SAHA, suberoylanilide hydroxamic acid.



T lymphocytes[48]. This suggests the possibility of augmenting the efficacy of immunotherapy by combining it with drugs that modulate epigenetic regulation (**Fig. 1**).

Despite the clinical successes achieved with DNA methylation inhibitors, there is still considerable room for improvement. The available DNA methylation inhibitors block DNA methylation by trapping DNMT enzymes on DNA, preventing methylation at other genomic loci . Notwithstanding the therapeutic benefits of simultaneously counteracting the broad hypermethylation of tumor suppressor genes characteristic of most cancers, global hypomethylation may lead to activation of oncogenes and/or increased genomic instability. Moreover, DNA hypomethylation can activate promoters within repetitive elements. For example, hypomethylation of long interspersed nuclear element-1 can activate an alternative transcript of the MET oncogene in bladder cancer[49]. Moreover, DNA methylation inhibitors have also been implicated in defects in memory-associated neural plasticity, suggesting a link between DNA methylation and neural plasticity associated with learning and memory[50].

Developing DNA methylation inhibitors that target specific genes or groups of genes would overcome these perceived risks of agents responsible for global DNA demethylation. Furthermore, because DNA methylation inhibitors act during the S phase of the cell cycle, they preferentially affect rapidly growing cells. This is advantageous when treating rapidly dividing cancer cells but may be less clinically useful in treating diseases that are not characterized by rapid cell cycling. Moreover, the observation that levels of DNA methylation return to pretreatment levels upon withdrawal of azanucleoside[11] suggests a continual need for DNMT inhibition. Thus, despite the clinical success of DNA methylation inhibitors, their lack of specificity, cell cycle dependency and need for continuous administration leave room for the development of better therapies.

## Histone modifications

Whereas DNA methylation is considered to be a very stable epigenetic modification, histone modifications are more labile. Levels of histone modifications are maintained by the balance between the activities of histone-modifying enzymes that add or remove specific modifications. As aberrant histone modification levels result from an imbalance in these modifying enzymes in diseased tissue, correcting the increased or decreased level of a particular enzyme should restore the natural equilibrium in the affected cells.

Cancer cells are characterized by dysregulation of histone methyltransferases and histone demethylases, overexpression of histone deacetylases (HDACs), and a global reduction in levels of histone acetylation[6,14,51–53]. HDAC inhibitors have long been studied in the clinical setting as potential therapies (**Fig. 2**), and recent clinical trials of these agents have been extensively reviewed elsewhere (see also **Tables 2** and **3**)[54]. HDAC inhibitors can also affect the acetylation

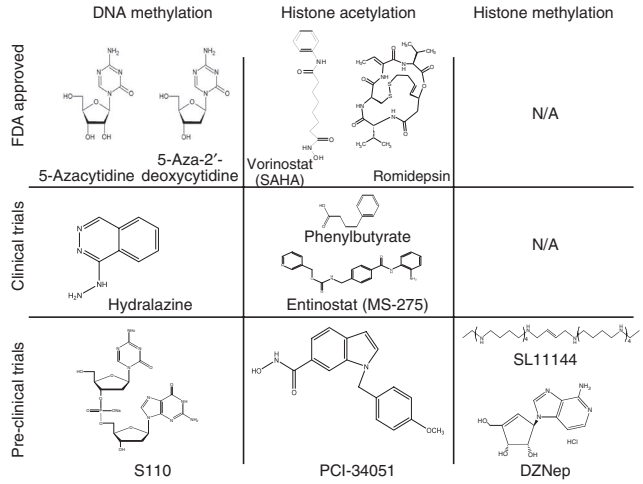


**Figure 2** Chemical structures of selected compounds that target epigenetic modifications. Several molecules that target epigenetic alterations in pathological states are currently at different stages of drug development. The nucleoside analogs 5-azacytidine and 5-aza-2′-deoxycytidine are approved by the US Food and Drug Administration (FDA) to treat high-risk MDS, and successful clinical results have been reported. The drug hydralazine is currently being investigated in clinical trials as a putative demethylating agent against solid tumors. S110, a dinucleotide containing 5-aza-CdR, has been shown *in vitro* to demethylate DNA and is more stable than 5-aza-CdR because it is less sensitive to deamination by cytidine deaminase. Targeting of histone acetylation has also been a successful example of epigenetic therapy. Several HDAC inhibitors are FDA approved, including the hydroxamic acid–based compound SAHA and the depsipeptide romidepsin, whereas others are currently in clinical trials for cancer (phenylbutyrate and entinostat) and neurologic diseases (entinostat). New molecules targeting specific HDACs are under preclinical investigation (such as PCI-34051, which targets HDAC8). More recently, significant effort is under way to find new molecules able to target histone methylation. To our knowledge, no drugs targeting histone methylation are FDA approved or in clinical trials. Even so, preclinical trials suggest antitumor activity of the oligoamine analog SL11144, which inhibits LSD1, and the *S*-adenosylhomocysteine hydrolase inhibitor DZNep, which depletes cellular levels of PRC2 components.

of proteins other than histones, potentially leading to more global effects[54]. Furthermore, because HDAC inhibitors only target ~10% of all acetylation sites[55], more work is necessary to understand the underlying basis for target specification of global and isoform-specific HDAC inhibitors. Substantial efforts are currently under way to find new molecules that can selectively inhibit specific HDACs[56,57] and thus avoid the side effects that occur with a global HDAC inhibitor, including cardiac toxicity[54] and deficits in hematopoiesis[58] and memory formation[59–61]. To date, specific inhibitors of HDAC6 (class II) and HDAC8 (class I) have been developed[56,57]. When combined with a better understanding of the pathophysiology of diseases associated with alterations in HDACs, the development of specific HDAC inhibitors will allow more rational therapy and potentially reduce side effects. For example, the HDAC inhibitor PCI-34051, which is derived from a low-molecular-weight hydroxamic acid scaffold, selectively inhibits HDAC8 and induces apoptosis in T-cell lymphomas but not other tumor or normal cells. This indicates that HDAC8 has an important role in the pathophysiology of this disease and suggests that therapy with an HDAC8-specific inhibitor(s) can reduce undesirable side effects[57]. Other HDAC inhibitors are selective to a group of HDAC isoforms, rather than a specific isoform, allowing their use for a wider range of diseases while minimizing side effects. For example, MGCD0103 (mocetinostat), which inhibits HDAC isoforms 1, 2 and 3 (class 1) and 11 (class 4), was shown in clinical trials to be tolerable and inhibit histone acetylation in patients with advanced solid tumors[62]. MGCD0103 was also shown to be safe and to have antileukemia effects[63]. Although the identification of additional specific HDAC inhibitors will increase specificity and the possibility of personalized treatments, it may also limit the likelihood of their successful incorporation into combinatorial therapies.

Histone methyltransferase and demethylase enzymes are generally more specific than HDACs in that they target fewer residues[64]. However, like HDACs, lysine and arginine methyltransferase enzymes also methylate proteins other than histones[65,66]. A great deal of effort is under way to find drugs able to revert specific histone methylation marks or to selectively target histone methyltransferases or histone demethylases. In this regard, a new class of oligoamine analogs was recently found that act as potent inhibitors of lysine-specific demethylase-1 (LSD1; **Fig. 2**). LSD1 targets the activating H3K4 mono- and dimethylation mark but can also target the repressive H3K9 dimethylation (H3K9me2) mark when complexed with the androgen receptor[51,67]. Treatment of colon cancer cells with LSD1 inhibitors (such as SL11144) increases H3K4 methylation, decreases H3K9me2, and restores expression of *SFRP2* (ref. 68), indicating context specificity of LSD1 and its inhibitors. LSD1 inhibition in neuroblastoma results in decreased proliferation *in vitro* and reduced xenograft growth[69]. Notably, LSD1 can also demethylate DNMT1, resulting in destabilization and loss of global maintenance of DNA methylation[70]. The ability of LSD1 to affect both histone and DNA methylation makes it a promising target for epigenetic therapy.

The repression mediated by the H3K27 trimethylation (H3K27me3) mark occurs through the actions of two multisubunit complexes, PRC1 and PRC2. The H3K27me3 mark deposited by EZH2 is recognized and bound by PRC1, which can further recruit additional proteins to establish a repressed chromatin configuration[6]. Gene promoters that are marked by PRC2 (that is, polycomb target genes) in embryonic stem cells have recently been shown to be far more likely than other genes to become methylated in cancer[15–17]. Similarly, polycomb targets in normal prostate cells also become methylated in prostate cancer[71]. Thus, alterations in chromatin structure do not always coincide with changes in gene expression associated with disease. Instead, DNA methylation replacement of polycomb repressive marks 'locks in' an inactive chromatin state through a process called epigenetic switching[71]. Although the mechanism underlying the predisposition of polycomb targets for DNA methylation is not fully understood, some links have recently been uncovered. CBX7, a component of the PRC1 complex, can directly interact with DNMT1 and DNMT3B at polycomb target genes[72].

Although drugs that target histone methylases and demethylases have considerable potential, more work is necessary to determine their specificities and the stabilities of the changes they effect. There are currently no such drugs in clinical trials. Preclinical studies suggest that the *S*-adenosylhomocysteine hydrolase inhibitor 3-deazaneplanocin A (DZNep) shows the most promise (**Fig. 2**). DZNep depletes cellular levels of PRC2 components (EZH2, EED and SUZ12) and consequently reduces H3K27me3 levels and induces apoptosis in breast cancer, but not normal, cells[73]. The effect of DZNep is similar to that observed when EZH2 is depleted by RNA interference, suggesting that this drug is more effective in cancers of the prostate and breast, which rely on abnormally high EZH2 expression levels[74]. In contrast, a subsequent study showed that DZNep also decreases H4K20me3. This demonstration that DZNep lacks specificity and acts more as a global histone methylation inhibitor underscores the need for further development of histone methylation inhibitors[75].

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