Published in final edited form as:

Mutat Res. 2008 December 1; 647(1-2): 44–51. doi:10.1016/j.mrfmmm.2008.07.006.

Chromatin, Cancer and Drug Therapies

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Abstract

The structure and organization of chromatin have attracted a great deal of attention recently because of their implications for the field of epigenetics. DNA methylation and the post-translational modifications that occur on histones can specify transcriptional competency. During cancer development, tumor suppressor genes become silenced by DNA hypermethylation and chromatin modifiers no longer perform in their usual manner. Current epigenetic therapy has been able to take advantage of the reversibility of these epimutations. Progress has been made in the treatment of hematological malignancies and some solid tumors. As the knowledge of how chromatin regulates gene expression is enhanced, improvements in the treatment of cancer can be made.

Keywords

Epigenetics; Chromatin; Cancer; DNA methylation inhibitors; HDAC inhibitors; Epigenetic Therapy

Introduction to Chromatin Structure

The organization of chromatin has been intensely studied and now the focus in the field of epigenetics has shifted to understanding the biological relevance and function of chromatin structure. Epigenetics, which is defined as the study of heritable changes in gene expression that occur without a change in the DNA sequence, provides insight into the extent by which chromatin structure exerts control on transcriptional regulation. Interpreting the patterns of post-translational histone modifications as well as DNA methylation and how these epigenetic mechanisms contribute to gene expression in a normal state and in cancer are key to developing drugs that can reverse abnormalities that occur during tumorigenesis.

Chromatin is comprised of DNA, histone proteins and non-histone proteins. The fundamental repeating unit of chromatin is the nucleosome, which consists an octamer of histones with two each of the four small and highly basic histones (H3, H4, H2A, and H2B) [1]. Approximately 146 bp of DNA are wrapped twice around each histone core providing a means for higher order packaging of DNA in the nucleus. The histone amino terminal tails that project out of the nucleosome core are subject to many post-translational modifications such as phosphorylation, ubiquitination, sumoylation, acetylation and methylation on specific amino acid residues [2],

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[3]. This review will highlight the most highly studied modifications, acetylation and methylation of histones H3 and H4 (Fig. 1).

Acetylation by histone acetyl transferases (HATs) occurs on the lysine residues of histone tails and is strongly correlated with active gene expression. The basic charges of the histone tails become neutralized upon acetylation. This causes increased accessibility for further modifications or access to the DNA for binding factors and transcriptional machinery [4–6]. Unlike acetylation, methylation of histones does not change the charge of the histone tails [7]. Lysine residues can accept up to three methyl groups, which are added by various histone methyltransferases (HMTs). The degree of methylation is informative for both the state of gene activity as well as which proteins/complexes might bind and read the message displayed by those marks [8],[9]. Methylated lysine residues may constitute either active or inactive marks. Active marks include histone H3 lysine 4 (H3K4), lysine 36 (H3K36) and lysine 79 (H3K79) [10–12]. The methylation marks on lysines 9 and 27 on histone H3 and lysine 20 of histone H4 are associated with an inactive chromatin state [13–15]. Interestingly, there is cross regulation between different marks such as the competition for lysine 9 on histone H3 between an inactive methyl mark and acetylation [16,17]. The distinct patterns of post-translational modifications make up the “histone code” and the precise combinations determine how the chromatin is read [18].

The position of nucleosomes on the DNA further adds to the complexity of chromatin structure. Nucleosome positioning and occupancy can also play a key role in regulating gene expression and the presence of a nucleosome at the transcription start site is commonly seen in inactive genes [19]. Studies have shown evidence of the loss of a nucleosome directly upstream of the transcription start site upon gene activation. This may allow greater access for binding of transcription complexes or factors [20]. It has been shown that a promoter of a gene with a basal level of transcription can already be depleted of nucleosomes which allows for quick induction upon stimulation [21]. Also, the reactivation of a completely silenced gene is associated with nucleosome loss [22]. These studies demonstrate the importance of nucleosomes in gene regulation.

DNA methylation influences gene regulation in concert with histone modifications and nucleosome positioning [23]. DNA methylation at the transcriptional start sites of genes is associated with inactivity and is important in imprinting, X inactivation and the silencing of retrotransposons. The 5 carbon on the cytosine ring in DNA can be modified by the placement of a methyl group by DNA methyltransferases (DNMTs). DNMT1 is referred to as the “maintenance” methylase due to its preference for hemimethylated CpG sites in DNA [24]. DNMT3a and DNMT3b are considered to be *de novo* methylases because they can methylate unmethylated DNA [24,25]. However, all three DNMTs have been shown to act cooperatively and the functional differences between the methylases may to a large extent be due to the genomic regions that they act upon [26,27]. Methylation occurs in the context of CpG dinucleotides, which are underrepresented in the genome possibly due to evolutionary depletion [28]. Regions of high CpG content are termed “CpG islands” and are found at the promoters of more than 50% of genes in the genome. CpG islands are often located at the promoter regions of housekeeping genes in an unmethylated state [29]. The DNA methylation mark can act both directly and indirectly to silence a gene by either inhibiting the binding of transcription factors or by possibly recruiting methyl-binding domain proteins (MBDs), which further recruit histone deacetylases (HDACs) [30].

MicroRNAs (miRs) are another mechanism used by the cell to regulate the expression of genes involved in differentiation, cell proliferation and apoptosis [31]. They are short RNAs 19–24 nucleotides in length that often bind to the 3'UTR of their target mRNA to either inhibit that mRNA's translation or cause its degradation [32]. MiR expression profiles differ depending

on cell type and like DNA methylation, they help to establish the cells identity. Currently more than 400 human miRs have been experimentally identified and are proposed to regulate more than 30% of all mRNAs post-transcriptionally [32,33].

Epigenetic Changes in Cancer

Epimutations in cancer can result in the activation of oncogenes, the silencing of tumor suppressors, and ultimately in the cell's ability to proliferate uncontrollably. These changes are often linked to the presence of altered levels of chromatin modifying enzymes and a shift in the genome-wide distribution of DNA methylation. Changes in histone marks work together with DNA methylation or independently to silence gene expression depending on the region of chromatin and the type of gene. Advances in our understanding of how these abnormalities occur will help in designing and improving drugs to target the factors that cause these changes during tumorigenesis.

Altered activity of the histone lysine methyltransferases can contribute to the deviant histone methylation patterns found in cancer. For example, histone lysine methylation on histone H3K9 and H3K27 are normally present at transcriptionally inactive or heterochromatic regions, yet they can be found at genes that are aberrantly repressed in cancer cells [34,35]. The methyltransferase MLL, which methylates H3K4, is involved in translocations that lead to the inappropriate expression of various homeotic (Hox) genes, which contributes to leukemic progression [36]. Methyltransferases within complexes well known for their suppressive activities are also up-regulated in cancer.

The Polycomb group (PcG) complexes are chromatin modifiers that are crucial to development, and have been implicated in the development of cancer [37]. These negative regulators of gene expression are very important in sustaining the repressive state of their target genes through the cell cycle [38]. Two of the PcG repressive complexes (PRC1 and PRC2) have both been shown to be involved in various cancers. Enhancer of zeste homologue 2 (EZH2), a component of PRC2 with H3K27 methyltransferase activity, is upregulated in mantle cell lymphoma, breast and prostate cancer [39–41]. RING1, a component of PRC1 that aids in the ubiquitylation of histone H2A lysine 119, is upregulated in prostate cancer [42].

The demethylation of histones is important in transcriptional regulation. Histone lysine methylation had been previously thought to be a very stable mark. However, the discovery of LSD1, a demethylase of mono- and dimethylated histone H3K4, showed that these chromatin marks are reversible [43]. LSD1's mechanism of action is through the amine oxidation of the methylated histone H3K4. Several histone lysine demethylases have been found since LSD1, including the Jumonji C domain (JmjC) proteins, which can specifically demethylate mono-, di- and trimethylated lysines [44]. Histone demethylases have been found to play a role in cancer progression as seen with JMJD2A, JMJD2B, and JMJD2C, which are expressed at high levels in prostate cancer [45].

Interestingly, LSD1 has been found to associate with HDACs, therefore HDAC inhibitors can potentially affect the function of demethylases [46]. Currently, histone demethylases have been identified which demethylate both active and inactive marks thereby functioning as both co-repressors and co-activators [47]. Therapeutic inhibition of specific demethylases may be a possible direction for the treatment of cancer, however there is still much to uncover about the precise functions and associations of histone demethylases.

The acetylation of histones is held at equilibrium by the action of HATs and HDACs and an imbalance of one or the other enzyme can lead to phenotypic changes in the cell. Alterations in HAT activity have been found in cancer, stressing the importance of strict regulation of histone acetylation. For example, a translocation involving MOZ and p300, both with HAT

activity, results in a fusion protein and is associated in leukemogenesis [48]. Errant HAT complexes such as these disturb normal epigenetic processes through inappropriate acetylation, altering chromatin organization and gene expression patterns. Inappropriate deacetylation can also contribute to cancer progression. HDACs are upregulated in various types of cancer, such as gastric, prostate, oral squamous cell, and lung [49–52]. Over-expression of HDACs can also lead to the transcriptional inactivation of tumor suppressors, such as p53 [53].

DNA methylation patterns are altered in the progression of cancer. Both the hypomethylation and hypermethylation of different regions of the genome play roles in contributing to tumorigenesis. During tumorigenesis, a genome-wide demethylation occurs and this can promote genomic instability possibly by activating silenced retrotransposons [54]. Global demethylation of repetitive sequences such as satellite DNAs can lead to increased chromosomal rearrangements further adding to genomic instability [55]. It is also possible that demethylation could lead to the activation proto-oncogenes, such as the R-Ras activation in gastric cancer [56,57].

Focal hypermethylation of CpG islands has been intensively studied in cancer. The list of genes found to have increased levels of methylation in their promoter regions accompanied by decreased expression in cancer has grown rapidly. Nearly all types of cancers have transcriptional inactivation of tumor suppressor genes due to DNA hypermethylation [58]. However, the exact mechanism responsible for the appearance of DNA methylation in a given promoter is not fully understood. Cancer cells can attain a growth advantage through the hypermethylation and silencing of genes that are involved in cell cycle regulation, DNA repair, cell signaling, and apoptosis. Additionally, the levels of DNMTs may also be important [59]. Over-expression of the DNA methyltransferases 1 and 3A was found in the bone marrow of patients with myelodysplastic syndrome (MDS) [60]. Upregulation of DNMTs has also been shown in prostate cancer cell lines and tissues [61].

MiRs have different expression profiles in cancer [62]. If a miR is found to be downregulated in cancer then its potential function as a tumor suppressor has been lost. Conversely, if it is upregulated the miR may be acting as an oncogene by downregulating a tumor suppressor gene. Little is known about the regulation of miRs, however it has now been shown that miRs can be regulated by DNA methylation and histone modifications as shown with miR 127 [63]. In this study miR 127 was reactivated in a cancer cell line upon treatment of both a DNA methylation and HDAC inhibitor. This demonstrates that miRs can be potential targets for epigenetic therapy.

As mentioned earlier, DNA methylation and histone modifications are interconnected and together can influence the regulation of genes [64]. However, it has been shown in some cases that changes in post-translational modifications play a greater role in gene expression than DNA promoter methylation [65] [59]. When there are few CpG sites within a promoter the impact of this methylation on gene expression may be less evident. Although there are examples of non-CpG island promoters where methylation does play a role in gene regulation [66], it is also possible that transcriptional regulation is not always influenced by DNA methylation, but rather is dictated by the surrounding chromatin configuration. Figure 2 depicts the two situations of many potential mechanisms by which a tumor suppressor may be shut down.

Cancer and Epigenetic Therapy

Epigenetic errors in cancer, unlike genetic lesions, can be reversed relatively easily through chemotherapeutic intervention, which makes epigenetic therapy promising. The goal of epigenetic therapy is to target the chromatin in rapidly dividing tumor cells and return it to a more “normal state” while only mildly disturbing the epigenome of healthy cells. This section will focus on the epigenetic drugs that are currently used in the clinic (Table 1) and their effects

on cancer cells. It is important to note both their benefits and shortcomings so that improvements can be made in the next generation of epigenetic drug therapies.

The nucleoside analogues 5-azacytidine (5-Aza-CR) and 5-aza-2'-deoxycytidine (5-Aza-CdR), known clinically as azacitidine (Vidaza®) and decitabine (Dacogen®), respectively, are FDA approved demethylating agents used to treat myelodysplastic syndrome (MDS) [67]. They differ from cytosine by a nitrogen substitution at the 5-carbon position. During replication these drugs are incorporated into DNA and their modified cytosine rings inhibit methylation by trapping DNMTs, thereby depleting the cell of these enzymes and resulting in the reduced methylation of cytosines in DNA synthesized after drug treatment [68,69]. In the study leading to the FDA approval of 5-Aza-CR, there was a 60% response rate of the patients with MDS [70]. It was also shown that 5-Aza-CR prolongs survival rate in high risk MDS patients [71]. Encouraging results were also obtained when MDS patients were treated with 5-Aza-CdR and there was a 70% overall response rate [72]. In addition to MDS, these drugs have proven to be useful in other hematological malignancies such as acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) [73].

These drugs potentially act by restoring normal cellular functions by allowing aberrantly hypermethylated tumor suppressor genes to become re-expressed, although the relationship between therapeutic activity and DNA methylation inhibition has not been formally proven. They have shown promising response rates in patients with MDS and CML along with a reversal of p15 hypermethylation in bone marrow [74–76]. Interestingly, 5-aza-CdR can restore drug sensitivity to cells that have become unresponsive to chemotherapy. For example, treatment of melanoma cell lines with 5-Aza-CdR restores sensitivity to cells that are unresponsive to chemotherapy by aiding in the re-expression of a crucial player in the apoptotic pathway, APAF-1 [77].

The use of these drugs raises questions regarding their potential to affect non-cancerous cells epigenetically. However, normal cells divide at a slower rate than malignant cells and incorporate less of these drugs into their DNA resulting in less of an effect on DNA methylation. Also, long-term negative effects of DNA methylation inhibitors in patients have not been found to date [78]. Drawbacks to these drugs are their chemical and *in vivo* liabilities as well as their acute hematological toxicities. A next generation DNA methylation inhibitor, such as zebularine, might possibly overcome these problems [79,80].

Other small molecule inhibitors such as RG108 or MG98 are not incorporated into DNA but instead bind to the catalytic site of DNMTs thereby causing inhibition of DNA methylation. RG108 (N-Phthalyl-L-tryptophan) has been shown to be minimally toxic in colon cancer cell lines and was successful at inhibiting DNMTs [81,82]. The antisense oligonucleotide MG98 (2'-O-CH₃-substituted phosphorothioate oligo deoxynucleotide) targets the 3' UTR of DNMT1 [83] and can cause a methylation decrease in cell lines and animal models. Phase I trials with MG98 did show DNA demethylation in patients, however the Phase II trials had no effect on methylation reduction in patients with renal cell carcinoma [84,85]. Although procainamide (4-Amino-N-(2-diethylaminoethyl)benzamide hydrochloride) is FDA approved for the use of cardiac arrhythmias, it can also reduce DNMT1's affinity for both DNA and S-adenosyl-methionine in a colon cancer cell line causing a decrease in DNA methylation [46]. Generally, these non-nucleoside analogue inhibitors are not as potent as the nucleoside analogues and therefore the need for improvement for these drugs still exists [86].

DNA methylation inhibitors are successful in affecting one epigenetic pathway that leads to the progression of cancer. HDAC inhibitors have also been proven to be useful in cancer treatment by allowing the re-establishment of acetylation to reactivate silenced genes [87]. HDAC inhibitors are divided into 4 groups based on their structures: hydroxamic acids, cyclic

peptides, short chain fatty acids, and benzamides. There are 18 HDAC isoenzymes that have been categorized into 4 classes. The challenge is in designing HDAC isoform-specific inhibitors and determining their potential clinical advantages over general inhibitors [82]. The specificities of the HDAC inhibitors used in the clinic vary from one to three classes of HDACs [85]. HDAC inhibitors have pleiotropic effects including inhibition of angiogenesis, induction of apoptosis and cell cycle arrest [88].

The hydroxamic acid HDAC inhibitors have been successful in treating both hematologic malignancies and solid tumors. X-ray crystallography has shown that the catalytic site of HDACs contains a zinc atom. The hydroxamic acid moiety of these HDAC inhibitors can fit into the catalytic site and bind to the zinc atom thereby inhibiting the HDAC [89]. Suberoylanilide hydroxamic acid (SAHA; vorinostat), a general inhibitor, targets HDACs from Class I and Class II by binding to the active site of the enzyme [90]. SAHA can be administered orally, is minimally toxic and has been FDA approved for the treatment of cutaneous T-cell lymphoma (CTCL). The overall response rate in a recent CTCL Phase IIb trial was 30% and those that did not respond still benefited from relief of pruritus early in the trial [91]. SAHA also is in phase II trials to treat solid tumors [85,92,93]. A recent use of SAHA in women with a recurrence of ovarian cancer showed a progression-free survival over 6 months [94]. Other hydroxamic acids, PXD101 ((E)-N-hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide) and LBH589 ((E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), have also been evaluated in clinical trials. PXD101 treatment of patients with advanced refractory solid tumors was shown to cause an increase in acetylation in their peripheral blood mononuclear cells, stabilize their disease, and was well tolerated [95]. LBH589 is best known for its role in hyperacetylation of histones H3, H4 and the protein Hsp90 [96]. LBH589 has shown clinical activity in cutaneous T-cell lymphoma (CTCL) and soon will be studied in chronic myeloid leukemia, and multiple myeloma [97].

Depsipeptide (FK228), an example of a cyclic peptide HDAC inhibitor, is more specific in that it exerts its effect on three of the Class I HDACs [85]. Depsipeptide has been recently shown to cause a decrease in methylation of DNA while increasing acetylation in lung, pancreatic, and colon cancer cell lines, however the mechanism is not well understood [98]. Depsipeptide has been shown to inhibit growth of human prostate cancer cells [99]. Concerns have arisen with this drug's potential cardiac toxicity [100], however none was observed in a phase I clinical trial to treat patients with MDS or AML [101].

Short chain fatty acids such as butyrate and valproic acid (2-Propylpentanoic acid) have the longest history of being used as HDAC inhibitors [59]. Valproic acid (VPA), originally used to treat epilepsy, has been used for the last decade as an anti-cancer drug since it can inhibit proliferation and induces differentiation in human neuroblastoma cells [102]. VPA is well tolerated, has low toxicity in adults, and is relatively stable [103]. Phenylbutyrate is in Phase I trials for MDS and was shown to be safe for treatment of solid tumors [104,105]. The shortcoming of these HDAC inhibitors is that a high concentration of drug is required for efficacy resulting in limited use in the clinic [106].

MS-275 (N-(2-aminophenyl)-4-[N-(pyridine-3-yl-methoxycarbonyl)aminomethyl]benzamide) and CI-994 (N-(2-aminophenyl)-4-acetylaminobenzamide) are two of the most well known synthetic HDAC inhibitors of the benzamide group. MS-275 can induce p21 expression and increase acetylation in prostate cancer cell lines and inhibit tumor growth in mouse xenograft models [107,108]. In Phase I trials patients with a variety of solid tumors and lymphoid malignancies showed increased levels of acetylation in peripheral blood mononuclear cells and the drugs were well tolerated [109]. CI-994 has undergone Phase I trials and can be used alone or in combination with other chemotherapeutic drugs to treat solid tumors in patients [82,110,111]. Neither of these drugs is as potent as the other classes of HDAC

inhibitors and seems to have the greatest effect when used in a combinatorial treatment [112]. The future directions for the development of epigenetic drugs will rely on the elucidation of their mechanisms and the downstream effects of treatment.

Many clinical trials are now studying the combination of either two epigenetic drugs or a non-epigenetic chemotherapeutic and an epigenetic drug in an effort increase response rates and maximize the efficacy of these drugs. Since HDAC inhibitors work primarily to increase acetylation, they may have a limited effect on genes that have been silenced by DNA methylation. However, HDAC inhibitors and DNA methylation inhibitors in combination can work synergistically to cause the re-expression of such genes. A study on colon cancer cell lines showed genes that were only expressed when the HDAC inhibitor and 5-Aza-CdR were coupled [113]. It was also found that DNA methyltransferase inhibitors could enhance the anti-tumor effects of depsipeptide in leukemic cells with the AML/ETO fusion protein [114]. Also, phenylbutyrate and 5-Aza-CdR have synergistic effects on reducing lung tumor formation in mice by more than 50% than with 5-Aza-CdR alone [115]. A similar study in xenograft hepatoma models only showed a decrease in tumor formation when treated with both SAHA and 5-Aza-CdR [116]. DNA methylation inhibitors and HDAC inhibitors are now used together in the clinic after garnering encouraging results *in vitro*.

For example, in humans a phase I trial involving MDS and AML patients that were treated with both sodium phenylbutyrate and Aza-CR showed reduced promoter methylation and increased global histone acetylation. The results from this trial suggest an increased response rate and it is hypothesized that in a phase II trial, using a longer exposure and lower dose of Aza-CR and HDAC inhibitor could further increase the response rate [117].

Often cells undergoing treatment with one epigenetic drug can have increased sensitivity to an additional drug. Pretreatment with an HDAC inhibitor can greatly increase cytotoxicity in various cell lines when followed by subsequent treatment of a chemotherapeutic drug [118]. This particular study suggests that pre-treatment causes the chromatin structure to become more open therefore increasing the efficiency of the drug to follow. Likewise, cisplatin resistant cells from head and neck cancer cell lines can be reprogrammed to become responsive after treatment with phenylbutyrate [119]. A phase I study in patients with solid tumors showed that CI-994 can be safely administered with paclitaxel and carboplatin and can cause a partial or complete response [111]. The increased sensitivity to other drugs after use of an epigenetic drug is encouraging since drug resistance does present a challenge in effective cancer treatment.

Additionally, it is important to target other enzymes that can disturb the epigenetic balance during carcinogenesis such as histone methyltransferases. Reagents that inhibit S-adenosylhomocysteine (SAH) hydrolase lead to an increase in SAH levels in the cell which inhibit methyltransferases, including histone methyltransferases [120,121]. While SAH hydrolase inhibitors have been used as anti-viral compounds, how they may be effective in cancer is in need of exploration [122].

The Future of Epigenetic Therapy

As the field of epigenetics advances, a better understanding is developing of the precise mechanisms by which DNA methylation and post-translational histone modifications play central roles in gene regulation. The therapeutics designed thus far have had encouraging results in counteracting the epimutations that occur during tumorigenesis. With the FDA approval of 5-Aza-CR, 5-Aza-CdR and SAHA, the use of epigenetic drugs has gained momentum and has proven useful in hematological malignancies and some solid tumors. Additionally, the combinatorial use of DNA methylation inhibitors and HDAC inhibitors in the clinic is gaining traction due to their synergistic effects in re-establishing the expression of tumor suppressor genes.

However, much work remains in designing drugs that will be more stable, less toxic and more specific in their enzyme inhibition. Expanding the use of these drugs to treat more types of solid tumors should also be possible. Broadening combinatorial drug therapies to include different permutations of the DNA methylation inhibitors, HDAC inhibitors and non-epigenetic chemotherapies will also be key in better cancer treatment. Fortunately, advancements in technology will help to further elucidate the understanding of epigenetic mechanisms. As a result, drugs which can better target chromatin modifiers that improperly function during carcinogenesis will be developed. Future epigenetic drugs can also be designed to target histone methyltransferases, histone demethylases or other chromatin modifiers not yet discovered. The rising interest in epigenetics research should therefore lead to improved cancer treatment.

Acknowledgements

We would like to thank Erika Wolff, Gangning Liang, Terry Kelly, Allen Yang, Christine Yoo, Tina Miranda and Jeffrey Friedman for their thoughtful input and time given to reading this review. Financial support is made possible by NIH grant RO1 CA83867.

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Fig. 1. Post-translational histone modifications on histone tails

Modifications made on the N-terminal tails of histones are important in establishing the activity state of chromatin. Many modifications are possible however only acetylation and methylation of a subset of lysine residues are depicted here. Active acetylation or methylation marks (green triangles or squares), can act to “loosen” chromatin to allow for access of transcriptional machinery while also serving as docking points for nucleosome remodeling complexes. Conversely, inactive marks such as methylation of specific residues can cause an inactive conformation of chromatin and can recruit repressive complexes.

Fig. 2. Examples of epigenetic changes that occur to inactivate a gene. a

b) Active genes are characterized by unmethylated CpG sites in their promoters (white circles), absence of a nucleosome (purple spheres) upstream of the transcription start site (black arrow), and active histone marks (green) placed by HATs and HMTs. b) During carcinogenesis, tumor suppressor genes become aberrantly methylated by DNMTs, and in turn MBDs that attach to the methylated CpG sites can recruit HDACs to aid in transcriptional repression. A nucleosome placed at the transcriptional start site, and histone methyltransferases additionally render the chromatin inaccessible. In this scenario, methylation plays a critical role in inactivating a tumor suppressor. However, in some cases c) methylation may not play a role (such as areas with less CpGs) and repression of the gene is dependent on chromatin remodeling factors such as PRC1 and PRC2.

Table 1

Epigenetic Drugs Used in the Clinic

Drug Name	Cancer	References
DNA Methylation Inhibitors		
5-azacytidine (FDA approved)	MDS, AML, CML	[67] [70] [71]
5-aza-2'-deoxycytidine (FDA approved)	AML, CML, MDS	[72] [73] [76]
MG98	Renal cell carcinoma	[83] [84] [85]
RG108	Colon cancer cell line	[81] [82]
Procainamide	Colon cancer cell line	[86]
HDAC Inhibitors		
SAHA (FDA approved)	CTCL, various solid tumors	[89] [91] [92] [94]
PXD101	Various solid tumors	[95] [97]
LBH589	CTCL	[96] [97]
Depsipeptide	Multiple cancer cell lines, MDS, AML	[98] [99] [100] [101]
Phenylbutyrate	MDS	[104] [105]
Valproic Acid	Neuroblastoma cells	[102] [103]
MS-275	Prostate cancer cell lines, various solid tumors and lymphoid malignancies	[107] [108] [109]
CI-994	Various solid tumors	[110] [111]