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TARGETING EPIGENETICS IN CANCER

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Abstract

Alterations of genes regulating epigenetic processes are frequently found as cancer drivers and may cause widespread alterations of DNA methylation, histone modification patterns or chromatin structure to disrupt normal patterns of gene expression. Because of the inherent reversibility of epigenetic changes, inhibitors targeting these processes are promising anti-cancer strategies. Small molecules targeting epigenetic regulators have recently been developed and clinical trials of these agents are underway for hematologic malignancies and solid tumors. In this review we describe how the writers, readers and erasers of epigenetic marks are dysregulated in cancer and summarize the development of therapies targeting these mechanisms.

Keywords

Epigenetics; DNA methylation; Histone acetylation; Histone methylation; epigenetic readers; anti-cancer therapies

INTRODUCTION

Epigenetics is the study of heritable changes in gene function that cannot be attributed to DNA sequence variations. Cellular identity and differences between cell types often rely upon systems in which there is no DNA variation. Instead, gene expression patterns are tightly controlled by the 3-dimensional architecture of chromatin and the action of multi-protein complexes especially RNA polymerase that transcribes DNA into RNA. In the nucleus, genomic DNA is wrapped around histones into nucleosome subunits that are condensed into chromatin. Highly condensed chromatin is termed heterochromatin and contains mostly inactive genes. In contrast, euchromatin has a more open structure and contains active genes. Chromatin structure is dynamically regulated by DNA methylation, nucleosome positioning and histone modifications (Figure 1).

In 1974 Kornberg and Thomas discovered that DNA was packaged into the nucleosome, consisting of approximately 147 base pairs (bp) of DNA wrapped around a histone octamer core and a linker histone wrapped around another 20 bp (1). Subsequent studies revealed that histone C- and N-terminal histone tails extended beyond the nucleosome core structure and

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are accessible to post-translational modification (2; 3). It had been proposed for many years that histones might affect gene expression, but it wasn't until Allis and coworkers demonstrated that histone acetylation was important for regulating mechanisms of gene expression that the significance of histone modification was realized (4). Studies have now revealed that a wide range of post-translational modifications can mark histones such as acetylation, methylation, ubiquitination or phosphorylation. Histone modifications bring about chromatin remodeling by two main mechanisms. First, marks such as acetylation or phosphorylation neutralize the positive charge of lysines, weakening interactions among nucleosomes and between DNA and histones to increase chromatin accessibility (5; 6). Second, histone modifications may serve as docking sites for additional histone modifying enzymes or specific chromatin factors that regulate chromatin architecture and/or gene expression.

DNA methylation usually occurs on cytosines that precede a guanine (CpG). The presence of endogenous palindromic CpG methylation patterns in the genome and transmission of these methylation marks through the germline has now been well established. The majority of CpG dinucleotides are concentrated within CpG-rich DNA regions termed "CpG islands" that are located near transcription start sites (TSSs) at ~70% of gene promoters (7). In addition, orphan CpG islands located far from TSSs and CpG dinucleotides in gene bodies or enhancers may be methylation targets that can regulate gene expression (8; 9). Hypermethylation of CpG islands may lead to silencing of the corresponding gene by precluding the binding of transcription factors and recruitment of methyl-CpG binding proteins that interact with repressive histone modifying enzymes (10; 11). Thus, DNA methylation plays a key role in regulating chromatin architecture and gene expression.

Epigenetic regulation is a dynamic and reversible process. Proteins that carry out these epigenetic modifications can be thought of as writers, readers and erasers. Epigenetic writers catalyze the addition of epigenetic marks onto either DNA or histones, most commonly on "tails" of histones that extend from the octamer structure. Readers recognize or are recruited to a specific epigenetic mark. Erasers remove epigenetic marks. In this review we will discuss how this network of epigenetic regulation has provided new targets for anti-cancer therapeutics.

EPIGENETIC DYSREGULATION IN CANCER

As the spectrum of mutations found in cancer has been elucidated in recent years by next generation sequencing, it is apparent that mutations, amplifications, deletions and rearrangement of genes affecting epigenetic regulation pathways frequently occur in cancer (12). Driver mutations in epigenetic regulators can lead to widespread alterations of DNA methylation or histone modification patterns that dysregulate chromatin structure and disrupt normal patterns of gene expression. DNA hypermethylation and/or repressive histone marks on a promoter can phenocopy loss of function mutations in tumor suppressor genes by silencing gene expression. Conversely, loss of DNA methylation or activating marks can significantly increase gene expression similar to what would be observed after oncogenic chromosomal translocations or gene amplification.

Unlike genetic events, epigenetic changes are reversible. Because of this inherent plasticity, deciphering how aberrant epigenetic mechanisms culminate in malignant transformation may yield novel insights into how targeting these mechanisms may be used as cancer therapy. DNA hypomethylating agents and histone deacetylase inhibitors are approved for some hematologic malignancies, including T-cell lymphoma (vorinostat 2006, romidepsin 2009), multiple myeloma (panobinostat, 2015) and myelodysplastic syndrome (MDS) (decitabine or azacitidine). Additional clinical trials of epigenetic therapies in hematologic malignancies and solid tumors as single agents and in combination with other therapies are underway.

TARGETING DNA METHYLTRANSFERASES WITH HYPOMETHYLATING AGENTS

Changes in both global and individual gene methylation patterns are often found in cancer and methylation patterns can distinguish tumor types. In general tumors show global DNA hypomethylation, due to loss of repeat region methylation and hypomethylation of specific loci (13). However, hypermethylation of specific CpG rich regions leading to silencing of tumor suppressors has been frequently reported in malignant transformation. For instance, the tumor suppressor gene Rb is silenced by hypermethylation in retinoblastoma, the cell cycle inhibitor p16 is often hypermethylated in colorectal, lung and breast carcinomas and the BRCA1 promoter was found hypermethylated in breast and ovarian cancers (14–16). Furthermore, aberrant methylation patterns outside CpG islands and hypomethylation patterns are equally important as hypermethylation in cancer. Hypomethylation of proto-oncogenes such as Hox11, c-Neu, Bcl-2, and Ras was reported in a variety of malignancies which may contribute to aberrant expression of these tumor promoting genes (17–20). The DNA methyltransferases (DNMTs), DNMT3A and DNMT3B are responsible for establishing *de novo* DNA methylation patterns that are maintained by DNMT1 (21; 22). Recurrent DNMT3A loss of function mutations have been reported in AML and are associated with subtle losses of DNA methylation whose functional significance remains to be ascertained (23–25).

The first compounds recognized as DNMT inhibitors (DNMTi) were the cytosine analogues 5′-azacytidine (Aza, Vidaza) and 5-aza-2′-deoxycytidine (Decitabine, Dacogen). Developed originally as high-dose cytotoxic anti-leukemia agents it is now understood that at low dose these compounds inhibit DNMT activity resulting in hypomethylation. These azanucleosides substitute nitrogen for carbon at the C-5 position of the pyrimidine ring and when incorporated into DNA irreversibly bind DNMT1 resulting in DNMT1 degradation and DNA demethylation (26; 27)(Figure 2). While decitabine is mostly incorporated into DNA about 80–90% of Aza is incorporated into RNA and evidence suggests that Aza's antineoplastic function and effect on gene expression may be due to its incorporation into both DNA and RNA (28). Studies from the 1980s showed the ability of DNMTi to reactivate silenced genes such as fetal globin genes (29; 30). Furthermore, the combination of DNMTi and HDAC inhibitors can synergistically activate genes (31). The ability of DNMTi to reactivate tumor suppressor genes was one motivation for the use of these agents in hematological and other malignancies. In 2004 and 2006 Aza and decitabine were approved

for treatment of MDS respectively (Table 1) (32; 33). Clinical trials remain ongoing for both compounds as single agents and in combination therapies for hematologic malignancies and solid tumors. In addition, a second-generation analogue, SGI-110, has been developed whose active metabolite is decitabine. This compound is a dinucleotide consisting of decitabine linked by a phosphodiester bond to deoxyguanosine that protects it from drug clearance by deamination (34). SGI-110 is currently being tested in clinical trials for AML, MDS, ovarian cancer and hepatocellular carcinoma (Table 1). These agents work slowly to cause clinical response but the molecular basis by which they exert anti-cancer activity remains uncertain. While hypomethylating agents have been shown to reverse promoter methylation and reactivate silenced tumor suppressor gene expression, other mechanisms may also be important (35). For instance, treatment with decitabine causes the formation of DNA-DNMT adducts and subsequent double-stranded DNA breaks resulting in G2 arrest (36). In addition, DNMTs have been found in complexes with histone modifying enzymes and a global increase of histone H3 and H4 acetylation has been observed after treatment with Aza (37–39). Furthermore, decitabine stimulates nuclear localization of IRF7 in colon cancer cells to cause expression of toxic endogenous retroviral sequences independent of promoter methylation (40).

Defects in demethylation of DNA can also lead to aberrant hypermethylation and altered expression of genes that drive neoplasia. Mutations in the enzymes IDH1 and IDH2 that normally catalyze decarboxylation of isocitrate to α-ketoglutarate occur in gliomas, AML, chondrosarcomas and cholangiocarcinoma (41–44). Mutant IDH1/2 reduces α-ketoglutarate to 2-hydroxygluterate (2-HG), a competitive inhibitor of the TET family of DNA hydroxylases (45). In addition, mutations and translocations of *TET2* have been observed in numerous hematologic malignancies and associated with poor prognosis in AML (46). TET enzymes normally convert 5′-methylcytosine to 5′-hydroxymethylcytosine, an important step in cytosine demethylation and inhibition of this mechanism results in DNA hypermethylation (47). Furthermore, 2-HG has been reported to inhibit α-ketoglutarate dependent Jumonji domain lysine demethylases and activate mTOR (48; 49). Compounds specifically targeting mutant IDH have been developed (AG-120 and AG-221) and are currently in clinical trials for patients with advanced hematologic malignancies (Table 1) (50). These agents would be expected to block the production of 2-HG by the mutant enzymes and allow DNA and histone methylation patterns to normalize.

TARGETING REVERSIBLE HISTONE ACETYLATION AND HISTONE DEACETYLASE

Acetylation of lysine on histone tails is highly dynamic and important for regulation of chromatin structure, transcription and DNA repair. Two competing enzyme families, histone lysine acetyltransferases (HATs) and histone deacetylases (HDACs), regulate histone acetylation. The about 30 known HATs are classified into two groups based on their capacity to acetylate nucleosomal histones. Type A HATs are located in the nucleus and acetylate chromatin bound histones and nuclear proteins. Type B HATs acetylate newly translated, but not nucleosomal, histones H3 and H4. Based on structural and functional homology the type A HATs are further categorized into families that include: GCN5, MYST, p300/CBP,

transcriptional coactivators and steroid receptor coactivators. HATs catalyze the transfer of an acetyl group from acetyl-CoA to the amino group of a histone lysine residue. Upon acetylation, the positive charge on lysine is neutralized diminishing the interaction of histones with DNA. In general this leads to a more open chromatin structure that is accessible to binding of proteins such as transcription factors. Thus, acetylation is associated with transcriptional activation while deacetylation is associated with gene repression. Numerous examples of chromosomal translocations (e.g. MLL-CBP and MOZ-CBP) or mutations (e.g. p300/CBP) involving type A HATs have been reported in hematologic malignancies and solid tumors (51–54). For instance, about 40% of diffuse large B-cell lymphomas and 41% of follicular lymphomas harbor deletions or mutations that inactivate p300/CBP (55). While type A HAT inhibitors have yet to enter clinical trials, the p300-HAT inhibitor C646 has been reported to specifically suppress growth of CBP-deficient lung and hematopoietic cancer cells (52). The only known type B HAT is a multiunit complex containing Hat-1 as its catalytic subunit that associates with the histone chaperones NASP and Asf1 in the nucleus to facilitate the deposit of histones onto DNA. In yeast, Hat-1 is required for telomeric silencing and repair of dsDNA breaks by homologous recombination (56; 57). In humans, Hat-1 is frequently amplified in cancer (cBioPortal.org) and IHC analysis of tissues from leiomyosarcoma and leiomyoma patients indicates increased Hat-1 correlates with worse survival (58). To date, no specific inhibitors of Hat-1 have been reported.

Many reports demonstrate that HDACs are overexpressed in cancers resulting in global loss of histone acetylation and silenced tumor suppressor gene expression. HDACs are divided into four classes based on their homology and structure. Classes I, II and IV are comprised of Zn-dependent HDACs while class III is made up of the NAD-dependent sirtuins. Class I HDACs (1, 2, 3 and 8) and class II HDACs (4, 5, 6, 7, 9 and 10) have been reported to play roles in tumorigenesis. Numerous synthetic or natural product HDAC inhibitors (HDACi) have been developed for cancers that display increased HDAC activity or HAT mutations. Based on their chemical structure, HDACi can be organized into groups consisting of hydroxamates, benzamides, cyclic peptides or short-chain fatty acids (Table 2). Because HDACs are Zn^{2+} dependent many HDACi target the Zn^{2+} ion in the active site of HDACs to inhibit their enzymatic activity. Nonselective broad spectrum HDACi that inhibit all zinc dependent HDACs include the hydroxamate class agents vorinostat, belinostat and panobinostat. Vorinostat induces cell cycle arrest, promotes apoptosis, sensitizes cells to other chemotherapy and has been approved to treat patients with cutaneous T-cell lymphoma (CTCL) (59). Belinostat was approved to treat peripheral T-cell lymphomas (PTCL) and panobinostat to treat multiple myeloma (MM). These drugs are also in clinical trials to treat solid tumors. Nonselective HDACi such as vorinostat can reverse aberrant epigenetic chromatin changes to reactivate tumor suppressor genes such as p21 (60). In addition, HDACs have multiple functions in the cell and also target non-histone proteins. For instance, acetylation enhances the activity of some transcription factors such as p53 and GATA-1 (61; 62). Therefore, HDACi work through many mechanisms to promote cell cycle arrest, induce differentiation and activate apoptosis pathways in cancer cells.

Selective HDACi include romidepsin that targets HDAC1 and 2 and has been approved for CTCL and PTCL patients. In addition, ricolinostat is an HDAC6 specific inhibitor in clinical

trials for patients with MM or lymphoma (63). HDAC6 is a cytoplasmic deacetylase that acts on tubulin, Hsp90 and binds to ubiquitinated proteins. Ricolinostat has been especially promising for MM since these cells have high Ig production and are dependent on clearance of misfolded/aggregated proteins. Blocking HDAC6 leads to the accumulation of unfolded ubiquitinated proteins that induce cell death. Many additional HDAC inhibitors are in different phases of clinical trials either as single agents or in combination (Table 2).

TARGETING HISTONE METHYLTRANSFERASES

Dynamic methylation of nucleosomal histones plays an important role in regulation of gene expression. The two major families of histone methyl transferases are lysine methyl transferases (KMTs) and protein arginine methyltransferases (PRMTs). In recent years mono-, di- or tri-methylation of histone lysine methylation has been characterized to alter affinity of reader proteins to the methylated histones (Figure 3). Specific lysine methylation marks on H3K4, H3K36 and H3K79 are associated with activation of gene expression while marks associated with repression are H3K9, H3K27 and H4K20 (64). Importantly, different methylation states (e.g. me0, me1, me2, me3) of the same lysine residue occur in different contexts and may have distinct functional consequences. For instance, H3K4me3 marks are found at active or poised transcription start sites while H3K4me1 is usually found at active enhancers (65). In addition, H3K9me1 is typically found at active genes but H3K9me3 can be associated with gene repression (64).

The identification of KMT genetic alterations in cancer has made small molecules that selectively inhibit oncogenic methyltransferases promising therapeutic agents. However, despite the abundance of research on the more than 50 KMTs relatively few selective inhibitors have been identified (Table 3). One promising epigenetic target is DOT1L, a H3K79 methyltransferase that has an important role in *MLL* (KMT2A) leukemias. Translocations of *MLL* are found in 5–10% of AMLs and tandem duplication of MLL is found in ~5% of de novo AML (66). *MLL* translocations result in fusion proteins that lack their catalytic SET domain and frequently contain elongation complex nuclear proteins such as AF9, AF10, ENL (67). This complex also contains DOT1L, and AML cells carrying these translocations display aberrant patterns of K79 methylation that activate homeobox gene expression (68). The DOT1L inhibitor Pinometostat (EPZ-5676) inhibits proliferation of cell lines with MLL rearrangements in association with decreases in H3K79me levels at Hox genes and is currently in clinical trials (69).

Enhancer of Zeste 2 (EZH2) is a catalytic component of the polycomb repressive complex 2 (PRC2) where it catalyzes di- and tri-methylation of H3K27 that represses transcription. EZH2 gain of function is associated with poor prognosis in breast and prostate cancer and an activating mutation in the enzymatic SET domain of EZH2 was observed in 22% of patients with diffuse large B-cell lymphoma (DLBCL) and 7 percent of follicular lymphomas (70–72). This EZH2 mutation increases the ability of the protein to create the repressive H3K27me3 mark and is associated with a global increase in H3K27 methylation in lymphoma cells. This in turn blocks expression of genes important for cell cycle control and B cell differentiation, contributing to lymphomagenesis. By contrast, loss of function EZH2 mutations have been reported in myeloid malignancies and T-ALL, associated with

widespread loss of the H3K27me3 mark suggesting that EZH2 may have either oncogenic and tumor suppressive properties depending on its cellular context and perhaps also due to the action of other co-occurring mutations (73). EZH2 is also frequently overexpressed in advanced cases of breast, prostate and other solid tumors, often in association with an epithelial to mesenchymal phenotype change (71; 72). In these cases an increase in H3K27me3 is not observed and there is evidence that in advanced prostate cancer, EZH2 contributes to gene activation by altering androgen receptor function in a manner that requires the enzymatic activity of the EZH2 protein (74).

The overexpression of EZH2 as well as its mutation motivated the development of small molecule inhibitors of EZH2 (EZH2i). EZH2i 3-Deazaneplanocin A (DZNep) is an adenosine analogue that binds to the enzyme in place of the S-adenosyl methionine (SAM) and causes degradation of the protein. DZNep induces cell cycle genes, inhibit HOXA9 expression and induce apoptosis of primary AML cells (75). In addition, DZNep in combination with panobinostat prolonged survival of mice xenografted with AML cells (75). More potent and selective SAM competitive EZH2 inhibitors EI1 and GSK126 are have been tested in cell culture and xenografts. EI1 treated cells display global loss of H3K27 methylation, and DLBCL cells carrying a gain of function EZH2 undergo apoptosis when treated with EI1 (76). Similarly, GSK126 inhibited EZH2, proliferation of mutant DLBCL cell lines and growth of these cells in xenografted mice (77). Tazemetostat (EPZ-6438) is another EZH2i that has shown promise in xenografts and is currently being evaluated in phase I/II trials as a single agent and in combination with dexamethasone (78–80). EZH2i may have uses in several situations including overexpression or mutation of the gene repressive protein EZH2, as well as tumors having loss of function mutations of gene activator proteins such as the H3K27 demethylase KDM6A (UTX) or inactivating mutations of the SWI/SNF proteins acting to restore a balance between forces mediating gene repression and activation (81; 82).

Other small molecule KMT inhibitors that have been developed as potential anticancer therapeutics include compounds targeting H3K9 KMTs (Table 3). Increased expression of the H3K9-specific KMT G9a was reported in lung cancer cell lines and treatment with inhibitor BIX-01294 reduces H3K9 methylation (83; 84). Similarly, SETDB1 is frequently amplified in melanoma and lung cancers and treatment with mithramycin down-regulates SETDB1 to inhibit proliferation (85; 86). In addition, the natural product chaetocin was identified as an inhibitor of SUV39H, a KMT that regulates erythroid and B-cell differentiation (87). These agents are currently being used for *in vitro* and *in vivo* preclinical studies.

PRMTs are frequently overexpressed in cancer and thus have also become attractive targets for anticancer strategies. The PRMT family has nine members (PRMT1-9) that methylate arginine to form monomethylarginine. Type I PRMTs (PRMT1-6 and 8) catalyze the formation of asymmetric dimethylarginine while type 2 PRMTs (PRMT5 and 9) form symmetric dimethylarginine (Figure 3). Histone arginine methylation marks can be activating (H4R3me2a, H3R2me2s, H3R17me2a, H3R26me2a) or repressive (H3R2me2a, H3R8me2a, H3R8me2s, H4R3me2s) and PRMT dysfunction has been observed in a variety of cancers. Overexpression of PRMT1 and PRMT4 (CARM1) was reported in NSCLC, and

PRMT4 can drive the expression of the c-Myc pathway by deregulating SWI/SNF in breast cancer (88; 89). Increased PRMT5 expression was observed in lymphoma, leukemia, glioblastoma and prostate cancer where it is reported to activate c-Myc and other oncogenic transcription factors (90). In addition, PRMT7 has been reported to inhibit E-cadherin expression and promote EMT in breast cancer (91). The only PRMT inhibitor in clinical trials is GSK3326595 (formerly EPZ015938) that is being evaluated for patients with solid tumors and non-Hodgkin lymphoma (92). Several inhibitors have shown promise in preclinical studies such as AMI-408 a PRMT1 inhibitor that suppressed the transformation function of MLL-GAS7 or MOZ-TIF2 fusions in AML models, and the PRMT5 inhibitor EPZ015666 that displays antitumor activity in xenografts of mantle cell lymphoma (93). In addition, the compound, MS023 was recently reported to inhibit asymmetric arginine dimethylation while increasing monomethylation and symmetric dimethylation, but its anti-cancer properties have yet to be characterized (94).

TARGETING HISTONE DEMETHYLATION

Two classes of lysine demethylases (KDMs) govern demethylation of histones: the amine oxidases that include lysine-specific demethylase 1 (LSD1) and the α-ketoglutarate-dependent Jumonji domain (JmjC) containing demethylases. The LSD-family KDMs only demethylate mono- and dimethylated lysines while the JmjC demethylases remove methyl from all three states of lysine methylation (95; 96). Target specificity of KDMs is often regulated by their participation in different complexes. Mutations or dysregulation of KDMs are reported in a variety of cancers, making these enzymes an attractive target for anti-cancer therapies.

The histone demethylase LSD1 (KDM1A) is highly expressed in several cancers and is specifically required for terminal differentiation of hematopoietic cells (97). LSD1 generally demethylates H3K4me1/2 thus repressing transcription, but when LSD1 interacts with the androgen receptor its enzymatic activity switches to H3K9me1/2 thereby stimulating transcription (98). LSD1 is also a substrate for G9a KMT and methylation of LSD1 stimulates recruitment of CDH during androgen-dependent gene expression (99). LSD1 has a C-terminal amine oxidase-like domain that is structurally related to monoaminoxidases (MAO). Thus, the MAO inhibitor tranylcypromine (TCP) inhibits LSD1, but its use is limited by indiscriminate anti-MAO activity (100). Several more selective TCP derivatives have been developed and some have entered clinical trials (Table 3). ORY-1001 has been shown to reduce H3K4me2 and LSD1 target gene expression and reduce tumor growth in xenografts (101). GSK2879552 promotes differentiation and inhibits proliferation of AML cells (101). In addition, LSD1 inhibitors GSK354 and GSK690 have recently been reported to inhibit cell growth in vitro (101). Interestingly, the small molecule HDAC inhibitor 4SC-202 has a dual function to inhibit LSD1 with similar potency and is in clinical trials for patients with advanced hematologic malignancies (102).

While four compounds are in clinical trials for LSD1, inhibitors of JmjC domain containing demethylases have been more difficult to develop. Most of the identified inhibitors are pan-specific metal chelators that are also competitive for cofactor 2-oxogluterate binding and only active in the low micromolar range. Two such compounds are GSK-J1 and its prodrug

GSK-J4 that inhibit KDM6A (UTX) and KDM6B (JMJD3) but also display lesser activity against KDM5A and 5B (103). These proteins are all implicated in cancer. For instance, inactivating mutations of KDM6A have been reported in AML, multiple myeloma and bladder cancer, while KDM6B is highly expressed in TALL and metastatic prostate cancer (81; 104). In addition, a KDM5A (JARID1) Nup98 fusion protein is found in 10% of pediatric acute megakaryoblastic leukemias (105). Recently, a potent and selective inhibitor of KDM5B, EPT1013182, has been reported to have antiproliferative effects in cell lines and inhibit growth in multiple myeloma xenograft models (106).

TARGETING READERS OF EPIGENETIC MARKS

Epigenetic reader proteins recognize and bind chromatin or histone modifications to either directly induce chromatin structural changes (e.g. compaction, remodeling) recruit secondary chromatin modifiers or serve as scaffold proteins for various nuclear processes such as transcription, replication or repair. Acetylated lysines are binding sites for proteins containing bromodomains such as the Bromodomain and Extra Terminal (BET) proteins BRD2, BRD3, BRD4 and BRDT. Readers that recognize methyl-lysine include MBT domain, Tudor domain, and Chromodomain proteins (107). Chromatin reader proteins can include several types of reader domains and binding at a specific chromatin site may depend on adjacent histone modifications.

BET family proteins are important for regulation of transcription and cell proliferation. BRD4 stimulates transcriptional elongation by recruitment of the P-TEFb complex to chromatin binding sites where it phosphorylates and increases the processivity of RNA polymerase II (108). In addition, recent studies suggest that BRD4 also recruits NSD methyltransferases to increase H3K36 methylation that activates gene expression (109). Several reports indicate that BET family members disruption is associated with cancer. BRD2 is overexpressed in B-cell lymphoma and translocations of BRD3 and BRD4 drive rare midline carcinomas (110). Recently small molecules have been developed that inhibit the tandem bromodomains of BET proteins (Table 4) (111; 112). One of the first BET inhibitors developed, JQ1, induced terminal differentiation of leukemic stem cells in primary AML samples and prevented ovarian carcinoma growth in xenografts (113; 114). Furthermore, JQ1 globally reduced androgen receptor (AR) target gene transcription and promoted apoptosis in cells with activated AR (115). In addition, dBET, a phthalimide-coupled JQ1, was demonstrated to induce BRD4 protein degradation and delay leukemia progression in mice (116). Recently, new classes of improved BET inhibitors have been developed. Three of these, I-BET762, CPI-0610 and OTX015 are in clinical trials for hematologic malignancies (117). In addition, TEN-010 is in clinical trials for NUT midline carcinomas and advanced solid tumors.

Inhibitors of methyl-lysine readers have also been developed but have not advanced beyond preclinical studies yet. L3MBTL1 and L3MBTL3 are members of the MBT proteins and their loss has been shown to contribute to hematopoietic malignancies. UNC125 binds to the aromatic cage of L3MBTL1 that is common to lysine methyl readers and inhibits L3MBTL1 peptide binding (118). Another MBT inhibitor, UNC1679, has been reported to have a much higher affinity for L3MBTL3 than L3MBTL1 and inhibits GFP-L3MBTL3 chromatin

binding in cells (119). Importantly, when take together these results suggest that targeting epigenetic readers may be a useful strategy to counteract the effect of aberrant histone acetylation/methylation profiles in cancer.

CONCLUSIONS AND FUTURE DIRECTIONS

Genome sequencing of patient tumor samples has revealed that alterations of genes regulating the cellular epigenetic state are frequently initiating events in cancer and subclones carrying these mutations are likely to persist after treatment. Thus, much recent work has been towards improving our understanding of the defective epigenetic mechanisms in cancer cell populations in order to develop more effective cancer therapies. Hypomethylating agents, HDAC inhibitors and agents that reverse cancer associated histone modifications have significantly increased our arsenal to treat cancers, particularly hematologic malignancies. In addition, emerging therapeutic strategies take advantage of crosstalk between different epigenetic mechanisms. For instance, cooperation between DNA methylation and histone deacetylation in gene expression has led to clinical trials that test combinations of HDAC inhibitors and DNA hypomethylating agents (120). These combination therapies are attractive over single agent therapies because they may synergistically reactivate silenced tumor suppressor gene expression, which allows the use of lower dosages that may reduce side effects and the likelihood of acquired resistance.

Many other mechanisms of epigenetic regulation have been described that may impact cancer. For example, in addition to lysine acetylation and methylation, histones are subject to other posttranslational modifications such as sumoylation, ubiquitination and/or phosphorylation but additional studies are necessary to determine the affect of these mechanisms on tumorigenesis. In addition, subunits of the SWI/SNF chromatin remodeler complex are frequently mutated in cancers (121). Cancer cells harboring SWI/SNF mutations become dependent on EZH2, and EZH2 inhibitors have proven effective in these cells (122). Furthermore, RNA as well as DNA is subject to covalent modification such as methylation and non-coding RNAs such as snRNAs involved in splicing, miRNAs involved in silencing and lncRNAs involved in gene expression have profound and heritable effects on gene expression. Therapeutic strategies may be developed to target these mechanisms as their significance in cancer becomes better understood.

As promising as the current epigenetic cancer therapies are, significant challenges remain. The precise role specific writers, readers and erasers play in different types of cancer at different stages of differentiation is not well understood, and no chemical inhibitors have been identified for many KDMs and KMTs that could be important targets in cancer. In addition, epigenetic writers, readers and erasers have targets in addition to those associated with chromatin. For instance, LSD1 can also demethylate non-histone proteins such as p53 and DNMT1. In addition, many proteins are regulated by acetylation and may be targets for KATs and HDACs including oncogenes and tumor suppressors such as MYC, p53 and PTEN (123). Thus, the effect of inhibiting epigenetic mechanisms may be due to cytoplasmic as well as nuclear processes. Furthermore, it remains uncertain whether the efficacy of agents targeting epigenetic mechanisms is specifically linked to any changes in gene expression. For instance it is not certain why cells harboring DNMT3A mutations that

causes hypomethylation are sensitive to hypomethylating agents. Many epigenetic therapies elicit a DNA damage response and may simply be functioning as cytotoxic agents. In the future, a better comprehension of how epigenetic mechanisms are disrupted in cancer could guide a more mechanistic based rationale for use of specific inhibitors as anticancer therapies.

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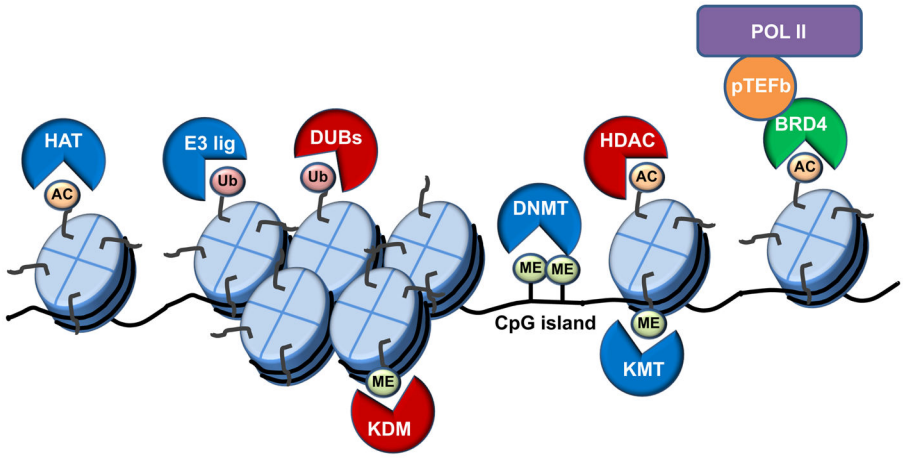

Figure 1.

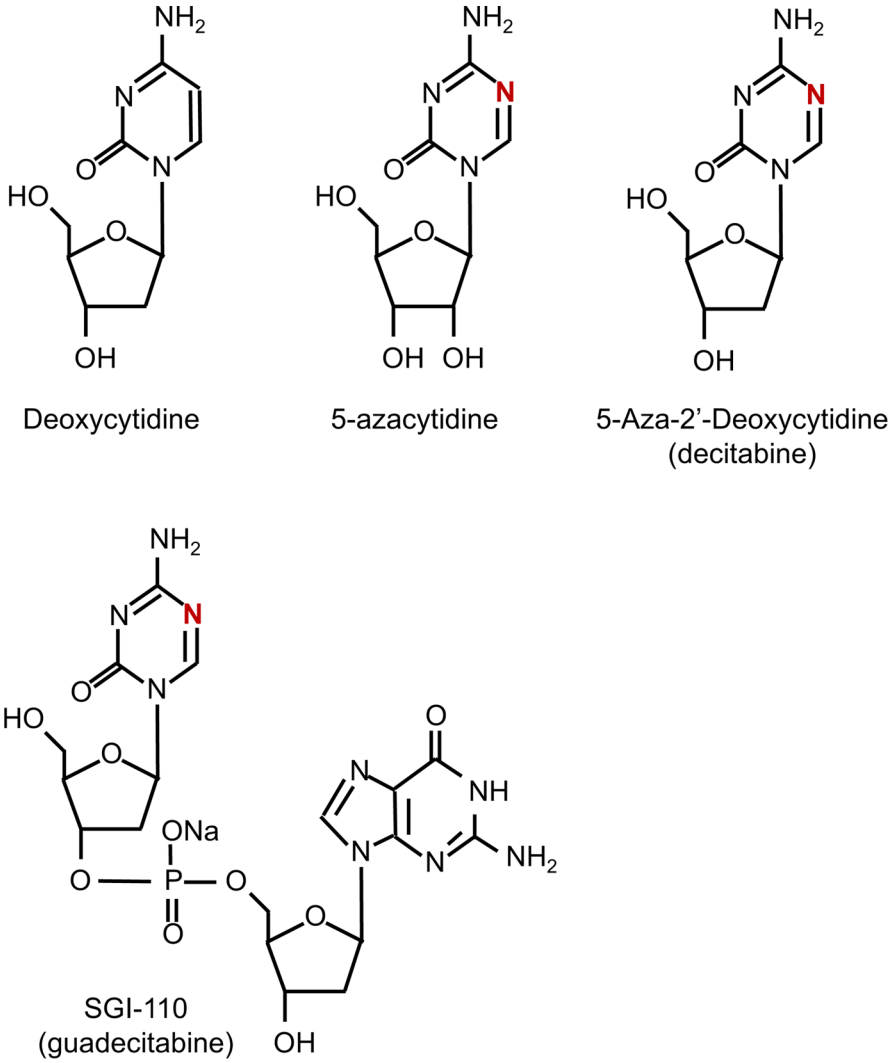

Figure 2.

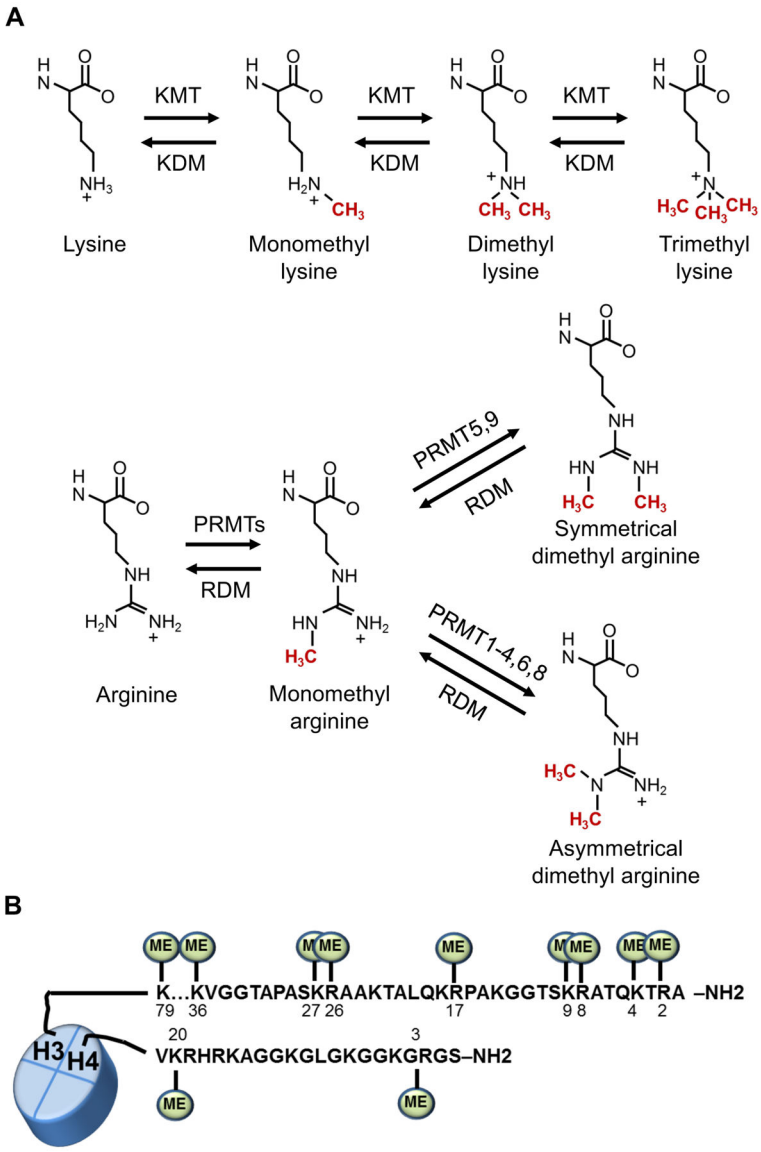

Figure 3.

Table 1

DNA methylation inhibitors in cancer therapy

Compound	Target	Clinical Status	Reference
5-Azacytidine (Vidaza)	DNMT1	Approved: MDS	(32)
5-Aza-2′-deoxycytidine (Decitabine)	DNMT1	Approved: MDS	(33; 124)
SGI-110 (Guadecitabine)	DNMT1	Clinical trials: MDS, AML, Ovarian, HCC	NCT01261312, NCT01752933
AG-120	Mutant IDH1	Clinical trials: AML	NCT02677922
AG-221	Mutant IDH2	Clinical trials: MDS, AML	NCT01915498

Abreviations: AML, Acute myelogenous leukemia; MDS, myelodysplastic syndrome; HCC, hepatocellular carcinoma

Table 2

Histone deacetylase inhibitors in cancer therapy

Compound	HDAC Target	Clinical Status	Reference
Hydroxamates:			
Abrexinostat	Class I, II	Clinical trials: HL, non-HL, CLL	(125)
Belinostat	Class I, II, IV	Approved: PTCL	(126)
CG200745	Class I, II, IV	Clinical trials: Solid tumors	(127)
CHR-3996	Class I	Clinical trials: Solid tumors	(128)
CUDC-101	Class I, II	Clinical trials: Squamous cell carcinoma	(129)
CUDC-907	Class I, II	Clinical trials: MM, lymphoma, solid tumors	(130)
Givinostat	Class I, II	Clinical trials: CLL, MM, HL	(131; 132)
MPT0E028	HDAC1, 2, 6	Clinical trials: Solid tumors	(133)
Panobinostat	Class I, II, IV	Approved: MM	(134; 135)
Pracinostat	Class I, II, IV	Clinical trials: AML, Prostate	(136)
Quisinostat	Class I, II	Clinical trials: Solid tumors, lymphoma, CTCL	(137)
Resminostat	Class I, II	Clinical trials: Colorectal, HCC, HL	(138)
Vorinostat (SAHA)	Class I, II, IV	Approved: CTLC	(139)
Benzamides:			
Chidamide	HDAC1, 2, 3, 10	Clinical trials: Breast, NSCLC	(140)
Entinostat	HDAC1, 2, 3	Clinical trials: NSCLC, solid tumors	(141)
Mocetinostat	Class I, IV	Clinical trials: Hematologic and solid tumors	(142)
Ricolinostat	HDAC6	Clinical trials: MM, lymphoma	(63)
Tacedinaline	Class I	Clinical trials: MM, lung, pancreatic	(143)
Cyclic Peptides:			
Romidepsin	HDAC1, 2	Approved: CTLC, PTCL	(144)
Fatty acids:			
AR-42	Class I, II	Clinical trials: AML, MM	(145)
Phenylbutyrate	Class I, II	Clinical trials: Solid, hematologic	(146)
Pivanex	Class I, II	Clinical trials: NSCLC, myeloma, CLL	(147)
Valproic acid	Class I, II	Clinical trials: Solid, hematologic	(148)

Abbreviations: ALL, Acute lymphocytic leukemia; AML, Acute myelogenous leukemia; CLL, Chronic lymphocytic leukemia; CTLC, cutaneous T-cell lymphoma; HCC, hepatocellular carcinoma; HL, Hodgkin's lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; NSCLC, Non-small cell lung cancer; PTCL, peripheral T-cell lymphoma

Table 3

Histone methylation/demethylation inhibitors in cancer therapy

Compound	Target	Cancer Type	Reference
HMT inhibitors:			
EPZ00477	DOT1L	MLL rearranged leukemias	(149)
Pinometostat (EPZ-5676)	DOT1L	Clinical trials: hematologic malignancies	(150) NCT01684150
SGC0946	DOT1L	MLL rearranged leukemias	(151)
DZNep	EZH2	Breast, colon, prostate	(152)
GSK126	EZH2	DLBCL	(77)
GSK343	EZH2	Ovarian	(77)
EPZ005687	EZH2	EZH2 mutant non-HL	(78)
EI1	EZH2	DLBCL	(76)
Tazemetostat (EPZ-6438)	EZH2	Clinical trials: Solid tumors, DLBCL, HL, non-HL	(79) NCT02889523
UNC1999	EZH2	DLBCL	(153)
BIX-01294	G9A	leukemia, bladder	(154)
BRD4770	G9A	Pancreatic	(155)
UNC0638	G9A	AML, breast	(156)
Chaetocin	SUV39H1	lymphomas	(87)
GSK3326595	PRMT5	solid tumors, non-HL	NCT02783300
AMI-408	PRMT1	AML	(93)
MS023	PRMT1,3,4,6,8		(94)
HDM inhibitors:			
ORY-1001	LSD1	Clinical trials: AML	(157)
GSK2879552	LSD1	Clinical trials: AML, Relapsed/Refractory Small Cell Lung Carcinoma	(157) NCT02034123 NCT02034123
GSK354, GSK690	LSD1	AML	(101)
NCD25, NCD38	LSD1	MDS	(158)
Tranylcypromine	LSD1	Clinical trials: AML, MDS	(157) NCT02273102
4SC-202	HDAC-LSD1	Clinical Trials: hematologic malignancies	(102) NCT01344707
GSK-J1, GSK-J4	JmjC domain proteins		(103)
EPT-103182	KDM5B	Hematologic and solid cancers	(106)

Abbreviations: AML, Acute myelogenous leukemia; DLBCL, Diffuse large B-cell lymphoma; HL, Hodgkin's lymphoma; MDS, myelodysplastic syndrome

Table 4

Inhibitors of epigenetic readers used as cancer therapy

Compound	Target	Cancer Type	Reference
JQ1	BET proteins	Prostate, AML with MLL translocations, MM, Nut midline carcinoma	(111; 113–115)
I-BET762	BET proteins	Clinical trials: hematologic malignancies, NUT midline carcinoma and solid tumors	(117) NCT01943851 NCT01587703
OTX015	BRD2, 3, 4	Clinical trials: AML	NCT01713582
CP-0610	BET proteins	Clinical trials: AML, MDS, MM, lymphoma	NCT01949883 NCT02158858 NCT02157636
TEN-010	BET proteins	Clinical trials: Nut midline carcinomas, solid tumors	NCT01987362
dBET-1	BET proteins	Leukemias	(116)
I-BET151	BET proteins	MM	(117)
GSK2801	BAZ2B		
UNC669	L3MBTL1		(118)
UNC1215	L2MBTL3		(119)

Abbreviations: AML, Acute myelogenous leukemia; MDS, myelodysplastic syndrome; MM, Multiple myeloma