Epigenetic cancer therapy: Proof of concept and remaining challenges

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Over the past few years several drugs that target epigenetic modifications have shown clinical benefits, thus seemingly validating epigenetic cancer therapy. More recently, however, it has become clear that these drugs are either characterized by low specificity or that their target enzymes have low substrate specificity. As such, clinical proof-of-concept for epigenetic cancer therapies remains to be established. Human cancers are characterized by widespread changes in their genomic DNA methylation and histone modification patterns. Epigenetic cancer therapy aims to restore normal epigenetic modification patterns through the inhibition of epigenetic modifier enzymes. In this review, we provide an overview about the known functional roles of DNA methyltransferases, histone deacetylases, histone methyltransferases, and demethylases in cancer development. The available data identify several examples that warrant further consideration as drug targets. Future research should be directed toward targeted enzyme inhibition and toward exploring interactions between epigenetic pathways to maximize cancer specificity.

Keywords:
- cancer; DNA methylation; drugs; epigenetics; histone modifications

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Abbreviations:
DNMT, DNA methyltransferase; **HDAC**, histone deacetylase.

Introduction

Epigenetic mechanisms regulate the interpretation of genetic information. As such, our knowledge of these mechanisms is essential for understanding the phenotypic plasticity of cells, both in the context of normal cellular differentiation and in human disease [1]. Research over the past two decades has identified two major levels of epigenetic modification: DNA methylation and covalent histone modifications [2, 3]. DNA methylation is mediated by a family of enzymes termed DNA methyltransferases (DNMTs) [4], while histone modification patterns are established and maintained by a diverse set of enzymes that add or subtract acetyl-, methyl-, and other modifications to various amino acids of histone proteins [5]. Both regulatory mechanisms cooperate to determine the expression potential of individual genes.

Epigenetic changes are increasingly recognized as a major characteristic of human cancers. This was first evidenced by biochemical analyses of tumor DNA, which revealed a 20% reduction in global DNA methylation levels [6]. In addition, later studies have shown that various cancer-associated genes, including tumor suppressor genes, can be hypermethylated during tumor formation [7]. Hypermethylation of promoter regions is strongly associated with gene silencing. Consequentially, the hypermethylation of tumor suppressor genes can result in the loss of the corresponding gene function. Because of the functional similarities between genetic mutations and these epigenetic alterations, the latter have also been termed epigenetic mutations, or epimutations. More recently, characteristic tumor-specific epimutations have also been demonstrated for histone modifications. These changes have been shown to occur both at the global and at the gene-specific level [8, 9]. It is commonly assumed that cancer cells accumulate a substantial number of epimutations that cooperate with genetic mutations to establish cancer cell-specific phenotypes characterized by high proliferation rates, apoptosis resistance, and increased cellular motility [10].

The prevalence of epigenetic mutations in human cancers and their general reversibility has fueled the development of drugs that target the enzymes that mediate epigenetic modifications. Two prominent examples are the cytosine analogs 5-azacytidine (azacytidine, Vidaza) and 2′-deoxy-5-azacytidine

Table 1. Overview of common DNMT and HDAC inhibitors and their clinical status

Group	Example	Alias	Specificity	Clinical status	Indication in monotherapy	Indication in combination therapy
Aza-nucleosides	5-Azacytidine	Azacytidine, Vidaza	DNMTs	Approved May 2004 for MDS	Myeloid leukemias	Myeloid leukemias (Belinostat PXD101), lung Ca (Entinostat)
	2′-Deoxy-5-azacytidine	Decitabine, Dacogen	DNMTs	Approved May 2006 for MDS	Myeloid leukemias	Myeloid leukemias (Vorinostat, LBH589, Romidepsin, VPA)
Hydroxamic acids	Trichostatin A	TSA	pan-HDAC	Preclinical		
	Suberoylanilide hydroxamic acid (SAHA)	Vorinostat, Zolinza	pan-HDAC	Approved Oct. 2006 for CTCL	NSCLC, soft tissue sarcoma, MDS, sickle cell disease	Ovarian Ca (Paclitaxel, Carboplatin), pancreatic Ca (radiation, proteasome inhibitor NPI-0052), liver Ca (Sorafenib), breast Ca (Lapatinib), brain metastasis (radiation), MDS (Decitabine)
	Panobinostat	LBH589	pan-HDAC	Phase I > II > III	Renal cell Ca, thyroid Ca, multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, SCLC, prostate Ca	Breast Ca (Lapatinib, Capecitabine), malignant glioma (Bevacizumab), prostate Ca (Docetaxel, Prednisone), head and neck Ca (Erlotinib), MDS (Decitabine), hepatocellular Ca (Sorafenib), NSCLC (Pemetrexed)
	Belinostat	PXD101	pan-HDAC	Phase I > II	Lymphoma, non-Hodgkin's lymphoma, peripheral T cell lymphoma, multiple myeloma	Acute leukemia and MDS (Bortezomib, Azacytidine), soft tissue sarcomas (Doxorubicin), thymic Ca (Cisplatin, Doxorubicin, Cyclophosphamide), NSCLC (Carboplatin, Paclitaxel, Bevacizumab), ovarian Ca (Carboplatin)
Cyclic tetrapeptides	Depsipeptide, Romidepsin	Istodax	HDAC 1 & 2	Approved Nov. 2009 for CTCL	Non-Hodgkin's lymphoma, lung Ca, colorectal Ca, head and neck Ca, lymphoma, MDS	Pancreatic Ca (Gemcitabine), leukemia, lymphoma (Bortezomib), multiple myeloma (Bortezomib, Dexamethasone), MDS (Decitabine)
Short-chain fatty acids	Valproic acid	VPA, Depakote	HDAC classes I, IIa	Phase I > II > III	Chronic lymphocytic leukemia, head and neck Ca	Brain metastasis (Etoposide), advanced Ca (Bevacizumab), chronic

Table 1. (*continued*)

Group	Example	Alias	Specificity	Clinical status	Indication in monotherapy	Indication in combination therapy
						myelogenous leukemia (Imatinib, Gleevec), MDS (Decitabine), lung Ca (Hydralazine), malignant melanoma (Karenitecin)
	Phenyl butyrate	4PBA	HDAC classes I, IIa	Phase I > II	Alpha 1-antitrypsin deficiency (liver disease)	Cystic fibrosis (Genistein, unconjugated isoflavones 100)
	AN-9	Pivanex	HDAC classes I, IIa	Phase I > II	Malignant melanoma, leukemia, lymphoma	NSCLC (Docetaxel)
Benzamides	Entinostat	MS-275, SNDX-275	HDAC 1 & 3	Phase I > II	Hodgkin's lymphoma, MDS, ER+ breast Ca	Leukemia (Clofarabine), lung Ca (Azacytidine), NSCLC (Erlotinib)
	Mocetinostat	MGCD0103, MG-0103	HDAC 1 & 3	Phase I > II	Chronic lymphocytic leukemia, lymphoma, MDS, non-Hodgkin's lymphoma	Leukemia (Azacytidine), solid tumors (Gemcitabine)
Synthetic benzamides	*N*-Acetyldinaline	CI-994, Tacedenaline	HDAC 1& 3	Phase I > II > III	Advanced myeloma,	Advanced NSCLC (Gemcitabine hydrochloride), pancreatic Ca (Gemcitabine hydrochloride)

For each chemical group at least one example is named, with corresponding enzyme specificity, clinical status, and indication used in mono- or combination therapy. MDS: myelodysplastic syndrome, CTCL: cutaneous T cell lymphoma, NSCLC: non small cell lung cancer, Ca: cancer. For further details see clinicaltrials.gov.

(decitabine, Dacogen), which are potent inhibitors of DNMTs (Table 1) and have been approved for the treatment of myelodysplastic syndrome, a pre-leukemic bone marrow disorder [11, 12]. Various additional molecules are currently being investigated as DNMT inhibitors in preclinical experiments [13]. Another prominent example for an epigenetic drug is the histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), which has been approved for the treatment of cutaneous T cell lymphoma [14]. Another HDAC inhibitor (Romidepsin, Istodax) has very recently been approved for the same indication. It is structurally unrelated to SAHA and belongs to the cyclic tetrapeptide group. As of today, there are at least 20 structurally different HDAC inhibitors in clinical trials, either in monotherapy or in combination therapy trials for hematological and solid tumors. These inhibitors can be separated into five different chemical groups (Table 1). It should be noted that combination therapies of HDAC inhibitors with other anticancer drugs or with radiation therapy have shown a wide range of synergistic effects, both in preclinical models and in early clinical trials [15]. It will be important to understand the mechanistic basis for these interactions to further increase the clinical use of HDAC inhibitors and other epigenetic drugs.

Despite these major achievements and substantial efforts in the development of epigenetic cancer therapies, proof of concept for a functional role of epigenetic mechanisms in cancer formation is still considered scarce. In this context, it is important to notice that the clinical effectiveness of the approved drugs does not prove the effectiveness of epigenetic therapy: azacytidine is not a specific inhibitor of DNMTs, but a nucleoside analog that affects many cellular pathways [16]. It is presently unclear to what extent the diverse and complex drug effects contribute to clinical responses. On the other hand, HDAC inhibitors, like SAHA, are often highly specific inhibitors, but the target enzymes are not specific for histones and include a wide range of non-histone proteins that are not involved in epigenetic regulation [17]. These issues emphasize the requirement for robust proof-of-concept data supporting the effectiveness of epigenetic cancer therapy.

Evidence for a functional role of DNA methylation in tumor development

DNMTs catalyze the methylation of cytosine residues in CpG dinucleotides and represent the best-known epigenetic modifier proteins. The mammalian DNMT family consists of four members (Fig. 1): DNMT1, DNMT2, DNMT3A, and DNMT3B [4]. DNMT1 is generally considered a maintenance methyltransferase that copies DNA methylation patterns from the parental to the newly synthesized strand in a process that is closely associated with DNA replication. In agreement with this

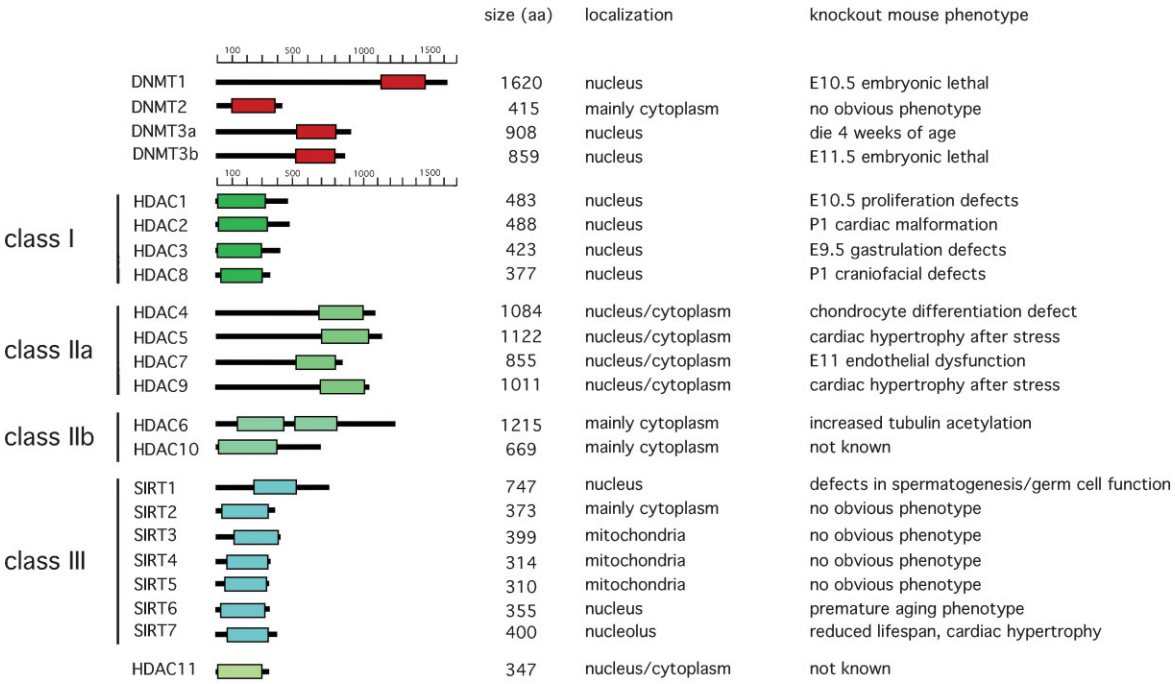

Figure 1. Schematic overview of known human DNA methyltransferases (DNMTs) and histone deacetylases (HDACs). HDACs are commonly grouped into four classes, based on their homology to yeast HDACs. Catalytic domains are indicated as boxes. The length of each enzyme is shown in amino acids (aa), subcellular protein localization and the phenotypes of knockout mice are also indicated.

notion, DNMT1 shows a strong preference for hemimethylated DNA and is unable to establish methylation marks *in vivo* [18]. This *de novo* methyltransferase activity is provided by a separate set of enzymes, DNMT3A and DNMT3B [19]. Interestingly, these enzymes have also been reported to have maintenance activities that overlap with DNMT1, which suggests that the functional specificities of DNMT enzymes might be more complex than initially thought [18]. The last member of the animal DNMT family, DNMT2, is unlikely to be involved in the establishment and/or maintenance of DNA methylation patterns. DNMT2 has been shown to act as a cytosine-5 RNA methyltransferase, which might potentially represent the evolutionary origin of eukaryotic DNMTs [20].

Only a few years after its initial description, it was suggested that DNMT1 is overexpressed in human cancer cells and might thereby contribute to cellular transformation [21, 22]. However, this finding has been discussed controversially [23] and there are no known oncogenic mutations in the DNMT1 gene. Importantly, proof-of-concept data were obtained from hypomorphic allele combinations of DNMT1 that cause reduced tumor burden in a mouse colon cancer model [24, 25]. Similarly, knockdown studies in human cancer cell lines implicate DNMT1 in the maintenance of cancer-specific methylation DNA patterns [26, 27]. Lastly, loss of DNMT1 by gene targeting in a human colon cancer cell line caused complex mitotic defects and cell death [28], which suggested an important function of the enzyme in cancer-relevant pathways. However, the tumor specificity of these effects still remains to be established.

Similar to DNMT1, there are presently no convincing published data demonstrating overexpression of DNMT3A or DNMT3B in human cancers. However, siRNA-mediated knockdown experiments have implicated DNMT3B in the maintenance of epimutations in a human lung cancer cell line [29]. In addition, overexpression of DNMT3B in the mouse intestine caused an increased intestinal tumor burden in a colon cancer model [30]. A later study showed that intestinal tumors from DNMT3B-overexpressing mice had acquired DNA-hypermethylation signatures that were similar to the epigenetic mutations observed in human colon cancer [31]. These findings suggest a specific role of DNMT3B in the etiology of human colon cancer and potentially also in other tumors.

Evidence for a functional role of HDACs in tumor development

Among the many known post-translational modifications of histone amino tails, histone acetylation was the first modification to be associated with gene regulation [32]. It is generally assumed that histone acetylation, mediated by histone acetyltransferases (HATs), opens up the chromatin structure, thus enabling DNA binding of transcription factors, while the removal of acetyl groups by HDACs causes chromatin condensation and restricted access to DNA. To date, 18 human genes that encode HDACs have been identified (Fig. 1). The majority of HDACs (class I and class II) have been shown to bind and modify hormone co-repressors and transcription factors, which probably explains their ability to regulate gene expression in chromatin-independent ways [33]. The third class of HDACs, the sirtuin (SIRT) enzymes, is defined by their homology to yeast Sir2 and has been mainly linked to aging [34]. However, high expression of SIRT1 has been reported in solid tumors and SIRT1 depletion has been shown to block cell proliferation and to induce apoptosis in cancer cells, but not in

normal cells [35, 36]. It was also shown that SIRT1 depletion induces re-expression of epigenetically silenced tumor suppressor genes [37], which suggested a functional role of SIRT1 in cancer development.

The roles of individual HDACs have been studied in considerable detail [38]. Many of these enzymes show complex activities in various developmental pathways (Fig. 1). Not surprisingly, functional redundancies have been reported between the most closely related isoforms HDAC1 and HDAC2 [39]. Tumor cells lacking HDAC1 and HDAC2 showed a distinct phenotype of nuclear bridging, nuclear fragmentation, and mitotic catastrophe and deletion of these two enzymes led to a complete block of tumor growth in mice [39]. In addition, HDAC2 has also been functionally linked to the etiology of colon cancer. Loss of the adenomatosis polyposis coli (APC) gene in mice led to c-myc-dependent increased expression of HDAC2 [40]. Reconstitution of APC reduced the expression of HDAC2 and induced apoptosis. Furthermore, inhibition of HDAC2 activity in tumor-bearing mice by valproic acid, a preferential class I HDAC inhibitor, reduced the number and the size of intestinal adenomas significantly [40]. It was later shown that HDAC2 knockout mice are viable, although they are approximately 25% smaller than their littermates [41]. Noticeably, however, the cell number and thickness of the intestinal mucosa were reduced, which is consistent with a role in intestinal tissue homeostasis. When the HDAC2 mutation was bred into the APC^{Min} mouse tumor model, tumor rates were significantly lower, suggesting an important role of HDAC2 for tumor development *in vivo* [41]. A mutated and truncated version of HDAC2 was also identified in sporadic carcinomas with microsatellite instability, as well as in tumors from hereditary nonpolyposis colorectal cancer syndrome patients [42]. This mutation caused loss of HDAC activity, which sequentially rendered tumor cells more resistant to antiproliferative and proapoptotic effects of HDAC inhibitor treatment [42]. These findings support a functional role of HDAC2 in tumor formation and suggest that the HDAC2 mutational status might be a useful predictor for HDAC inhibitor therapy response.

The functional links between HDAC2 and cancer as well as the high drugability of HDACs in general have fuelled the development of HDAC inhibitors (Table 1). Vorinostat, the first approved HDAC inhibitor, has shown pharmacological activity against different tumor cell lines *in vitro* and various tumor entities can thus be potentially targeted by the drug. For example, vorinostat has been shown to induce cell cycle arrest and differentiation in breast cancer cell lines. Withdrawal of vorinostat caused the cells to re-enter the cell cycle and reverse to a less differentiated phenotype [43]. Panobinostat (LBH-589), which is structurally similar but biochemically more potent than vorinostat, is currently being tested in clinical trials against various hematological and solid tumors [44, 45]. Several other hydroxamic acid based HDAC inhibitors including belinostat (PDX101), NVP-LAQ824, as well as the benzamides entinostat (MS-275) and mocetinostat (MGCD0103) are in phase I and phase II clinical trials [46, 47] that include patients with a wide variety of hematological and solid neoplasms (Table 1, we only highlight the most advanced inhibitors for each chemical group).

The mode of action of HDAC inhibitors is frequently associated with transcriptional reactivation of tumor suppressor genes, such as p21 [48]. Inhibition of HDAC1 by treatment of multiple myeloma cells with Vorinostat can reactivate p21 expression and thereby prevent tumor cell proliferation [49]. While these and similar findings seemed to indicate the existence of a central epigenetic pathway that can be targeted by HDAC inhibitors, more recent publications have raised questions regarding the overall substrate specificity of HDACs. A comprehensive and quantitative proteomic analysis of lysine acetylation sites identified 3,600 acetylation sites on 1,750 proteins [17]. Treatment of cells with the HDAC inhibitors SAHA and MS-275 revealed that the drugs mainly target large macromolecular complexes contributing to regulation of almost all nuclear functions and to a surprisingly large array of cytoplasmic functions [17]. The regulatory scope of HDACs therefore appears much broader than previously thought and comparable with that of other major post-translational modifications [17, 50]. It is now clear that HDAC inhibitors induce hyperacetylation of many non-histone protein substrates and that the effects of HDAC inhibitors in cancer therapies may therefore not be mediated through epigenetic changes in histone acetylation [33]. Further mode-of-action research will be required to substantiate the classification of HDAC inhibitors as epigenetic drugs.

Histone methylation: A modification with specific epigenetic functions?

Research over the past ten years has identified many additional histone modifications, which include phosphorylation, methylation, ubiquitination, and sumoylation [5]. Among these, histone lysine methylation has been attributed a more specific function in epigenetic regulation. Histone lysine residues can be mono-, di-, or tri-methylated by histone methyltransferases. Based on the sequence and structure of their catalytic domain, methyltransferases can be divided into three groups: SET domain-containing and Dot1-like lysine methyltransferases [51] as well as arginine methyltransferases [52].

Histone H3 lysine 9 methylation is a crucial epigenetic mark of heterochromatin and transcriptional silencing. Downregulation of histone H3 lysine 9 methyltransferase G9a and Suv39h1 induces centrosome disruption and chromosome instability in cancer cells [53]. Knockout mice of the H3K9 tri-methylating enzymes show genomic instability and develop B cell lymphomas [54]. The first inhibitor of a lysine-specific histone methyltransferase, chaetocin, was identified as a fungal metabolite from *Chaetomium minutum*. Chaetocin was demonstrated to be specific for SU(VAR)3-9 enzymes and only decreased methylation at histone H3 lysine 9 [55]. However, tumor growth inhibition by chaetocin remains to be shown.

G9a is the predominant histone H3 lysine 9 methyltransferase in euchromatin, required for overall mono- and dimethylation of H3K9 [56] as well as H3K27 [57]. G9a has been implicated in the epigenetic silencing of tumor suppressor genes [58] as well as in the targeting of *de novo* DNA methylation in embryonic stem cells [59]. Knockout mouse embryos die around E9.5–12.5 and show severe growth retardation,

thus establishing an essential function of G9a in normal cells [56, 60]. Overexpression of G9a has been observed in prostate, breast, lung, and colon cancer cell lines [61, 62]. Overexpression of a catalytically inactive version carrying a single amino acid substitution (H1166K) showed severe growth inhibitory effects in cancer cells [63] and thus suggests a functional role of G9a in tumor formation. Of note, a high-throughput screen against a preselected chemical compound library was performed to identify inhibitors of G9a [64]. One hit, BIX-01294, caused a moderate reduction in histone H3 lysine 9 dimethylation in bulk histone preparations. Treatment of different cell lines with twofold IC_{50} concentrations of BIX-01294 did not induce detectable morphological changes, and global drug-induced histone methylation changes appeared rather small [64]. As such, additional work will be required to establish G9a inhibitors as drug candidates for further development.

Enhancer of Zeste 2 (EZH2) is another SET domain-containing histone methyltransferase. EZH2 catalyzes the di- and trimethylation of histone H3 lysine 27 (H3K27me2/me3) [65]. This histone methyltransferase activity is required for early mouse development, since knockout mice die during embryogenesis [66]. Interestingly, recent findings also revealed the expression of a mutant EZH2 variant in human follicular lymphoma and B cell lymphoma [67]. An upregulation of EZH2 has been observed in a wide variety of tumors, such as prostate cancer, lymphomas, colorectal and gastric cancer, and bladder and breast cancer, and it was suggested that EZH2 could be an oncogene [68]. EZH2 is overexpressed in localized prostate cancers with a higher risk of disease recurrence after radical prostatectomy [69] and highly overexpressed in metastatic castration-refractory prostate cancer [69, 70]. Knockdown of EZH2 inhibited growth and cell invasion of myeloma and prostate cancer cells [69, 71, 72], whereas overexpression promoted colony formation, anchorage-independent growth, and cell invasion as well as xenograft tumor growth [71]. EZH2 is also overexpressed in breast cancers and overexpression is associated with poor clinical outcome [73–75]. EZH2 knockdown decreased proliferation and delayed G2/M cell-cycle transition and led to an upregulated expression of the tumor suppressor gene BRCA1 [76]. EZH2 currently appears to be the best-characterized histone methyltransferase target for tumor therapy. However, important questions with respect to tumor specificity of EZH2 expression and function still need to be clarified in future studies. First attempts for the pharmacological inhibition of EZH2 have been reported [77]. However, the corresponding drug, 3-deazaneoplanocin A (DZNep), does not specifically inhibit EZH2 but rather induces the degradation of EZH2 and associated proteins through a mechanism that has not been fully understood yet [77–79].

Lastly, it should also be noted that histone methylation can be enzymatically reversed by histone demethylases [80]. The lysine-specific demethylase 1 (LSD1) was the founding member for enzymes that directly reverse histone H3K4 and/or H3K9 modifications by an oxidative demethylation reaction using flavin as cofactor [81, 82]. The more recently discovered Jumonji enzymes also demethylate histone lysine residues, but use a Fe^{2+}- and 2-oxoglutarate-dependent dioxygenase mechanism [83]. LSD1 homozygous knockout mice show early embryonic lethality. Knockdown of LSD1 abrogated androgen-induced transcriptional activation and cell proliferation in prostate cancer cells [82]. More recent studies have suggested a functional role of LSD1 in neuroblastoma. LSD1 expression correlated with adverse outcome and was inversely associated with differentiation in neuroblastic tumors [84]. Knockdown of LSD1 *via* small interfering RNAs decreased cellular growth, induced expression of differentiation-associated genes, and increased H3K4 methylation. It was also shown that LSD1 expression was significantly upregulated especially in bladder, lung, and colon cancer samples when compared to adjacent non-cancer tissue, and that knockdown of LSD1 suppressed proliferation of bladder and lung cancer cells [85]. These data suggest that LSD1 may be a candidate target for therapeutic intervention for various types of cancer.

Exploiting interactions between DNA methylation and histone modifications: Directions for future research

The identification of tumor-specific epigenetic pathways represents a critically important step toward the establishment of targeted epigenetic cancer therapies. One possibility is the targeting of defined DNMTs with specific oncogenic functions [31]. Another possibility is the discovery of tumor-specific functions for enzymes with specific histone modification activities (see above). A third option is the identification of tumor-specific interactions between epigenetic pathways. A classical example for this is provided by the interaction between DNMTs and HDACs through methyl-CpG binding proteins (Fig. 2A). It was shown that DNA methylation cooperates with histone deacetylation in the epigenetic silencing of cancer-related genes [86]. In agreement with this notion, co-treatment of cancer cells with DNMT inhibitors and HDAC

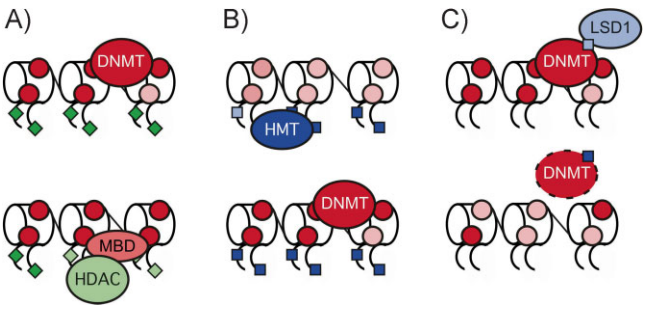

Figure 2. Known interactions between DNA methylation and histone modification systems. Histone octamers are shown with specific modifications and associated enzymes. **A**: DNA methylation (dark red circles) by DNMTs causes binding of methyl-CpG binding domain (MBD) proteins, which recruit HDACs to induce transcriptional repression by deacetylation (light green diamonds indicate deacetylated histone tails). **B**: Interaction between histone lysine methylation and DNA methylation. Genes marked by H3K27 methylation (dark blue squares, HMT: histone methyltransferase) become *de novo* DNA methylated. **C**: Interaction between histone lysine-specific demethylase 1 (LSD1) and DNMT1. LSD1 stabilizes DNMT1 by lysine demethylation (light blue square). Depletion of LSD1 causes loss of DNA methylation through decreased DNMT1 protein stability.

inhibitors showed synergistic effects in gene activation [87]. However, the combination of DNMT inhibitors and HDAC inhibitors has not yet been reported to provide a clear clinical benefit.

While the clinical application potential of the interaction between DNA methylation and histone hypoacetylation remains to be established, the results from preclinical experiments clearly suggest crosstalk between epigenetic silencing systems that warrants further investigation. A particular interesting finding in this context is the interaction between histone lysine methylation and DNA hypermethylation. Several independent studies have shown that genes that are marked by bivalent chromatin structures (*i.e.* the presence of both H3K4 and H3K27 methylation marks) in embryonic stem cells have a high probability of becoming *de novo* methylated in cancer [88–90]. The mechanistic details of these interactions are only beginning to be elucidated (Fig. 2B). The available data, however, raise the intriguing possibility that cancer-specific epigenetic mutations reflect the stem cell origin of tumors. As such, targeting of the interaction between bivalent chromatin structures and DNA hypermethylation might represent a highly specific approach toward erasing cancer-specific epigenetic mutations.

A third example for an interaction between a DNMT and a histone-modifying enzyme has been provided recently. It was shown that a targeted deletion of the LSD1 histone lysine demethylase causes progressive loss of DNA methylation [91]. Interestingly, this loss correlates with decrease in DNMT1 protein stability, which appears to be mediated by lysine hypermethylation of the DNMT1 protein in LSD1-deficient cells (Fig. 2C). These results uncover another, direct link between DNA methylation and histone modification systems that may be relevant for cancer epigenetics and needs to be explored in further studies.

Conclusion

While epigenetic drugs and biomarkers are becoming increasingly important for the clinical management of cancer, clear proof-of-concept data for the clinical efficacy of epigenetic approaches remains to be established. We have identified three major points that need to be considered in this context: (i) the low drug specificity of the established DNMT inhibitors azacytidine and decitabine, (ii) the lack of histone specificity for the HDAC family of proteins, and (iii) the current lack of specific drugs against targets with specific epigenetic activities in the clinical practice. As such, further work is required to establish unambiguous proof of concept for epigenetic cancer therapies. Because of their specific role in epigenetic gene regulation, the development of rationally developed DNMT inhibitors represents an important focus of future activities. Another area is the identification of histone modifying enzymes with specific epigenetic functions and the development of molecularly targeted drugs against these factors. It is hoped that these drugs, either as single agents or in combination will prove to be clinically effective against a multitude of cancers and other diseases influenced by epigenetic lesions.

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