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## Histone Lysine-Specific Methyltransferases and Demethylases in Carcinogenesis: New Targets for Cancer Therapy and Prevention

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### Abstract

Aberrant histone lysine methylation that is controlled by histone lysine methyltransferases (KMTs) and demethylases (KDMs) plays significant roles in carcinogenesis. Infections by tumor viruses or parasites and exposures to chemical carcinogens can modify the process of histone lysine methylation. Many KMTs and KDMs contribute to malignant transformation by regulating the expression of human *telomerase reverse transcriptase (hTERT)*, forming a fused gene, interacting with proto-oncogenes or being up-regulated in cancer cells. In addition, histone lysine methylation participates in tumor suppressor gene inactivation during the early stages of carcinogenesis by regulating DNA methylation and/or by other DNA methylation independent mechanisms. Furthermore, recent genetic discoveries of many mutations in KMTs and KDMs in various types of cancers highlight their numerous roles in carcinogenesis and provide rare opportunities for selective and tumor-specific targeting of these enzymes. The study on global histone lysine methylation levels may also offer specific biomarkers for cancer detection, diagnosis and prognosis, as well as for genotoxic and non-genotoxic carcinogenic exposures and risk assessment. This review summarizes the role of histone lysine methylation in the process of cellular transformation and carcinogenesis, genetic alterations of KMTs and KDMs in different cancers and recent progress in discovery of small molecule inhibitors of these enzymes.

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### CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

## Keywords

Histone lysine-specific methyltransferases; histone lysine-specific demethylases; cell transformation; carcinogenesis; gene mutations; inhibitors

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## 1. INTRODUCTION

Epigenetic alterations have been shown to play a fundamental role during the process of carcinogenesis and thus epigenetic regulatory enzymes are increasingly recognized to be important targets for cancer prevention and therapy. DNA methylation and histone modifications are the two major epigenetic modifications involved in transcriptional regulation. DNA methylation is mediated by a group of DNA methyltransferase (DNMTs), while covalent histone modifications are more complex and controlled by diversified groups of enzymes that regulate histone methylation, acetylation, ubiquitylation, phosphorylation and sumoylation [1, 2]. So far, four drugs, including DNMT inhibitors 5-azacytidine (Vidaza) and decitabine (20-deoxy-5-azacytidine, Dacogen) and the histone deacetylase (HDAC) inhibitors suberoylanilide hydroxamic acid (SAHA, Zolinza) and romidepsin (Istodax) have been approved by the FDA for treatment of cancer [4–6]. However, these DNMT inhibitors (e. g. 5-azacytidine and 20-deoxy-5-azacytidine) are nucleoside analogs and have low specificity to DNMTs and tumor cells, whereas histone deacetylases have broad substrate specificity including many non-histone proteins that are not involved in epigenetic regulation [7, 8]. As such, there are currently no established epigenetic biomarkers that can accurately predict patients' responses to DNMT and HDAC inhibitors in the clinic. Therefore, the “proof of principle” of “epigenetic therapies” using DNMT and HDAC inhibitors for treating cancer, as well as their tumor specificity, remains to be established. To support the potential effectiveness of epigenetic therapies, there is urgent need to identify novel and more specific targets of epigenetic regulation and their inhibitors.

In contrast to HDACs and DNMTs that globally regulate gene expression across different types of cells, recent studies have indicated that histone lysine methyltransferases (KMTs) and histone lysine demethylases (KDMs) may alter gene expression that was specific to particular normal and cancer cell types [9, 10]. In addition, genetic alterations in KMTs and KDMs such as chromosomal translocations, gene mutations, fusion proteins, and resultant aberrant expression, are frequently observed in cancer [11–16]. These genetic alterations in KMTs and KDMs have recently been linked to their oncogenic properties *via* and loss of tumor suppressing functions, as well as linked to the developmental plasticity of cancer cells [11–16]. Moreover, infections by tumor viruses or parasites and exposure to carcinogens affect the levels of histone lysine methylation and the associated patterns of gene expression, which leads to tumorigenic transformation [17, 18]. Taken together, these observations support that targeting aberrant KMTs and KDMs in cancer may achieve a higher degree of specificity in epigenetic therapy and accomplish prevention by blocking tumor specific epigenetic alterations or mutations. It is also conceivable that genetic alterations in KMTs and KDMs could serve as patient stratification biomarkers for future potential treatment with specific inhibitors of KMTs and KDMs. In this review, we summarize functional roles of KMTs and KDMs in cellular transformation and carcinogenesis and their genetic alterations in cancers, as well as the inhibitors of KMTs and KDMs. We will also discuss the challenges and opportunities for developing personalized medicine by targeting histone lysine methylation in appropriate patients.

## 2. HISTONE LYSINE METHYLTRANSFERASES AND DEMETHYLASES (KMTs AND KDMs)

So far, there are more than 50 human KMTs and 30 KDMs that have been identified [19, 20]. KMTs catalyze the transfer of one to three methyl groups from S-adenosylmethionine (SAM) to specific lysine residues on histones. H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20 are most commonly reported lysine residues which can become mono-, di-, or trimethylated. According to recent findings, H3K9, H3K27, and H4K20 methylation is associated mainly with repressed transcription, whereas methylation of H3K4 and H3K36 is associated with activated transcription [21]. The classification of KMTs and KDMs and mechanisms of histone lysine methylation are summarized as (Figs.1 and 2).

Based on the sequence and structure of their catalytic domain, KMTs can be classified into two families: DOT1 like (DOT1L) and SET-domain-containing lysine methyltransferases [22]. Here we discuss five groups of KMTs that target different histone lysine marks. They have been reported to be current or potential drug targets in cancers and include: **1.** Mixed-lineage leukemia gene 1 (MLL1)/KMT2A and SET and MYND domain containing protein 3 (SMYD3 /KMT3E) (H3K4 me); **2.** Variegation 3–9 homolog 1 and 2 (SUV39H1 and 2)/KMT1A/B and G9a/KMT1C (H3K9 me); **3.** EZH2/KMT6A (H3K27 me); **4.** Nuclear receptor-binding SET domain protein 2 (NSD2)/MMSET/WHSC1 and SMYD2 (H3K36 me); **5.** DOT1L/KMT4 (H3K79 me).

Lysine-specific demethylase 1 (LSD1) was the first discovered KDM which revealed that the process of histone lysine methylation is reversible [23]. Up to now, there are two major families of KDMs that have been identified [24]. LSD1 belongs to KDM1 family that includes two members so far: KDM1A/LSD1 and KDM1B/LSD2. The LSD1 demethylase family removes a single methyl group *via* an amine oxidation process in the presence of a FAD cofactor. Because the amine oxidation process requires a protonated nitrogen at the  $\epsilon$ -amino group of lysine, LSD1 cannot remove a trimethyl group from the methylated lysine. The second KDM family is Jumonji C (JmjC) domain containing protein family, which catalyzes the hydroxylation of a lysine methyl group *via* an  $\alpha$ -keto-glutarate and Fe(II)-ion dependent reaction. There are seven subgroups in JmjC family with a total of 14 KDMs (KDM2A/B, KDM3A/B, KDM4A–D, KDM5A–D and KDM6A/B). Aberrant regulation of KDMs is also involved in cancer progression, however they have been much less extensively studied than KMTs. KDMs and KMTs work coordinately to maintain normal global histone lysine methylation levels and then regulate gene expression patterns.

## 3. HISTONE LYSINE METHYLATION IN CELL IMMORTALIZATION AND TRANSFORMATION

The process of neoplasia begins with cell transformation. Virus infection (e.g. human papilloma virus, Epstein-Barr virus, etc.), transfection with oncogenes (e.g. mutant Ras), and exposure to ionizing radiation or chemical carcinogens (e.g. cigarette smoke, nickel, arsenic, and chromium) can initiate and/or promote the process of cell transformation, leading to uncontrolled cell growth. The predominant mechanism for carcinogen induced cell transformation is currently associated with genetic damage with DNA alterations leading to point mutations of genes, translocations of genetic material between chromosomes, and gene duplication with amplification. However, the transition from normal cells to transformed cells requires an extensive reconfiguration of the genome's expression program. The chain of events from carcinogen exposure to cell transformation is still largely unknown. Emerging evidence indicates that epigenetic mechanisms, including histone lysine

methylation, are also involved in the process of cellular transformation or oncogenic reprogramming.

### 3.1. Infection, Carcinogen Exposure and Histone Lysine Methylation

In addition, infection by oncogenic viruses or *Theileria* parasites, or exposure to carcinogens, such as 2-amino-1-methyl-6-phenylimidazo [4, 5  $\beta$ ] pyridine (PhIP), 4-aminobiphenyl (4-ABP), nickel, chromate, and arsenite, can alter the expression of histone lysine methyltransferases in normal cells, leading to cellular transformation [17, 25–33]. Anderton *et al* [26] showed that oncogenic Epstein-Barr virus infection induced the expression of the H3K27me3 demethylase, KDM6B, in primary B cells. Hyland *et al* [27] reported that there were elevated levels of EZH2/KMT6A and KDM6A in human papillomavirus type 16 E6/E7-transformed keratinocytes. SMYD3/KMT3E dimethylates and trimethylates H3K4. SMYD3 has been shown to be the only upregulated histone methyltransferase upon *Theileria* infection and is associated with trimethylation of H3K4 at the MMP-9 promoter [17].

Bradley *et al.* [29] showed that carcinogens PhIP and 4-ABP increased nuclear localization of LSD1 and reduced mono-methylation of H3K4 in human mammary epithelial cells (HMECs). Costa and his associates [30–33] published a series of papers, showing that nickel, chromate, and arsenite increased di- and tri-methylated H3K4 and di-methylated H3K9, while arsenite decreased mono-methyl H3K4. The increase of H3K9me2 by arsenite is associated with increased expression of G9a/ KMT1C protein and mRNA [32]. In addition, nickel has been shown to inhibit JMJD1A/KDM3A activity [33, 34]. These results argue that KMTs and KDMs may be potential targets for oncogenic infections- and chemical carcinogen- mediated transformation. Potential mechanisms of immortalization and transformation that are associated with altered expression of KMTs and KDMs are discussed below.

### 3.2. Cell Immortalization

Activation of telomerase is essential for cellular immortalization and malignant transformation. The lack of telomerase activity in most normal cells is mainly due to the stable repression of the telomerase catalytic subunit, encoded by the human *telomerase reverse transcriptase* (*hTERT*). Atkinson *et al.* [35] reported that highly trimethylated H3-K4 was associated with active transcription of the *hTERT* gene in telomerase-proficient tumor cells. The *hTERT* is a direct target gene of a H3K4 demethylase SMYD3 [36]. Overexpression of SMYD3 in non-transformed cells results in the induction of *hTERT* expression and the increased cell proliferation and colony formation, whereas down-regulation of SMYD3 expression by small interference RNA (siRNA) leads to growth inhibition or apoptosis of colorectal carcinoma and hepatocellular carcinoma cells [36, 37]. Inhibition of LSD1 by a pharmacological inhibitor or siRNA also induces the expression of *hTERT* in both normal human fibroblasts and cancer cells due to increased binding of LSD1 to *hTERT* proximal promoter and induction of dimethylated H3K4 [38]. Not dead yet (Ndy)-1/KDM2B and Ndy-2/KDM2A have Jumonji C-dependent histone H3K36 dimethyl demethylase or H3K4 trimethyl-demethylase activities. Pfau *et al.* [39] showed that overexpression of Ndy1 or 2 promoted the immortalization of mouse embryo fibroblasts (MEFs) in the absence of replicative senescence, and that knockdown of Ndy1 and expression of dominant-negative Ndy1 mutants induced senescence. The Ndy1 induced immortalization is dependent on JmjC domain and JmjC domain-mediated histone demethylation [40]. Taken together, these results highlight a potential mechanism of epigenetic control and modulation of the *hTERT* promoter *via* histone lysine methylation during the process of cellular immortalization.

### 3.3. Cellular Transformation

Histone methyltransferases can form a fusion partner gene or interact with proto-oncogenes or other methyltransferases to immortalize normal cells and transform non-malignant cells. The MLL1/KMT2A that encodes a H3K4 methyltransferase is frequently translocated in leukemia [41]. Due to chromosome translocation, the MLL1 N terminus can be 'fused' to the C terminus of over 50 different partners, leading to the loss of the H3K4 methyltransferase domain [41]. Many MLL1 fusion partners result in leukemic transformation by the involvement of transcriptional regulation through chromatin remodeling. The MLL1 fusion partners AF4, AF5, AF9, AF10 and ENL interact with the Dot1-like protein (DOT1L)/KMT4 that methylates H3K79 [41]. In addition, indirect interaction with DOT1L/KMT4 may be possible through common binding proteins, which has been documented between histone sites and many other MLL1 fusion partners including ABI1, EEN, EPS15 and ELL [41]. In the absence of DOT1L/KMT4, MLL1-AF10 has been shown to be unable to transform haematopoietic progenitors [42, 43]. Daigle *et al.* reported that EPZ004777, a potent and selective inhibitor of DOT1L/KMT4, selectively killed MLL1 rearranged MV4-11 and MOLM-13 cells by blocking cellular H3K79 methylation and inhibiting leukemogenic gene (e.g. Homeobox A9, HOX A9) expression [44]. These results suggest that DOT1L/KMT4 has emerged as an important mediator of MLL1-fusion-mediated leukemic transformation, and acts as a "driver" target for treatment of MLL1 rearranged leukemia. Although cancer cells carry multiple genetic and epigenetic abnormalities, they can be highly dependent on the activity of a single oncogene for continued cell proliferation and survival. This phenomenon is called "oncogene addiction" [45]. Therefore, identification of the state of oncogene addiction, i.e. the 'Achilles' heel,' due to genetic alterations of KMTs and KDMs would be important for development of effective targeted therapy in specific cancers.

Nuclear SET domain containing protein 2 (NSD2) alone has been shown to transform p19ARF<sup>-/-</sup> MEFs in an H3K36me<sub>2</sub>-dependent manner [46]. In addition, the expression of catalytically active NSD2 in t(4;14)-negative myeloma cells can form xenograft tumors in nude mice [46]. Mutant NSD1 with inactivation of H3K36 methyltransferase activity inhibits myeloid progenitor immortalization *via* HOX A gene activation [47]. Ecotropic viral integration site 1(EVI1) is an oncoprotein aberrantly expressed in acute myeloid leukemia and myelodysplastic syndrome cells [48]. SUV39H1/KMT1A or G9a/KMT1C can interact with EVI1 leading to the enhancement of EVI1 transcriptional repression [48]. Mulligan *et al.* reported that knockdown of G9a by siRNA induced transformation of HMECs expressing hTERT and SV40 Large T [49]. EZH2/KMT6A that catalyzes H3K27 methylation can transform the growth of a normal prostate epithelial cell line both *in vitro* and *in vivo*, and that siRNA knockdown of EZH2/KMT6A inhibits the proliferation of human papillomavirus positive cancer cells [50, 51]. These results suggest that EZH2/KMT6A also has oncogenic properties. A model that transformation is driven by increased H3K27 methylation as a consequence of EZH2/KMT6A overactivity has therefore been proposed recently [50, 51].

Jhdm1b/KDM2b encoding a Jumonji domain family H3K36me<sub>2</sub> and H3K4me<sub>3</sub> demethylase is first identified as a hotspot for proviral insertion in murine tumors generated by random mutagenesis of Moloney murine leukemia virus, and ectopic expression of Jhdm1b/KDM2b is sufficient to transform hematopoietic progenitors [52]. WHSC1L1/NSD3 has been found to be one of the most potently transforming oncogenes based on the number of altered phenotypes expressed by the cells. Conversely, knockdown of WHSC1L1/NSD3 in 8p11-12-amplified breast cancer cells results in cell growth inhibition [53].



This body of accumulated evidence shows that histone lysine methylation plays an essential role in the process of cellular transformation *via* oncogenic reprogramming. This observation suggests that genetic alterations of KMTs and of KDMs may drive the process of oncogenesis in a variety of circumstances and that the KMTs and KDMs are important targets for cancer prevention and treatment.

## 4. HISTONE LYSINE METHYLATION AND DNA METHYLATION IN CARCINOGENESIS

Cancer prevention and therapy can be greatly improved by detection at its early stages. Epigenetic alterations, including DNA and histone lysine methylation, are detectable in the broad regions of normal tissues during the early stages of carcinogenesis before the initiation of tumorigenesis.

### 4.1. Carcinogen Induced Histone Methylation in Experimental Animals

Carcinogens, like N-methyl-N-nitrosurea (MNU), have been shown to modify amino acids and lead to predominantly methylated lysine and arginine residues in H3 histone proteins [54, 55]. In a well-established 17 $\beta$ -estradiol-induced mammary carcinogenesis in female August Copenhagen Irish (ACI) rats, epigenetic changes, including an increase in H3K9 and H3K27 trimethylation and *de novo* CpG island methylation at the *Rassf1a* promoter, as well as loss of global DNA methylation and *LINE-1* hypomethylation and H4K20 trimethylation, have been found in the mammary gland tissue as early as 2 days after exposure to 17 $\beta$ -estradiol [56]. In tamoxifen-induced hepatocarcinogenesis, female Fisher 344 rats fed with tamoxifen for different numbers of weeks showed that global DNA hypomethylation is increased and *de novo* DNA methyltransferases DNMT3a and DNMT3b, as well as H4K20 trimethylation, are progressively decreased in liver at all-time points compared to non-target tissues (*i.e.* mammary gland, pancreas and spleen) [57, 58]. In 2-acetylaminofluorene (2-AAF)-induced preneoplastic livers of rats, increased H3K9 and H3K27 trimethylation in the promoter regions of *Rassf1a*, *p16(INK4a)*, *Socs1*, *Cdh1*, and *Cx26* tumor suppressor genes, and early *Rassf1a* and *p16(INK4a)* promoter CpG island hypermethylation, as well as a decrease of global and *LINE-1*-associated H4K20 trimethylation with time also are detected [59, 60]. In the methyl-deficient model of endogenous hepatocarcinogenesis in rats, the methyl-deficient diet results in a progressive loss of H4K20 and H3K9 trimethylation, which is accompanied by a decreased expression of Suv4-20h2/KMT5C and RIZ1/KMT8 and an increased expression of Suv39-h1/KMT1A/B in liver tumors [61]. These observations indicate that carcinogen-induced changes in histone methylation precede known promotion mechanisms and phenotypic alterations during the process of carcinogenesis.

### 4.2. Histone Lysine Methylation and Gene Silencing

DNA methylation is associated with histone modifications. The functional link between patterns of DNA methylation and histone methylation is first demonstrated by the observation that DNMT1 inhibitor 5-aza-2'-deoxycytidine (5-Aza-CdR, decitabine) treatment of cancer cells leads to depletion of DNA methylation, a loss of H3K9 methylation, and a corresponding increase in H3K4 methylation [62, 63]. DNA methylation patterns are established and maintained by three DNA methyltransferases: DNMT3a, DNMT3b, and DNMT1. It has been shown that DNMT3a and its accessory protein DNMT3L contain an H3K4me0-interacting ATRX-Dnmt3-Dnmt3L (ADD) domain and H3K4 methyltransferase MLL1 contains a CpG-interacting Cys-X-X-Cys (CXXC) domain [64]. The interactions through these protein domains may couple H3K4 methylation reaction to unmethylated DNA [64]. In addition, H3K9 methyltransferase SETDB1 contains a putative methyl-CpG-binding domain (MBD) that also potentially links H3K4 methylation reaction to unmethylated DNA [64]. Ubiquitin-like with PHD and RING finger domains 1

(UHRF1) plays a role in maintaining DNA methylation in mammalian cells by targeting DNMT1 to DNA replication foci [65]. UHRF1 has been found to bind hemimethylated DNA, H3K9me3, and G9a/KMT1C [65]. In addition, persistent transcriptional repressive histone modifications, such as H3K9me3 and H3K27me3, are associated with DNMT1-mediated DNA methylation recovery after DNMT1 inhibitor treatment [66]. Furthermore, targeted deletion of LSD1 in embryonic stem cells results in a progressive loss of DNA methylation *via* degradation of DNMT1 protein [67].

Bivalent chromatin structures that are present with both activating H3K4 trimethylation and repressive H3K27 trimethylation [68] have also been identified in human tumors. Bivalent chromatin structures signify an important molecular characteristic of embryonic stem cells. In addition, genes affected by *de novo* DNA methylation during tumorigenesis are pre-marked by H3K27 trimethylation, suggesting an instructive role of H3K27 trimethylation in the establishment of cancer specific DNA methylation patterns [69–71]. The relationship between H3K27 trimethylation and *de novo* DNA methylation therefore may reflect the presence of a stem cell-like epigenetic program in cancer cells [72].

However, enriched H3K27 trimethylation at promoter regions can also lead to a tumor suppressor silencing mechanism that is independent of DNA methylation. Vallot *et al.* [73] have recently shown that multiple regional epigenetic silencing (MRES) is associated with progression of carcinoma *in situ* in bladder. MRES occurs not through DNA methylation, but *via* histone H3K9 and H3K27 methylation and histone H3K9 hypoacetylation. Taken together, these results indicate that histone lysine methylation may play a crucial role in tumor suppressor gene inactivation during the early stages of carcinogenesis *via* DNA methylation-dependent and /or -independent mechanisms.

## 5. ALTERNATIONS IN CANCER

Deregulation of KMTs and KDMs and their signaling networks in cancer can occur through several mechanisms that include gene mutations, amplification, translocations and aberrant expression. Understanding of these mechanisms will be helpful in identifying cancer specific targets and developing more effective epigenetic therapies. In this regard, we will summarize alterations of KMTs and KDMs in cancer classified by underlying mechanisms as Table 1.

### 5.1. Mutations

Recent next-generation sequencing studies have greatly facilitated discovery of mutations of KMTs and KDMs in cancers and precancerous conditions. Acquired EZH2/ KMT6A mutations have been identified in a variety of cancers [74–78]. Heterozygous mutations at the tyrosine 641 (Y641), which is predicted to be replaced with a histidine, in the SET domain of the EZH2/KMT6A demonstrate enhanced catalytic activity for mono- to di- and di- to trimethylation, whereas wild-type EZH2/KMT6A shows greatest catalytic activity for monomethylation of H3K27 and relatively weak efficiency for the subsequent reactions [79–81]. Therefore, heterozygous Y641 mutants, when co-expressing with wild-type EZH2/ KMT6A, increase levels of H3K27me3 and may be functionally equivalent to EZH2/ KMT6A overexpression [79–81]. Distinct from those of both wide-type EZH2/KMT6A and Y641 mutants, another EZH2 A677G mutant that has been identified in lymphoma cell lines and primary human B-cell lymphoma tumor specimens is equally efficient for methylating H3K27me0, H3K27me1, and H3K27me2 [82–85]. In contrast to description above, EZH2 mutations in myelodysplasia-myeloproliferative neoplasms – precancerous conditions - (10 to 13%), myelofibrosis (13%), and various subtypes of myelodysplastic syndromes (6%) are inactive and spread throughout the gene, which comprise missense, nonsense, and premature stop mutations [86–88]. All nonsense and stop codon mutations are predicted to result in a



loss of EZH2/KMT6A methyltransferase function as the catalytic SET domain lies at the far C terminus [89]. Patients who are diagnosed with myeloid neoplasms and harbor EZH2 inactive mutations exhibit a poor survival as compared to those without mutations [90]. Taken together, these results suggest that either loss or gain of EZH/KMT6A function are associated with tumorigenesis.

*UTX/KDM6A* encoding an H3K27 demethylase was the first identified mutated histone demethylase gene in human cancer [91]. Mutations of *UTX/KDM6A* commonly occur in a wide variety of cancers, including leukemia, renal cell carcinoma (RCC), breast adenocarcinoma, lung cancer, pancreatic adenocarcinoma, bladder cancer, prostate cancer, and others [78, 91–95]. Point mutations affecting the functional jumonji C (JmjC) domain of UTX inactivate its H3K27 demethylase activity [92]. In addition, *UTX/KDM6A* has been reported as the most frequently mutated gene in transitional cell carcinoma (TCC) of the bladder (mutated in 21% of TCCs) and the second most frequently mutated gene (next to TP53) in lung cancer [93]. *UTX/KDM6A* mutations are present at the C-terminus and the N-terminus of the UTX protein but mostly in the region adjacent to the JmjC domain required for UTX activity [78]. Therefore, most mutations in *UTX/KDM6A* are thought to cause loss of function

MLL2/ KMT2D and MLL3/KMT2C are complexed with UTX, which function in a concerted transcriptional regulatory mechanism for many developmental genes, including the HOX gene family. This occurs by involvement of H3K27 demethylation and H3K4 methylation [96]. The HOX gene family collectively controls segment specificity and cell fate in the developing embryo. Each MLL family member is thought to target different subsets of HOX genes. MLL2 also is known to regulate the transcription of a diverse set of genes [97]. Somatic inactivating mutations in both MLL2 and MLL3 are found in various cancers, including lung cancer, RCC, medulloblastoma, glioblastoma, head and neck squamous cell cancers, pancreatic ductal adenocarcinoma, melanoma, leukemias, bladder cancer, Opisthorchis viverrini-related cholangiocarcinoma (CCA), and colorectal cancer [94, 98–100]. The observed pattern of monoallelic somatic inactivation of MLL2 in these cancers suggests a role for MLL2 as a haploinsufficient tumor suppressor. Frameshift mutations and deletion of MLL3 are found in more aggressive cancers [101]. In addition, targeted inactivation of MLL3 in mice results in ureteral epithelial tumors, and spontaneous tumorigenesis was exacerbated in p53 (+/–) mice [102]. These results suggest that MLL3 also functions as a tumor-suppressor gene.

H3K4 demethylase, JARID1C/KDM5C, has also been found to be mutated in human pancreatic cancers and RCC [94], supporting the importance of H3K4 methylation status in cancer. Additionally, mutations affecting a H3K36 methyltransferase *SETD2/KMT3A*, a H3K9 demethylase *KDM3B*, and a H3K4 demethylase *JARID1C/KDM5C*, in cancers have been reported and are associated with distinct gene expression patterns [94, 103–106]. NSD1 is mutated in several cancers, including carcinoma of the upper aerodigestive tract [107]. Multiple distinct and spatially separated inactivating mutations in *SETD2/KMT3A*, *PTEN*, and *JARID1C/KDM5C* are identified within a single tumor, suggesting potential convergent phenotypic evolution [104].

## 5.2. Chromosome Translocations

Besides gene mutations, other abnormalities implicate histone-modifying enzymes. NSD2/MMSET/WHSC1 is frequently overexpressed in multiple myelomas due to a recurrent t(4;14)(p16;q32) chromosomal translocation [108]. *NSD1/KMT3B* and *NSD3* are fused with nucleoporin-98 (NUP98) due to the recurring t(5;11)(q35;p15.5) translocation in AML and in therapy and radiation-associated myelodysplastic syndrome with t(8;11)(p11;p15), respectively [109].

The *MLL1/KMT2A* gene may be with one of over 60 distinct partner genes through chromosomal translocations in various human acute leukemias, resulting in the formation of multiple MLL1 fusion protein with transforming activities [110]. The most frequent translocations are the t(4;11) and t(11;19) translocations, which are associated with the expression of MLL1–AF4 and MLL1–ENL, respectively in ALL [110]. Other translocations, such as the t(9;11), t(6;11), t(x;11) and t(11;19), result in expression of MLL1–AF9, MLL1–AF6, MLL1–FKHR-L1, and MLL1–ELL fusion proteins in AML, myelodysplasia or etoposide-therapy-related acute leukemia, respectively [110]. Different fusions can lead to different histone lysine methylation pathways during MLL1-induced leukemic transformation, which adds complexity to drug development efforts for targeting MLL1-mediated methylation in leukemia [110]. *Mll* knockout mice studies have significantly improved the understanding of the molecular consequences of 11q23 translocations [111]. Loss of *MLL1* results in embryonic lethality and defects of axial skeleton and hematopoietic system, as well as abnormal HOX gene expression, which is known to play roles in oncogenesis and attenuated H3K4 methylation [111]. The efficacy of conventional chemotherapies for leukemia may be explained by translocations in the *MLL* locus at 11q2. Patients with certain cytogenetic abnormalities do not effectively respond these chemotherapies [112]. MLL1 has also been shown to be required for *multidrug resistance 1* (MDR1) gene promoter methylation and the activation of MDR1 is accompanied by increased H3K4 methylation. This result suggests that MLL1 be served as a new target for circumvention of tumor multidrug resistance [113].

### 5.3. KMT and KDM Gene Amplification and Over-expression

*NSD3* that maps to chromosome band 8p12 is amplified in several tumor cell lines and primary breast carcinomas, which is associated with poor prognosis [114]. NSD2/ MMSET overexpression has been reported in more than 15 different cancers compared to their respective normal tissues. In most cancer types, the expression levels of NSD2 positively correlate with tumor aggressiveness and prognosis [115]. In neuroblastoma, chemotherapy and retinoic acid can decrease the expression levels of NSD2, and NSD2 levels are associated with drug responses, suggesting the potential of NSD2 as a therapeutic target for a subset of neuroblastomas with unfavorable prognosis [116]. For some cases of myelodysplastic syndrome and AML, *MLL1/KMT2A* is present in increased copy number, which occurs as the result of additional copies of chromosome 11 or through *MLL1* amplification [117]. The amplification of *MLL1* is associated with the upregulation of at least some of the genes that are consistently expressed in leukemias with MLL1 rearrangements [117].

*SMYD3/KMT3E* that is responsible for H3K4 di- and trimethylation is frequently up-regulated in colorectal carcinoma (CRC), hepatocellular carcinoma (HCC), liver and breast cancers [118, 119]. The SMYD3-gene-transfected cells have showed increased proliferation rate and become more resistant to cell death, *via* transcription of several oncogenes (e.g. *Wnt10b*) and genes associated with cell adhesion (i.e. *N-Myc*, *CrkL*, *L-selectin*, *CD31* and *galectin-4*) [118, 119]. Human cancer cells expressing both the full-length and an N-terminal cleaved form of SMYD3 exhibit higher methyltransferase activity than that of the full-length protein [120]. This result indicates that the N-terminus may play an important role in regulating enzymatic activity. SMYD2/KMT3C can methylate both histone (H2B, H3, and H4) and nonhistone proteins p53 and Rb [121, 122]. Overexpression of SMYD2 has been reported in the esophageal cell line KYSE150 and esophageal squamous cell carcinoma (ESCC) primary tumor samples. Genetic knockdown of SMYD2 leads to decreased ESCC cell proliferation *via* apoptosis and cell cycle regulation [123]. Taken together, these results make SMYD2 and 3 attractive targets for the development of novel anticancer drugs.

Several H3K9 methyltransferases, such as Suv39h1/ KMT1A, Suv39h2/KMT1B, G9a/ KMT1C, and SETDB1/ KMT1E, are over-expressed in tumor versus normal tissues [124–126]. SETDB1/KMT1E has been found frequently amplified in malignant melanoma, and its expression accelerates melanoma formation in zebrafish [127]. Levels of G9a are significantly elevated in HCC compared to non-malignant liver tissues [126].

EZH2 overexpression has also been reported in various cancer types, such as prostate cancer, breast carcinoma, bladder carcinoma, lung cancer, pancreatic cancer, colon cancer, and others, whereas EZH2 expression is rarely detectable in all tested normal tissues [128]. The function of EZH2 in carcinogenesis is commonly associated with increased cell proliferation. In addition, EZH2 expression increases the invasiveness and metastatic potential of cancer cells and is associated with aggressive tumor subgroups [129, 130].

Expression levels of LSD1 are significantly elevated in human bladder cancer tissues compared to nonneoplastic bladder tissues [131, 132]. Tumor tissues with early stage bladder cancer exhibit particularly high LSD1 expression [131]. This result suggests that LSD1 may be one of the factors involved in the initiation process of carcinogenesis. LSD1 overexpression in prostate cancer, lung cancer and colorectal cancer cells, as well as estrogen receptor (ER)-negative tumors, has also been observed [132, 133]. In prostate cancer, the overexpression of LSD1 is associated with disease recurrence during therapy [133, 134].

The other family members of KDMs, such as JARID1A/KDM5A, JARID1B/KDM5B, JARID1C/KDM5C, JMJD2B/KDM4B, JMJD2C/KDM4C and JMJD3/KDM6B, are also up-regulated in various types of cancers [135–147]. Enhanced JARID1A expression has been shown to contribute to drug resistance in NSCLC cell lines, while JARID1B is thought to be required for melanoma maintenance [144]. JARID1B has also been reported to be highly expressed in 90% of ductal breast carcinomas and associated with aggressive phenotype of breast and prostate cancer [145–146]. JARID1C plays a role in the pathogenesis of human papillomavirus (HPV)-associated cancers as JARID1C has been identified as one of the papillomavirus E2 protein-dependent regulators for HPV oncogene expression [147]. The overexpression of JMJD2C increases the expression of Mdm2 oncogene but decreases the expression of p53, which is dependent on its demethylase activity [140]. The colocalization of JMJD2C with androgen receptor and LSD1 in both normal prostate and prostate carcinomas suggests that these two demethylases may cooperatively regulate androgen receptor-dependent gene transcription [141]. Few studies have shown the relation of JMJD3 with cancer progression, but in one report, JMJD3 has been shown to be upregulated in prostate cancer with higher expression in metastatic prostate cancer [148]. Preclinical studies have shown that inhibition of some of these enzymes can suppress tumorigenesis [143]. Therefore, a rationale for developing specific inhibitors for demethylases for targeted cancer therapy has been established [143].

In contrast to the above described KDMs that normally function as oncogenes, JHDM1B/ KDM2B and UTX/KDM6A are more likely to be tumor suppressors. JHDM1B is responsible for removal of methyl groups from H3K36 me1/2, but not H3K36 me3. The expression of JHDM1B is significantly down-regulated in the most aggressive type of the primary brain tumors, glioblastoma multiforme, compared to normal brain tissue [149]. UTX can specifically remove methyl groups from H3K27 me2/3 and, thereby counteract PcG-mediated histone modification by EZH2 [150, 151]. Altered expression of UTX and UTX-bound target genes are commonly seen in human cancers. One study has linked the inactivation of UTX to transcriptional silencing of the RB pathway [152]. However, the detailed mechanism of UTX-mediated tumor suppression remains unclear.

Alterations of KMT or KDM activities lead to changes in global histone methylation levels and dysregulation of specific gene expressions. Global histone methylation levels have been analyzed between normal and cancer cells in the cultured cells and clinical specimens. For example, global loss of H4K20 me3 has been shown to be one of the hallmarks of human cancer [153]. Immunohistochemical studies of a well-characterized series of human breast carcinomas have demonstrated that H3K4 me2 and H4K20 me3 levels are significantly lower in tumor tissues than those in normal tissues, and correlate with clinicopathologic factors [154]. In prostate cancer, global levels of H3K4 me1 and H3K9 me2 and me3 are significantly reduced in cancer tissues compared to normal tissues. High levels of H3K4 me3, H3K9 me2 and me3, and H3K27 me1, me2 and me3 are associated with increased clinical-pathological parameters such as serum PSA, capsular invasion, seminal vesicle infiltration, lymph node involvement, and Gleason score [155, 156]. Global histone lysine methylation levels, including H3K4me1, H3K4me3, H4K20me1, H4K20me2, and H4K20me3, are lower in bladder cancer than in normal urothelial tissues and inversely correlated with pathological stages of human urinary bladder cancer [157]. In lung cancer, lower levels of H3K4 me2 appear to predict significantly poorer survival probabilities of patients [158, 159]. These observations suggest that specific and novel multiple histone methylation patterns are associated with cancer and may have important biological significance.

## 6. INHIBITORS OF KMTs AND KDMs

The emerging fundamental roles of altered KMTs and KDMs in cell transformation, carcinogenesis and tumorigenesis have implicated that development of inhibitors for these enzymes is a new frontier for drug discovery. However, so far, only a few compounds targeting KMTs and KDMs are available for preclinical and clinical development due to their toxicity. Most small molecule inhibitors for KMTs and KDMs are mainly used in basic research. Some of the first-generation inhibitors for KMTs and KDMs are derived from natural products. Small molecule inhibitors of KMTs and KDMs are summarized in Table 2.

### 6. 1. KMT Inhibitors

KMT enzymes catalyze the transfer of one to three methyl groups from S-adenosylmethionine (SAM) to specific lysine residues on histones. Targeting the cofactor (SAM) binding site of protein methyltransferases appears to be the first approach for KMT inhibition. Sinefungin A, natural product isolated from *Streptomyces spp.*, is the first SAM-competitive and nonselective inhibitor of KMTs identified [160]. Another natural KMT inhibitor, Chaetocin, was discovered by random screening of compound libraries. Chaetocin is a fungal metabolite and has been found to be an inhibitor of the *Drosophila melanogaster* Suv39 family including Suv39h1 with an IC<sub>50</sub> of 0.8 μM [161]. Chaetocin does not inhibit E(Z) or SET7/9 at concentrations below 90 μM, suggesting its potential selectivity against certain KMTs. A recent attempt at total synthesis of (+)-chaetocin enantiomers has showed that they also have inhibitory activity towards G9a with IC<sub>50</sub>s of 2.4 μM and 1.7 μM, respectively [162]. Chaetocin has also been reported to exhibit anti-myeloma activity, and can inhibit Suv39h1 in acute myeloid leukemia cells with hypermethylated tumor suppressor genes [163]. These results support the potential of developing chaetocin H3K9 methyltransferase inhibitors as therapeutics to target reactivation of silenced genes. Gliotoxin analogs with a disulfide bond, which are isolated from the fungus *Penicillium sp. strain JMF034*, show potent inhibitory activity of G9a and Suv39h1 without affecting SET7/9 [164].

In another high throughput screen against a preselected chemical library, a highly selective small inhibitor of G9a, BIX-01294 (diazepinquinazolin-amine derivative) has been identified to lower bulk H3K9 me2 levels in mouse ES cells and fibroblasts, with levels

restored upon removal of the inhibitor [165]. BIX-01294 binds at the protein substrate channel of G9A and GLP1. However, BIX-01294 is unable to induce significant changes in cellular morphology and its potency in affecting global histone methylation appears to be very limited. Recently, second-generation inhibitors, such as E72, UNC321, UNC0638, and UNC0646 that are based on a 7-alkoxyamine tethered to the quinazoline core, have been developed with a marked improvement of potency and specificity against G9a/GLP. Among them, UNC0646 has also demonstrated improved potency of this quinazoline series in cell-based assays [166]. In addition, Yuan *et al* [167] reported that BRD4770, a compound from a focused library of 2-substituted benzimidazoles as a potential SAM mimetic, reduced cellular levels of di- and trimethylated H3K9, induced senescence and inhibition of cell growth in the pancreatic cancer cell line PANC-1.

The DOT1L inhibitor EPZ004777 is also a SAM analogue and binds to the SAM binding site. However, EPZ004777 shows more than 1,000 fold greater selectivity over other histone methyltransferases, including EZH2, SETD7, and WHSC1. EPZ004777 has been shown to kill mixed lineage leukemia cells with little effect on non-MLL-translocated cells [44]. In addition, EPZ004777 increases the survival of mice bearing tumors with MLL translocation [168]. By using structure- and mechanism-based design, Yao *et al* [169] identified several potent and specific DOT1L inhibitors with IC<sub>50</sub> values as low as 38 nM.

EZH2 is essential for cancer stem cell self-renewal. A potent SAM hydrolase inhibitor, 3-Deazaneplanocin A (DZNep), has been shown to selectively inhibit EZH2, leading to H3K27 demethylation and induction of apoptosis in breast cancer cells but not in normal breast epithelial cells [170]. In addition, DZNep demonstrates promising anti-tumor activity *in vivo*, and inhibits cancer cell invasion and tumor angiogenesis in prostate and brain cancers, respectively. Unlike DZNep that inhibits EZH2 *via* degradation of Polycomb Repressive Complex 2 (PRC2), GSK-343 and GSK-126 directly inhibit EZH2 methyltransferase activity by competing with the co-factor SAM with potencies of Ki<sup>app</sup> = 0.5–4 nM [171, 172]. Both GSK343 and GSK126 are cell-active and over 1000-fold selective for EZH2 versus 20 other KMTs [171, 172].

SMYD2 exhibits oncogenic properties by repressing the functional activities of p53 and retinoblastoma protein. Therefore, SMYD2 is an attractive drug target for the development of small-molecule inhibitors. In a high-throughput screening assay, a potent and selective SMYD2 inhibitor, AZ505, has been identified and structural analysis shows that AZ505 is bound in the peptide binding groove of SMYD2 [173, 174].

## 6. 2. KDM1A/AOF2/LSD1 Inhibitors

LSD1 utilizes flavin adenosine dinucleotide (FAD) as a cofactor to demethylate mono- and dimethylated H3K4 [175]. LSD1 is also involved in the demethylation of H3K9 when associated with nuclear transcriptional factors (e.g. estrogen and androgen receptors), as well as in the demethylation of non-histone proteins such as p53, Stat3 and DNMT1 [175]. Monoamine oxidase (MAO) A and B and LSD1 show homology in their catalytic site in a flavin-dependent manner. MAO A and B are established targets for neurological disorders [176]. A large number of inhibitors have been synthesized and tested for their specificity toward MAO A or B [176, 177]. Recently a number of MAO inhibitors, such as clorgyline, pargyline, tranlycypromine and phenelzine have also been tested for LSD1 inhibitory activities [178]. Tranlycypromine demonstrates the highest potency with IC<sub>50</sub> of 21 μM [179]. Further study shows that tranlycypromine treatment reduces neuroblastoma xenograft growth, but causes substantial toxicity *in vivo* [20]. By analysis of structure-activity relationships and subsequent extension of the chemical structure further into the lysine substrate pocket, a more potent and selective tranlycypromine analogue against LSD1 (S2101) has been designed and synthesized [180]. Pargyline inhibits LSD1 activity when it



is associated with the androgen receptor complex, suggesting that the inhibitory activity of pargyline may be due to the conformational change of LSD1 [181]. Willmann *et al* [182] recently reported that Namoline, a  $\gamma$ -pyrone from a focused, natural product inspired library, inhibited LSD1 with an  $IC_{50}$  of about 50  $\mu$ M, and resulted in silencing of AR-regulated gene expression and a growth inhibitory effect on prostate cancer cells. Additionally, polyamine analogues (bisguanidine and biguanidine types) have been characterized as potent non-competitive inhibitors of LSD1 and shown to reactivate aberrantly silenced genes to suppress colon cancer development [183–185]. Based on molecular modeling, Wang *et al* [186] developed a novel small molecule LSD1 inhibitor (i.e. CBB1007) that inhibited the proliferation of pluripotent cancer cells including teratocarcinoma, embryonic carcinoma, and seminoma, or embryonic stem cells with minimum growth-inhibitory effects on non-pluripotent cancer or normal somatic cells. These results indicate that LSD1 inhibitors may be a new class of epigenetic drugs for cancer prevention and treatment.

### 6. 3. JmjC-Domain Demethylase Inhibitors

Jumonji domain-containing lysine demethylases use  $Fe^{2+}$  and 2-oxoglutarate (2-OG) as co-substrates/co-factors. Specific inhibitors against JmjC-domain demethylases are designed to compete with 2-oxoglutarate and bind to the catalytic iron in the active site with selectivity over other human 2-OG oxygenases. N-oxalylglycine (NOG) as a 2-oxoglutarate analogue is a weaker inhibitor of JMJD2C and the catalytic core of JMJD2A [187]. Another 2-OG analog, 2, 4-pyridindicarboxylic acid (PDCA), inhibits the Jumonji domain-containing demethylases and other 2-OG-dependent oxygenases such as HIF prolyl hydroxylase 1 (HPH1; also known as EGLN2) and HPH2 (also known as EGLN1) [188]. More selective compounds have also been developed by extending the chemical structure of the Jumonji domain-containing demethylase inhibitor template to the iron in the substrate binding pocket [187]. These compounds, such as metal-chelating hydroxamic acids, have increased potency [187]. However, the molecular and physicochemical properties of the compound may limit its bioavailability [187]. Recently, a new generation of cell permeable Jumonji domain-containing demethylase inhibitors with subtype selectivity and more drug-like properties have been identified through high-throughput screening, which include 8-hydroxyquinolines and 2,2'-bipyridines [189]. In addition, some flavonoid- and catechol-type molecules, as well as HDAC inhibitor SAHA, have also been found to be JmjC KDM inhibitors [190].

Based on the crystal structures and the enzymatic mechanism of Jumonji C domain-containing histone demethylases (JHDMs), Wang *et al*. [191] designed and synthesized a small molecule inhibitor of JHDM, called methylstat, which contains a methyllysine mimic (substrate mimic), an  $\alpha$ -ketoglutarate mimic (co-factor mimic) and a linker. Methylstat exhibits selective inhibitory activity against trimethyl-specific JHDMs *in vitro* and prevents myogenesis through inhibition of H3K27me3-demethylase UTX [191]. By studying high-resolution crystal structures of histone peptides in complex with JMJD3 in the presence of cofactor analogues and by determining the required JMJD3 residues for substrate recognition, Kruidenier *et al*. [192] have further identified a more potent and selective inhibitor (GSK-J1) of the H3K27 histone demethylases JMJD3 and UTX ( $IC_{50}$  = 60 nM for human JMJD3). GSK-J1 does not inhibit other chromatin-modifying enzymes or protein kinases. These JHDM inhibitors would be very useful tools for understanding roles of JMJD3 and UTX in gene transcription and epigenetic inheritance.

## 7. PERSPECTIVE AND CONCLUSION

An enormous amount of evidence recently has been emerging that histone lysine methylation plays a crucial role in cancer initiation and development. Infection by oncogenic viruses or parasites, or exposure to chemical carcinogens, can directly affect lysine histone methylation leading to cellular transformation, suggesting that certain KMTs and KDMs



may be direct targets of oncogenic viruses and chemical carcinogens. However, the epigenetic mechanisms for the involvement of histone lysine methylation in cellular transformation and early carcinogenesis remain largely unexplored. Understanding the epigenetic mechanisms that lie behind the window of operational reversibility during early carcinogenesis, may allow chemopreventive strategies to be implemented to revert or halt these early carcinogen or infectious agent-induced changes prior to the development of cancer.

Recently, with second-generation sequencing, profound genetic alterations in KMTs and KDMs, including gene mutation, translocation and amplification, have been observed in a wide variety of cancers. As mutations or other genetic alterations (i.e. gene translocation and amplification) in KMTs and KDMs have the potential to reprogram the whole genome by altering the expression of hundreds of genes all at once, these diverse mutations or other genetic alterations can be associated with a uniform phenotype that may not be distinguishable on clinical grounds. Therefore, development of molecular diagnostics from genetic alterations of KMTs and KDMs could be useful for further stratifying cancer patients into subgroups for future selection of targeted therapies. In addition, cancer-specific epigenetic drug targets may be identified or validated through ongoing functional studies that investigate the mechanisms underlying the phenotypic plasticity of cancer cells.

However, function roles of KMTs and KDMs in cancer appear to be complex and there exists a cross-talk among them. Currently there is no single unique methylation mark that can predict a specific type of cancer. Regulation of gene activation/repression not only depends on the position of lysine methylation, but also the number of methylation residues. In addition, due to intratumor heterogeneity, multiple mutations or genetic alterations may co-exist and single tumor biopsy sampling may not be sufficient to portray tumor mutational landscapes. Such biological complexity points to challenges in developing targeted epigenetic therapies in the future. If multiple epigenetic targets must be inhibited to effectively prevent or treat cancer, then single-target screening strategies may ultimately fail, and screening a natural compound with the potential to affect many signaling pathways at once may be more productive.

Due to the wide diversity of chemical structures in natural products and the interaction between natural products and cellular targets during long-term process of natural evolution, natural products often provide a higher “hit rate” to targets than a random approach. Natural products therefore have served as a stepping stone for developing more specific KMT and KDM inhibitors. However, natural products have the limitation of being promiscuous inhibitors affecting a wide range of enzymatic targets. Further chemical modification of natural products for developing more selective inhibitors is often required. Targeted approaches have advantages of known mechanisms of action and selectivity for specific patient populations.

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## Glossary

<b>2-AAF</b>	2-acetylaminofluorene
<b>4-ABP</b>	4-aminobiphenyl
<b>ACI</b>	August Copenhagen Irish

<b>ADD</b>	ATRX-Dnmt3-Dnmt3L
<b>AR</b>	androgen receptor
<b>AML</b>	acute myelogenous leukemia
<b>CCA</b>	cholangiocarcinoma
<b>CRC</b>	colorectal carcinoma
<b>CXXC</b>	Cys-X-X-Cys
<b>DNMT</b>	DNA methyltransferase
<b>DZNep</b>	3-Deazaneplanocin A
<b>DOT1L</b>	DOT1 like
<b>ESCC</b>	esophageal squamous cell carcinoma
<b>EVI1</b>	Ecotropic viral integration site 1
<b>EZH2</b>	enhancer of zeste homolog 2 ( <i>Drosophila</i> )
<b>FAD</b>	flavin adenosine dinucleotide
<b>H3K4 me</b>	histone H3 lysine 4 mono-methylation
<b>H3K4me2</b>	histone H3 lysine 4 di-methylation
<b>H3K4me3</b>	histone H3 lysine 4 tri-methylation
<b>H3K9 me</b>	histone H3 lysine 9 mono-methylation
<b>H3K9me2</b>	histone H3 lysine 9 di-methylation
<b>H3K9me3</b>	histone H3 lysine 9 tri-methylation
<b>H3K27 me</b>	histone H3 lysine 27 mono-methylation
<b>H3K27me2</b>	histone H3 lysine 27 di-methylation
<b>H3K27me3</b>	histone H3 lysine 27 tri-methylation
<b>H3K36 me</b>	histone H3 lysine 36 mono-methylation
<b>H3K36me2</b>	histone H3 lysine 36 di-methylation
<b>H3K36me3</b>	histone H3 lysine 36 tri-methylation
<b>H3K79 me</b>	histone H3 lysine 79 mono-methylation
<b>H4K20me1</b>	histone H4 lysine 20 mono-methylation
<b>H4K20me2</b>	histone H4 lysine 20 di-methylation
<b>H4K20me3</b>	histone H4 lysine 20 tri-methylation
<b>HCC</b>	hepatocellular carcinoma
<b>HDAC</b>	histone deacetylase
<b>HPH1</b>	Hypoxia inducible factor prolyl hydroxylase 1
<b>HMECs</b>	human mammary epithelial cells
<b>HPV</b>	human papillomavirus
<b>HOX A9</b>	Homeobox A9
<b>hTERT</b>	human telomerase reverse transcriptase

<b>JHDMs</b>	Jumonji C domain-containing histone demethylase
<b>JmjC</b>	Jumonji C
<b>KDM</b>	demethylases
<b>KMT</b>	histone lysine methyltransferases
<b>LSD1</b>	Lysine-specific demethylase 1
<b>MBD</b>	methyl-CpG-binding domain
<b>Ndy</b>	Not dead yet
<b>MAO</b>	monoamine oxidase
<b>MEFs</b>	mouse embryo fibroblasts
<b>MLL</b>	Mixed-lineage leukemia gene
<b>MNU</b>	N-methyl-N-nitrosurea
<b>MRES</b>	multiple regional epigenetic silencing
<b>NOG</b>	N-oxalylglycine
<b>NSD2</b>	Nuclear receptor SET domain containing protein 2
<b>NUP98</b>	nucleoporin-98
<b>2-OG</b>	2-oxoglutarate
<b>PDCA</b>	2, 4-pyridindicarboxylic acid
<b>PhIP</b>	2-amino-1-methyl-6-phenylimidazo [4, 5 $\beta$ ] pyridine
<b>RCC</b>	renal cell carcinoma
<b>PRC2</b>	Polycomb Repressive Complex 2
<b>SAHA</b>	suberoylanilide hydroxamic acid
<b>SAM</b>	S-adenosylmethionine
<b>SMYD3</b>	SET and MYND domain containing protein 3
<b>SUV39H</b>	Variegation 3–9 homolog
<b>siRNA</b>	small interference RNA
<b>TCC</b>	transitional cell carcinoma
<b>UTX</b>	Ubiquitously transcribed tetratricopeptide repeat, X chromosome

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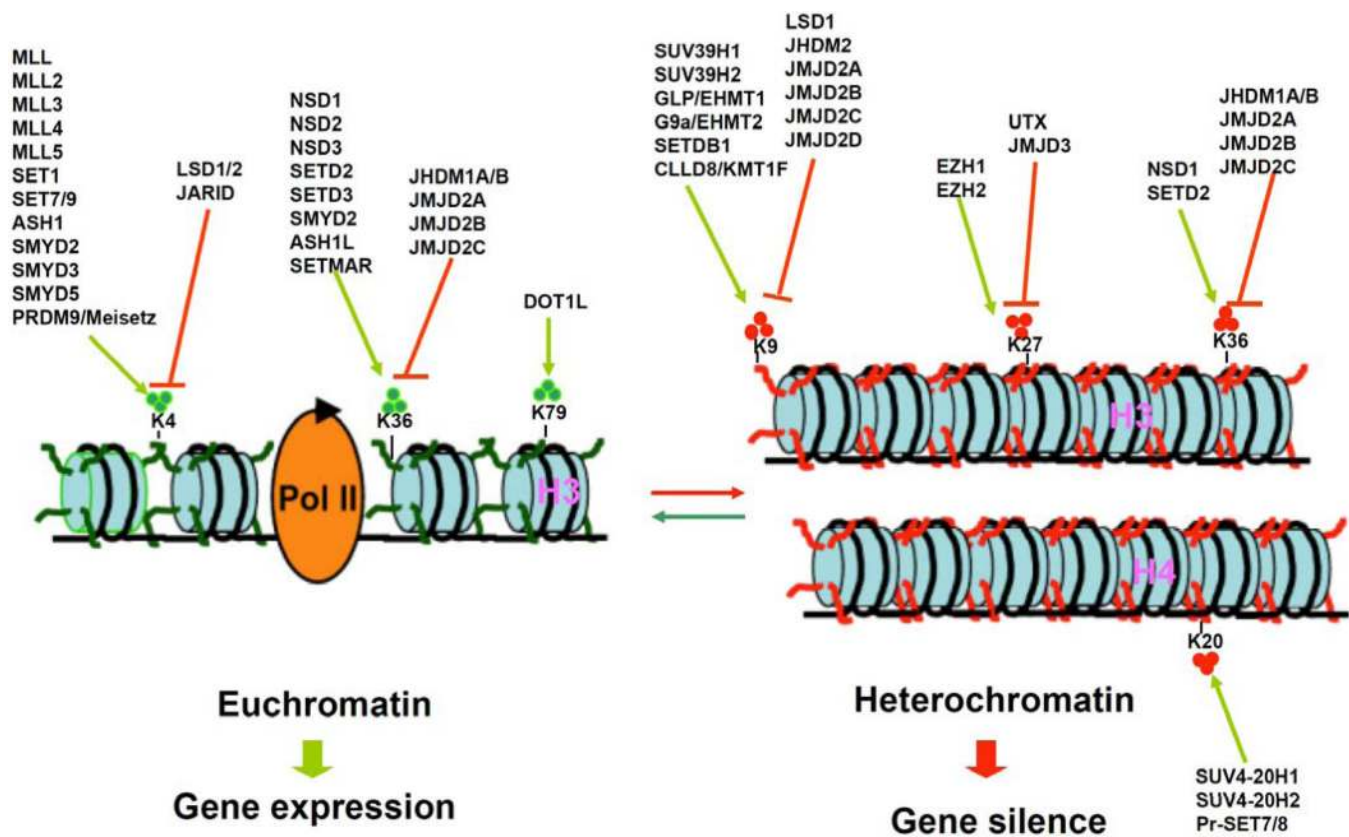


Fig 1.

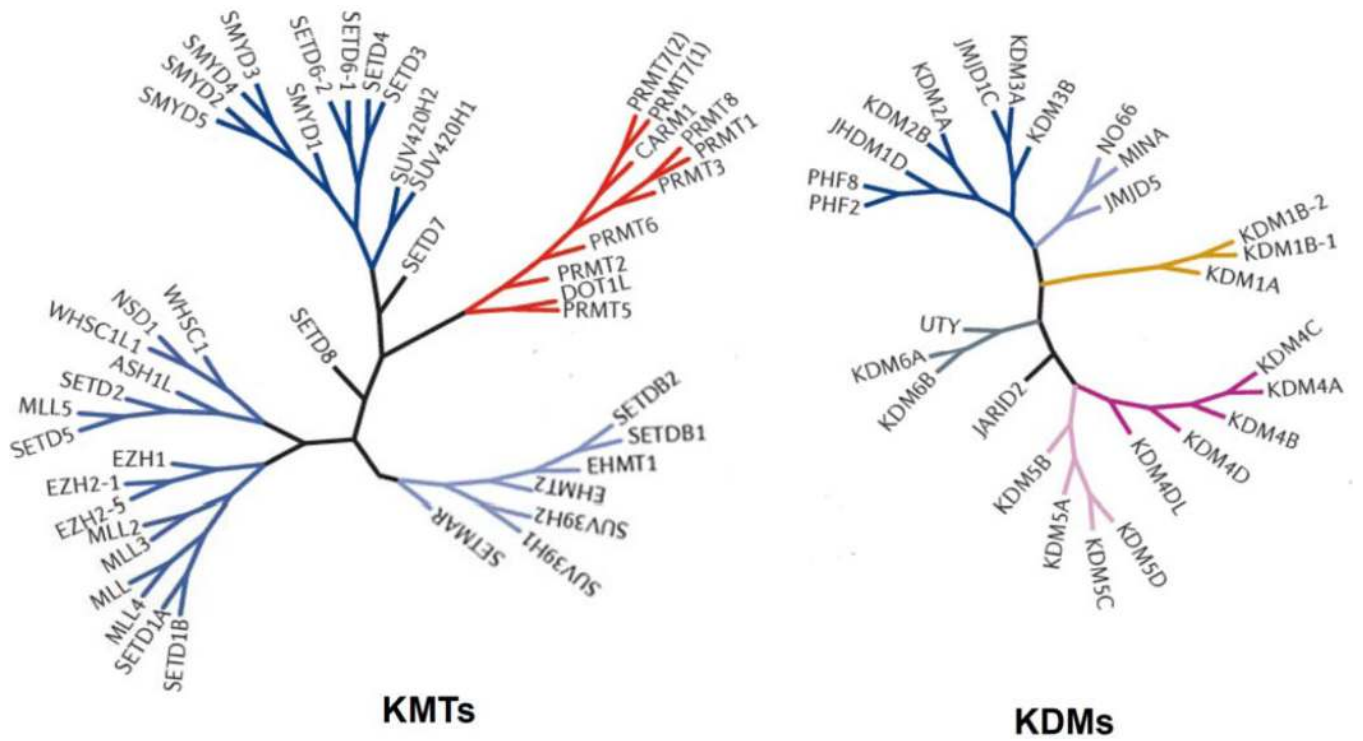


Fig 2.

Table 1

Alterations of KMTs and KDMs in cancer

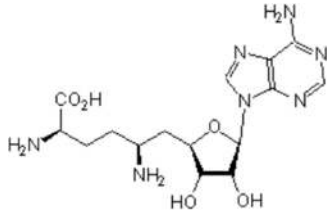
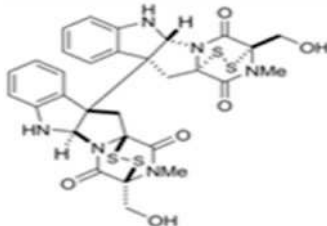
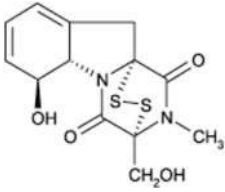
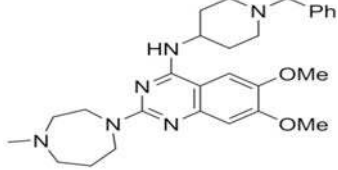
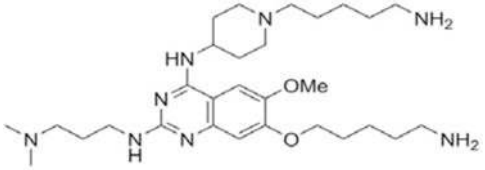
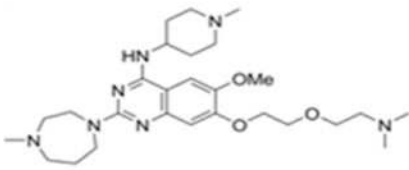
Enzymes	Genetic Alteration	Substrates	Function	Cancer
<b>KMT2C MLL3</b>	Mutations and deletion [93, 100, 101]	H3K4me1, me2, and me3	A co-activator complex of nuclear receptors	Medulloblastoma, Opisthorchis viverrini-related cholangio-carcinoma, acute myeloid leukemia, glioblastoma, melanoma, and colorectal, pancreatic, lung, bladder and breast cancers [93, 100, 101].
<b>KMT2D MLL2</b>	Recurrent mutations, duplications and translocations [93, 95, 98–100]	H3K4me3	A coactivator for Estrogen Receptor (ER) $\alpha$ [195]	Medulloblastoma, non-Hodgkin lymphoma, multiple myeloma, renal cell carcinoma, lung cancer, prostate cancer [93, 95, 98–100]
<b>KMT3A SETD2</b>	truncating or missense mutations [94, 103–106]	H3K36me3	Transcription activation, and binds to the promoters of adenovirus 12 E1A gene	Early T-cell precursor acute lymphoblastic leukaemia, clear cell renal carcinoma [94, 103–106]
<b>KMT3B NSD1</b>	Mutations, fuse to NUP98, microdeletion, and minimal common regions of gain [107]	H3K36me1, me2, and me3	Transcriptional intermediary factor	Carcinoma of the upper aerodigestive tract, acute myeloid leukemia, ganglioglioma, lung adenocarcinoma in never smokers, acute lymphoblastic leukemia, neuroblastoma, and Wilms tumor [107].
<b>KMT6A EZH2</b>	Mutations and overexpression [74–90]	H3K27me1, me2, and me3	Repressing expression of HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes	A wide variety of cancers [74–90]
<b>KMT8 PRDM2 /RIZ1</b>	Inactivating mutation and deletion, silencing [194–197]	H3K9me3	An ER co-activator [198]	hepatocellular carcinoma, oral squamous cell carcinomas, prostate cancer, breast cancer cells [194–197]
<b>KDM3B JMJD1B</b>	cryptic deletion or overexpression [105]	H3K9me1 and me2	Synergistic interaction with CBP, inducing leukemogenic oncogene <i>lmo2</i> expression [199]	myeloid malignancies, prostate cancer [105, 199]
<b>KDM5C JARID1C</b>	inactivating mutations [94]	H3K4me3	Transcriptional repression of neuronal genes	clear cell renal carcinoma [94]
<b>KDM6A UTX</b>	Inactivating mutations [78, 91–95]	H3K27 me2, and me3	HOX expression, recruitment of the PRC1 complex and monoubiquitination of histone H2A	Squamous cell carcinomas, acute myeloid leukemias (AML), glioblastoma, breast, bladder and colorectal cancers [78, 91–95]
<b>KMT2A MLL1</b>	Translocation, overexpression [110–112, 117]	H3K4me1	HOX and E2F3-dependent gene expression	Breast cancer cells, prostate cancer, and glioblastoma multiforme [110–112, 117]
<b>NSD3 WHSC1L1</b>	Translocation, amplification [114, 201]	H3K36 me1 and me2	Gene expression in cell growth, motility, cell cycle, and apoptosis [202]	Leukemia, pancreatic ductal adenocarcinoma, breast and lung cancers [114, 201, 202]
<b>NSD2 WHSC1 MMSET</b>	Translocation, overexpression [115,116]	H3K36me1 and me2	Interact with $\beta$ -catenin [203]	multiple myeloma, bladder cancer neuroblastoma [115,116]

Enzymes	Genetic Alteration	Substrates	Function	Cancer
<b>KMT1C G9a</b>	Over expression [126]	H3K9me1 and me2, H3K27me	DNA methylation, gene expression silence, and cell cycle [126, 214, 215]	Hepatocellular carcinoma [126]
<b>KMT1E SETDB1</b>	Amplification [127]	H3K9me3	Silencing of euchromatic genes and interaction with DNMT3A [212]	Melanoma [127]
<b>KMT2E MLL5</b>	Over expression [216]	H3K4me and me2	A coactivator of RAR-alpha and cell cycle regulator	AML, HPV16/18-associated cervical cancers [204, 205]
<b>KMT3C SMYD2</b>	Over expression [123]	H3K4me H3K36me2	Rb and p53 methylation and cell cycle [121, 122]	esophageal squamous cell carcinoma [123]
<b>KMT3E SMYD3</b>	Over expression [118–120]	H3K4me2 and me3, H4K5me, H4K20me	MMP9 expression [217]	colorectal carcinoma, hepatocellular carcinoma, and breast cancer [118–120]
<b>KDM1A LSD1</b>	Over expression [131–134]	H3K4me and me2, H3K9me	A coactivator of androgen receptor and snail/sluc [133, 218]	Bladder, prostate, lung and colorectal cancer [131–134]
<b>KDM3A JMJD1A</b>	Over expression [207, 208]	H3K9me1 and me2	HOXA1 and CCND1 expression and G1S transition [207, 208]	Bladder and lung cancers [207, 208]
<b>KDM5A JARID1A</b>	Overexpressed and gene fusion [219–221]	H3K4me2 and me3	Transcription of HOX and repression of CXCL12 genes [143]	Gastric cancer, leukemia and cervical cancer [219–219]
<b>KDM2B FBXL10</b>	Down-regulation [211]	H3K36me2 H3K4me3	Binds the transcribed region of ribosomal RNA and represses the transcription of ribosomal RNA [149].	A hotspot for proviral insertion in murine tumors, Human brain tumors [211].
<b>KDM4B JMJD2B</b>	Over expression [209, 210]	H3K9me2 and me3	A co-factor of estrogen receptor [223]	Bladder, lung and gastric cancer [209, 210]
<b>KDM5B JARID1B</b>	Over expression the copy number gain [144–146, 188]	H3K4 me1, me2 and me3	Growth regulation, co-regulation of the E2F/RB1, BRCA1 and HOXA5 [223]	Bladder, prostate, breast cancer melanoma, lung cancer [144–146, 188]
<b>KDM6B JMJD3</b>	Overexpression [148]	H3K9 and H3K36	HOX gene expression and inflammatory response [150, 224]	Prostate cancer [148]

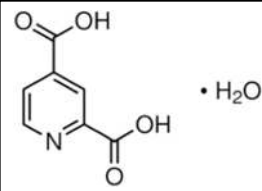
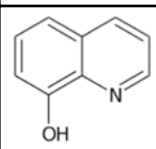
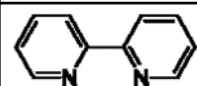
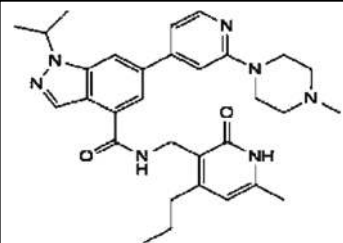
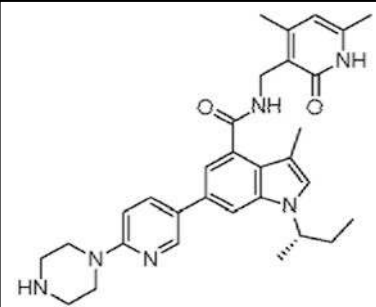
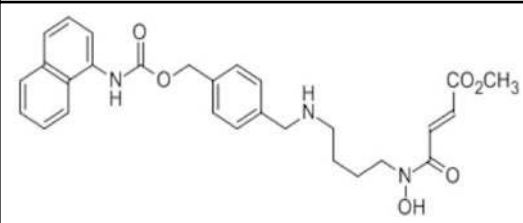


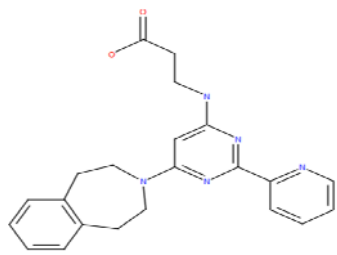
Table 2

## KMT and KDM inhibitors

Agent	Chemical Structure	Targets	Potency	Refs.
Sinefungin A		S-adenosyl-L-methionine (SAM, AdoMet) methyltransferase	Ki = 1.3 $\mu$ M	[160]
Chaetocin		SU(VAR)3-9	Ki = 0.6 $\mu$ M	[161]
Gliotoxin		G9a	Ki = 6.4 $\mu$ M	[164]
BIX-01294		G9a, GLP	G9a: Ki = 180 nM GLP: Ki = 34 nM	[165,166]
E72		GLP	Ki = 100 nM	[211]
UNC0321		G9a, GLP	G9a: Ki < 15 nM GLP: Ki = 15 nM	[166]

Agent	Chemical Structure	Targets	Potency	Refs.
UNC0638		G9a, GLP	G9a: $K_i < 15$ nM GLP: $K_i = 19 \pm 1$ nM	[166]
UNC0646		G9a	$K_i = 6$ nM	[212]
EPZ004777		DOT1L	$K_i = 0.3$ nM	[212]
AZ505		SMYD2	$K_i = 0.3$ $\mu$ M	[212]
trans-2-Phenylcyclopropylamine		LSD1	$K_i = 242$ $\mu$ M	[179]
Polyamine analogues		LSD1	$K_i < 2.5$ $\mu$ M	[183–185]
CBB1007		LSD1	$K_i = 5.27$ $\mu$ M	[186]
N-oxalylglycine		JMJD2C JMJD2A	JMJD2C: $K_i = 500$ $\mu$ M	[187]

Agent	Chemical Structure	Targets	Potency	Refs.
			JMJD2A: $K_i = 250 \mu\text{M}$	
2,4-pyridindicarboxylic acid (PDCA)		KDM5B KDM4A/C	$K_i = 3 \pm 1 \mu\text{M}$	[188]
8-hydroxyquinoline		JMJD2	$K_i = 28 \mu\text{M}$	[189]
2,2'-bipyridine		JMJD2E	$K_i = 6 \mu\text{M}$	[190]
GSK-343		EZH2	$K_i = 4\text{nM}$	[172]
GSK-126		EZH2	$K_i = 3\text{nM}$	[171]
Methylstat		JMJD3, JMJD2E, JMJD2C	$K_i = 4.3 \mu\text{M}$	[191]

Agent	Chemical Structure	Targets	Potency	Refs.
GSK-J1	 <p>The chemical structure of GSK-J1 is a complex molecule. It features a central pyridine ring. One nitrogen atom of the pyridine ring is substituted with a 2-phenyl-1,2,3,4-tetrahydroquinoline group. The other nitrogen atom of the pyridine ring is substituted with a 2-pyridin-4-yl group. A propyl chain is attached to the 4-position of the pyridine ring, with a carboxylic acid group at the end of the chain.</p>	JMJD3, UTX	Ki = 60nM	[192]