Roles of histone methyl-modifying enzymes in development and progression of cancer

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Review Article

Roles of histone methyl-modifying enzymes in development and progression of cancer

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Retroviral insertional mutagenesis in mice is considered a powerful forward genetic strategy to identify disease genes involved in cancer. Our high-throughput screens led to frequent identification of the genes encoding the enzymes engaged in histone lysine methylation. Histone methylation can positively or negatively impact on gene transcription, and then fulfill important roles in developmental control and cell-fate decisions. A tremendous amount of progress has accelerated the characterization of histone methylations and the enzymes that regulate them. Deregulation of these histone methyl-modifying enzymes has been increasingly recognized as a hallmark of cancer in the last few years. However, in most cases, we have only limited understanding for the molecular mechanisms by which these enzymes contribute to cancer development and progression. In this review, we summarize the current knowledge regarding some of the best-validated examples of histone lysine methyltransferases and demethylases associated with oncogenesis and discuss their potential mechanisms of action. (*Cancer Sci* 2013; 104: 795–800)

A great body of evidence supports that epigenetic changes are responsible for cancer development.[1,2] There are different types of mechanisms in the field of epigenetics: DNA methylation, post-translational modifications of histones, chromatin remodeling and noncoding RNAs. The earliest demonstration of an epigenetic link to cancer was derived from the studies about DNA methylation. Evidence showing the role of DNA hyper- or hypo-methylation in oncogenesis has been accumulating for more than 20 years. However, the role of the other mechanisms is an emerging area of interest. During the past decade, the focus has shifted to studies of post-translational modifications of histones, because alterations in the balance of histone modifications lead to deregulated gene expression and are associated with cancer. The nucleosome, the basic building block of chromatin, is composed of two copies of each histone: H2A, H2B, H3, and H4. The amino-terminal tails of histones are subject to posttranslational modifications (Fig. 1a), which include acetylation, methylation, phosphorylation, ubiquitylation and SUMOylation. These modifications influence the structure of chromatin and show different functional outputs depending on the type, the site and the degree of modifications. The modification pattern of histone has been linked to gene function during development and tumorigenesis.

Identification of genes involved in cancer gives us crucial information about the molecular mechanism of cancer development. Genetic screens for mutations contributing to tumor formation in model organisms facilitate the efficient identification of cancer genes in an *in vivo* setting. Retroviral insertional mutagenesis in mice is one of the potent cancer gene discovery tools.[3] Previously we have accomplished high-throughput cloning of retroviral integration sites from the tumors of murine leukemia virus (MuLV)-infected mice.[4] This led to the identification of hundreds of candidate cancer genes including many genes encoding histone lysine methyltransferases (KMTs) and demethylases (KDMs). In this review, we discuss the current state of knowledge regarding histone lysine methylation and especially highlight new insights into the role of KDMs in the course of cancer development.

Frequent Identification of Histone-Modifying Enzymes by Retroviral Insertional Mutagenesis

Slow transforming retroviruses such as MuLV can efficiently induce tumors in mice by integrating into host genome and deregulating the expression of proto-oncogenes or inactivating the expression of tumor suppressor genes (Fig. 1b).[3] Thus the retroviral integration sites in the tumors provide powerful molecular tags for cancer gene discovery. The ability to find oncogenic mutations has been accelerated by technical advances including PCR-based cloning of retroviral integration sites, the availability of mouse genome sequence, and high-throughput DNA sequencing.[5] Many of the identified mutations in these screens are not restricted to the tumor formation in mice, but also play a causal role in the development of human cancers.[5]

High-throughput retroviral tagging allowed identification of novel cancer-inducing loci involved in the development of particular types of tumors, or that collaborate with known oncogenic pathways.[4,6–8] We have established the Retroviral Tagged Cancer Gene Database (RTCGD, http://variation.osu.edu/rtcgd/) containing a collection of retroviral insertional screens.[9] The RTCGD creates the opportunity for multiple datasets to be compared and is periodically updated with the supply of recently reported retroviral integration sites. During our high-throughput screens, many genes encoding KMTs and KDMs (Fig. 1c, as indicated with red letters) have been identified as potential oncogenes or tumor suppressor genes.[4,8] The result provided us the opportunity to explore the functions of these enzymes in tumor initiation and progression.

Histone Modifications in Cancer

Historically, histone acetylation is the most extensively studied modification on histone amino-terminal tails.[10] Acetylation of lysine residues on histone H3 and H4 leads to the formation of an open chromatin structure, which induces transcriptional activation. Acetylation is regulated by the competing activities of two enzyme families, histone lysine acetyltransferases (HATs) and deacetylases (HDACs). Mutations or chromosomal

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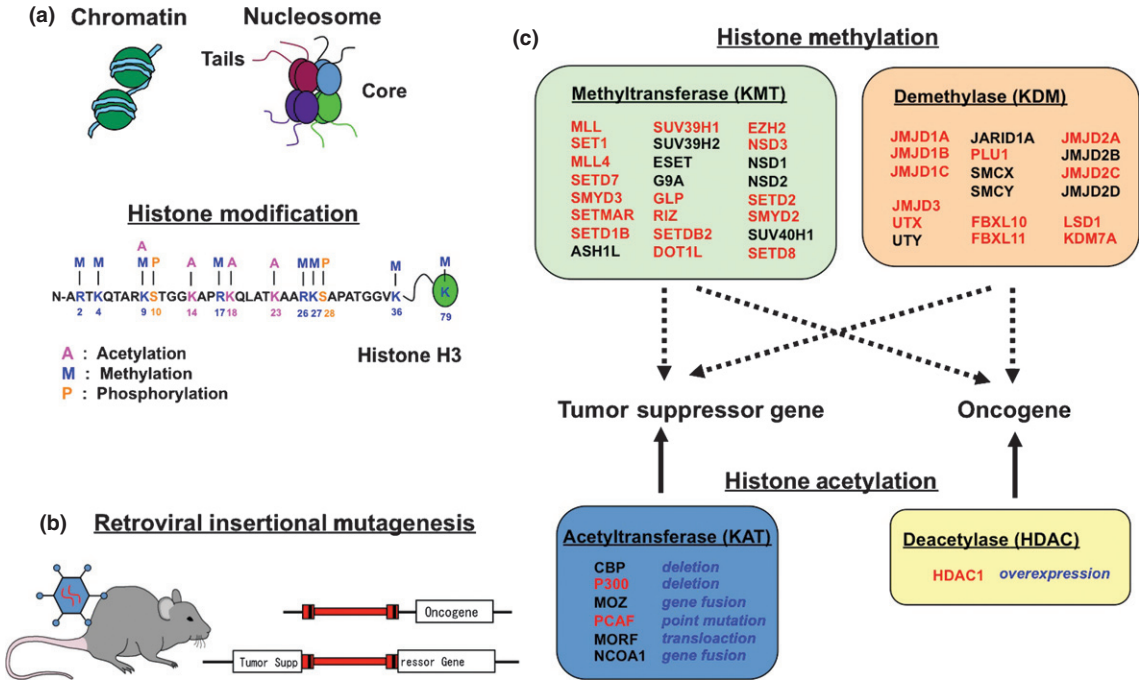

Fig. 1. Retroviral insertional mutagenesis demonstrated the involvement of histone-modifying enzymes in cancer development. (a) Histone modifications. Chromatin is the macromolecular complex of DNA and histone proteins, and the basic unit of chromatin is the nucleosome. It contains a histone octamer, with two each of histones H2A, H2B, H3 and H4. The amino-terminal tails of histones are subject to posttranslational modifications including acetylation, methylation and phosphorylation, which play a critical role in the regulation of DNA-based biological processes. (b) Retroviral insertional mutagenesis. Retroviruses induce tumors by randomly integrating into the host genome. The mechanism leading to cancer is thought to be the activation of proto-oncogenes or inactivation of tumor suppressor genes. (c) Histone modifying enzymes identified by retroviral tagging. Using high-throughput analysis, we found many genes encoding histone methyltransferases and demethylases (indicated with red letters) as potential oncogenes or tumor suppressor genes. Histone acetylation has been unambiguously associated with cancer, but the importance of histone methylation in cancer is just beginning to be uncovered.

translocations of HAT genes including Gcn5/PCAF, p300/CBP and the MYST families are detected in various solid tumors and hematological cancers.[10] On the other hand, HDAC overexpression is frequently observed in various cancers.[11] Histone lysine deacetylases are implicated in cancer due to their aberrant transcriptional silencing of tumor suppressor genes. As a result, the enzymatic inhibitors of HDACs have been developed as anti-cancer drugs.[12]

In contrast, histone methylation had been thought to be an irreversible process for a long time. The discovery of KMTs and KDMs has resulted in a completely different view of histone methylation in which this modification is dynamic.[13,14] Reversible histone lysine methylation is implicated in diverse biological processes including cellular proliferation, differentiation, DNA repair and recombination.[13] The importance of the tight regulation of histone methylation is indicated by emerging links of histone methylation to human disease such as cancer.[15,16]

Regulation of Histone Lysine Methylation

Histone lysine methylation is associated with activated or repressed transcription of individual genes depending on the residue and the degree, since lysine residues can be mono-, di- or tri-methylated (me1, me2 or me3).[14] Moreover, a given methylated mark is often linked to a specific position of the gene, either around the transcription start site (TSS) or in the coding region, regulating transcriptional initiation or elongation. In general, methylation of lysine-4 of histone H3 (H3K4) around TSS and methylation of H3K36 and H3K79 on the coding region are associated with active transcription.[17] Methylation of H3K9 and H3K27 on promoters correlates with transcriptional repression.[17]

A large number of KMTs and KDMs have been identified so far (Fig. 2). Two classes of KMTs, the SET domain-containing enzymes and the DOT1-like proteins, have been shown to methylate specific lysine residues of histones.[16] Similarly, two families of KDMs have been identified, the flavin adenine dinucleotide (FAD)-dependent amine oxidases (LSD1/KDM1A) and the Jumonji C (JmjC) domain-containing demethylases that are Fe (II) and α-ketoglutarate-dependent enzymes. KDMs also have exquisite substrate specificity.[13,14]

KMTs in Cancer

The roles of KMTs in cancer have been extensively reviewed[18]; therefore, in this review, we will focus on EZH2/KMT6 methyltransferase, since it is often found as a key player in human malignancies.[18] EZH2 is the catalytic component of the polycomb repressor complex 2 (PRC2), which is primarily responsible for H3K27 methylation. Gene-expression studies demonstrated EZH2 upregulation in a number of tumors such as prostate, breast, lung and colon cancer.[18] Downregulation of microRNA-101, a negative regulator of EZH2, has been also reported as a cause of EZH2 overexpression in prostate cancer.[19] In B cell lymphoma, gain-of-function mutations in EZH2 have been discovered, supporting the notion that EZH2 is an oncogene.[20] Importantly, EZH2-mediated tri-methylation of H3K27 was proposed as a mechanism of tumor-suppressor gene silencing in cancer that is independent of promoter DNA methylation.[21] In addition, overexpression of EZH2 frequently correlates with advanced cancer and poor prognosis.[22] However, deep-sequencing projects of cancer genomes have recently identified coding mutations within EZH2 in various myeloid and lymphoid neoplasms, suggesting a tumor-suppressive role for EZH2 in these cell lineages.[23]

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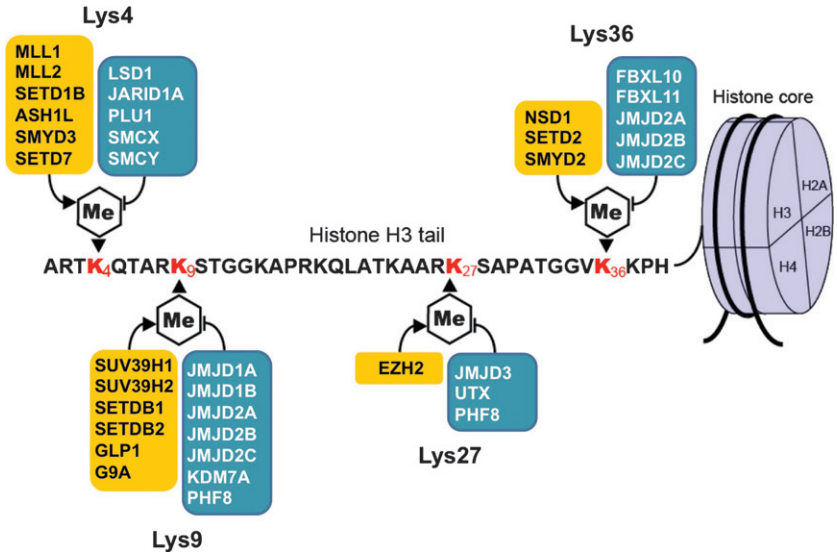

Fig. 2. Dynamic regulation of histone lysine methylation. The amino-terminal tails of the histone H3 and four most characterized lysine residues are shown. The methylation at lysine 4 (K4) and K36 correlates with transcriptionally active genes, whereas methylation at K9 and K27 is linked to transcriptional repression. Histone lysine methyltransferases (KMTs) and demethylases (KDMs) for various lysine residues are shown in the yellow and blue boxes, respectively.

Table 1. Connections between histone demethylases and human cancer

Name	Synonyms	Alteration	Associated human cancer	Reference
KDM1A	LSD1, AOF2	Overexpression	Prostate cancer, neuroblastoma, lung cancer, colorectal carcinoma and bladder cancer	(26,27,28)
		Downregulation	Breast cancer	(29)
KDM2B	FBXL10, JHDM1B	Overexpression	Pancreatic ductal adenocarcinoma and various leukemia	(59,60)
		Downregulation	Glioblastoma multiform	(61)
KDM3A	JMJD1A, JHDM2A	Overexpression	Malignant colorectal cancer and renal call carcinoma	(62,63)
KDM4A	JMJD2A	Overexpression	Lung and breast cancer	(56,57)
KDM4B	JMJD2B	Overexpression	Estrogen receptor-positive breast cancer, bladder and lung cancer	(43,58)
KDM4C	JMJD2C, GASC1	Amplification	Esophageal cancer, breast cancer, medulloblastoma, primary mediastinal	(37,39,40,41)
		Overexpression	B cell and Hodgkin lymphoma	
KDM5A	JARID1A, RBP2	Overexpression	Gastric cancer and breast cancer	(48,49)
		Translocation	Acute myeloid leukemia	(64)
KDM5B	JARID1B, PLU1	Overexpression	Breast cancer, prostate cancer and bladder and lung cancer	(44,45,46,47)
		Downregulation	Malignant melanoma	(65)
KDM6A	UTX	Mutation	Multiple cancers including multiple myeloma, esophageal squamous cell carcinomas, renal cell carcinomas and chronic myelomonocytic leukemia	(31,32,33)
KDM6B	JMJD3	Downregulation	Lung, liver carcinomas and various hematopoietic malignancies	(34,35)
PHF8	JHDM1F	Overexpression	Prostate cancer	(66)

KDMs in Cancer

Recent studies provide increasing genetic evidence suggesting that mutation or deregulation of KDMs may be a critical determinant in cancer development. This has been described in several reviews,[15,16,24] and we will therefore limit the discussion here to some key examples. Table 1 provides an overview for KDMs and their association with human cancer.

LSD1/KDM1A. LSD1/KDM1A is the first protein reported to possess histone demethylase activity in mammalian cells. Especially, there is a growing interest in LSD1 as a potential target for cancer therapies.[25] Expression levels of LSD1 are significantly elevated in prostate, bladder, lung, colorectal cancer and neuroblastoma.[26–28] Knockdown or chemical inhibition of LSD1 was shown to inhibit the proliferation of several cancer cell lines that overexpress LSD1.[25,27] LSD1 was reported to regulate the expression of genes involved in chromatin regulations such as chromatin remodeling and assembly, thereby contributing to oncogenesis.[27] However, a recent paper demonstrated that overexpression of LSD1 in certain breast cancers suppressed metastasis and reduced tumor growth.[29] These results suggest that the role of LSD1 in tumorigenesis is significantly dependent on its cellular context.

KDM6 family. Just as EZH2 H3K27 methyltransferase is found aberrant in many cancers, H3K27 demethylases are mutated and deregulated. There are two histone demethylases, UTX/KDM6A and JMJD3/KDM6B, specific for H3K27me3/me2.[30] Inactivating mutations in the UTX gene were the first cancer-associated mutations identified in the KDM family and have been found in many cancers such as multiple myeloma, esophageal squamous cell carcinoma, renal cell carcinoma and myelomonocytic leukemia.[31–33] The expression of JMJD3 was also shown to be decreased in subsets of human cancers, indicated by data-mining analysis using published microarray data.[34,35] Therefore, both of the mammalian H3K27 demethylases are suggested to possess tumor-suppressive characteristics.

We have identified UTX as one of the candidate tumor suppressor genes targeted by retroviral integrations in our screens. We first examined the effects of UTX overexpression and found significant decrease of cell growth dependent on intact catalytic activity.[36] The growth inhibition by UTX was due to aberrant transcriptional activation of retinoblastoma tumor suppressor gene RB and its related RBL2 through

H3K27 demethylation. Thus RB pathway was shown to be one of the downstream targets of UTX-mediated cell growth control.

Overexpression of JMJD3 similarly led to cell cycle arrest, but JMJD3 was shown to activate the expression of INK4A-ARF locus through its demethylase activity.[34,35] Moreover, JMJD3 expression itself was induced by activation of the RAS-RAF pathway, suggesting that it could be involved in oncogene-induced senescence. Depletion of JMJD3 enabled cells to overcome the senescence, an important barrier to tumorigenesis. Therefore, although both UTX and JMJD3 can antagonize EZH2 activity and switch from a repressive to an active chromatin status, they play distinct roles in cancer development by regulating different target genes.

KDM4 family. Interestingly, oncogenic potential of the genes in the KDM family was described long before the characterization of their demethylase activity. One of the examples is JMJD2C/KDM4C, which was originally named "gene amplified in squamous cell carcinoma 1 (GASC1)", because of its amplification in esophageal cancer cells.[37] JMJD2C catalyzes the removal of H3K9me2/3 and H3K36me2/3[38] and is also overexpressed in osteosarcoma, medulloblastomas, breast cancer, primary mediastinal B-cell and Hodgkin lymphoma.[39–41] Knockdown of JMJD2C inhibited proliferation of several cancer cell lines, while forced expression could induce transformed phenotypes in immortalized mammary epithelial cells.[38,39]

We have also identified JMJD2C as an oncogene candidate by our screens. To uncover the function of JMJD2C in oncogenesis, we searched for the critical downstream target genes regulated by JMJD2C and discovered MDM2 oncogene.[42] JMJD2C increased the expression of MDM2, a negative regulator of TP53. Increased levels of MDM2 correlated directly with decreased TP53 product in the cells, suggesting that the MDM2 oncogene might mediate JMJD2C-related tumorigenesis.

Overexpression of JMJD2B/KDM4B, another member of KDM4 family, was also reported in bladder and lung cancer.[43] Microarray analysis revealed that JMJD2B could regulate the expression of genes involved in the cell cycle pathway leading to oncogenesis, and that CDK6 (cyclin-dependent kinase 6) was one of the important downstream targets.[43] Although the exploration of the genomic targets and cellular functions of KDM4 family members has just started, this family is thought to be one of the feasible molecular targets for cancer therapy.

KDM5 family. Another example for early-described oncogene product is PLU1/KDM5B demethylase. PLU1 was originally identified in a screen for genes regulated by c-ErbB2 oncogene,[44] and is highly expressed in various types of tumors such as breast, prostate, bladder and lung cancers.[44–46] Increased expression of PLU1 caused proliferation in breast cancer and depletion of PLU1 inhibited tumor growth in a mouse mammary tumor model. KDM5 family of enzymes can demethylate H3K4me3/me2, and function as transcriptional repressors.[47] PLU1 regulates the expression of cellular genes that control cell proliferation including BRCA1 tumor suppressor, E2F1 and E2F2 genes.[46,47] Another KDM5 family member, JARID1A/KDM5A was also found overexpressed in gastric and breast cancer.[48,49] Interestingly, KDM5 family demethylases are proposed to play an important role in malignant progression of cancer. This will be discussed in the following section.

KDMs in the Process of Tumor Progression

Although accumulating evidence reveals the role of KDMs in tumor initiation, a potential connection between KDMs and tumor progression is yet to be fully investigated. In malignant progression, it is a common event that patients who receive extended chemotherapy respond well to it initially, but a subpopulation of cancer cells becomes drug-resistant. One of the KDMs, JARID1A/KDM5A, has been shown to be associated with drug resistance in lung cancer cells.[50] Knockdown of JARID1A reduced the number of drug resistant cells, but its overexpression decreased the sensitivity of the whole population to drug treatment.[50] This finding implicates that a distinct chromatin status established by JARID1A is required for the maintenance of the drug-tolerant subpopulation during tumor progression.

We focused on another member of KDM5 family, PLU1/KDM5B, since PLU1 is one of the oncogene candidates identified by our screens. In addition to the role in tumor initiation, PLU1 was suggested to be associated with malignant progression of prostate cancer.[45] However, the mechanism by which PLU1 contributed to tumor progression remained unclear. At first, we examined whether ectopic expression of PLU1 could affect the invasive potential of the cells.[51] Overexpression of PLU1 enhanced the invasive potential of the weakly invasive cells dependent on its demethylase activity (Fig. 3a,b). Among the target genes transcriptionally repressed by PLU1, we selected KAT5/TIP60 gene as a good candidate, since KAT5 was considered a tumor suppressor gene and reported to be more frequently downregulated in advanced carcinoma.[52,53] Our mechanistic investigations proved that the regulation of KAT5 by PLU1 was responsible for the PLU1-induced cell invasion. Moreover, we found that CD82/KAI1, which was activated by KAT5, might be a candidate effector of cell invasion promoted by PLU1 (Fig. 3c). KAI1 is a member of the tetraspanin family of type III membrane proteins and a metastasis suppressor gene for human malignancies including

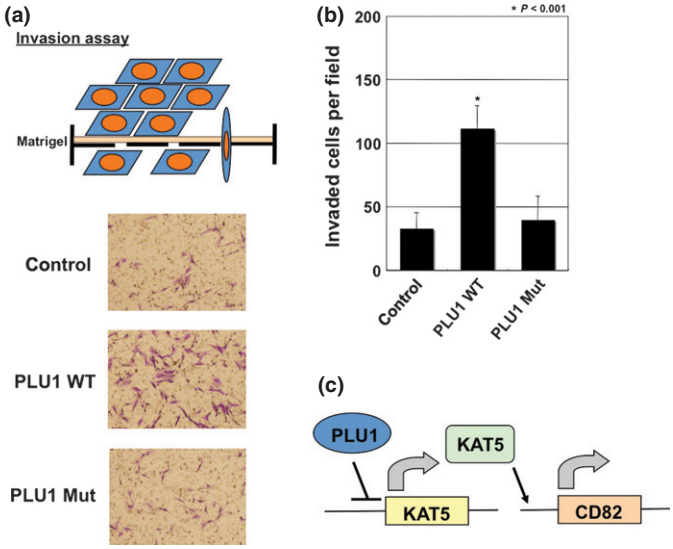

Fig. 3. Overexpression of PLU1 increased the invasive activity of the cells. (a) A modified Boyden chamber assay was used to determine if the cells invade through membranes coated with Matrigel. MCF10A cells were infected with the control retrovirus or the retrovirus expressing wild-type (WT) PLU1 or the mutant (Mut), and then invasion assays were performed. Representative fields of invaded and stained cells are shown. (b) PLU1 enhanced cell invasion dependent on its demethylase activity. Cells that invaded through the membrane to the lower surface were fixed, stained, and counted. $P < 0.001$ versus control. (c) Gene expression cascade underlying PLU1-induced cell invasion. Mechanistic investigations suggested that PLU1 repressed the downstream target gene KAT5/Tip60 to lead to the downregulation of CD82/KAI1, a metastasis suppressor, thereby promoting the invasive potential.

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prostate, breast, pancreatic and lung cancers.[54,55] Thus our study demonstrated a novel role of KDM that could contribute to cell invasion, one of the important features of cancer aggressiveness.

Future Perspectives

Recently deregulation of histone methylation in cancer has received increasing attention and consequently the list of KMTs and KDMs associated with human cancers has been steadily growing. However, in most cases, our understanding of the molecular mechanisms that contribute to cancer development is still quite limited. Many studies have evaluated the expression of KMTs and KDMs and chromatin modifications in cancer, but it is mostly unclear that these alterations are the causative events or the effects of the disease itself. In the various stages for malignant progression of tumors, we have only recently become aware that KMTs and KDMs are implicated in migration, invasion, epithelial-to-mesenchymal transition (EMT) and drug resistance of cancer cells. To gain deeper insights into the causes as well as the consequences of aberrant histone methylation will definitely extend our comprehension of cancer development. Furthermore, due to the reversibility of histone methylation, the approaches that target KMTs and/or KDMs in cancer cells could be undoubtedly the next trend in cancer therapies.

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Disclosure Statement

The authors have no conflict of interest.

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