

Protein Methylation and Demethylation in Cancer

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ABSTRACT

Epigenetic modifications are heritable chromatin alterations that contribute to the temporal and spatial interpretation of the genome. The epigenetic information is conveyed through a multitude of chemical modifications, including DNA methylation, reversible modifications of histones, and ATP-dependent nucleosomal remodeling. Deregulation of the epigenetic machinery contributes to the development of several pathologies, including cancer. During the last decade, we saw an explosion of studies investigating the role of protein methylation/demethylation of histones. Protein methylation and demethylation are catalyzed by protein methyltransferases and protein demethylases. Their substrates have been shown to play important roles in cancers. Although the underlying mechanisms of tumorigenesis are still largely unknown, growing evidence is starting to link aberrant regulation of methylation to tumorigenesis. This review focuses on summarizing the recent progress in understanding of the function of protein lysine and arginine methylation/demethylation. We also discuss the potential and the caveats of targeting protein methylation for the treatment of cancer.

Key words: Epigenetics; Neoplasm; Histone modification; Protein methylase; Protein demethylase

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INTRODUCTIONS

Almost every nucleated cell in an organism contains the identical complement of genomic DNA, yet different cell types express and repress different arrays of genes as the basis for tissue and organ differentiation. Whether a specific gene is transcribed or repressed is determined by the conformational state of the chromosomal DNA-protein complex referred to as chromatin, and by the ability to recruit transcription factors and allied proteins to specific promoter sites^[1]. Both chromatin conformation and transcription factor recruitment are controlled by epigenetic enzyme-mediated covalent modification of the DNA and the protein components of chromatin. Genomic DNA is modified by methylation of the C5 position of cytosine within CpG dinucleotide sites of genes by the DNA methyltransferase (DNMT) class of enzymes^[2]. The structural proteins of chromatin are referred to as histones; there are a number of posttranslational modifications of histones which are mediated by specific enzymes that impact chromatin conformation and transcription factor recruitment^[3]. Specific amino acid residues of histones are modified by covalent attachment of methyl groups, acetyl groups, phosphate groups and ubiquitin. These groups can be added and removed by specific classes of enzymes: protein methyltransferases (PMTs); protein demethylases (PDMs); histone acetyl transferases (HATs); histone deacetylases (HDACs); protein kinases; protein phosphatases; protein ubiquitin ligases (E3s) and protein ubiquitin carboxyl-terminal hydrolases (UCHs).

During the last decade, we saw an explosion of studies investigating the role of protein methylation/demethylation of histones. ‘Ying-Yang’ posttranslational modifications are important to

fine-tuning the activity of these histone proteins. Protein methylation and demethylation are catalyzed by protein methyltransferases and protein demethylases. Their substrates have been shown to play important roles in cancers. Although the underlying mechanisms of tumorigenesis are still largely unknown, growing evidence is starting to link aberrant regulation of methylation to tumorigenesis (Table 1). In this review, we aim to summarize the recent progress in understanding of the function of protein lysine and arginine methylation/demethylation. We also describe the potential and the caveats of targeting protein methylation for the treatment of cancer.

PROTEIN LYSINE METHYLATION

Protein lysine methylation has gained tremendous attention since the

discovery of SUV39H1 as the first histone lysine methyltransferase in 2000^[4]. Following the discovery, numerous proteins have been found to possess methyltransferase activity, such as G9a/GLP^[5,6], MLLs^[7], EZH2^[8], SET2^[9], SET7/9^[10], DOT1^[11,12], and PR-SET7 (also known as SETD8)^[13]. These enzymes catalyze the transfer of methyl group from the co-factor S-adenosyl-L-methionine (SAM) to the lysine residues of histones. More recently, many non-histone proteins have been identified as substrates for these enzymes, hence the name protein lysine (K) methyltransferases (PKMTs) (Table 2). It is worth pointing out that PKMTs play important roles in other biological processes including developmental biology and stem cell differentiation. However, here we will focus on the implications of PKMTs in cancers, especially.

Table 1 Genetic alterations in the cancers associated with the protein methylases and demethylases.

Target	Genetic alterations	Cancer type
Methylase		
EZH2	1) Heterozygous activating mutations occurring at Y641, A677 & A687 that result in hypermethylation of H3K27me3 2) Deletion of miR-101 leads to EZH2 overexpression 3) Deletion of SNF5 leads to EZH2 dependency	1) Lymphoma 2) Prostate cancer 3) Malignant rhabdoid tumor
DOT1L	1) 11q23 chromosomal translocations fusing MLL1 (without its catalytic SET domain) to DOT1L binding partners such as AF4, AF9, AF-10 and ENL leading to aberrant H3K79 methylation. 2) CALM-AF10 and SET-NUP214 fusions are known to mis-target DOT1L	Leukemia
NSD1	t(5;11)(q35;p15.5) translocation create NSD1-NUP98 fusions	Acute myeloid leukemia
WHSC1	t(4;14)(p16;q32) chromosomal translocations that places WHSC1 gene under the control of the IGH promoter and results in the overexpression of WHSC1	Multiple myeloma
WHSC1L1	1) t(8;11)(p11.2;p15) chromosomal translocations fuses WHSC1L1 to NUP98 2) 8p11-12 focal amplifications	1) Acute myeloid leukemia Breast cancer 2) Squamous cell lung cancer
SETDB1	1q21 amplifications	Melanoma
SMYD2	1q32 amplifications	Esophageal squamous cell carcinoma
Demethylase		
JMJD2C	1) 9p23-24 amplifications 2) t(9;14)(9p24.1q32) translocations creating fusions to IGH	1) Esophageal squamous cell carcinoma Squamous cell lung Cancer 2) Medulloblastoma Basal breast cancer
LSD2	6p22 amplifications	Urothelial carcinomas
UTX	Inactivating mutations of UTX lead to pro-oncogenic hypermethylation of H3K27me3 and dependence on EZH2	Multiple blood and solid cancers

Table 2 Summary of histone lysine methyltransferases and demethylases.

Mark	Methylation	Demethylation	Catalytic specificity of the demethylase
H3K4	MLL1/KMT2A	LSD1/KDM1	me2/me1→me0
	MLL2/KMT2B	JARID1A/RBP2/KDM5A	me3/me2/me1→me0
	MLL3/KMT2C	JARID1B/PLU-1/KDM5B	me3/me2/me1→me0
	MLL4/KMT2D	JARID1C/SMCX/KDM5C	me3/me2→me1
	MLL5/KMT2E	JARID1D/SMCY/KDM5D	me3/me2→me1
	hSET1A/KMT2F	NDY1/JHDM1B/FBXL10/KDM2B	me3→me2
	hSET1B/KMT2G		
	ASH1/KMT2H		
	SET7-9/KMT7		
H3K9	SUV39H1/KMT1A	LSD1/KDM1	me2/me1→me0
	SUV39H2/KMT1B	JHDM2A/JMJD1A/KDM3A	me2/me1→me0
	G9a/KMT1C	JHDM2B/5qNCA/KDM3B	me2
	EuHMTase/GLP/KMT1D	JHDM2C/TRIP8/KDM3C	me2
	ESET/SETDB1/KMT1E	JMJD2A/JHDM3/KDM4A	me3/me2→me1
	CLL8/KMT1F	JMJD2B/KDM4B	me3/me2→me1
	RIZ1/KMT8	JMJD2C/GASC1/KDM4C	me3/me2→me1
		JMJD2D/KDM4D	me3/me2/me1→me0
H3K27	KMT6/EZH2	UTX/KDM6A	
		JMJD3/KDM6B	me3/me2→me1
H3K36		UTY	me3/me2→me1
	SET2/KMT3A	NDY2/JHDM1A/FBXL11/KDM2A	
	NSD1/KMT3B	NDY1/JHDM1B/FBXL10/KDM2B	me2/me1→me0
	SMYD2/KMT3C	JMJD2A/JHDM3/KDM4A	me2/me1→me0
		JMJD2B/KDM4B	me3/me2→me1
H3K79	DOT1L/KMT4	JMJD2C/GASC1/KDM4C	me3
	PR-SET7-8/KMT5A		
H4K20	SUV4-20H1/KMT5B		
	SUV4-20H2/KMT5C		

Adapted from Kampranis SC *et al* Adv Cancer Res 2009

Methylation of lysines residues is known to occur usually on histone H3 (K4, K9 and K27) and H4 (K20). As mentioned above, the SUV39 protein was the first histone methyltransferase to be discovered^[4]. The methyltransferase activity of SUV39 is directed against lysine 9 of histone H3 and its catalytic domain resides within a highly conserved structure, the SET domain. The sequences within the SET domain are not however sufficient for enzymatic activity. Methylation is only seen when two flanking cystein-rich sequences (PRE-SET and POST-SET) are fused to the SET domain. Use of the simple modular architectural research tool (SMART) indicates that there are 73 entries in the human database which possess a SET domain. In contrast, there are 6 SET domain proteins in *Saccharomyces cerevisiae*, 11 in *Schizosaccharomyces pombe*, 41 in *Drosophila* and 37 in *Caenorhabditis elegans*. Previously characterized human proteins possess a SET domain showing that they can be grouped into four classes (Figure 1). The classification is based on the similarity between the human SET domains as primary and their relationship to SET domains in yeast (*S. cerevisiae*) as secondary. Two groupings show similarity to either yeast SET1 or SET2, thus defining two of the classes. Another class has SUV39 as its defining member and a fourth family represents homologues of the RIZ SET domain. The four families described may subdivide to further classes when more information is available. Overall, the subdivisions indicate that enzymes with sequence similarity in their SET domain also have other structural features (i.e. domains) in common. Figure 2 illustrates protein lysine methylation/demethylation and their biological roles in terms of transcriptional activity, Figure 3 summaries methylase and demethylase at the major 6 lysines of histone tail according to genetic regulatory activity. Next topic is a description of the defining features of each family emphasizing wherever possible, their links to chromatin and transcriptional regulation.

The SET1 family

Two uncharacterized homologous human proteins, designated here as hSET1A and hSET1B are highly related in their SET domain to yeast SET1 (ySET1) that has the capacity to methylate lysine 4 of histone H3. Other related members in this family include proteins of the polycomb and trithorax (trx) group of genes, whose function is linked to the epigenetic regulation of regulated genes in development.

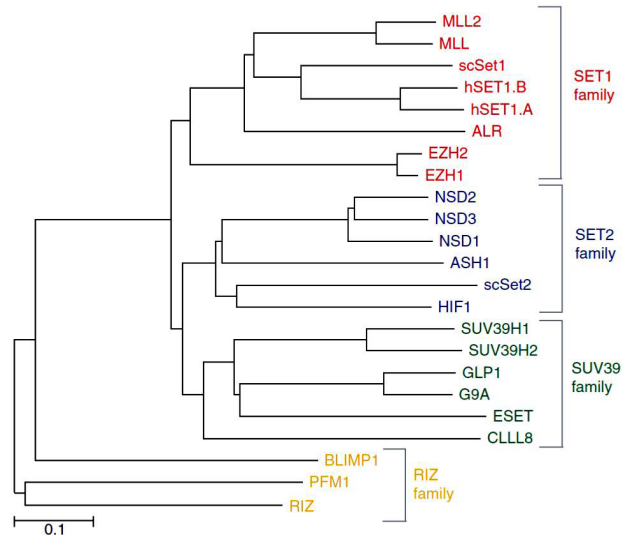


Figure 1 A dendrogram showing the relationship between some of the more characterized human SET-domain proteins. The comparison is based on the homology within the SET-domain. The Clustal W program was used to generate the figure. On the right are the four families defined by the homologues. (Adapted from Tony Kouzarides et al, Current Opinion in Genetics & Development 2002).

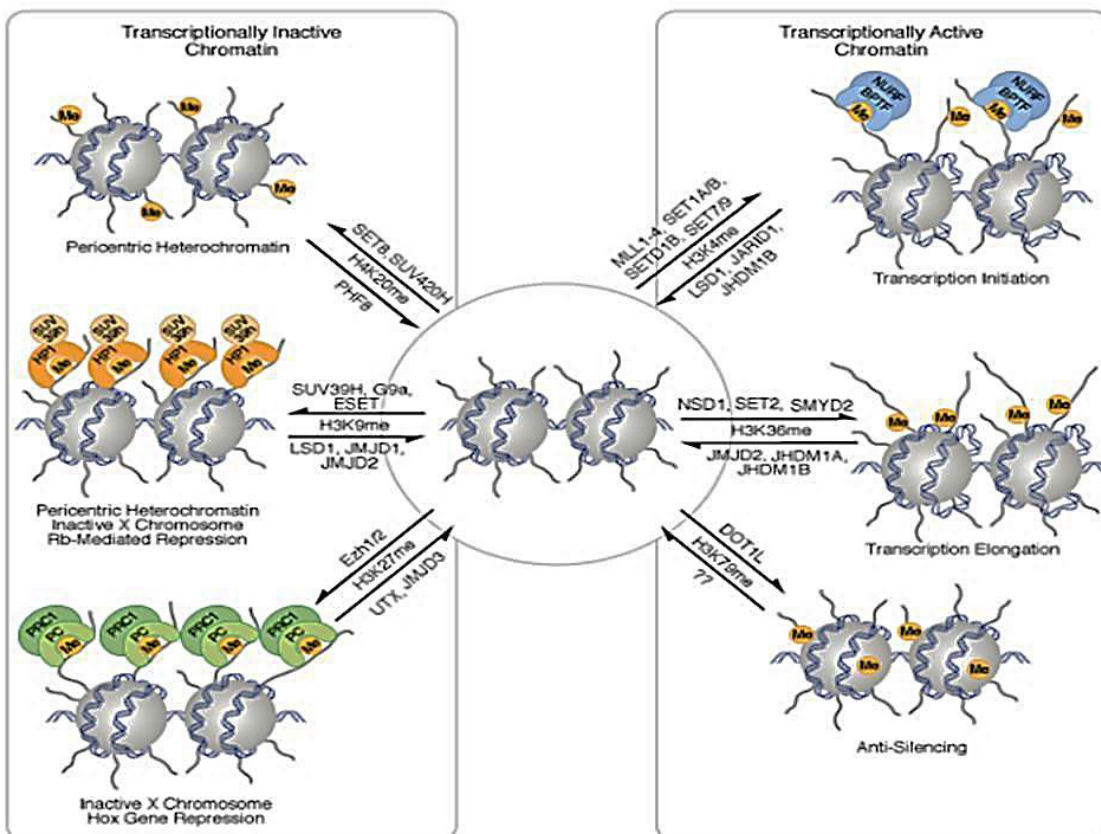


Figure 2 Summaries of protein lysine methylation/demethylation and their biological roles in terms of transcriptional activity.

They include the polycomb proteins EZH1 and EZH2 (also known as ENX1 and ENX2), the TRX proteins MLL (also known as TRX1, HRX, ALL1, HTRX), MLL2 (also known as TRX2, HRX2) and the related protein ALR. A defining structural feature of this family is a SET domain at the very carboxyl terminus of the protein that is mostly followed by a POST-SET domain. The two proteins that do not fit this bill are EZH1 and EZH2. These have no POST-SET domain and may therefore represent a subset of this family, or a distinct family.

EZH2 is one of the first PKMTs implicated in human cancers^[14,15]. Its expression is highly correlated with the metastasis of various cancers, such as prostate and breast cancers. EZH2 is the enzymatic subunit of polycomb repressive group 2 (PRC2) that methylates histone H3 at K27^[16]. However, the underlying mechanisms of oncogenic effect of EZH2 are not fully understood. It is also unclear whether H3K27 methylation is required for the role of EZH2 in tumorigenesis since EZH2 may have other substrates beyond histone H3^[17]. The only connection to chromatin regulation comes from the fact that EZH2 is part of a deacetylase complex^[18] and it binds a protein homologous to the ATPase SNF2^[19]. The TRX-related MLL proteins on the other hand have been studied extensively, mainly as a consequence of their link to cancer^[20]. Many translocations have been found between MLL and other proteins that play a causative role in leukemia. The most notable translocations (in a chromatin context) are fusions of MLL to the CBP acetylase and to AF10, a protein whose *Drosophila* homologue associates with HP1^[21]. Interestingly, MLL has a bromo-domain (an acetyl-lysine binding structure), a link which may be relevant to CBP. In addition, it has a MBD domain that may allow it to recognize methylated DNA^[22].

DOT1L performs H3K79 methylation, a modification that is associated with transcription elongation. One of its pathological roles is the mis-regulation of the Hox gene expression through interacting with AF9, a fusion partner of MLL. The misregulation can lead to leukemogenesis^[23].

Another promising therapeutic target for cancer is **SMYD2**. A recent paper has shown that SMYD2 is involved in maintaining an undifferentiated status of MLL-AF9-induced acute myeloid leukemia (AML)^[24]. Although the mechanism underlying this leukemia maintenance is unclear, SMYD2 has been shown to methylate p53^[25] and Rb^[26] which are the most important tumor suppressors. In addition, SMYD2 is reportedly overexpressed in esophageal squamous cell carcinoma^[27]. Knockout mice for SMYD2 have been generated^[28]. Future work needs to address whether SMYD2 knockout mice are resistant to tumorigenesis in response to oncogenic insults at various tissues. In addition, the epigenetic role of SMYD2 in cells is largely unknown. One report showed that SMYD2

methylates histone H3K36^[29], while another report suggested that SMYD2 is an H3K4 methyltransferase^[30]. These observations merit further studies to elucidate the mechanisms underlying this dual-substrate specificity.

The SUV39 Family

The most striking feature in this group of proteins is the presence of a PRE-SET domain in all members, and indeed the absence of such a domain in any protein outside the SUV39 family. Given the necessity of this domain for enzymatic activity^[4], it is suggested that this domain may provide specificity necessary for the SET domain to methylate lysine 9 of histone H3, rather than any other lysine. Consistent with this idea, three members of this family (in addition to SUV39H1) have been identified as histone H3 lysine 9 methyltransferases: SUV39H2^[31], G9A^[32] and ESET^[11]. The chromo-domain present in SUV39 is perhaps surprisingly not present in any other SET domain protein. However, the function of this domain is still unclear. Although it does not bind methylated lysine 9, (in contrast to the HP1 chromo-domain), it is still possible that it may recognize other methylated lysines in histones or in other proteins.

SUV39H1 and its homolog SUV39H2 are required for heterochromatin formation. Double knockout of SUV39h1 and SUV39h2 mice are subject to genomic instability^[33]. SUV39h1-dependent senescence has been shown to protect mice from Ras-driven invasive T-cell lymphoma^[34]. Based on these studies, SUV39H1 appears to play a tumor-suppressive function. Controversially, SUV39H1-mediated H3K9me has been linked to gene silencing of the tumor suppressor genes, such as p15INK4B and E-cadherin, in AML^[35]. Therefore, it is highly possible that the default function of SUV39H1 is to maintain genome stability by limiting the acute activation of oncogenes while its dysregulation could cause tumor formation.

G9a and **GLP** belong to one new group of methyltransferases that methylate p53. They have a wide range of biological and pathological functions. From the cancer perspective, G9a and GLP regulate the apoptotic function of p53. The di-methylation of p53-K373 by G9a and GLP decreases the transcriptional activity of p53. Interrogation of Oncomine database reveals that G9a is overexpressed in various tumors, further suggesting its oncogenic effects. Indeed, the overexpression of G9a was shown to increase metastasis and invasion in lung cancer^[36]. However, the ultimate outcome of inhibition of G9a/GLP could be complicated by the fact that they methylate other histone and non-histone substrates^[37]. G9a and GLP are largely responsible for H3K9 mono- and di-methylation. Recently, a distinguishing feature of cancer cell lines, i.e. the loss of G9a-dependent large block of H3K9me2, was observed^[38]. This suggests that the loss of G9a activity or its substrate H3K9me2 confers growth or survival advantage to cancer cells. Therefore, whether the inhibition of G9a/GLP can decrease the growth or increase the apoptosis of cancer cells requires further investigation.

Two proteins, **ESET** and **CLLL8** have what appears to be an expanded SET (ESET) domain. Close analysis reveals that this is as a result of a large insertion of sequence (>300 residues for ESET and >170 for CLLL8) between two highly conserved blocks within the SET domain. In all other proteins, these two sub domains are found directly adjacent to each other. This insertion evidently does not seem to affect either enzymatic activity or specificity. One possible scenario is that this sequence forms a 'hinge domain' that may regulate activity of the enzyme following the binding of proteins. Interestingly, both ESET and CLLL8 contain a MBD domain, a structure that is potentially capable of recognizing methylated

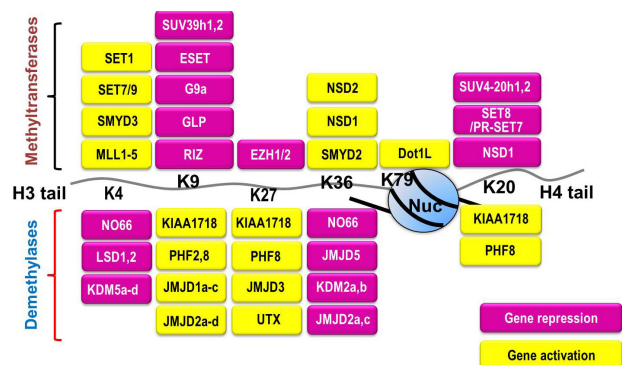


Figure 3 Illustration of the methylase and demethylase at the 6 major lysines of histone tail according to genetic regulatory activity.

DNA^[39]. This structure may be able to direct the lysine methylase activity of ESET to DNA methylated promoters.

LYSINE DEMETHYLATION

Like other protein modifications, lysine methylation is also subject to its counter modification, demethylation (Table 2). For histones, the first reported demethylase is lysine-specific demethylase 1 (LSD1, also known as BHC110)^[40,41]. However, LSD1 can only demethylate mono- or di-methylated lysines. Shortly after the discovery of LSD1, a second family of enzymes, Jmj C-domain containing proteins, was found to have demethylation activity for tri-methylated, as well as mono- and di-methylated lysines^[42]. These enzymes are referred to as protein lysine demethylases (PKDMs). The roles for PKDMs in human diseases, including cancer and neurological disorders, are beginning to be delineated^[43,44]. The clinical relevance of demethylase inhibitors has not been demonstrated using a small molecule; however, a few interesting inhibitors have been disclosed. Demethylases of the LSD1/KDM1 family share some sequence and structural similarities to amine oxidases^[45] and monoamine oxidase. Inhibitors, such as tranlycypromine, have been shown to inhibit LSD 1 by forming a covalent adducts between the flavin cofactor and the inhibitor^[46]. The Jmj C-domain containing demethylase (JHDM) family conforms to a different catalytic mechanism, relying on an active site iron and a 2-oxoglutarate cofactor. Analogs of the 2-oxoglutarate cofactor have been shown to be inhibitors of recombinant enzyme and to increase methylation in cell systems^[47].

The Amine Oxidase Family

LSD1 may serve as a viable target for therapeutic intervention in cancers. It decreases the activity of p53. However, recent studies have discovered some controversial roles of LSD1. LSD1 has been shown to demethylate p53 and decrease the apoptotic effect of p53, suggesting that it can act as an oncogene. Indeed, the overexpression of LSD1 is observed in prostate cancer^[48] and also correlates with poor prognosis of neuroblastoma^[49]. Several reports have also shown that LSD1 has a potential role in the repression of E-cadherin, a molecule mediating the cell–cell junction, and cell migration^[50-52]. The expression of E-cadherin is inversely correlated with metastasis. All of these studies suggest that LSD1 is a putative onco-protein. However, one report proposes that LSD1 can suppress the metastasis of breast cancer by repressing tumor growth factor (TGF)-beta1 signaling^[53]. Unfortunately, the exact cause of this controversy is at present unknown. It is possible that the function of LSD1 is regulated by other binding partners and the ultimate effect is context-dependent. LSD2, the homolog of LSD1, was recently shown to have demethylation activity toward H3K4me2^[54]. So far, there is no report to indicate that LSD2 plays a role in tumorigenesis.

The Jmj-domain Family

JMJD2c (also known as GASC1) is a member of the Jmj C-domain containing protein family. The members of this family, as described above, can demethylate mono-, di- and/or tri-methylated lysines. JMJD2c was characterized as an H3K9me3/me2 demethylase. The overexpression of JMJD2c was observed in esophageal squamous carcinoma^[55]. It regulates androgen receptor-mediated gene expression^[56]. Therefore, it could also play an important role in androgen receptor-dependent prostate cancer, although this hypothesis needs to be tested formally. Very recently, functional interplay between JMJD2c and JAK2, a histone tyrosine kinase^[57], has been revealed in B cell lymphoma and Hodgkin's lymphoma^[58].

These findings provide a mechanistic rationale for testing the co-inhibition of JAK2 and JMJD2c in cancers. It is important that JMJD2c and LSD1 also should cooperate in androgen receptor-regulated gene expression^[56]. These observations fuel the idea of investigating the effects of LSD1 and JMJD2c inhibition in prostate cancer.

The RIZ family

The RIZ (retinoblastoma-interacting zinc finger) protein is the most characterized member of this eponymous family^[59] that contains two other proteins, BLIMP and PFM1. This is a homogeneous family in the sense that they have very similar structural features. They all have a SET domain towards the amino terminus, they have no PRE or POST-SET, and possess a number of zinc-finger motifs clustered towards the carboxyl terminus. Close inspection of the SET domain of these three proteins reveals striking sequence changes in a highly conserved motif (NHSC) which, when mutated, abolishes catalytic activity^[4]. The histidine is invariant in all other SET domains and may represent a catalytic residue. In fact, they are not protein lysine demethylase. However, the changes in this motif in members of the RIZ family suggests that they may possess an altered substrate specificity or indeed may not possess methyltransferase activity and may, therefore, function as antagonists for the active enzymes. The ability of some SET domains to bind histones^[60] may be relevant to this point.

Current information concerning the RIZ family suggests that they are transcriptional regulators controlling differentiation and that they may be involved in cancer. The RIZ protein can bind and stimulate the activity of estrogen receptors^[61] and can repress the activity of SP1-site containing promoters^[62]. Both of these functions rely on an intact SET domain. There are two spliced variants of RIZ one of which, RIZ1, contains the SET domain, whereas the other, RIZ2, does not. Mice lacking RIZ1 develop diffuse large B-cell lymphomas and other tumours. Given that RIZ2 is still expressed in these mice, a role of the SET domain in tumor predisposition is suggested. The BLIMP1 protein (B-cell induced maturation protein 1) is a transcriptional repressor implicated in the silencing of the c-myc, IFN- β and CIITA genes^[63-65]. Little is known about PFM1, but its profile suggests that it is involved in differentiation. Like RIZ, it has alternate mRNA products, one of which probably lacks a SET domain, and maps to a chromosomal location frequently deleted in tumours^[66].

ARGININE METHYLATION

Apart from lysine methylation, arginine (R) methylation has been also known to play certain roles in cancer (Table 3). The history of arginine methylation was recently surveyed^[67]. Several arginine residues are also modified by methylation. These include, R2, R8, R17, and R26 of histone H3, and R3 of histone H4. Arginine residues may undergo mono-methylation, symmetric di-methylation, or asymmetric di-methylation. There are five known arginine methyltransferases that have a highly conserved catalytic domain. PRMT1, PRMT3 and PRMT4/CARM1 are classified as Class I enzymes as they can catalyze the formation of asymmetric di-methylated arginine whereas PRMT5/JBP1 is classified as a class II enzyme as it catalyzes symmetric di-methylation. The PRMT2 protein has not yet been established as an enzyme^[68]. H3R2 is asymmetrically di-methylated by CARM1/PRMT4^[69] and PRMT6^[70]. H3R8 is methylated by PRMT5^[71], while H3R17 and H3R26 are asymmetrically di-methylated by CARM1/

Table 3 Summary of histone arginine methyltransferases and demethylases/deiminases.

Mark	Methylation	Demethylation	Catalytic specificity of the demethylase
H3R2	CARM1	PAD4/PADI4	me1/me0→citrulline
	PRMT6	JMJD6	me2→me1
H3R8	PRMT5		
H3R17	CARM1		
H3R26	CARM1		
H4R3	PRMT1	PAD4/PADI4	me1/me0→citrulline
	PRMT5	JMJD6	me2(s)/me2(a)/me1→me0

Adapted from Kampranis SC *et al* Adv Cancer Res 2009.

PRMT4, which also methylates H3R2^[69]. Finally, H4R3 is mono-methylated by PRMT172 and di-methylated, both symmetrically and asymmetrically by PRMT5^[73]. The methylation of specific arginine residues contributes to the regulation of cell fate. For example, ectopic expression of CARM1 in mouse blastomers increases the levels of arginine methylation and promotes the dramatic upregulation of the pluripotency genes NANOG and SOX2. This, in turn, promotes the cycling of pluripotent cells and the expansion of the inner cell mass of the blastocyst^[74].

The molecular mechanisms by which arginine methylation contributes to chromatin structure and transcriptional regulation are not yet clear. However, it has been shown that arginine methylation may regulate the modification or recognition of neighboring histone residues. Thus, it has been shown that methylation of H3R2 prevents the tri-methylation of H3K4 and vice versa^[70]. Furthermore, it has been shown that asymmetric methylation of H3R2 inhibits the association of the TFIID subunit TAF3 with H3K4me3^[75].

Arginine methylation of histones

Methylation of histones by arginine methyltransferases has been known for some time, but only recently has this modification been validated as a functional event because the methylation of arginine on histones was not easily detectable by standard bulk-histone sequencing. A breakthrough came when CARM1 (now known also as PRMT4) was isolated as a two-hybrid interacting partner of GRIP1, a p160 family co-activator of nuclear hormone receptors^[76]. CARM1/PRMT4 acted as a co-activator of nuclear receptor activity using an arginine methyltransferase domain capable of methylating specifically histone H3 *in vitro*. This was the first clue that methylation of histones at arginine may be a stimulating event for transcription. Confirmation of this has come recently using antibodies that specifically recognize the major methylation site by CARM1, Arg17 of histone H3. These antibodies, when used in chromatin immunoprecipitation assays, identify the presence of a methyl group on Arg17 only when nuclear receptor regulated promoters are active^[77,78]. Arginine methylation thus represents a histone modification that correlates with the active state of transcription, much like acetylation. Interestingly, CARM1/PRMT5 co-operates with the p300 acetylase to stimulate transcription by nuclear receptors^[79], raising the possibility of cross talk between methylation and acetylation.

The PRMT1 methyltransferase has also been shown to mediate methylation of histones *in vivo*. Antibodies raised against methylated Arg3 of H4 (the site of PRMT1 methylation) recognizes bulk-purified histones^[72,80]. That PRMT1 is the major Arg3 methyltransferase is established from the fact that Arg3 is not methylated in PRMT1^{-/-} cell lines. Target promoters at which Arg3 of H4 is methylated have not yet been identified. However, a good candidate would be nuclear receptor stimulated genes as PRMT1 is found associated with p160 co-activators and can co-operate with CARM1/PRMT4 to stimulate

such genes^[81]. As with PRMT4/CARM1, methylation by PRMT1 has been shown to have a functional connection to acetylation on histone H4. Wang *et al*^[72] showed that methylation of Arg3 can augment the subsequent acetylation of H4, indicating once again the cross talk between two stimulating modifications.

Arginine methylation within non-histone proteins

Although this review has a focus on methylation of histones no review on arginine methyltransferases can be complete without a mention of non-histone methylated proteins, since these have been the primary focus of research since the discovery of methylation over thirty years ago. These studies may give clues to the function of histone methylation. Well-studied substrates include heterogeneous nuclear ribonucleoproteins hnRNPs, which are involved in pre-mRNA splicing and RNA transport. Methylation in these proteins can occur at RGG. In the case of a yeast hnRNP, Np13p, methylation affects its nuclear-cytoplasmic localization^[82,83]. Other methylated substrates include snRNPs (SmD1 and SmD3) which are methylated at GRG motifs^[84] and myelin basic protein^[68].

Methylation of two non-histone proteins links arginine methylation to signal transduction pathways. The first is **Sam68**, a substrate for the Src kinase, whose methylation within a proline rich motif leads to diminished binding to SH3 containing signaling proteins^[85]. The second substrate is **STAT1**, a transcription factor involved in interferon-induced signal transduction. Methylation of STAT1 takes place on Arg 31 and is mediated by PRMT1. This methylation augments the transcriptional activation capacity of STAT1, at least partly, by inhibiting its interaction with a negative regulator PIAS1^[86]. A modification of the CBP/p300 acetylase was recently discovered which directly links arginine methylation to transcription regulation via a non-histone protein^[87]. The CARM1 protein methylates CBP/p300 near the KIX domain which is necessary for the insertion for CBP/p300 with the CREB transcription factor. The net result is that methylation prevents activation by CREB through displacing CBP/p300.

PROTEIN METHYLATION AS IMPLICATED TARGETS IN CANCER

The central role of epigenetic abnormalities in tumor evolution has prompted efforts for the development of therapeutic approaches that aim to reverse epigenetic changes. The cytosine analogs 5-azacytosine (azacytidine) and 2-deoxy-5-azacytidine (decitabine) are currently the most advanced drugs for epigenetic cancer therapies. These DNA methyltransferase inhibitors have shown remarkable efficiency in the derepression of epigenetically silenced tumor suppressor genes^[88,89]. Here we will focus on the clinical implications and the recent progress in the discovery of selective PKMT antagonists in cancers. It is worth pointing out that PKMTs play important roles in other biological processes including developmental biology and stem cell differentiation.

Antagonist of protein lysine methyltransferase

Since Greiner *et al*^[90] discovered the first selective, non-nucleoside inhibitor of recombinant *Drosophila* Su(var)3-9, **chaetocin** in 2005, the antagonist discovery effort has quickly gained momentum and a number of new antagonists have emerged. Herein, we describe selective PKMT antagonists with an emphasis on most recently discovered compounds. PRMT antagonists were recently surveyed and are not included in this review^[91]. **Chaetocin** was found to antagonize SUV39H1, the human ortholog of *Drosophila* Su(var)3-9,

as well as other H3K9 PKMTs, including *Neurospora* DIM5 and mouse G9a. It was selective over non-H3K9 PKMTs, such as H3K27 PKMT dE(z) complex, H3K4 PKMT SET7/9, and H4K20 PKMT SETD8^[90]. Interestingly, a total synthesis report found natural (+)- and synthetic (-)-**chaetocin** to be equipotent against G9a^[92]. In addition, it was found that **chaetocin** inhibited thioredoxin reductase^[93]. Like other members of the epidithiodiketopiperazine class^[94], chaetocin is cytotoxic. Despite its cytotoxicity, it was reported that chaetocin-treated *Drosophila* SL-2 cells at an antagonist concentration of 0.5 μ M showed marked reduction in cellular levels of di- and trimethylated H3K9 without apparent changes in cellular levels of methylated lysines (such as H3K27, H3K36, H3K79, and H3K4)^[90].

Discovery of **BIX01294**, the first selective small molecule inhibitor for G9a and GLP, by Jenuwein and co-workers^[95] was an important advance in the PKMT antagonists' discovery field, as this compound was the first PKMT antagonist that blocks protein-protein interactions^[96]. **BIX01294** had good in vitro potency against G9a and GLP and was selective over other H3K9 PKMTs (SUV39H1 and SETDB1), H3K4 PKMT SET7/9^[96]. Unfortunately **BIX01294** was toxic in cellular assays at concentrations above 4.1 μ M. Mechanistically, unlike chaetocin, **BIX01294** did not inhibit G9a in a SAM-competitive manner but rather occupied the histone peptide binding pocket, which was evidenced by the X-ray crystal structure of BIX01294 and GLP in the presence of SAH (S-adenosyl-L-homocysteine)^[95,96]. This crystal structure revealed that **BIX01294** did not bind in the SAM-binding site nor did it interact with the lysine binding channel^[96].

By elaborating the 7-methoxy moiety of the quinazoline template, it was discovered that a series of new analogs interacted with the lysine-binding channel, including **UNC0224**, a 7-fold more potent G9a inhibitor in the G9a ThioGlo assay when compared with BIX01294^[97,98]. A high-resolution (1.7Å) X-ray co-crystal structure of G9a and UNC0224 (PDB: 3K5K) showed that the 7-dimethylamino propoxy side chain of UNC0224 only partially occupied the lysine binding channel of G9a^[95,98], and thus space remained to accommodate a longer side chain or larger aminocapping group.

The most potent G9a antagonist to date, **UNC0321** was a result of further side chain manipulations^[99]. Because **UNC0321** likely reached the detection limits of the biochemical assays, Morrison Ki values were determined using an endoprotease-coupled microfluidic capillary electrophoresis assay^[100]. **UNC0321** (Morrison Ki = 63 pM) was about 40-fold more potent than UNC0224 (Morrison Ki = 2.6 nM) and 250-fold more potent than BIX01294 (Morrison Ki = 16 nM)^[99]. While highly potent in biochemical assays, **UNC0321** was less potent in cellular assays in comparison with BIX01294, prompting the development of analogs with higher cellular potency.

UNC0638, specifically designed to increase lipophilicity and cell membrane permeability while maintaining high in vitro potency, was indeed found to have excellent in vitro potency (Morrison Ki G9a = 3.7 nM; Ki = 3.0 nM) and was > 100-fold selective over a wide range of epigenetic and non-epigenetic targets^[101]. Mechanism of action studies revealed that **UNC0638** was competitive with the peptide substrate and non-competitive with the co-factor SAM. The MOA findings were confirmed by X-ray crystal structure of the G9a-UNC0638-SAH complex (2.56Å resolution; PDB: 3R1W). The combination of high potency, excellent selectivity, low cell toxicity and robust on-target activities in cells makes **UNC0638** an excellent chemical probe of G9a/GLP for cellular studies. Most recently, **UNC0646** and **UNC0631**, close analogs of UNC0638, were reported to have comparable cellular potency and toxicity and could serve as alternative tool compounds for investigating specific cellular

systems^[102]. For example, **UNC0646** had an outstanding toxicity/function ratio in MCF7 (470), 22RV1 (510), and IMR90 (360) cells, making this compound potentially more useful for studying G9a biology in these specific cell lines^[102].

Chang *et al.*^[103] also developed a potent G9a and GLP antagonist **E72** based on the quinazoline template with binding affinities determined by isothermal titration calorimetry (Kd GLP = 136 nM; G9a = 164 nM). A brief selectivity study showed **E72** was inactive against SUV39H2 with no inhibition at 5 μ M^[103]. The X-ray co-crystal structure of the GLP-E72 complex in the presence of SAH (2.19Å, PDB: 3MO5) showed that E72 analogous to UNC0224 with G9a occupies both the surface of the peptide binding groove and the lysine binding channel. In three separate cell types, E72 was significantly less toxic than BIX01294 at compound concentrations of 10 μ M and was able to reactivate K-ras-mediated epigenetic silencing of the Fas gene in NIH 3T3 cells^[103].

Most recently, Daigle *et al.*^[104] discovered a highly potent and selective SAM-competitive DOT1L antagonist **EPZ004777** (Ki = 0.3 nM), which is a co-factor product (SAH) mimic. EPZ004777 was > 1000-fold selective for DOT1L over CRM1, EHMT2, EZH1, EZH2, PRMT1s, PRMT5, PRMT8, SETD7, and WHSC1, all of which are SAM-utilizing methyltransferases. **EPZ004777** was found to selectively kill off cells bearing MLL translocation. In addition, in vivo administrations of **EPZ004777** led to extension of survival in a mouse MLL xenograft model^[104]. Subsequently, Yao and co-workers^[105] showed that protecting the N6 position in SAH does not affect binding to DOT1L, but seems to instill selectivity against CARM1, PRMT1, G9a, and SUV39H2. The best compound in their series, compound 1 had an IC₅₀ of 0.11 μ M against DOT1L and was proposed to be capable of covalently binding to the histone^[105].

Recently Ferguson *et al.*^[106] described a potent antagonist of SMYD2, **AZ505** (Ki = 0.30 μ M) identified by high throughput screening. As seen from its crystal structure in complex with SMYD2, **AZ505** occupies the peptide binding groove and is peptide substrate competitive. Furthermore, this compound was shown to be > 83 fold selective for SMYD2 over a panel of PKMTs (SMYD3, DOT1L, EZH2, GLP, G9a, and SET7/9)^[106]. Given the broad roles of SMYD2 in cancers, it will be interesting to test the effect of this inhibitor and its analogs in cancer cells.

Antagonists of protein lysine demethylase

Several groups have investigated polyamine analogs for their ability to inhibit recombinant LSD1 in vitro and in vivo. Among these compounds, the best were **polyamines 2 and 3**, which antagonized LSD1 activity by 85% and 82%, respectively, at 10 μ M^[107,108]. Subsequently, Huang *et al.*^[109] investigated polyamines, such as PG11150, as antagonists of LSD1. It was found that colorectal cancer cells treated with polyamine PG11150 should display re-expression of multiple aberrantly silenced tumor suppressor genes. In addition, PG11144 the trans-isomer of PG11150, displays a marked decrease in tumor growth and increases H3K4me2 levels in the mouse xenograft model, without significant overall toxicity, when administered in combination treatments alongside a known DNMT inhibitor 5-azacytidine. Interestingly, when used alone PG11144 displayed antitumor activity, while polyamine 2 did not produce a similar effect without the accompanying DNMT inhibitor. However, selectivity of these inhibitors for LSD1 over monoamine oxidase (MAO)-A, MAO-B and the newly discovered LSD2 is yet to be addressed.

A high degree of homology exists between the catalytic sites of MAO-A, B, and LSD1; thus, one might reasonably expect that many of the existing **monoamine oxidase inhibitors** could antagonize

LSD1. Recognizing this, researchers tested a known non-selective MAO inhibitor PCPA (trans-2-phenylcyclopropylamine) and indeed found it to have LSD1 inhibitory activity ($K_i = 357 \mu\text{M}$; LSD1)^[110,111]. MAO and LSD inhibition is fairly insensitive to stereochemistry as has been shown^[112,113]; thus (+)-racemates of trans-tranylcypromine and its derivatives are commonly used for testing and in the clinic^[111]. Inhibition of LSD1 by tranylcypromine has been shown to proceed via ring-opening of the cyclopropyl moiety followed by formation of a covalent adduct with the C(4) of the FAD co-factor. PCPA displays no apparent selectivity for LSD1 over MAO-A, or MAO-B, which prompted a number of groups to investigate LSD1-selective derivatives based on its core structure.

Antagonists based on the **tranylcypromine** scaffold include compound 4 ($K_i=8 \mu\text{M}$) discovered in 2008^[114], S2101 ($K_i=0.6 \mu\text{M}$, LSD1; $K_i=110 \mu\text{M}$, MAO-A; $K_i=17 \mu\text{M}$, MAO-B) in 2010^[115], compound 5 ($K_i=1.9 \mu\text{M}$, LSD1; $K_i=290 \mu\text{M}$, MAO-A) in 2009^[116], and compound 6 ($K_i=6 \mu\text{M}$, LSD1) in 2010^[112]. These inhibitors affect histone H3K9 and H3K4 methylation levels in cells, mediated by the inhibition of LSD1-catalyzed demethylation.

The functional interaction between LSD1 and HDAC has been reported by Lee *et al.*^[117]; they found that LSD1 and HDAC enhanced the activity of each other. Given the fact that HDAC inhibitors have already been approved for cutaneous T-cell lymphoma treatment, this molecular mechanism raises an interesting hypothesis that LSD1 and HDAC inhibitors could cooperatively inhibit tumorigenesis.

JMJD2 demethylases are antagonized by **analogs of the co-factor 2-OG**, including N-oxalylglycine (NOG), pyridine decarboxylases, and the related bipyridyl derivative 12. Other chemotypes that are also presumed to bind to the active site Fe (II) include catechols, hydroxamic acids, and tri-carboxylic acid cycle intermediates, such as succinate and fumarate^[118-120]. Antagonist compound 13 and 14, designed on the basis of the crystal structure of NOG in complex with JMJD2A (PDB ID 2OQ6), are equipped with an appendage intended to engage a large subpocket adjacent to the active site. Hamada *et al.*^[121] have demonstrated inhibition of JMJD2A, 2C and 2D activity by 14 and its analogs in vitro and in vivo. Importantly, only the methyl ester prodrug of 14 was active in cellular assays presumably due to poor cell permeability of the free acid-containing analogs. Rose *et al.*^[122] produced a crystal structure of an analog of their best antagonist, compound 13 in complex with JMJD2A (PDB ID 2WWJ) confirming the predicted binding mode for their N-oxalyl-D-tyrosinyl derivatives. Compound 13 exhibited some selectivity against prolyl hydroxylase domain-containing protein 2 (PHD2) in biochemical assays, while 14 appeared to inhibit other Fe(II)/ α -ketoglutarate-dependent oxygenases indiscriminantly^[121]. Interestingly, while NOG itself selectively inhibits PHD1/2 over JMJD2C/2A, its analog 15 is selective for JMJD2C and JMJD2A^[123]. This selectivity is presumably based on the presence of a methylene group next to the carbonyl of the hydroxamate moiety, and its affinity for JMJD2 is dependent on the length of the linker leading to the tertiary amino group. Another JMJD-selective inhibitor was recently discovered^[124]. Compound 16 was designed to incorporate both a substrate mimic and a methyllysine mimic. This inhibitor is more than 9000-fold selective for the Jmj C-domain containing enzymes over PHDs. Its methyl ester prodrug methylstat is cell active and may have potential for anticancer chemotherapy^[124].

A high-throughput assay based on time-resolved fluorescence resonance energy transfer was reported recently to screen the antagonists for LSD1 and JMJD2c^[125]. Numerous antagonists for these two enzymes have been identified through the assay. Because LSD1 and JMJD2c cooperate in regulating the gene expression

in prostate cancer, these inhibitors will be extremely valuable for testing the synergistic effects of co-inhibition of these two enzymes in cancer. Because of the common mechanism underlying the demethylation reaction of JmjC domain-containing proteins, the specificity of these inhibitors needs to be rigorously tested in the future.

CONCLUDING REMARKS

Cancer is a genetic disease. Emerging evidence, however, is challenging this concept by showing that non-genetic heritable changes affecting chromatin structure and gene expression may play an equally important role in the biology of the cancer cell. Based on this evidence, tumor development and evolution depends on the combination of genetic and epigenetic changes. Actually, the two of courses are not independent. Thus, epigenetic silencing of genes involved in the response to DNA damage may give rise to genetic instability and the accumulation of genetic changes in cancer cells. Genetic changes affecting genes involved in epigenetic regulation, on the other hand, may alter the epigenome of the cancer cell.

In this review, we have focused on one part of histone methylation, namely the protein methylation and demethylation especially in terms of associated enzymes that regulate the status of methylation of histones. However, it is clear that epigenetic mechanisms are interdependent and integrated into the regulatory machinery of the cell. These epigenetic regulators can be modified by the complex signaling pathways that become deregulated in the cancer cell. Future studies on the cancer epigenome and its regulation will redefine our understanding of the cancer cell and may reveal new targets for therapeutic intervention.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

- 1 Kouzarides T. (2007). Chromatin modifications and their function. *Cell* 128, 693-705.
- 2 Okano M, Bell D.W., Haber D.A., Li E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99, 247-257.
- 3 Strahl B.D., Allis C.D. (2000). The language of covalent histone modifications. *Nature* 403, 41-45.
- 4 Rea S., Eisenhaber F., O'Carroll D., Strahl B.D., Sun Z.W., Schmid M., Opravil S., Mechtler K., Ponting C.P., Allis C.D., Jenuwein T. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593-599.
- 5 Tachibana M., Sugimoto K., Nozaki M., Ueda J., Ohta T., Ohki M., Fukuda M., Fukuda M., Takeda N., Niida H., Kato H., Shinkai Y. (2002). G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev* 16, 1779-1791.
- 6 Tachibana M., Ueda J., Fukuda M., Takeda N., Ohta T., Iwanari H., Sakihama T., Kodama T., Hamakubo T., Shinkai Y. (2005). Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-

- K9. *Genes Dev* 19, 815–826.
- 7 Milne T.A., Briggs S.D., Brock H.W., Martin M.E., Gibbs D., Allis C.D., Hess J.L. (2002). MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell* 10, 1107–1117.
 - 8 Cao R., Wang L., Wang H., Xia L., Erdjument-Bromage H., Tempst P., Jones R.S., Zhang Y. (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 298, 1039–1043.
 - 9 Strahl B.D., Grant P.A., Briggs S.D., Sun Z.W., Bone J.R., Caldwell J.A., Mollah S., Cook R.G., Shabanowitz J., Hunt D.F., Allis C.D. (2002). Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol Cell Biol* 22, 1298–1306.
 - 10 Wang H., Cao R., Xia L., Erdjument-Bromage H., Borchers C., Tempst P., Zhang Y. (2001). Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol Cell* 8, 1207–1217.
 - 11 van Leeuwen F., Gafken P.R., Gottschling D.E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 109, 745–756.
 - 12 Feng Q., Wang H., Ng H.H., Erdjument-Bromage H., Tempst P., Struhl K., Zhang Y. (2002). Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr Biol* 12, 1052–1058.
 - 13 Nishioka K., Rice J.C., Sarma K., Erdjument-Bromage H., Werner J., Wang Y., Chuikov S., Valenzuela P., Tempst P., Steward R., Lis J.T., Allis C.D., Reinberg D. (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol Cell* 9, 1201–1213.
 - 14 Varambally S., Dhanasekaran S.M., Zhou M., Barrette T.R., Kumar-Sinha C., Sanda M.G., Ghosh D., Pienta K.J., Sewalt R.G., Otte A.P., Rubin M.A., Chinnaiyan A.M. (2002). The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419, 624–629.
 - 15 Kleer C.G., Cao Q., Varambally S., Shen R., Ota I., Tomlins S.A., Ghosh D., Sewalt R.G., Otte A.P., Hayes D.F., Sabel M.S., Livant D., Weiss S.J., Rubin M.A., Chinnaiyan A.M. (2003). EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA* 100, 11606–11611.
 - 16 Margueron R., Reinberg D. (2011). The Polycomb complex PRC2 and its mark in life. *Nature* 469, 343–349.
 - 17 Su I.H., Dobenecker M.W., Dickinson E., Oser M., Basavaraj A., Marqueron R., Viale A., Reinberg D., Wülfing C., Tarakhovskiy A. (2005). Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell* 121, 425–436.
 - 18 van der Vlag J., Otte A.P. (1999). Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. *Nat Genet* 23, 474–478.
 - 19 Cardoso C., Timsit S., Villard L., Khrestchatsky M., Fontes M., Colleaux L. (1998). Specific interaction between the XNP/ATR-X gene product and the SET domain of the human EZH2 protein. *Hum Mol Genet* 7, 679–684.
 - 20 Caldas C., Aparicio A. (1999). Cell memory and cancer — the story of the trithorax and Polycomb group genes. *Cancer Metastasis Rev* 18, 313–329.
 - 21 Linder B., Gerlach N., Jäckle H. (2001). The Drosophila homolog of the human AF10 is an HP1-interacting suppressor of position effect variegation. *EMBO Reports* 2, 211–216.
 - 22 Caldas C., Myeong-Hee K., MacGregor A., Cain D., Aparicio S., Wiedemann L.M. (1998). Isolation and characterization of a Puffer fish MLL (mixed lineage leukaemia)-like gene (fMLL) reveals evolutionary conservation in vertebrate genes related to Drosophila trithorax. *Oncogene* 16, 3233–3241.
 - 23 Okada Y., Feng Q., Lin Y., Jiang Q., Li Y., Coffield V.M., Su L., Xu G., Zhang Y. (2005). hDOT1L links histone methylation to leukemogenesis. *Cell* 121, 167–178.
 - 24 Zuber J., Rappaport A.R., Luo W., Wang E., Chen C., Vaseva A.V., Shi J., Weissmueller S., Fellmann C., Taylor M.J., Weissenboeck M., Graeber T.G., Kogan S.C., Vakoc C.R., Lowe S.W. (2011). An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. *Genes Dev* 25, 1628–1640.
 - 25 Huang J., Perez-Burgos L., Placek B.J., Sengupta R., Richter M., Dorsey J.A., Kubicek S., Opravil S., Jenuwein T., Berger S.L. (2006). Repression of p53 activity by Smyd2-mediated methylation. *Nature* 444, 629–632.
 - 26 Saddic L.A., West L.E., Aslanian A., Yates JR 3rd, Rubin S.M., Gozani O., Sage J. (2010). Methylation of the retinoblastoma tumor suppressor by SMYD2. *J Biol Chem* 285, 37733–37740.
 - 27 Komatsu S., Imoto I., Tsuda H., Kozaki K., Muramatsu T., Shimada Y., Aiko S., Yoshizumi Y., Ichikawa D., Otsuji E., Inazawa J. (2009). Overexpression of SMYD2 relates to tumor cell proliferation and malignant outcome of esophageal squamous cell carcinoma. *Carcinogenesis* 30, 1139–1146.
 - 28 Diehl F., Brown M.A., van Amerongen M.J., Novoyatleva T., Wietelmann A., Harriss J., Ferrazzi F., Böttger T., Harvey R.P., Tucker P.W., Engel F.B. (2010). Cardiac deletion of Smyd2 is dispensable for mouse heart development. *PLoS One* 5, e9748.
 - 29 Brown M.A., Sims R.J. 3rd, Gottlieb P.D., Tucker P.W. (2006). Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. *Mol Cancer* 5, 26.
 - 30 Abu-Farha M., Lambert J.P., Al-Madhoun A.S., Elisma F., Skerjanc I.S., Figeys D. (2008). The tale of two domains: proteomics and genomics analysis of SMYD2, a new histone methyltransferase. *Mol Cell Proteomics* 7, 560–572.
 - 31 Strahl B.D., Grant P.A., Briggs S.D., Sun Z.W., Bone J.R., Caldwell J.A., Mollah S., Cook R.G., Shabanowitz J., Hunt D.F., Allis C.D. (2002). Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol Cell Biol* 22, 1298–1306.
 - 32 Wang H., Cao R., Xia L., Erdjument-Bromage H., Borchers C., Tempst P., Zhang Y. (2001). Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol Cell* 8, 1207–1217.
 - 33 Peters A.H., O’Carroll D., Scherthan H., Mechtler K., Sauer S., Schofer C., Weipoltshammer K., Pagani M., Lachner M., Kohlmaier A., Opravil S., Doyle M., Sibilia M., Jenuwein T. (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* 107, 323–337.
 - 34 Braig M., Lee S., Loddenkemper C., Rudolph C., Peters A.H., Schlegelberger B., Stein H., Dörken B., Jenuwein T., Schmitt C.A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 436, 660–665.
 - 35 Lakshminikuttamma A., Scott S.A., DeCoteau J.F., Geyer C.R. (2010). Reexpression of epigenetically silenced AML tumor suppressor genes by SUV39H1 inhibition. *Oncogene* 29, 576–588.
 - 36 Chen M.W., Hua K.T., Kao H.J., Chi C.C., Wei L.H., Johansson G., Shiah S.G., Chen P.S., Jeng Y.M., Cheng T.Y., Lai T.C., Chang J.S., Jan Y.H., Chien M.H., Yang C.J., Huang M.S., Hsiao M., Kuo M.L. (2001). H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. *Cancer Res* 70, 7830–7840.
 - 37 Rathert P., Dhayalan A., Murakami M., Zhang X., Tamas R., Jurkowska R., Komatsu Y., Shinkai Y., Cheng X., Jeltsch A. (2008). Protein lysine methyltransferase G9a acts on nonhistone targets. *Nat Chem Biol* 4, 344–346.
 - 38 Wen B., Wu H., Shinkai Y., Irizarry R.A., Feinberg A.P. (2009). Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet* 41, 246–250.
 - 39 Ng H.H., Bird A. (1999). DNA methylation and chromatin modification. *Curr Opin Genet Dev* 9, 158–163.

- 40 Shi Y, Lan F, Matson C, Mulligan P, Whetstine J.R., Cole P.A., Casero R.A. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119, 941–953.
- 41 Hakimi M.A., Dong Y., Lane W.S., Speicher D.W., Shiekhattar R. (2003). A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes. *J Biol Chem* 278, 7234–7239.
- 42 Tsukada Y, Fang J, Erdjument-Bromage H, Warren M.E., Borchers C.H., Tempst P, Zhang Y. (2006). Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 439, 811–816.
- 43 Shi Y. (2007). Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* 8, 829–833.
- 44 Schulte J.H., Lim S., Schramm A., Friedrichs N., Koster J., Versteeg R., Ora I., Pajtlar K., Klein-Hitpass L., Kuhfittig-Kulle S., Metzger E., Schüle R., Eggert A., Buettner R., Kirfel J. (2009). Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 69, 2065–2071.
- 45 Yang M., Culhane J.C., Szewczuk L.M., Jalili P., Ball H.L., Machius M., Cole P.A., Yu H. (2007). Structural basis for the inhibition of the LSD1 histone demethylase by the antidepressant trans-2-phenylcyclopropylamine. *Biochemistry* 46, 8058–8065.
- 46 Ueda R., Suzuki T., Mino K., Tsumoto H., Nakagawa H., Hasegawa M., Sasaki R., Mizukami T., Miyata N. (2009). Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J Am Chem Soc* 131, 17536–17537.
- 47 Hamada S., Kim T.D., Suzuki T., Itoh Y., Tsumoto H., Nakagawa H., Janknecht R., Miyata N. (2009). Synthesis and activity of Noxalylglycine and its derivatives as Jumonji C-domain containing histone lysine demethylase inhibitors. *Bioorg Med Chem Lett* 19, 2852–2855.
- 48 Kahl P., Gullotti L., Heukamp L.C., Wolf S., Friedrichs N., Vorreuther R., Sollleder G., Bastian P.J., Ellinger J., Metzger E., Schüle R., Buettner R. (2006). Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence. *Cancer Res* 66, 11341–11347.
- 49 Schulte J.H., Lim S., Schramm A., Friedrichs N., Koster J., Versteeg R., Ora I., Pajtlar K., Klein-Hitpass L., Kuhfittig-Kulle S., Metzger E., Schüle R., Eggert A., Buettner R., Kirfel J. (2009). Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 69, 2065–2071.
- 50 Shi Y., Sawada J., Sui G., Affarel B., Whetstine J.R., Lan F., Ogasawa H., Luke M.P., Nakatani Y., Shi Y. (2003). Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* 422, 735–738.
- 51 Lin T., Ponn A., Hu X., Law B.K., Lu J. (2010). Requirement of the histone demethylase LSD1 in Snail-mediated transcriptional repression during epithelial–mesenchymal transition. *Oncogene* 29, 4896–4904.
- 52 Lin Y., Wu Y., Li J., Dong C., Ye X., Chi Y.I., Evers B.M., Zhou B.P. (2010). The SNAG domain of Snail1 functions as a molecular hook for recruiting lysine-specific demethylase 1. *EMBO J* 29, 1803–1816.
- 53 Wang Y., Zhang H., Chen Y., Sun Y., Yang F., Yu W., Liang J., Sun L., Yang X., Shi L., Li R., Li Y., Zhang Y., Li Q., Yi X., Shang Y. (2009). LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* 138, 660–672.
- 54 Fang R., Barbera A.J., Xu Y., Rutenberg M., Leonor T., Bi Q., Lan F., Mei P., Yuan G.C., Lian C., Peng J., Cheng D., Sui G., Kaiser U.B., Shi Y., Shi Y.G. (2010). Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol Cell* 39, 222–233.
- 55 Cloos P.A., Christensen J., Agger K., Maiolica A., Rappsilber J., Antal T., Hansen K.H., Helin K. (2006). The putative oncogene GASC1 demethylates tri and dimethylated lysine 9 on histone H3. *Nature* 442, 307–311.
- 56 Wissmann M., Yin N., Muller J.M., Greschik H., Fodor B.D., Jenuwein T., Vogler C., Schneider R., Günther T., Buettner R., Metzger E., Schüle R. (2007). Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nat Cell Biol* 9, 347–353.
- 57 Dawson M.A., Bannister A.J., Gottgens B., Foster S.D., Bartke T., Green A.R., Kouzarides T. (2009). JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature* 461, 819–822.
- 58 Rui L., Emre N.C., Kruhlak M.J., Chung H.J., Steidl C., Slack G., Wright G.W., Lenz G., Ngo V.N., Shaffer A.L., Xu W., Zhao H., Yang Y., Lamy L., Davis R.E., Xiao W., Powell J., Maloney D., Thomas C.J., Möller P., Rosenwald A., Ott G., Muller-Hermelink H.K., Savage K., Connors J.M., Rimsza L.M., Campo E., Jaffe E.S., Delabie J., Smeland E.B., Weisenburger D.D., Chan W.C., Gascoyne R.D., Levens D., Staudt L.M. (2010). Cooperative epigenetic modulation by cancer amplicon genes. *Cancer Cell* 18, 590–605.
- 59 Buyse I.M., Shao G., Huang S. (1995). The retinoblastoma protein binds to RIZ, a zinc-finger protein that shares an epitope with the adenovirus E1A protein. *Proc Natl Acad Sci USA* 92, 4467–4471.
- 60 Katsani K.R., Arredondo J.J., Kal A.J., Verrijzer C.P. (2001). A homeotic mutation in the trithorax SET domain impedes histone binding. *Gen Dev* 15, 2197–2202.
- 61 Steele-Perkins G., Fang W., Yang X.H., Van Gele M., Carling T., Gu J., Buyse I.M., Fletcher J.A., Liu J., Bronson R., Chadwick R.B., de la Chapelle A., Zhang X., Speleman F., Huang S. (2001). Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein methyltransferase superfamily. *Gen Dev* 15, 2250–2262.
- 62 Xie M., Shao G., Buyse I.M., Huang S. (1997). Transcriptional repression mediated by the PR domain zinc finger gene RIZ. *J Biol Chem* 272, 26360–26366.
- 63 Lin Y., Wong K., Calame K. (1997). Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. *Science* 276, 596–599.
- 64 Ren B., Chee K.J., Kim T.H., Maniatis T. (1999). PRD1-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. *Gen Dev* 13, 125–137.
- 65 Piskurich J.F., Lin K.I., Lin Y., Wang Y., Ting J.P., Calame K. (2000). BLIMP-1 mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nat Immunol* 1, 526–632.
- 66 Yang X.H., Huang S. (1999). PFM1 (PRDM4), a new member of the PR-domain family, maps to a tumor suppressor locus on human chromosome 12q23–q24.1. *Genomics* 61, 319–325.
- 67 Huang J., Berger S.L. (2008). The emerging field of dynamic lysine methylation of non-histone proteins. *Curr Opin Genet Dev* 18, 152–158.
- 68 McBride A.E., Silver P.A. (2001). State of the Arg: protein methylation at arginine comes of age. *Cell* 106, 5–8.
- 69 Schurter B.T., Koh S.S., Chen D., Bunick G.J., Harp J.M., Hanson B.L., Henschen-Edman A., Mackay D.R., Stallcup M.R., Aswad D.W. (2001). Methylation of histone H3 by coactivator-associated arginine methyltransferase 1. *Biochemistry* 40, 5747–5756.
- 70 Guccione E., Bassi C., Casadio F., Martinato F., Cesaroni M., Schuchlantz H., Luscher B., Amati B. (2007). Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. *Nature* 449, 933–937.
- 71 Dacwag C.S., Ohkawa Y., Pal S., Sif S., Imbalzano A.N. (2007). The protein arginine methyltransferase Prmt5 is required for myogenesis because it facilitates ATP-dependent chromatin remodeling. *Mol Cell Biol* 27, 384–394.
- 72 Wang H., Huang Z.Q., Xia L., Feng Q., Erdjument-Bromage H., Strahl B.D., Briggs S.D., Allis C.D., Wong J., Tempst P., Zhang Y. (2001). Methylation of histone H4 at arginine 3 facilitating tran-

- scriptional activation by nuclear hormone receptor. *Science* 293, 853–857.
- 73 Ancelin K., Lange U.C., Hajkova P., Schneider R., Bannister A.J., Kouzarides T., Surani M.A. (2006). Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat Cell Biol* 8, 623–630.
- 74 Torres-Padilla M.E., Parfitt D.E., Kouzarides T., Zernicka-Goetz M. (2007). Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature* 445, 214–218.
- 75 Vermeulen M., Mulder K.W., Denissov S., Pijnappel W.W., van Schaik F.M., Varier R.A., Baltissen M.P., Stunnenberg H.G., Mann M., Timmers H.T. (2007). Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* 131, 58–69.
- 76 Chen D., Ma H., Hong H., Koh S.S., Huang S.M., Schurter B.T., Aswad D.W., Stallcup M.R. (1999) Regulation of transcription by a protein methyltransferase. *Science* 284, 2174–2177.
- 77 Ma H., Bauman C.T., Li I.H., Strahl B.D., Rice R., Jelnek M.A., Aswad D.W., Allis C.D., Hager G.L., Stallcup M.R. (2001). Hormone-dependent, CARM1-directed arginine-specific methylation of histone H3 on a steroid regulated promoter. *Curr Biol* 11, 1981–1985.
- 78 Bauer U.T., Daujat S., Nielsen S.J., Nightingale J., Kouzarides T. (2002). Methylation of arg17 of histone H3 is linked to gene activation. *EMBO Reports* 3, 39–44.
- 79 Stallcup M.R., Chen D., Koh S.S., Ma H., Lee Y.H., Li H., Schurter B.T., Aswad D.W. (2000). Co-operation between protein-acetylating and protein-methylating co-activators in transcriptional activation. *Biochem Soc Trans* 28, 415–418.
- 80 Strahl B.D., Briggs S.D., Brame C.J., Caldwell J.A., Koh S.S., Ma H., Cook R.C., Shabanowitz J., Hunt D.F., Stallcup M.R., Allis C.D. (2001). Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. *Curr Biol* 11, 1–5.
- 81 Koh S.S., Chen D., Lee Y.H., Stallcup M.R. (2001). Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. *J Biol Chem* 276, 1089–1098.
- 82 Shen E.E., Henry M.F., Weiss V.H., Valentini S.R., Silver P.A., Lee M.S. (1998). Arginine methylation facilitates the nuclear export of hnRNP proteins. *Genes Dev* 12, 679–691.
- 83 Yun C.Y., Fu X.D. (2000). Conserved SR protein kinase functions in nuclear import and its action is counteracted by Arginine methylation in *Saccharomyces cerevisiae*. *J Cell Biol* 150, 707–718.
- 84 Brahm H., Raymackers J., Union A., de Keyser F., Meheus L., Luhrmann R. (2000). The C-terminal RG dipeptide repeats of the spliceosomal sm proteins D1 and D3 contain symmetrical dimethylarginines, which form a major B-cell epitope for anti-sm autoantibodies. *J Biol Chem* 275, 17122–17129.
- 85 Bedford M.T., Frankel A., Yaffa M.B., Clarke S., Leder P., Richard S. (2000). Arginine methylation inhibits the binding of proline-rich ligands to Src homology 3, but not WW, domains. *J Biol Chem* 275, 16030–16036.
- 86 Morwen K.A., Tang J., Zhu W., Schurter B.T., Shual K., Herschman H.R., David M. (2001). Arginine methylation of STAT1 modulates IFN- α /beta-induced transcription. *Cell* 104, 731–741.
- 87 Xu W., Chen H., Du K., Asahara H., Tini M., Emerson B.M., Montminy M., Evans R.M. (2001). A transcriptional switch mediated by cofactor methylation. *Science* 294, 2507–2511.
- 88 Constantinides P.G., Jones P.A., Gevers W. (1977). Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment. *Nature* 267, 364–366.
- 89 Santi D.V., Garrett C.E., Barr P.J. (1983). On the mechanism of inhibition of DNA-cytosine methyltransferases by cytosine analogs. *Cell* 33, 9–10.
- 90 Greiner D., Bonaldi T., Eskeland R., Roemer E., Imhof A. (2005). Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3–9. *Nat Chem Biol* 1, 143–145.
- 91 Yost J.M., Korboukh I., Liu F., Gao C., Jin J. (2011). Targets in epigenetics: inhibiting the methyl writers of the histone code. *Curr Chem Genomics* 5(Suppl 1), 72–84.
- 92 Iwasa E., Hamashima Y., Fujishiro S., Higuchi E., Ito A., Yoshida M., Sodeoka M. (2010). Total synthesis of (+)-chaetocin and its analogues: their histone methyltransferase G9a inhibitory activity. *J Am Chem Soc* 132, 4078–4079.
- 93 Tibodeau J.D., Benson L.M., Isham C.R., Owen W.G., Bible K.C. (2009). The anticancer agent chaetocin is a competitive substrate and inhibitor of thioredoxin reductase. *Antioxid Redox Signal* 11, 1097–1106.
- 94 Gardiner D.M., Waring P., Howlett B.J. (2005). The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. *Microbiology (UK)* 151, 1021–1032.
- 95 Kubicek S., O’Sullivan R.J., August E.M., Hickey E.R., Zhang Q., Teodoro M.L., Rea S., Mechtler K., Kowalski J.A., Homon C.A., Kelly T.A., Jenuwein T. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell* 25, 473–481.
- 96 Chang Y., Zhang X., Horton J.R., Upadhyay A.K., Spannhoff A., Liu J., Snyder J.P., Bedford M.T., Cheng X. (2009). Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. *Nat Struct Mol Biol* 16, 312–317.
- 97 Collazo E., Couture J.F., Bulfer S., Trievel R.C. (2005). A coupled fluorescent assay for histone methyltransferases. *Anal Biochem* 342, 86–92.
- 98 Liu F., Chen X., Allali-Hassani A., Quinn A.M., Wasney G.A., Dong A., Barsyte D., Koziarzki I., Senisterra G., Chau I., Siarheyeva A., Kireev D.B., Jadhav A., Herold J.M., Frye S.V., Arrowsmith C.H., Brown P.J., Simeonov A., Vedadi M., Jin J. (2009). Discovery of a 2,4-diamino-7-aminoalkoxyquinazoline as a potent and selective inhibitor of histone lysine methyltransferase G9a. *J Med Chem* 52, 7950–7953.
- 99 Liu F., Chen X., Allali-Hassani A., Quinn A.M., Wigle T.J., Wasney G.A., Dong A., Senisterra G., Chau I., Siarheyeva A., Norris J.L., Kireev D.B., Jadhav A., Herold J.M., Janzen W.P., Arrowsmith C.H., Frye S.V., Brown P.J., Simeonov A., Vedadi M., Jin J. (2010). Protein lysine methyltransferase G9a inhibitors: design, synthesis, and structure activity relationships of 2,4-diamino-7-aminoalkoxy-quinazolines. *J Med Chem* 53, 5844–5857.
- 100 Wigle T.J., Provencher L.M., Norris J.L., Jin J., Brown P.J., Frye S.V., Janzen W.P. (2010). Accessing protein methyltransferase and demethylase enzymology using microfluidic capillary electrophoresis. *Chem Biol* 17, 695–704.
- 101 Vedadi M., Barsyte-Lovejoy D., Liu F., Rival-Gervier S., Allali-Hassani A., Labrie V., Wigle T.J., Dimaggio P.A., Wasney G.A., Siarheyeva A., Dong A., Tempel W., Wang S.C., Chen X., Chau I., Mangano T.J., Huang X.P., Simpson C.D., Pattenden S.G., Norris J.L., Kireev D.B., Tripathy A., Edwards A., Roth B.L., Janzen W.P., Garcia B.A., Petronis A., Ellis J., Brown P.J., Frye S.V., Arrowsmith C.H., Jin J. (2011). A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nat Chem Biol* 7, 566–574.
- 102 Liu F., Barsyte-Lovejoy D., Allali-Hassani A., He Y., Herold J.M., Chen X., Yates C.M., Frye S.V., Brown P.J., Huang J., Vedadi M., Arrowsmith C.H., Jin J. (2011). Optimization of cellular activity of G9a inhibitors 7-aminoalkoxy-quinazolines. *J Med Chem* 54, 6139–6150.
- 103 Chang Y., Ganesh T., Horton J.R., Spannhoff A., Liu J., Sun A., Zhang X., Bedford M.T., Shinkai Y., Snyder J.P., Cheng X. (2010). Adding a lysine mimic in the design of potent inhibitors of histone lysine methyltransferases. *J Mol Biol* 400, 1–7.
- 104 Daigle S.R., Olhava E.J., Therkelsen C.A., Majer C.R., Sneeringer C.J., Song J., Johnston L.D., Scott M.P., Smith J.J., Xiao Y., Jin L., Kuntz K.W., Chesworth R., Moyer M.P., Bernt K.M., Tseng J.C.,

- Kung A.L., Armstrong S.A., Copeland R.A., Richon V.M., Pollock R.M. (2011). Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell* 20, 53–65.
- 105 Yao Y., Chen P., Diao J., Cheng G., Deng L., Anglin J.L., Prasad B.V., Song Y. (2011). Selective inhibitors of histone methyltransferase DOT1L: design, synthesis, and crystallographic studies. *J Am Chem Soc* 133, 16746–16749.
- 106 Ferguson A.D., Larsen N.A., Howard T., Pollard H., Green I., Grande C., Cheung T., Garcia-Arenas R., Cowen S., Wu J., Godin R., Chen H., Keen N. (2011). Structural basis of substrate methylation and inhibition of SMYD2. *Structure* 19, 1262–1273.
- 107 Sharma S.K., Wu Y., Steinbergs N., Crowley M.L., Hanson A.S., Casero R.A., Woster P.M. (2010). (Bis)urea and (bis)thiourea inhibitors of lysine-specific demethylase 1 as epigenetic modulators. *J Med Chem* 53, 5197–5212.
- 108 Huang Y., Greene E., Stewart T.M., Goodwin A.C., Baylin S.B., Woster P.M., Casero R.A. Jr. (2007). Inhibition of lysine-specific demethylase 1 by polyamine analogues results in reexpression of aberrantly silenced genes. *Proc Natl Acad Sci USA* 104, 8023–8028.
- 109 Huang Y., Stewart T.M., Wu Y., Baylin S.B., Marton L.J., Perkins B., Jones R.J., Woster P.M., Casero R.A. Jr. (2009). Novel oligoamine analogues inhibit lysine-specific demethylase 1 and induce reexpression of epigenetically silenced genes. *Clin Cancer Res* 15, 7217–7228.
- 110 Culhane J.C., Wang D., Yen P.M., Cole P.A. (2010). Comparative analysis of small molecules and histone substrate analogues as LSD1 lysine demethylase inhibitors. *J Am Chem Soc* 132, 3164–3176.
- 111 Schmidt D.M., McCafferty D.G. (2007). trans-2-Phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1. *Biochemistry* 46, 4408–4416.
- 112 Benelkebir H., Hodgkinson C., Duriez P.J., Hayden A.L., Bulleid R.A., Crabb S.J., Packham G., Packham G., Ganesan A. (2011). Enantioselective synthesis of tranlycypromine analogues as lysine demethylase (LSD1) inhibitors. *Bioorg Med Chem* 19, 3709–3716.
- 113 Binda C., Valente S., Romanenghi M., Pilotto S., Cirilli R., Karytinis A., Ciossani G., Botrugno O.A., Forneris F., Tardugno M., Edmondson D.E., Minucci S., Mattevi A., Mai A. (2010). Biochemical, structural, and biological evaluation of tranlycypromine derivatives as inhibitors of histone demethylases LSD1 and LSD2. *J Am Chem Soc* 132, 6827–6833.
- 114 Gooden D.M., Schmidt D.M., Pollock J.A., Kabadi A.M., McCafferty D.G. (2008). Facile synthesis of substituted trans-2-arylcyclopropylamine inhibitors of the human histone demethylase LSD1 and monoamine oxidases A and B. *Bioorg Med Chem Lett* 18, 3047–3051.
- 115 Mimasu S., Umezawa N., Sato S., Higuchi T., Umehara T., Yokoyama S. (2010). Structurally designed trans-2-phenylcyclopropylamine derivatives potently inhibit histone demethylase LSD1/KDM1. *Biochemistry* 49, 6494–6503.
- 116 Ueda R., Suzuki T., Mino K., Tsumoto H., Nakagawa H., Hasegawa M., Sasaki R., Mizukami T., Miyata N. (2009). Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J Am Chem Soc* 131, 17536–17537.
- 117 Lee M.G., Wynder C., Bochar D.A., Hakimi M.A., Cooch N., Shiekhattar R. (2006). Functional interplay between histone demethylase and deacetylase enzymes. *Mol Cell Biol* 26, 6395–6402.
- 118 Cloos P.A., Christensen J., Agger K., Maiolica A., Rappsilber J., Antal T., Hansen K.H., Helin K. (2006). The putative oncogene GASC1 demethylates tri and dimethylated lysine 9 on histone H3. *Nature* 2006, 442: 307–311.
- 119 Rose N.R., Ng S.S., Mecinovic J., Lienard B.M., Bello S.H., Sun Z., McDonough M.A., Oppermann U., Schofield C.J. (2008). Inhibitor scaffolds for 2-oxoglutarate-dependent histone lysine demethylases. *J Med Chem* 51, 7053–7056.
- 120 Sakurai M., Rose N.R., Schultz L., Quinn A.M., Jadhav A., Ng S.S., Oppermann U., Schofield C.J., Simeonov A. (2010). A miniaturized screen for inhibitors of Jumonji histone demethylases. *Mol Biosyst* 6, 357–364.
- 121 Hamada S., Kim T.D., Suzuki T., Itoh Y., Tsumoto H., Nakagawa H., Janknecht R., Miyata N. (2009). Synthesis and activity of N-oxalylglycine and its derivatives as Jumonji C-domain-containing histone lysine demethylase inhibitors. *Bioorg Med Chem Lett* 19, 2852–2855.
- 122 Rose N.R., Woon E.C., Kingham G.L., King O.N., Mecinovic J., Clifton I.J., Ng S.S., Talib-Hardy J., Oppermann U., McDonough M.A., Schofield C.J. (2010). Selective inhibitors of the JMJD2 histone demethylases: combined nondenaturing mass spectrometric screening and crystallographic approaches. *J Med Chem* 53, 1810–1818.
- 123 Hamada S., Suzuki T., Mino K., Koseki K., Oehme F., Flamme I., Ozasa H., Itoh Y., Ogasawara D., Komaarashi H., Kato A., Tsumoto H., Nakagawa H., Hasegawa M., Sasaki R., Mizukami T., Miyata N. (2010). Design, synthesis, enzyme-inhibitory activity, and effect on human cancer cells of a novel series of jumonji domain-containing protein 2 histone demethylase inhibitors. *J Med Chem* 53, 5629–5638.
- 124 Luo X., Liu Y., Kubicek S., Myllyharju J., Tumber A., Ng S., Che K.H., Podoll J., Heightman T.D., Oppermann U., Schreiber S.L., Wang X. (2011). A selective inhibitor and probe of the cellular functions of Jumonji C domain-containing histone demethylases. *J Am Chem Soc* 133, 9451–9456.
- 125 Yu V., Fisch T., Long A.M., Tang J., Lee J.H., Hierl M., Chen H., Yakowec P., Schwandner R., Emkey R. (2011). High-throughput TR-FRET assays for identifying inhibitors of LSD1 and JMJD2C histone lysine demethylases. *J Biomol Screen* 17, 27–38.

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