
Histone Methyltransferases: Opportunities in Cancer Drug Discovery

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9.1 Introduction

Posttranslational modifications on the core nucleosomal histones play an important role in the regulation of chromatin structure and function. These modifications include methylation of lysine and arginine residues, acetylation and ubiquitinylation of lysine residues, and phosphorylation of serine residues. The histone modifications are referred to as “marks,” and the enzymes that catalyze the deposition of the marks are “writers,” while the enzymes that remove the marks are “erasers.” Proteins that recognize specific histone posttranslational marks or series of marks are referred to as “readers.” Thus, the methyltransferases “write” the methyl marks on lysine and arginine residues. The methyl marks on lysine residues can exist in a mono-, di-, or trimethylation state, while arginine residues are either mono- or dimethylated, with the dimethylation existing in an asymmetrical or symmetrical state (Fig. 9.1a). Mass spectrometry studies have identified specific sites of lysine and arginine methylation found on histone H3 and histone H4 (Fig. 9.1b). The pattern of modifications present on the histones is associated with specific functional states of the chromatin. For example, trimethylation of histone H3 lysine 4 (H3K4me3) is found in areas of chromatin that are either poised for transcription or actively transcribed (Bernstein et al. 2005, 2006). H3K4me3 is found in combination with trimethylation of histone H3 lysine 27 (H3K27me3) in areas that are poised for transcription. Histone H3K27me3 is generally associated with transcriptional silencing. Our understanding of the role of

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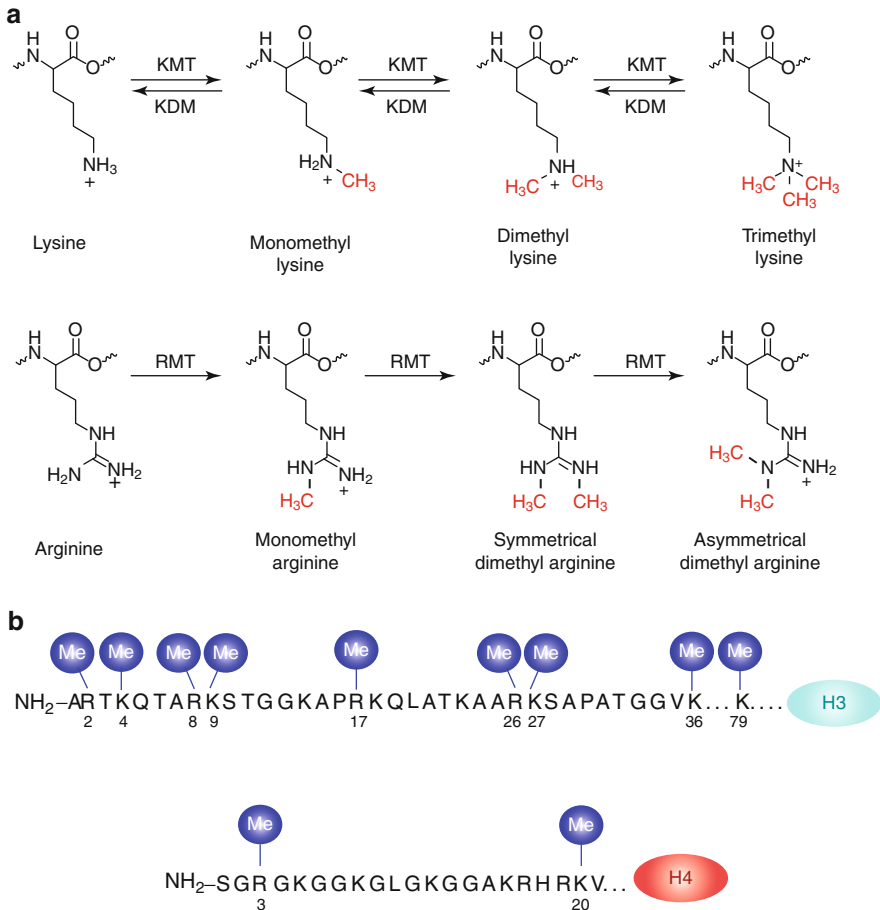


Fig. 9.1 Methylation of lysine and arginine residues in proteins. **(a)** Lysine methyltransferases mono-, di- or trimethylate lysine residues while lysine demethylases remove these modifications. Arginine methyltransferases monomethylate and asymmetrically or symmetrically dimethylate arginine residues and there are no known enzymes that directly remove these modifications. **(b)** Sequences of human histone H3 and H4 N-terminal tails and known sites of methylation

these posttranslational modifications of histones has increased tremendously in the past few years. The function and regulation of methyl marks on histone lysine and arginine residues are discussed in the sections in this review.

Histone methyltransferases are divided into two major families based upon their amino acid substrate: the lysine methyltransferases and arginine methyltransferases (Copeland et al. 2009; Bedford and Richard 2005; Richon et al. 2011). The lysine methyltransferases (KMTs) consist of over 50 enzymes, and all of the enzymes in this class share a common catalytic domain referred to as the S(u(var)3–9, Enhancer of Zeste, TriThorax, or SET domain except for one enzyme, DOT1L. The catalytic domain of DOT1L shares structural homology with the arginine methyltransferases (RMTs), based on the observation that the cofactor S-adenosyl methionine (SAM)

displays a common extended conformation in DOT1L and the RMTs (Richon et al. 2011). This is in contrast to the U shape conformation that SAM adopts in the catalytic domain of the SET domain KMTs (Schubert et al. 2003). Nine enzymes that catalyze arginine methylation have been described and include PRMT1, PRMT2, PRMT3, CARM1, PRMT5, PRMT6, PRMT7, PRMT8, and PRMT10 (Bedford and Richard 2005; Di Lorenzo and Bedford 2011). The RMTs are divided into two major groups. The type I enzymes (PRMT1, PRMT2, PRMT3, CARM1, PRMT6) catalyze either monomethylation or asymmetric dimethylation, and the type II enzymes catalyze symmetric dimethylation (PRMT5 and PRMT7). PRMT7 has also been shown to catalyze monomethylation of specific substrates and is the sole member of the type III enzyme group (Miranda et al. 2004). Two additional, related putative RMTs have been described (PRMT9 and PRMT11), but have not been biochemically characterized (Di Lorenzo and Bedford 2011). A systematic survey of the human genome for RMT-related enzymes was performed, and an additional 33 putative enzymes were identified that contained related methyltransferase domains (Richon et al. 2011).

Alterations in histone lysine and arginine methylation and associated protein methyltransferases have been observed in a wide variety of cancers (Table 9.1). These alterations include translocations, mutations, amplification, and aberrant recruitment of methyltransferases and demethylases. One example is EZH2, a histone H3K27 methyltransferase that is altered in several cancer types through a variety of different mechanisms that leads to increased H3K27me₃ levels. The increase in H3K27me₃ is associated with repression of gene expression that is believed to result in the development of cancer.

The identification of genetic alterations in methyltransferases in cancer leads to a strategy for targeting patient populations with these alterations using small molecules designed to selectively inhibit the oncogenic methyltransferase. Drug discovery efforts have been initiated, and several small molecule inhibitors of this class of enzymes have been identified. The current state of the development of the methyltransferase inhibitors is summarized in Sect. 4.

9.2 Histone Methylation, Methyltransferases, and Cancer Association

9.2.1 Lysine Methylation

9.2.1.1 H3K4 Methylation

The regulation of methylation on lysine 4 on histone H3 (H3K4) is complex, with at least 20 enzymes reported to catalyze the addition or removal of methylation at this site. Methylation of H3K4 is almost exclusively associated with transcriptionally active chromatin (Sims and Reinberg 2006; Strahl et al. 1999), and high levels of H3K4 mono-, di-, and trimethylation can be found at or near transcription start sites (Barski et al. 2007). There is a strong relationship between the state of methylation and transcription; trimethylation is found at the 5' end of active genes and correlates positively with active transcription, RNA polymerase II occupancy, and histone acetylation (Bernstein et al. 2005; Santos-Rosa et al. 2002; Schneider et al. 2004).

Table 9.1 Examples of alterations of histone methyltransferases in cancer

Histone mark	Methyltransferase	Cancer association	References
<i>Lysine</i>			
H3K4	MLL	Translocated in <i>MLL-rearranged</i> leukemias; results in loss of SET domain	Hess (2004), Krivtsov and Armstrong (2007), Krivtsov et al. (2008)
	MLL4	Amplified in solid tumors	Hess (2004)
	SMYD3	Overexpressed in breast, liver, colon, and cervical cancer	Hamamoto et al. (2004, 2006), Luo et al. (2009), Wang et al. (2008a)
H3K9	G9a	Increased expression in transformed lung cell lines	Watanabe et al. (2008)
	SETDB1	Amplified and overexpressed in melanoma	Ceol et al. (2011)
H3K27	EZH2	Amplified and overexpressed in multiple solid tumors; mutated in lymphomas and MDS	Simon and Lange (2008), Sarma et al. (2008), Bracken et al. (2003), Cao et al. (2008b), Kirmizis et al. (2003), Kleer et al. (2003), van Haften et al. (2009), Varambally et al. (2002), Morin et al. (2010), Sneeringer et al. (2010), Yap et al. (2011)
H3K36	WHSC1/NSD2, MMSET	Translocated and overexpressed in myeloma	Chesi et al. (1998), Lauring et al. (2008), Stec et al. (1998)
	WHSC1L1/NSD3	Amplified in lung cancer and breast cancer; translocation with NUP98	Angrand et al. (2001), Rosati et al. (2002)
H3K79	DOT1L	Mislocalized by <i>MLL</i> translocation in leukemia	Hess (2004), Krivtsov and Armstrong (2007), Krivtsov et al. (2008), Okada et al. (2005)
<i>Arginine</i>			
H4R3	PRMT1	Recruited to AML1-ETO oncogenic translocation in leukemia	Shia et al. (2012)
H3R8	PRMT5	PRMT5 and H3R8 methylation levels are elevated in lymphoid cancer cells	Pal et al. (2004, 2007)
H3R17	CARM1	Increased expression correlates with androgen independence in human prostate carcinoma, elevated expression in breast tumors	Frietze et al. (2008), El Messaoudi et al. (2006), Majumder et al. (2006)

Dimethylation of H3K4 often co-localizes with H3K4me3 in discrete zones 5–20 nucleosomes long proximal to actively transcribed genes. On its own, the dimethyl H3K4 mark has been found on genes that are maintained in a transcriptionally poised state (Bernstein et al. 2005; Schneider et al. 2004; Ruthenburg et al. 2007).

Unlike in yeast, where Set1 is the only H3K4 methyltransferase, humans have at least 13 confirmed or putative methyltransferases which regulate the addition of methyl groups at H3K4 (MLL1, MLL2, MLL3, MLL4, MLL5, Ash1, Set7/9, Set1A, Set1B, SMYD1, SMYD2, SMYD3, and PRDM9) (Ruthenburg et al. 2007; Abu-Farha et al. 2008; Hamamoto et al. 2004; Kouzarides 2007; Tan et al. 2006). Additionally, at least 7 H3K4 demethylases (LSD1, LSD2, JHMD1B, JARID1A, JARID1B, JARID1C, and JARID1D) have been reported to date (Kouzarides 2007; Lim et al. 2010a). The diverse array of human enzymes that have evolved to regulate the methylation status of the H3K4 indicates that exquisite control must be maintained over this mark in all cell types. In general, H3K4 methyltransferases are activators of gene expression, and H3K4 demethylases are repressors. The dynamic balance between these opposing classes of enzymes indicates the precision with which H3K4 methylation is regulated in diverse cell types.

While there is some overlap of function between H3K4 methyltransferases, the context-specific expression and activity of each enzyme is crucial to cell fate during differentiation and development (Eissenberg and Shilatifard 2010), cell functions, e.g., DNA repair, that maintain the status quo and in specialized processes, such as V(D)J recombination, which support physiological integrity (Shilatifard 2008). For example, the MLL family of H3K4 methyltransferases is known to exert control over the expression of *HOX* genes, and recent work from Shilatifard (2008) has described the phenomenon of redundancy versus specialization among this family in the activation of these gene clusters. Experiments performed using wild-type or *Mll1* knockout MEF cells indicate that of 10,041 genes associating with H3K4me₃, only 5% show a reduction in H3K4me₃ in the *Mll1* knockout MEFs (Wang et al. 2009). Subsequent expression profiling indicated that the knockout of *Mll1* resulted in the enhanced expression of 2,265 genes and reduced expression of 2,459 or a positive or negative regulation of 3% of all genes (Wang et al. 2009). Overall this analysis supported the concept that a substantial overlap in H3K4 methyltransferase activity does exist, but precise control over the expression of a small subset of genes is dependent on MLL. Cell-type-specific expression of MLL family methyltransferases has also been observed. For example, MLL3 and MLL4 are observed to have minimal roles in *HOX* gene regulation in fibroblasts (Wang et al. 2009). The methylation product of the methyltransferases also adds to the level of regulation of H3K4me₃ observed; during oogenesis, MLL2 controls levels of trimethylation but has no effect on monomethylation, indicating that a different methyltransferase produces H3K4me₁, and MLL2 activity is coordinated to catalyze the trimethyl state (Andreu-Vieyra et al. 2010). Similar target and product specificity exists among the H3K4 demethylases. LSD1 and LSD2 are observed to primarily act upon mono- and dimethylated H3K4, and JARID family demethylases are specific for di- and trimethyl H3K4 (Lim et al. 2010a). Beyond cell- and temporal-specific expression of H3K4 methyltransferases, another level of regulation may involve accessory proteins. For example, enzymatic activity may be nonexistent or greatly reduced when the SET domain-containing subunit is present on its own; however, activity is stimulated greatly when present as part of a complex. The identity of the other partners in the complex differs for each member of the MLL family and presumably dictates the specificity and the context in which methylation occurs at the target H3K4 residue (Eissenberg and Shilatifard 2010).

H3K4 methylation is associated with an open chromatin configuration which enables access of the chromatin to transcription factors and machinery. It is becoming apparent that protein modules capable of “reading” these methyl marks are responsible for attracting effector proteins containing these modules to chromatin sites bearing H3K4, leading to a variety of outcomes (Ruthenburg et al. 2007). Methyl-lysine binding modules are generally part of the Royal Family of proteins and include MBT, Agenet, Tudor, Chromo, and PWWP domains. Additionally, methyl-lysine recognition motifs can be found in the PHD family and WD40 repeat protein WRD5 (Maurer-Stroh et al. 2003). The key lysine methylation recognition features of these domains are an aromatic electron-rich cage, interacting with the lysine cation, with additional charge neutralization and H-bonding by 0–2 acidic functionalities depending on the methylation state of lysine (Guo et al. 2009; Kaustov et al. 2011). The number of proteins that contain H3K4 methyl-lysine recognition modules far outnumbers the enzymes that modulate methylation of this residue. The ING-family of proteins contain SHD fingers that preferentially recognize H3K4me_{2/3}, and members of this family usually bind to and affect the activity of histone acetyltransferases and deacetylases (Adams-Cioaba and Min 2009; Feng et al. 2002). The recruitment of effector proteins to H3K4me₃ often stimulates gene expression, as is the case for the PHD finger of human bromodomain and PHD domain transcription factor (BPTF), the largest subunit of the NURF complex, which is responsible for remodeling chromatin at active loci (Li et al. 2006; Wysocka et al. 2006). However, there are examples of H3K4me₃ effector modules that repress gene expression upon recruitment. In response to DNA damage, the PHD finger of ING2, a critical component of the mSin3a-HDAC1 deacetylase complex, binds H3K4me₃ in proximity to the promoters of proliferation genes and shuts off transcription (Shi et al. 2006). The binding of effector proteins to H3K4me₃ can also impact other neighboring marks. The demethylases PHF8 and JHDM1D harbor a PHD finger, allowing them to dock onto the target nucleosome. However, the length and rigidity of a linker region separating the Jumonji domain from the PHD finger dictates whether the enzyme is a H3K9 (PHF8) or H3K27 (JHDM1D) demethylase (Horton et al. 2010). Unlike ING-family and BPTF PHD fingers, there are even recognition modules that selectively bind to unmodified lysine. A component of the LSD1 complex, BHC80, binds unmethylated H3K4, prevents remethylation and aids in the propagation of the H3K4 demethylation (Lan et al. 2007). Similarly, DNMT3L is recruited to unmethylated H3K4 by a PHD-like domain to maintain a repressive effect on gene expression at nucleosomes not demarcated by H3K4 methylation (Ooi et al. 2007).

Histone H3K4 methylation is associated with oncogenesis at multiple levels. However, given the complexity of enzymes that add or remove methyl groups from H3K4, and the diverse array of effector proteins that bind to H3K4, selecting the appropriate targets for initiating drug discovery is challenging (Copeland et al. 2009). SMYD3 is an example of a H3K4 methyltransferase where there appears to be a link between overexpression and tumor progression (Hamamoto et al. 2004). SMYD3 contains a SET domain and a MYND-type zinc-finger domain that is common among developmental proteins (Hamamoto et al. 2004). SMYD3 forms a

complex with RNA Pol II (Hamamoto et al. 2004), and its overexpression is associated with increased expression of oncogenes (e.g., N-Myc, CrkL, Wnt10b, RIZ, and hTERT), cell cycle control genes (e.g., cyclin G1 and CDK2), and regulators of signal transduction (e.g., STAT1, MAP3K11, and PIK3CB) (Luo et al. 2009; Liu et al. 2007). SMYD3 has been shown to be overexpressed in hepatic, colorectal, cervical, and breast cancers (Hamamoto et al. 2004, 2006; Luo et al. 2009; Wang et al. 2008a). In the case of breast cancers, recent evidence suggests that SMYD3 directly interacts with the ligand-binding domain of the estrogen receptor α (ER α), enhancing the levels of di- and trimethylation of H3K4 at ER α target genes (Kim et al. 2009), ultimately increasing their expression. RNAi-mediated knockdown of the SMYD3 has been shown to decrease invasiveness and proliferation and in some cases induce apoptosis.

MLL is another example of an H3K4 methyltransferase implicated in cancer. Rearrangements of the *MLL* gene have been identified in both pediatric and adult leukemias and are correlated with poor prognosis (Hess 2004; Krivtsov and Armstrong 2007; Krivtsov et al. 2008). The *MLL* rearrangements result in loss of the SET or catalytic domain while retaining the DNA-targeting domain. The translocation results in aberrant expression of a subset of the *HOX* genes, causing a block of hematopoietic differentiation. Targeting of the fusion proteins or mediators of the fusion partners could represent a novel and effective way to treat these leukemias. Several *MLL* translocation partners (AF4, AF9, AF10, and ENL) recruit another lysine methyltransferase, DOT1L (Okada et al. 2005). DOT1L catalyzes methylation of histone H3K79 and is described in a subsequent section. In addition to alterations in *MLL*, amplification of *MLL4* is also observed in solid tumors (Hess 2004).

The histone H3K4 demethylases may also be important targets for therapeutic intervention using small molecules. LSD1 is overexpressed in prostate cancer, breast cancer, neuroblastoma and bladder cancer, and loss of H3K4me3 is postulated to result in the silencing of tumor suppressor gene expression (Lim et al. 2010a, b; Schulte et al. 2009; Shi and Whetstine 2007). Increasing local nonmethylated H3K4 not only impedes the recruitment of transcription factors but also enhances the activity of DNA methyltransferases proximal to the demethylated mark. Indeed, the use of LSD1 inhibitors in combination with a DNA methyltransferase inhibitor led to inhibition of the growth of human colon cancer xenografts (Huang et al. 2009). Similar to LSD1, JARID1B is found overexpressed in breast, testis, esophageal, and prostate cancers (Lim et al. 2010a; Barrett et al. 2007; Xiang et al. 2007). In addition to alterations in specific enzymes, alterations in H3K4 trimethylation have been described to be associated with cancer. For example, H3K4 trimethylation has been found to be associated with chromosome breakpoints in leukemia (Barski et al. 2007). While pursuing inhibitors of the reader domains that recognize H3K4 methylation is also a possibility (Kireev et al. 2010; Wigle et al. 2010), this chapter will focus on the opportunities presented by the histone methyltransferases.

9.2.1.2 H3K9 Methylation

Lysine methylation at H3K9 is generally associated with transcriptional silent chromatin and is an example of another mark where multiple enzymes regulate the

methylation status. There are at least 7 methyltransferases (G9a, GLP, Suv39H1, Suv39H2, SETDB1, SETDB2, and PRDM2) and 7 demethylases (LSD1, JHDM2a, JHDM2b, JHDM3A, JMJD2B, JMJD2C, and JMJD2D) known to act upon this mark (Kouzarides 2007). Unlike H3K4, where there is no reported acetylation, the H3K9 mark is also prominently acetylated. Also, the serine (10) of histone H3 can become phosphorylated by the kinase Aurora B (Jeong et al. 2010).

Studies in organisms ranging from yeast to human show that H3K9 methylation is a hallmark of facultative and constitutive heterochromatin (Krishnan et al. 2011). High-resolution genomic mapping used to generate transcription start site (TSS) alignment has shown that both H3K9me2 and H3K9me3 were higher in chromatin of silent genes compared to transcriptionally active genes in a region of 10 kb surrounding the TSS. H3K9me1 localized to more active promoters surrounding the TSS (Barski et al. 2007). In addition, the di- and trimethylation states of H3K9 are implicated in the recruitment of DNA methyltransferases, which are also associated with areas of heterochromatin (Cheng and Blumenthal 2010).

Two of the most well-studied enzymes that catalyze methylation of H3K9 are G9a (also known as EHMT2) and G9a-like protein (GLP or EHMT1). G9a and GLP catalyze the mono- and dimethylation of H3K9 (Wu et al. 2010), but not trimethylation of H3K9. Unlike other methyltransferases, G9a and GLP do not rely on cross talk between neighboring marks to methylate H3K9, only the presence of a un- or monomethylated lysine (Rathert et al. 2008). DNA methyltransferases 3A and 3B are physically recruited to chromatin by G9a bound through its ankyrin repeats, facilitating the spread of heterochromatin (Xin et al. 2003). Functionally, G9a and GLP appear to be essential to early development, and knockouts of either in mouse embryos are lethal (Tachibana et al. 2002, 2005). In addition, G9a plays an essential role in embryonic stem cell differentiation through H3K9me2-mediated silencing of *Oct3/4* (Feldman et al. 2006). There is substantial redundancy between G9a and GLP target genes, but specialization does exist. G9a knockdown leads to a global reduction of H3K9me2 at 67 % of promoters (Wagschal et al. 2008).

The Suv39 family of methyltransferases catalyze di- and trimethylation of H3K9, and like G9a and GLP, they interact with DNA methyltransferases to facilitate the formation of heterochromatin (Loyola et al. 2009). Suv39 members are essential in recruiting Heterochromatin Protein 1 (HP1), a chromodomain-containing protein selectively recognizing trimethyl lysine that is essential in the stability of telomeres and centromeres. In addition to the Suv39 enzymes, SETDB1 has also been shown to catalyze di- and trimethylation of H3K9 (Schultz et al. 2002; Wang et al. 2003). The accessory protein mAM stimulates the conversion of di- to trimethylation of H3K9 by SETDB1 (Wang et al. 2003). SETDB1 is localized at the 3' ends of zinc-finger genes and appears to play an important role in gene silencing induced at these promoters (Friedtze et al. 2010). Demethylation of the H3K9 mark is carried out mainly by two families: JMJD1 family proteins are specific for H3K9 mono- and dimethylation and members of JMJD2 family demethylate di- and trimethylated H3K9 (Shi and Whetstine 2007; Cloos et al. 2006; Klose et al. 2006).

Overexpression of G9a, Suv39H1 and SETDB1 has been linked to oncogenic transformation in a variety of cancers, including lung and prostate cancer (Watanabe

et al. 2008). However, despite reports of the overexpression of G9a in cancer, there is a lack of definitive proof to this end. Indeed, small molecules that are nanomolar or sub-nanomolar inhibitors of G9a and GLP (Kubicek et al. 2007; Liu et al. 2009a, 2010) can reverse H3K9me2 in cells and regulate G9a target gene expression; however, these changes do not correlate with inhibition of proliferation. G9a may also contribute to the development of cancer by methylating and inactivating the p53 tumor suppressor protein (Huang et al. 2010). Recently, it has been shown that Suv39H1 overexpression may be oncogenic through its association and recruitment of DNMT1 (Kang et al. 2007).

Recurrent amplification and overexpression of SETDB1 has been described in melanoma (Ceol et al. 2011). Ceol et al. show that enforced expression of SETDB1 can accelerate melanoma onset in a zebrafish model, thus supporting the role of SETDB1 as an oncogene in melanoma.

There are also genetic alterations in the H3K9 demethylases that have been described in cancer. For example, recurrent amplifications and overexpression of JMJD2C/GASC1 have been found in squamous cell cancer cell lines and the basal-like subtype of breast cancer (Cloos et al. 2006; Yang et al. 2000; Liu et al. 2009b). Liu et al. show that GASC1 induces transformed phenotypes and regulates the expression of genes critical for stem cell self-renewal when ectopically expressed in immortalized breast cells.

9.2.1.3 H3K27 Methylation

Methylation of lysine 27 on histone H3, in particular trimethylation, is associated with gene silencing. The deposition of methyl groups onto H3K27 is catalyzed by the Polycomb-Repressive Complexes 2 (PRC2) containing either EZH1 or EZH2 as the catalytic subunit of the complex. EZH2-PRC2 is noted for being the prominent complex in actively dividing cells, while EZH1-PRC2 is ubiquitously expressed (Margueron et al. 2008). The core PRC2 complex is composed of EED, RbAp48, Suz12, and EZH2 (Cao and Zhang 2004a). Demethylation of H3K27 is principally carried out by UTX and JMJD3 and results in the activation of gene expression (Agger et al. 2007).

PRC2 catalyzes the mono- through trimethylation of H3K27 (Margueron et al. 2008; Cao et al. 2002). In vivo, H3K27me2 is the most abundant form, accounting for roughly 50 % of H3K27 methylation, with H3K27me1 and H3K27me3 representing 10 and 35 % of H3K27 methylation, respectively (Peters et al. 2003). Of these methylation states, the trimethylation of H3K27 (H3K27me3) is associated with increased DNA methylation in proximity to nucleosomes bearing this mark. This leads to chromatin condensation and ultimately the repression of genes that are located in these heterochromatic regions (Schlesinger et al. 2007). The establishment of the H3K27me3 is important during processes such as X-inactivation, germ-line development, and stem cell pluripotency, which require sustained gene suppression (Cao and Zhang 2004b). PRC2 has important roles in stem cell biology; there is strong overlap between binding of PRC2 to regions that are populated by genes controlled by the transcription factors Oct4, Sox2, and Nanog, which are critical to pluripotent stem cell generation and maintenance (Boyer et al. 2005,

2006). Loss of PRC2 derepresses embryonic stem cell differentiation genes (Boyer et al. 2006), and knockdown of Oct4 disrupts PRC2 association with target genes (Squazzo et al. 2006), indicating that PRC2 activity is important in inhibiting differentiation. Interestingly, H3K27me3 can sometimes co-localize with H3K4me3, an activating mark. In this chromatin state, termed “bivalent chromatin,” H3K27me3 is the dominant mark, and gene expression is repressed; however, the presence of the H3K4me3 is believed to maintain the genes in a poised state for activation of transcription upon demethylation by UTX or JMJD3 (Simon and Lange 2008).

The EED subunit of the PRC2 complex contains a WD40 domain that preferentially recognizes and binds to the higher methylation states of lysines in H3K9, H3K27, and H1K26, all of which contain a conserved ARKS sequence (Xu et al. 2010). Since H3K9me3 and H3K27me3 are repressive marks, their recognition by EED may facilitate the propagation of H3K27me3 across regions where gene expression is silenced. Additional proteins, such as PHF1, are not essential to EZH2 catalytic activity but may associate with PRC2 to enhance its activity. Loss of PHF1 correlates with a concomitant decrease in H3K27me3 at the HOXA6, HOXA9, and HOXA11 promoters. PHF1 contains a Tudor domain and two PHD fingers that most likely target PRC2 to discrete loci via recognition of adjacent methylated lysines (Cao et al. 2008a; Sarma et al. 2008). Also, it has been shown that large intervening noncoding RNAs (lincRNAs) interact with PRC2 and control the localization of H3K27 methylation via recognition of complementary DNA sequences (Gupta et al. 2010). The deposition of H3K27me3 is recognized by Polycomb-Repressive Complex 1 (PRC1), a complex with a core composition of Polycomb (PC), Polyhomeotic (PH), BMI-1, and RING1, which is thought to contribute to Polycomb silencing (Levine et al. 2002).

H3K27 trimethylation is strongly implicated in cancer, and several mechanisms leading to increased H3K27me3 (PRC2 overexpression, *UTX* loss of function mutations and PRC2 subunit overexpression) are associated with many human cancers (Simon and Lange 2008; Sarma et al. 2008; Bracken et al. 2003; Cao et al. 2008b; Kirmizis et al. 2003; Kleer et al. 2003; van Haafden et al. 2009; Varambally et al. 2002). As a result, PRC2, specifically its catalytic subunit EZH2, is one of the most pursued epigenetic targets. Overexpression and amplification of EZH2 (or other PRC2 subunits) is believed to silence genes that promote differentiation. One of the first reports of the involvement of PRC2 in cancer was a gene profiling study of metastatic and localized prostate cancer in which EZH2 scored as the most significant gene upregulated in metastatic cancer (Varambally et al. 2002). PRC2 correlates with poor outcome in prostate cancers and can be used as a prognostic indicator. Similarly, EZH2 overexpression in breast cancers is well documented and correlates with the aggressiveness of the tumor (Bracken et al. 2003; Kleer et al. 2003).

In addition to overexpression, somatic mutations in the catalytic domain of EZH2 have been described in diffuse large B-cell lymphoma (Morin et al. 2010). The mutations result in a single amino acid change in the catalytic SET domain of EZH2 at Y641 (Y641F, Y641N, Y641S, Y641H) in follicular lymphoma (FL) and germinal center B-cell-like (GCB) subtypes of diffuse large B-cell lymphoma (DLBCL). Initial biochemical experiments using recombinant PRC2 having these

mutations demonstrated that mutant EZH2 complexes were unable to methylate a peptidic substrate corresponding to the H3K27 mark, while the wild-type enzyme methylated the same substrate. Morin et al. concluded, therefore, that the mutants appeared to be loss-of-function variants (Morin et al. 2010). However, subsequent studies using nucleosome, histone, and peptide substrates revealed that point mutations of Y641 to F, N, S, or H resulted in a change in the substrate specificity of EZH2 from unmethylated lysine to the dimethylated lysine. The Y641 mutants are much more proficient at catalyzing trimethylation than the wild-type enzyme (Sneeringer et al. 2010; Yap et al. 2011). Considering that these mutations were discovered as heterozygous, and both mutant and wild-type enzyme are expressed, it is believed that the coordinated activities of wild-type and mutant EZH2 drive H3K27 trimethylation and thus promote the development human lymphomas (Sneeringer et al. 2010; Yap et al. 2011). These findings suggest that lymphoma patients bearing the EZH2 mutation may be sensitive to small molecule inhibitors of EZH2. Ultimately, this may provide a framework for further genetic analysis of histone methyltransferase mutations, as catalytic site mutations that change the substrate specificity of the SET domain are not unprecedented (Wu et al. 2010; Del Rizzo et al. 2010).

9.2.1.4 H3K36 Methylation

H3K36 methylation is primarily an activating mark that is catalyzed by at least 6 methyltransferases (NSD1, NSD2, NSD3, SMYD2, SETD2, and SETMAR) and erased by at least 5 demethylases (JHDM1a, JHDM1b, JHDM3A, JMJD2B, and JMJD2C) (Kouzarides 2007; Lee et al. 2005; Li et al. 2009). Methylation at H3K36 competes with acetylation, as it is also recognized as a target for acetyltransferases. H3K36 trimethylation is generally observed downstream of transcriptional start sites, within the coding region of genes, and it peaks around the 3' end (Barski et al. 2007). In yeast, H3K36 dimethylation is scarce or absent in upstream gene regulatory regions, telomeres, mating loci, and regions transcribed by RNA polymerase III and is predicted to be restricted to areas in contact with RNAP II. Localization of H3K36me2 is highly conserved from yeast to humans and is speculated to demarcate regulatory from coding regions in a similar fashion (Rao et al. 2005). It has been shown that SMYD2 associates with the Sin3A HDAC complex to generate H3K36me2 and restrain cell proliferation (Brown et al. 2006). WHSC1 (also known as NSD2) preferentially mono- and dimethylates H3K36 in the context of nucleosomes, but interestingly, in the absence of DNA, it can have its substrate preference changed to H4K44, a mark that has not been proven to exist in vivo (Li et al. 2009). While the NSD methyltransferases can only catalyze mono- or dimethylation, SETD2 can catalyze mono-, di-, and trimethylation (Yuan et al. 2009), and it is the key enzyme regulating trimethylation of H3K36.

Dysregulation of H3K36 methylation is a molecular determinant of several cancers. Overexpression of NSD3 (also known as WHSC1L1) resulting from amplification within chromosome 8p12 is observed in several tumor cell lines and breast carcinomas (Angrand et al. 2001). Chromosomal translocations that result in a fusion product of NSD methyltransferases to nucleoporin 98, NUP98-NSD1

(Jaju et al. 2001; Wang et al. 2007) or NUP98-NSD3 (Rosati et al. 2002), are defined genetic mutations associated with leukemogenesis. The NUP98-NSD1 fusion is believed to induce acute myeloid leukemia (AML) through the binding of genomic elements in proximity to Hox genes, maintaining H3K36 methylation and leading to overexpression of proto-oncogenes such as *HoxA7*, *HoxA9*, *HoxA10*, and *Meis1* (Wang et al. 2007). Inactivating mutations in the NSD SET domain of the NUP98 fusions abolished the oncogenic potential of the chimera (Wang et al. 2007). In myeloma, t(4:14) translocations have been found to drive overexpression of NSD2 leading cancer cell proliferation and adhesion (Chesi et al. 1998; Lauring et al. 2008; Stec et al. 1998). The alterations in the NSD proteins cause methylation of H3K36 in genomic regions which do not normally contain this modification, resulting in aberrant gene expression and transformation. Therefore, one can envision small molecules that inhibit the catalytic activity of NSD family methyltransferases being useful in the treatment of several hematological malignancies.

9.2.1.5 H3K79 Methylation

H3K79 methylation is catalyzed by DOT1L. Unlike other histone modifications that are situated in the accessible N-terminal histone tails, H3K79 methylation occurs on the core of histone H3. DOT1L is the only known lysine methyltransferase that lacks a canonical SET domain (Dillon et al. 2005; Gao and Liu 2007), and structurally, the catalytic domain of DOT1L is more closely related to arginine methyltransferases than to lysine methyltransferases (Richon et al. 2011; Wu et al. 2010; Min et al. 2003). The mechanism of depletion of the H3K79 methylation mark appears to require replacement of histone H3 since no H3K79 demethylases have been described. DOT1L was originally identified for its role in telomere silencing (Singer et al. 1998), and it was later discovered that it possessed H3K79 methylating activity (Ng et al. 2002a, b; van Leeuwen et al. 2002). DOT1L-mediated methylation of H3K79 is considered to enhance gene expression (Steger et al. 2008). The catalytic activity of DOT1L is highly dependent on the presence of ubiquitinated histone H2B, and it is only active in the context of nucleosomal substrates (Ng et al. 2002b; Shahbazian et al. 2005).

DOT1L is linked to oncogenic transformation in MLL-rearranged leukemias. DOT1L is recruited by MLL fusion partners, resulting in the mis-targeting of DOT1L and aberrant H3K79 methylation. This leads to increased transcription of MLL fusion target genes which then block differentiation and promote proliferation (Krivtsov et al. 2008; Okada et al. 2005; Bitoun et al. 2007). MLL translocations are found in approximately 5–10 % of acute leukemias (Slany 2009) and involve fusion of the N-terminal domain of MLL to partners such as AF10, AF4, AF9, ENL, ELL, and AF6 (Krivtsov and Armstrong 2007). DOT1L associates with the MLL fusions through the partner-binding domains. In these fusions, the DNA binding of MLL is retained; however, it lacks the SET domain. This concept of MLL fusion-mediated DOT1L recruitment was reinforced by an artificial DOT1L-MLL fusion which also was capable of inducing leukemic transformation (Okada et al. 2005). Therefore, patients with MLL-rearranged leukemias capable of recruiting DOT1L are an

example of a genetically defined cancer population that may be candidates for treatment with small molecule inhibitors of DOT1L (Barry et al. 2010).

9.2.1.6 H4K20 Methylation

Methylation of H4K20 is catalyzed by at least 4 enzymes (SETD8, Suv420H1, Suv420H2, and NSD1), and there are currently no known demethylases for this mark (Kouzarides 2007). Top-down mass spectrometry analysis has revealed that H4K20me₂ is the dominant modification, with H4K20me₁ and H4K20me₃ being less abundant (Pesavento et al. 2008; Yang et al. 2008). H4K20me₂ is widely distributed and is observed to be bound by the Tudor domain of 53BP1 (Botuyan et al. 2006), which is involved in initiating the DNA damage response (Yang et al. 2008; Sanders et al. 2004). H4K20me₁, catalyzed by SETD8, is localized to the chromatin of active genes (Oda et al. 2009). H4K20me₁, however, is a known target of malignant brain tumor domain proteins such as L3MBTL1, which result in chromatin compaction (Kalakonda et al. 2008). Additionally, monomethylation is seen in X-inactivation (Kohlmaier et al. 2004). H4K20me₁ appears to be either activating or repressive depending on the context. H4K20me₃, catalyzed primarily by Suv420H1 and Suv420H2, is found in pericentromeric chromatin and is thought to be a silencing mark (Schotta et al. 2004).

Several cancers have decreased H4K20 trimethylation. In non-small cell lung cancer, loss of H4K20me₃ increases as the disease progresses, and this correlates negatively with survival (Van Den Broeck et al. 2008). Additionally, a study comparing a panel of normal cell lines to cancer cell lines found cancer cell lines displayed a loss of H4K20me₃ (Tryndyak et al. 2006). Additionally, a mouse model of skin cancer indicates that this loss of H4K20me₃ occurs early in tumorigenesis (Fraga et al. 2005). Therefore loss of H4K20me₃ may serve as a biomarker in early cancer detection.

9.2.2 Histone Arginine Methylation

Methylated histone arginine residues have also been linked to oncogenic signaling events and cancer phenotypes. The next section outlines the roles of specific arginine methylation sites and their association with cancer.

9.2.2.1 H3R8me₂s and H4R3me₂s

The two most studied histone arginine methyl modifications linked to human cancer are H3R8me₂s and H4R3me₂s and both are catalyzed by PRMT5 (Pal et al. 2004). Arginine methylation at these sites is associated with transcriptional repression. In fact, PRMT5 can be recruited to specific promoters by a variety of transcriptional repressors, including Snail and AJUBA proteins (Hou et al. 2008). Hypermethylation of H3R8 and H4R3 has been observed in several lymphoid cancer cell lines, such as mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (B-CLL), and in tumor samples from MCL patients. Furthermore, these methyl marks have been associated with the repression of multiple tumor suppressor genes, including ST7, NME1 (Pal et al. 2004, 2007), and RB

family members (Wang et al. 2008b). Importantly, RNAi-mediated knockdown of PRMT5 in these lymphoid cancer cell lines reduced proliferation, diminished hypermethylation of H3R8 and H4R3, and restored expression of tumor suppressor genes.

The mechanism governing increased methylation of H3R8 and H4R3 in lymphomas and leukemias appears to be increased PRMT5 protein translation. Surprisingly, PRMT5 mRNA is generally lower in transformed lymphoid cancer cell lines when compared to “normal” cells of the same lineage. However, PRMT5 protein translation efficiency is greatly enhanced in transformed B cells and MCL cell lines due to aberrant expression of certain subsets of PRMT5-targeting microRNAs (miRNAs) (Pal et al. 2007). In addition to enhanced translational efficiency, PRMT5 enzymatic activity is regulated by phosphorylation of its co-stimulatory protein, MEP50. Increased phosphorylation of MEP50 by cyclin D1/cdk4 complexes is associated with increased PRMT5 activity. These data suggest that PRMT5 may play an important role in cyclin D1-driven cancers (Aggarwal et al. 2010).

9.2.2.2 H4R3me2a

Asymmetric dimethylation of histone H4 on Arg-3 (H4R3me2a) is generally associated with gene promoters undergoing active transcription. H4R3me3a formation is catalyzed by at least three different type I arginine methyltransferases, PRMT1, PRMT6, and PRMT8 (Di Lorenzo and Bedford 2011). Of these RMTs, PRMT1 is viewed to be the predominant cellular asymmetric arginine methyltransferase, accounting for as much 85 % of physiological protein arginine methylation (Tang et al. 2000).

H4R3me2a has been linked to oncogenic signaling in several contexts. Notably, PRMT1 has been reported to be a key component of a novel MLL oncogenic transcriptional complex, which includes MLL-EEN, CBP, and the bridging protein Sam68 (Cheung et al. 2007). Using chromatin immunoprecipitation, Cheung et al. demonstrated that H4R3me2a promoter levels correlate with the expression of key MLL-EEN target genes, such as HOXA9. Additionally, these authors directly fused PRMT1 to the MLL protein and demonstrated that this fusion product increased self-renewal potential in primary hematopoietic cells. However, an enzymatically inactive point mutant version of PRMT1 was not able to bolster self-renewal capacity in this assay. Finally, this group demonstrated that shRNA-mediated knockdown of PRMT1 abrogated MLL-EEN-mediated transformation of primary hematopoietic cells. In summary, these data support the link between the H4R3me2a mark and MLL-EEN-mediated oncogenic signaling.

Recently, PRMT1 was reported to interact with a splice isoform of the oncogenic fusion protein AML-ETO (AE9a), the characteristic translocation product of t(8;21)-positive acute myeloid leukemia (Shia et al. 2012). PRMT1 directly methylates AE9a on Arg-142 and is recruited to AE9a target gene promoters, where it increases methylation on H4R3 (H4R3me2a). Genetic knockdown of PRMT1 decreased the levels of H4R3me2a at AE9a target gene promoters in AML-ETO translocation-positive cell lines. Most notably, knockdown of PRMT1 also decreased the self-renewal capacity of AE9a in colony growth assays, consistent with an important role for PRMT1 and H4R3me2a in t(8;21) leukemogenesis.

Interestingly, dimethylation of H4R3 can repress or promote gene expression at the same promoter depending on whether the modification is symmetric or asymmetric.

Symmetric dimethylation of H4R3 by the type II protein arginine methyltransferase PRMT5 leads to recruitment of DNMT3A to the β -globin locus via direct binding of DNMT3A to H4R3me2s (Zhao et al. 2009). Subsequently, DNMT3A methylates CpG sites leading to transcriptional repression of the β -globin locus.

On the other hand, asymmetric methylation of H4R3 (H4R3me2a) by the type I protein arginine methyltransferase PRMT1 leads to repression of gene expression at the β -globin locus. The H4R3me2a mark, catalyzed by PRMT1, recruits the histone acetyltransferase KAT2B (PCAF) to the β -globin locus control region, and in turn, KAT2B acetylates H3K9 and K14 (Li et al. 2010). Acetylation of these sites is associated with increased transcription at the β -globin locus. Thus, the activities of PRMT5 and PRMT1 at the same histone H3 arginine residue have opposing functions on gene expression. Within the context of lymphomas, such as mantle cell and B-CLL, where the H4R3me2s mark is increased at key tumor suppressor promoters, it remains an open question as to whether H4R3me2a is concurrently downregulated.

9.2.2.3 H3R17me2a

Increased asymmetric dimethylation of H3R17, known to be catalyzed by CARM1, has been observed in breast (Frietze et al. 2008; El Messaoudi et al. 2006) and prostate (Majumder et al. 2006) cancers. CARM1 is recruited to nuclear hormone receptor complexes via interactions with members of the p160 steroid co-activator family (Chen et al. 1999). Upon hormone stimulation, CARM1 is recruited to p160-nuclear hormone receptor complexes and increases H3R17 dimethylation at androgen- and estrogen-responsive promoters. These signaling events lead to increased transcription of several proliferation genes, such as *E2F1*, *CCNA1*, *CCNE1*, *CCNE2*, and *CDC25A*. Disruption of CARM1 in both prostate and breast cancer cell lines decreases H3R17 methylation at hormone-responsive promoters and leads to decreased cancer cell proliferation. It should be noted that increased expression of nuclear CARM1 has been reported to correlate with the progression of prostate cancer malignancy (Majumder et al. 2006; Hong et al. 2004). However, these studies did not investigate whether H3R17me2a levels also correlate with disease progression.

9.2.2.4 H3R26me2a

Although not as well characterized as H3R17me2a, H3R26me2a is also generated by CARM1. This mark has been linked to oncogenic signaling by virtue of the observation that the CARM1/NCOA3 complex is recruited to the promoters of a number of E2F-driven target genes, including *CCNE1*, *DHFR*, and *CDC6* (El Messaoudi et al. 2006). Recruitment of the CARM1/NCOA3 complex to said promoters increases the levels of both H3R17me2a and H3R26me2a.

9.2.2.5 Nonhistone Substrates of Protein Arginine Methyltransferases in Cancer

The importance of nonhistone substrates in PRMT-mediated oncogenic signaling pathways is becoming increasingly clear. These substrates include a variety of signaling molecules such as transcription factors, transcriptional co-activators, chromatin modulating proteins, and other DNA-binding proteins. One of the most compelling examples of these nonhistone substrates is the tumor suppressor p53.

Jansson and colleagues identified in the oligomerization domain of p53 three arginine residues which appear to be methylated by PRMT5 (Jansson et al. 2008). Disruption of PRMT5 reduces methylation of these residues and promotes a p53-mediated apoptotic response following DNA damage. Thus, PRMT5 may have a role in regulating p53-mediated cell fate (cell cycle arrest versus apoptosis) following a DNA damage stimulus.

As mentioned previously, the CARM1 has been shown to be recruited to nuclear hormone receptors via its interaction with the p160 steroid co-activator family of proteins. As a part of these transcriptional complexes, CARM1 can methylate not only nucleosomes (H3R17) but also its p160-binding partner NCOA3 (Feng et al. 2006; Naeem et al. 2007). Interestingly, this methylation event, which is increased by hormone stimulation, leads to disassembly of the NCOA3 transcriptional complex and downregulation of hormone-dependent transcription. Thus, the role of CARM1 in nuclear hormone receptor signaling is complex, as CARM1 can activate proliferative transcriptional pathways via methylation of nucleosomes (H3R17), while simultaneously attenuating hormone-dependent transcription by methylating NCOA3. The question as to how the balance of these stimulatory and inhibitory pathways becomes shifted in favor of proliferation in certain types of breast and prostate cancer is an area for future investigation.

9.2.2.6 DNA Damage Response Machinery and PRMT1

PRMT1 has been linked to asymmetric arginine methylation of number of DNA damage checkpoint machinery proteins. Recently, it was reported that PRMT1 methylates BRCA1 in a region that contains multiple lysine and arginine methylation sites and that arginine methylation of BRCA1 can be detected in multiple breast cancer lines and breast tumor samples (Guendel et al. 2010). Knockdown of PRMT1 in breast cancer cell lines caused a change in the binding pattern of BRCA1 to target promoters. It remains unclear exactly how BRCA1 arginine methylation correlates with cancer phenotypes. PRMT1 has also been reported to methylate MRE11, a member of the DNA double-strand break repair complex (Boisvert et al. 2005a). While methylation of MRE11 did not appear to affect formation of the MRE11-RAD40-NBS1 complex, mutation of arginine residues in the GAR (glycine-arginine rich) domain diminished its exonuclease activity. Like MRE11, 53BP1 has also been reported to contain a GAR motif and to be methylated by PRMT1 (Boisvert et al. 2005b). Mutation of arginine residues within the 53BP1 GAR motif abrogated its ability to localize to sites of DNA damage. Taken together, these observations suggest a role for PRMT1 in regulating DNA damage pathways which have critical importance to oncogenic signaling.

9.3 Mechanism of Methyltransfer

The KMT and RMT share a common mechanism to transfer the methyl group from a universal methyl donor, S-adenosyl-L-methionine (SAM) to the nitrogen atom of lysine and arginine side chains, respectively (Fig. 9.1). The transfer of the methyl group results in the production of S-adenosyl-L-homocysteine (SAH).

9.3.1 SET Domain Lysine Methyltransferases

As discussed above, the SET domain class of lysine methyltransferases includes enzymes known to methylate several sites on histone H3 (K4, K9, K27 & K36) and at least one site on histone H4 (K20). The KMTs can transfer 1, 2, or 3 methyl groups to lysine utilizing SAM as a methyl donor. The mechanism for methyl transfer has been extensively studied, and several crystal structures of KMTs bound to SAM/SAH, and a peptide substrate have been solved. The substrate specificity of the KMTs is not only specific to the lysine site on the histone but also depends on the methylation state of the lysine. Mutations and different binding proteins can change the substrate preference of some of the KMTs.

The SET domain family of methyltransferases is reported to contain 51 human proteins (Copeland et al. 2009; Richon et al. 2011). Generally the SET methyltransferases are thought to function as part of larger complexes such as the PRC2 complex. The accessory proteins act to target and regulate activity while the SET methyltransferase is the catalytic engine of the complex.

The SAM-binding motif contained in the SET domain of the KMTs has both highly conserved and divergent regions. In the highly conserved region, a NHS sequence is maintained for the majority of SET proteins. The histidine backbone carbonyl accepts a hydrogen bond from the N-6 of SAM, while the backbone amine of the same histidine donates a hydrogen bond to the N-7 of SAM (Dillon et al. 2005). The interaction with the conserved histidine has been seen in all of the co-crystal structures of SET methyltransferases with SAM or SAH. The remaining SAM-binding pocket can vary significantly between subfamilies, but all contain interactions with the amino acid portion of SAM. It is worth noting that while the interactions vary, the 3-dimensional configuration of SAM/SAH in the SET methyltransferase co-crystals are superimposable on each other and completely distinct from any of the other SAM-utilizing methyltransferases.

One of the most studied KMT is SET7/9 (SETD7). It has been the focus of several mechanistic papers regarding the process of transferring a methyl group from SAM to the lysine. There is consensus that the reacting lysine is initially protonated when binding to the KMT. While some disagreement exists on the nature of the base, the mechanism involves the deprotonation of lysine to give the neutral amine which then attacks the methyl of SAM in a S_N2 -like fashion. Guo et al. (Guo and Guo 2007) have suggested that the conserved tyrosine could be the base that deprotonates the lysine, while others have suggested water acts as the base. Xiao et al. (2005) pointed to bulk solvent to be the base, while Dirk et al. (2007) suggested a water molecule in the active site as the base. Zhang and Bruice (2007) favor the water being the base but point out that the water molecule in the active site is not basic enough to deprotonate the lysine and there is not access to bulk solvent in the active site. They indicate that a water channel forms upon binding the substrate, allowing the proton to be shuttled to bulk solvent. The water channel is proposed to form only when the lysine lacks a methyl group. Once the lysine is methylated, the methyl blocks the channel and prevents subsequent methylation, thus giving SET7/9 its specificity of catalyzing the 0–1 H3K4 methylation.

The lysine-binding portion of the active site of the SET domain has been shown to consist of an aromatic cage containing 1–3 tyrosine residues which can accept hydrogen bonds from the lysine. The aromatic cage stabilizes the charged species and lowers the pKa of the lysine, while the tyrosines play a role in proper alignment of lysine (Zhang and Bruce 2008). Mutational studies with GLP and G9a (Wu et al. 2010; Rathert et al. 2008) and with SET7/9 (Collins et al. 2005) have shown that mutation of one of the aromatic cage tyrosines to phenylalanine, and in one case to alanine, allows the enzyme to further methylate the lysine. Additionally for G9a, it was shown that mutating an aromatic cage phenylalanine to tyrosine restricted the number of methyl groups it could transfer, allowing only the unmethylated lysine to be an effective substrate. The two positions which control the ability of the SET to use methylated lysines as substrates have been termed the Phe/Tyr switch.

While initially these switches were identified by performing *in vitro* mutational studies, recently similar mutations have been described in follicular and germinal center B-derived diffuse large B-cell lymphomas (Morin et al. 2010). In these lymphomas, EZH2 was found to be mutated at Y641 (which corresponds to one of the residues identified previously as a Phe/Tyr switch) resulting in a change to several other amino acids (Morin et al. 2010). Enzymatic analysis of these mutant enzymes showed a change in substrate preference from H3K27me $0 > 1 > 2$ for the wild-type enzyme to $2 > 1 > 0$ for the Y641 mutants (Sneeringer et al. 2010; Yap et al. 2011). These change-of-function mutants led to elevated cellular levels of H3K27me₃, which contributes to lymphomagenesis. Interestingly, all of the Y641 mutants displayed the same pattern of changes in substrate utilization, and all of the changes could be ascribed to transition state interactions with the enzyme (Sneeringer et al. 2010).

9.3.2 Arginine Methyltransferases

The arginine methyltransferases (RMTs) can be divided into two major subgroups, type I and II (Di Lorenzo and Bedford 2011). The type I RMTs consist of PRMT 1, 3, 4, 6, and 8 and type II of PRMT 5 and 7. Additionally, PRMT7 has been described to be a type III RMT on certain substrates (Fig. 9.2). The type I RMTs catalyze both the formation of monomethylated arginine (MMA) intermediates and asymmetrical dimethylated arginines. The type II RMTs catalyzes the formation of MMA and symmetrical dimethylated arginines. The type III RMT catalyzes only the formation of MMAs. In each case, SAM is utilized as the methyl donor as depicted in scheme A. The majority of methylated arginines found *in vivo* appear to be DMAs (dimethylated arginines) (Zhang and Cheng 2003).

A number of RMTs including PRMT1 and 3 and CARM1 (PRMT4) have been found to exist in dimeric or oligomeric states (Zhang and Cheng 2003); this has led groups to speculate on whether the *in vivo* observation of preponderant DMA versus MMA is due to the dimeric/oligomeric structure leading to a processive mechanism. Kolbel et al. (2009), however, have demonstrated that PRMT1 and PRMT3 act *via* a distributive mechanism (Fig. 9.3). This was based on the analysis of

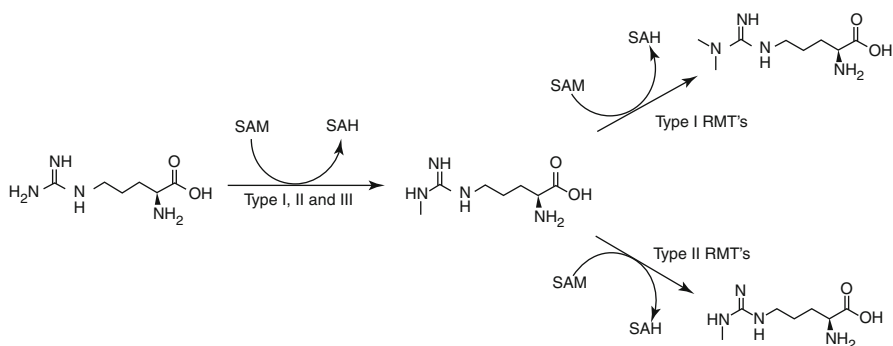


Fig. 9.2 Methylation of arginine residues by types I, II, and III RMTs

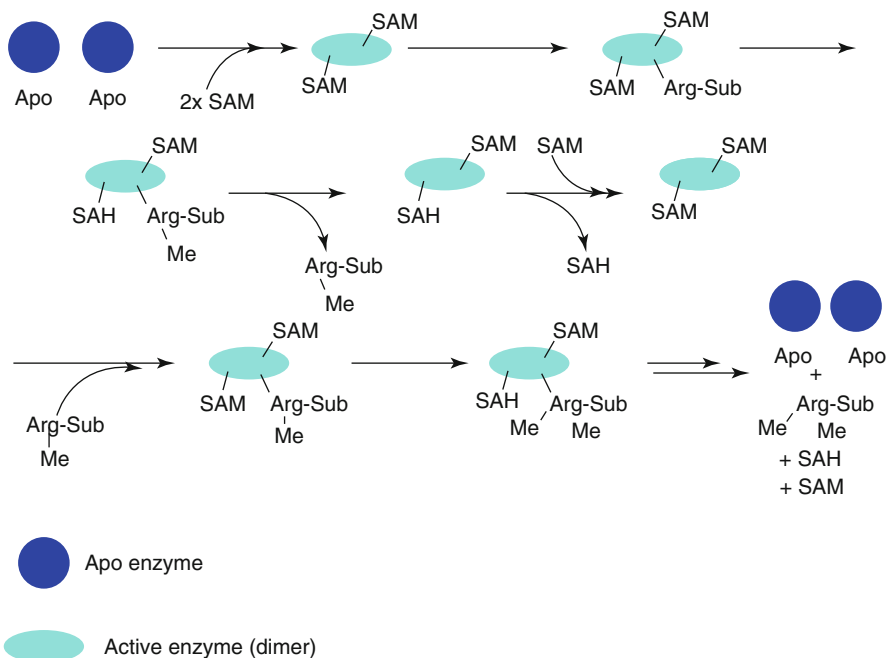


Fig. 9.3 Distributive mechanism of arginine methylation by type I RMTs

multiple kinetic experiments, including the observation of a Poisson product distribution highly indicative of a distributive mechanism whereas a processive mechanism should result in a product profile biased towards dimethylated species. The authors suggest that release of the methylated arginine substrate is obligatory and rate determining.

Additional work is necessary to understand why DMA products predominate in vivo as the distributive mechanism does not appear to account for this finding.

Experiments with peptide substrates suggest little change in K_m and k_{cat} values for MMA residues over the corresponding unmethylated counterparts, i.e., the MMA arginine residues are not preferred substrates; however, further work with physiological substrates is required. Alternative explanations include that the RMTs are components of multi-protein complexes, where the other proteins may influence the substrate specificity of the RMT. CARM1, for example, is found in a complex of at least ten proteins called the nucleosomal methylation activator complex (NUMAC) (Xu et al. 2004). Interestingly, recombinant CARM1 in the absence of some, if not all, of the other proteins of the NUMAC complex preferentially methylates free histone H3 rather than the nucleosome. CARM1 activity has also been reported to be modulated by phosphorylation resulting in a decrease in RMT activity (Higashimoto et al. 2007). Neighboring residues on histone H3, such as H3K18, have been demonstrated to affect the rate of methylation on H3R17 by CARM1. For example, CARM1 activity on H3R17 is increased when H3K18 is acetylated ($\sim 5\times$). This is due to an increase in k_{cat} rather than K_m (Daujat et al. 2002). It is interesting to note that for CARM1 substrates, the +1 residue is neutral in known protein substrates except for histone H3. For example, the following CARM1 substrates contain a neutral +1 residue; PABP1 R455, R460; HuR R206, R217; HuD R225, R236; p300 (KIX domain) R580, R604; and CBP (post-KIX domain) R714, R742, R768 (Yue et al. 2007). This suggests a potential electrostatic sensing mechanism to explain the preference of a DMA product on H3R17. However, this does not provide an explanation for other substrates of CARM1. This example also highlights the inherent complexity of histone modifications, as posttranslational modification of specific residues can affect the methylation status of other residues. This may be important for drug discovery as changes in methylation status may be gene-specific due to the effect of other posttranslational modifications of chromatin. Therefore, it may be very difficult to detect changes from a methyltransferase inhibitor when measuring global methylation levels.

9.3.2.1 Structure and Catalytic Mechanism of RMTs

The X-ray crystal structure of PRMT1, PRMT3, and CARM1 in various states has been obtained (Zhang and Cheng 2003; Yue et al. 2007; Zhang et al. 2000). The CARM1 X-ray structure was obtained in the presence and absence of SAH, and it was found that upon binding to SAH, significant structural reorganization of the protein occurs resulting in encapsulation of SAH (and presumably SAM) and the formation of the substrate-binding pocket. The authors conclude that the structure is consistent with an ordered mechanism in which SAM binding occurs first and the intermediate MMA must be released from the active site prior to the replenishing of SAM, an observation which is consistent with a distributive mechanism (Yue et al. 2007).

Insight from the structures of PRMT1, PRMT3, and CARM1 has led to the proposal of a catalytic mechanism (Fig. 9.4). When the substrate binds to the RMT-SAM complex, a conserved carboxylate (for CARM1, this is Glu 267, E153 in PRMT1, E355 in PRMT3) interacts with one of the nitrogen atoms in the guanidine group of the arginine residue, resulting in increased localization of positive charge on the $N_{\eta 1}$ nitrogen allowing the $N_{\eta 2}$ nitrogen to react with SAM (Zhang and Cheng 2003; Yue et al. 2007; Zhang et al. 2000). This carboxylate residue has been shown

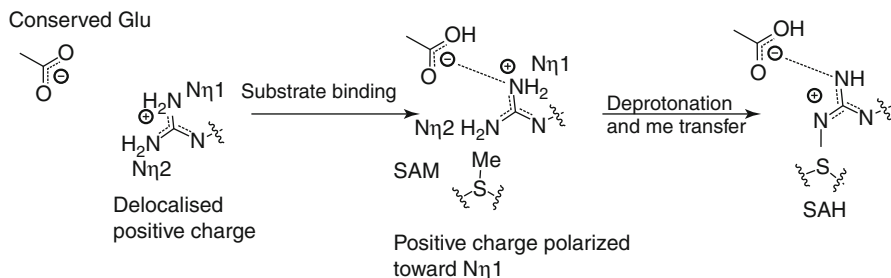


Fig. 9.4 Proposed catalytic mechanism of RMTs

to be critically important in catalysis, as the mutant Glu267Asn is catalytically inactive (Chen et al. 1999; Yue et al. 2007). It is likely, however, that the guanidine group is charged and needs to be deprotonated before reacting with SAM. The authors point out that the timing of the deprotonation step is unknown but is likely to occur *via* a His-Asp (CARM1-H415/D166, PRMT1-H476/D233) coupled proton relay similar to serine proteases (Zhang et al. 2000; Fersht and Sperling 1973). The His and Asp residues are also somewhat conserved throughout the RMTs (Zhang et al. 2000). It has been proposed that deprotonation is likely to be rate determining as in other S_N2 enzymatic reactions (Yue et al. 2007). If the His-Asp couple is involved in the rate-determining step, then it is likely to be highly sensitive to the electronic state of neighboring residues (e.g., difference in *k*_{cat} for H3K18 and acetylated H3K18).

The protein crystal structures have also provided a hypothesis for the selectivity differences between type I and II RMTs. The type I RMTs produce asymmetric dimethylated arginine residues. One possible explanation for this is due to the sulfur atom of M337 (PRMT3), which is only 3.6 Å away from N η 1, essentially sterically blocking this nitrogen. The N η 2 has space to accommodate a monomethyl group which allows for the asymmetrical DMA product formation (Zhang et al. 2000).

9.4 Inhibitors of Histone Methyltransferases

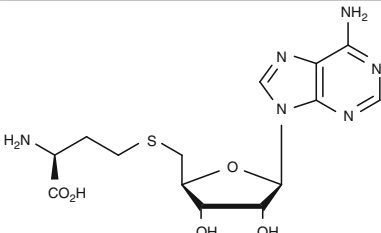
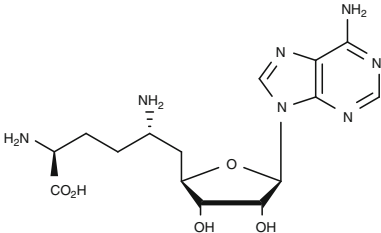
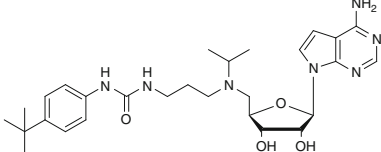
Despite the discovery of the first HMT over 10 years ago (Rea et al. 2000) and the description of close to 100 related proteins (Richon et al. 2011), there has been a paucity of literature describing potent and selective inhibitors not based on SAM, SAH, or Sinefungin (Table 9.2). The majority of reports have focused on inhibitors of CARM1, G9a, and GLP.

9.4.1 Lysine Methyltransferase Inhibitors

9.4.1.1 G9a (EHMT2) and GLP (EHMT1)

The majority of published work on chemical inhibitors of SET domain-containing HMTs has focused on GLP and G9a. The first drug-like small molecule inhibitor

Table 9.2 Lysine and arginine methyltransferase inhibitors: nucleoside analogs

Compound name	Compound structure	Primary target potency (IC ₅₀)	Comments
SAH		Product of reaction for all PMTs. IC ₅₀ s range from 0.1 to 20 μM	Nonselective
Sinefungin		Natural product analog of SAM/SAH. IC ₅₀ s range from 0.1 to 20 μM	Nonselective
EPZ004777		DOT1L inhibitor IC ₅₀ of 0.4 nM; >1,000-fold selectivity against other PMTs	Selective DOT1L inhibitor

that acted on these two enzymes was Compound (I) and is known as BIX-01294 (Kubicek et al. 2007). Compound (I) was identified *via* high-throughput screening and is closely related to the α -adrenoceptor antagonist bunazosin (Weidinger 1995) (Table 9.3). Compound (I) demonstrated an IC₅₀ of 1.7 μM for G9a and 38 μM for GLP. Two independent groups have replicated the IC₅₀ for G9a but found the compound to be significantly more active at GLP with an IC₅₀ ~0.7 μM (Liu et al. 2009a; Chang et al. 2010). The authors attribute the discrepancy to the fact that the original data was not performed under linear assay conditions. Compound (I) appears to be selective against other HMTs as only minimal activity was observed at PRMT1, ESET, SET7/9, Suv420H1, and SUV39H1.

Compound (I) is uncompetitive with SAM, suggesting that it only binds to the SAM-G9a complex and not the free enzyme. These data are consistent with the X-ray structure of Compound (I) bound to GLP (~80 % sequence similarity to G9a) where the small molecule is bound in the substrate-binding pocket and SAH is also bound (Chang et al. 2009).

In mouse embryonic stem cells treated with 4.1 μM of (I) the H3K9me2 mark was reduced by 20 % with a concomitant rise in unmethylated H3K9 and no change in H3K9me3 or H3K9me1 levels. No changes on the global methylation status of H3K27, H3K36, or H4K20 were observed indicating that the inhibitor selectively inhibits G9a/GLP in cells. The inhibitor (I) did reduce H3K9me2 in G9a null ES

cells, and the authors ascribe this finding to the activity on GLP which also affects H3K9 methylation. This explanation is even more likely as the work of other groups has shown that Compound (I) is essentially equipotent against GLP. Therefore, the current evidence suggests that Compound (I) is a dual GLP/G9a inhibitor.

ChIP analysis demonstrated that Compound (I) affected H3K9me2 levels at target genes such as *Mage-a2*. Approximately 60 % loss of the H3K9me2 mark at the *Mage-a2* promoter was observed in mouse ES cells that were treated with 4.1 μ M Compound (I). This inhibition was reversible in that the mark returned to original levels within 48 h once the inhibitor was removed.

Two independent groups (Liu et al. 2009a; Chang et al. 2010) expanded upon the crystal structure findings of the GLP-Compound (I)-SAH complex, hypothesizing that an appropriately placed lysine mimic added to Compound (I) would result in improved activity at G9a and or GLP. The hypothesis was derived from the observation that when the X-ray structures of GLP-(I) and GLP-peptide substrate were compared, it was noted that Compound (I) resembled the bound conformation of histone H3 Lys4 to Arg8 but leaving the lysine-binding channel unoccupied. Using this observation, both Chang et al. and Liu et al. replaced the 7-OMe with an aminoalkoxy groups. The first result of the Liu et al. effort was UNC-0224 (III) which demonstrated improved potency compared to (I) (see Table 9.3). It was also found to be inactive in inhibiting other HMTs including SETD7, SET8/PreSET7, PRMT3, and the H3K9 demethylase JMJD2E.

A high-resolution (1.7Å) X-ray crystal structure of the G9a-UNC0224 (III) complex was obtained. On the basis of this structural information, the authors indicate the improved potency of UNC-0224/Compound (III) over Compound (I) is explained by interactions gained from the additional lysine mimetic moiety. In particular, the electrostatic interaction of the protonated dimethyl amine and Leu1086 and the π -cation interaction of the protonated dimethyl amine with Tyr 1154 add to the affinity.

Key structural insights were identified from this analysis, and these are illustrated in Fig. 9.5. Two features of particular note are that (1) the bulk of UNC-0224 (III) occupies the histone peptide-binding site as in the GLP-(I) structure and (2) the lysine-binding channel is not fully occupied by the amino-propoxy lysine mimic. This last observation suggested the potential for further improvement in potency with a longer lysine mimetic. This hypothesis was confirmed by the synthesis of Compound (IV) which shows improved potency against G9a (Table 9.3).

The inhibitor (IV) demonstrated a high Hill slope suggesting that the potency limit of the assays used had been reached (Liu et al. 2009a). Using microfluidic capillary electrophoresis, the K_i values of 2.6 nM and 63 pM were established for Compound (III)/UNC0224 and Compound (IV), respectively, compared to 16 nM for Compound (I). This analysis shows that Compound (IV) has greatly improved potency over both Compound (I)/BIX-01924 and Compound (III)/UNC0224.

Vedadi et al. describe the chemical probe UNC0638 (Vedadi et al. 2011) (Table 9.3). UNC0638 shares common features with (III)/UNC0224 and (IV) but interestingly the basic and polar 1-methyl-1,4-diazepane ring system has been replaced with the neutral and lipophilic cyclohexyl ring. This modification was presumably made in order

Table 9.3 Examples of G9a inhibitors

Compound name	Compound structure	Primary target potency (IC ₅₀)	Comments
Compound (I)/ BIX-01294		SAM noncompetitive inhibitor of G9a (0.2– 1.7 μM) and GLP (0.03–38 μM)	
Compound (II) bunazosin			Alpha- adrenoceptor antagonist
Compound (III)/ UNC-0224		K _i 2.5 nM	Reported as essentially equipotent against GLP
Compound (IV)		K _i 63 pM	Reported as essentially equipotent against GLP
Compound (V)/ UNC0638		K _i 2.5 nM; G9a inhibitor (IC ₅₀ <15 nM)	Inhibitor reduces H3K9Me2 in MDA-MB231 cells with an IC ₅₀ of 81 nM
Compound (VI)		G9a inhibitor (IC ₅₀ 100 nM)	Essentially equipotent against GLP

to improve cell permeability. Indeed, Compound (V) showed reduction of H3K9me2 in MDA-MB-231 cells with an IC₅₀ of 81 nM and with a better separation of methyl mark effects and cell toxicity compared to Compound (I).

Chang et al. (2010) also found that a longer chain at the seven position resulted in improved potency. This change combined with replacement of the diazepane ring and benzyl groups of Compound (I) with a 3-diemethylaminopropyl and a 5-aminopentyl chain, respectively, led to Compound (VI) (Table 9.3). The cumulative effect of these changes is 7-fold improvement in affinity for GLP relative to Compound (I) under identical assay conditions.

It was noted from the (VI)-GLP-SAH X-ray structure that the sulfur atom of SAH was only 4.7Å from the nitrogen of the lysine mimetic, very close to the distance seen in the GLP-SAH-H3K9me1peptide substrate structure of 4.2Å (Chang et al. 2010). Superposition of the (VI)/GLP complex with the GLP/H3K9me1 peptide side-chain complex suggested that the 5-aminopentoxy side chain was appropriately positioned to be a substrate for methylation. Mono-, di-, and trimethylated derivatives of (VI) were detected by mass spectrometry following overnight incubations of nearly equimolar ratio of (VI) and GLP. Despite the nonphysiological conditions of this experiment, it does provide evidence that the inhibitors may also be substrates.

9.4.1.2 DOT1L

EPZ004777 has been reported as a potent and selective inhibitor of DOT1L (Daigle et al. 2011). This molecule is an aminonucleoside analog that was shown to be SAM competitive with an IC_{50} of 0.4 nM for DOT1L biochemical activity and greater than 1,000-fold selectivity against other KMTs and RMTs (Table 9.2). EPZ004777 causes dose-dependent reduction of histone H3K79me2 in cultured cells and inhibits proliferation of leukemia cells bearing the *MLL* translocation. It is noteworthy that cells lacking the *MLL* translocation were insensitive with respect to antiproliferative effects despite undergoing a reduction in the H3K79me2 mark. EPZ004777 also demonstrated in vivo efficacy in a mouse model of disseminated *MLL*-rearranged leukemia, at doses that were well tolerated. These data demonstrate the high specificity of targeting DOT1L for *MLL*-rearranged leukemias.

9.4.2 Arginine Methyltransferase Inhibitors

9.4.2.1 CARM1

One of the first non-SAM like small molecule inhibitors of CARM1 was described by Purandare et al. (2008). High-throughput screening efforts identified Compound (VII) with a CARM1 IC_{50} of 1.8 μ M (Table 9.4). Progression of this hit led to the identification of Compound (VIII) with a 10-fold improvement in CARM1 IC_{50} , 0.08 μ M (Purandare et al. 2008). Compound (VIII) was also tested for activity against PRMT1 and PRMT3, and IC_{50} values of >25 μ M were obtained for each enzyme. No cellular data was reported, presumably because the series was hampered by poor permeability. Compound (VIII) scored poorly in a PAMPA assay with a permeability of less than 0.015 mm/s. An improvement in the permeability was observed when the secondary amide was replaced with the bio-isosteric 1,3,4-oxadiazole ring. This change led to an increase of approximately 10-fold in permeability with no loss of CARM1 inhibitory activity.

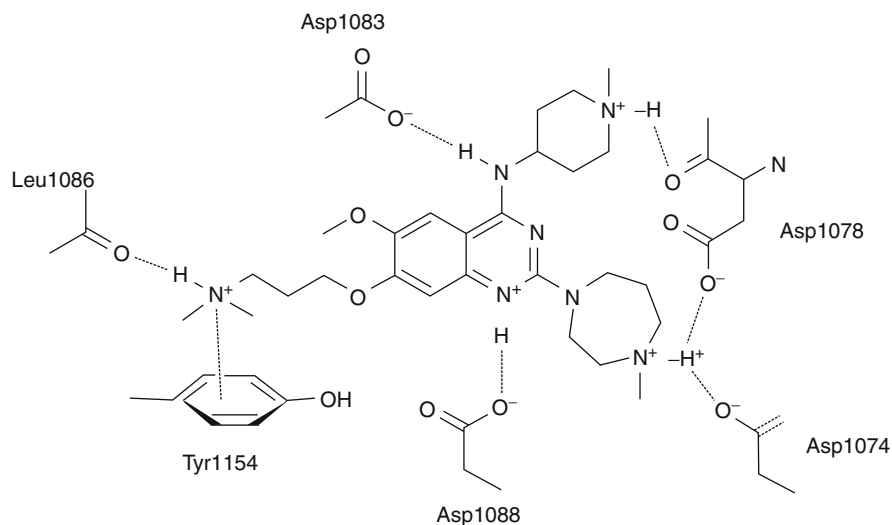


Fig. 9.5 Key interactions of compound (III)/UNC0224 with G9a

Further optimization of the oxadiazole (IX) led to the discovery of (X) which improved CARM1 potency (IC_{50} 0.04 μ M) and permeability to 0.267 μ m/s (Huynh et al. 2009) (Table 9.4). The compound was further characterized for ADMET properties and found to not inhibit P-450 enzymes (human hepatocyte assay-HHA) or to activate the human pregnane-X receptor (hPXR).

Allan et al. have also developed a related series of thienyl pyrazoles of which Compound (XI) is representative (Allan et al. 2009). They found that the series was of similar potency as earlier compounds described above. Compound (XI) was also selective over the RMT PRMT1 and the KMT SETD7 (IC_{50} >100 μ M for each enzyme). Compound (XI), however, was reported to lack cellular activity in two different cell types after 48 h of exposure to the inhibitor at a concentration of 5 μ M. A number of reasons for the lack of cell activity are possible, such as cell permeability (the authors report that the thienyl-amide series also possessed poor permeability similar to the phenyl-amide series (e.g., Compound (VIII)) (Huynh et al. 2009). It is also possible that the compounds lack sufficient biochemical potency to elicit cellular effects. Alternatively, the lack of changes in global levels of H3R26 methylation may be due to redundancy due to other RMTs. In light of this, Compounds (XI) and (XII) were evaluated in a series of functional assays. The assays targeted both estrogen dependent transcription, estrogen growth and androgen dependent transcription. Neither Compound (XI) nor (XIII) demonstrated any effect in these assays consistent with the lack of methylation changes observed previously.

Therrien et al. (2009) also noted the poor PK profile of the phenyl-amide and thienyl-amide series, low oral bioavailability in rats and low exposure in mice when dosed by intraperitoneal injection. Compound (XII) which is representative of the phenyl-amide series was characterized with extremely high clearance (1,433 ml/

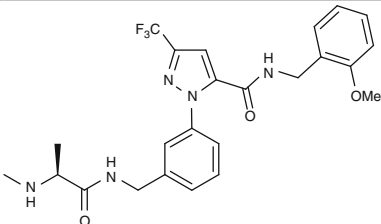
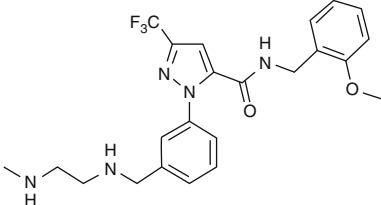
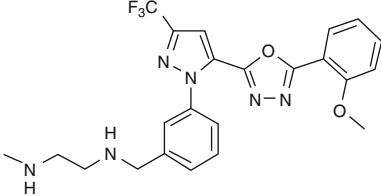
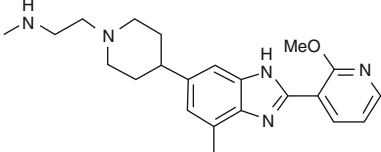
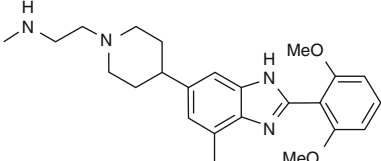
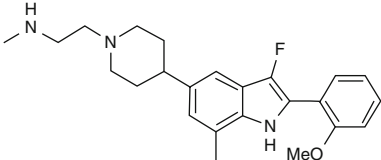
min/kg) and a short $t_{1/2}$ (<0.25 h) when dosed by intravenous injection in rats. The high clearance precluded the accurate measurement of oral bioavailability, volume of distribution and half-life. Therrien et al. hypothesized that the high clearance was due to the presence of the (S)-alanine benzylamide present in both chemical series.

Table 9.4 Examples of CARM1 inhibitors

Compound name	Compound structure	Primary target potency (IC_{50})	Comments
Compound (VII)		CARM1 IC_{50} 1.8 μ M	
Compound (VIII)		CARM1 IC_{50} 80 nM	PAMPA <15 nm/s
Compound (IX)		CARM1 IC_{50} 60 nM	PAMPA 106 nm/s
Compound (X)		CARM1 IC_{50} 40 nM; >100-fold for PRMT1 and PRMT3	PAMPA 267 nm/s; hPXR (EC50) >25 μ M; HHA IC_{50} >100 μ M; improved permeability
Compound (XI)		CARM1 IC_{50} 60 nM	

(continued)

Table 9.4 (continued)

Compound name	Compound structure	Primary target potency (IC ₅₀)	Comments
Compound (XII)		CARM1 IC ₅₀ 60 nM	Cl 1,433 ml/min/kg
Compound (XIII)		CARM1 IC ₅₀ 200 nM; >100 μM for PRMT1 and SET7/9	Cl 20 ml/min/kg; Vdss 0.2 kg; t _{1/2} 2.1 h; F4 %
Compound (XIV)		CARM1 IC ₅₀ 590 nM	Cl 37 ml/min/kg; Vdss 0.6 kg; t _{1/2} 1.8 h; F3 %
Compound (XV)		CARM1 IC ₅₀ 840 nM	X-ray complex of Sinefungin-CARM1-inhibitor
Compound (XVI)		CARM1 IC ₅₀ 70 nM	>300-fold selective for CARM1 compared to PRMT1 and PRMT3
Compound (XVII)		CARM1 IC ₅₀ 81 nM	>100-fold selective for CARM1 compared to PRMT1 and PRMT3

After SAR exploration into the replacement of this moiety, it was found that a N¹-benzyl-N²-methylethane-1,2 diamine unit could adequately replace the alanine amide group with minimal loss in CARM1 potency, as exemplified by Compound

(XIII). The PK profile of Compound (XIII) was evaluated in rats and was found to have a much reduced clearance (20 ml/min/kg) which resulted in an improved $t_{1/2}$ of 2.1 h. The volume of distribution and oral bioavailability, however, remained low: 1.4 l/kg and 4 %, respectively.

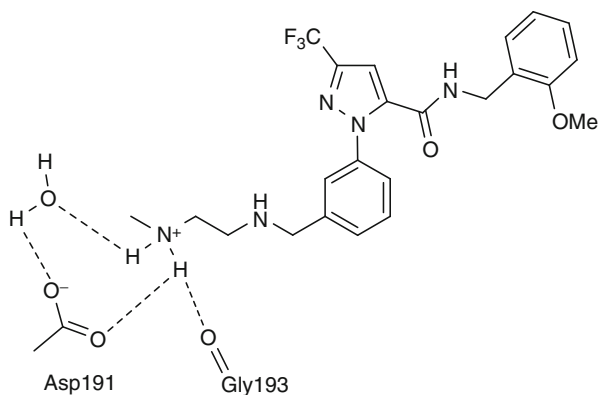
The low oral bioavailability may be due to the amide functionality, as both Therrien et al. (2009) and Huynh et al. (2009) indicate that this group confers low permeability in both the phenyl and thienyl series. To address these concerns, Therrien et al. (2009) synthesized Compound (XIV) with the 1,3,4-oxadiazole ring which had been shown to improve permeability (Huynh et al. 2009). As can be seen from Compound (XIV), bio-steric replacement did not improve oral bioavailability or increase the volume of distribution; however, no PAMPA data was reported for this analog, so it is unclear if this analog had improved permeability.

Troffer-Charlier et al. also proposed a binding mode based on docking (XIII) into a published X-ray structure of CARM1-SAH (Troffer-Charlier et al. 2007). A graphical representation of the critical interaction of the N¹-benzyl-N²methylethane-1,2 diamine group is shown schematically in Fig. 9.6. The diamine group is proposed to bind to a water molecule as well as the carboxylate group of Asp191 and the backbone carbonyl of Gly193. A significant SAR effort around the diamine side chain was undertaken, with small changes producing large changes in activity. It was proposed that the linker needs to be flexible in order to adopt the required conformation to interact with the water molecule, Asp 191 and Gly193, while avoiding a potentially energetically costly interaction with the positively charged Arg 169 (which interacts with the carboxylate of SAH). No kinetic or crystallographic data has been reported on this diamine series to date to support the idea that these compounds bind in the SAM-binding site.

Wan et al. also found a benzo[d]imidazole hit (XV) *via* high-throughput screening which inhibited CARM1 with an IC₅₀ of 0.84 μM (Wan et al. 2009). This series also contains a diamine moiety similar to that in the Compounds (XIII) and (XIV), though in this series a tertiary amine is incorporated into the diamine, and no such corresponding analog was reported (Allan et al. 2009; Therrien et al. 2009). Therefore, it is unclear if the two series are related in regard to how they interact with CARM1.

Wan et al. performed a hit to lead effort on (XV) and found most changes to the molecule were detrimental (Wan et al. 2009). The C-2 substituent on the benzo[d]imidazole, however, proved to be a fruitful area to analog resulting in Compound (XVI) with over 10-fold improvement in CARM1 IC₅₀. Further studies from Wan et al. led to the discovery of the indole Compound (XVII) (presented at ACS Symposium, Boston, 2010). Compound (XVI) is a potent CARM1 inhibitor and inhibits histone H3 methylation (IC₅₀ 0.081 μM). It was reported to inhibit the methylation of nonhistone CARM1 substrates (PABP and HuR) with similar potency. This group also performed additional pharmacology on the indole compound (XVII) and found that the compound is metabolically stable in mouse, rat, and human microsomes and displays no inhibition of P-450 enzymes but does, however, possess poor permeability as measured in Caco-2 and PAMPA experiments. The compound also is an hERG inhibitor which is likely due to the basic side chain

Fig. 9.6 Proposed binding of compound (XIII) in the SAM binding pocket of CARM1



coupled with high lipophilicity (clogP ~4.9). A co-crystal of Compound (XVII) with Sinefungin and CARM1 was also presented. Unlike the model proposed by Therrien et al. (2009), it was found that the inhibitor was bound in the substrate-binding pocket rather than the SAM-binding pocket which was occupied by Sinefungin.

No cellular data was reported for any of the new analogs. This remains an outstanding issue for the CARM1 inhibitors described to date, as poor permeability is observed in multiple series and until robust cellular effects are observed, it is unclear whether these compounds are appropriate tools for examining the role of CARM1 mediated biology.

9.5 Summary and Future Perspectives

The HMTs are a promising new class of targets for cancer. Members of the enzyme family have demonstrated strong cancer relevance, and recent genome studies have linked genetic alterations in members of this family with specific cancer indications. Until recently, very few small molecule inhibitors have been described, and currently the first inhibitors are entering evaluation in clinical trials. The field has begun identifying inhibitors of these novel enzymes, initially with nucleoside or natural product-derived inhibitors. The development of a rich set of selective inhibitors with diverse chemical properties will enable the field to evaluate the potential of these targets in both preclinical and ultimately clinical studies. Many questions remain, but with the increasing biological understanding of these enzymes and their role in cancer and other diseases, it is likely that this field will rapidly evolve over the coming years to provide important new therapeutics for cancer treatment.

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