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Histone methylation modifiers in cellular signaling pathways

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Abstract

Histone methyltransferases and demethylases epigenetically regulate gene expression by modifying histone methylation status in numerous cellular processes, including cell differentiation and proliferation. These modifiers also control methylation levels of various non-histone proteins, such as effector proteins that play critical roles in cellular signaling networks. Dysregulated histone methylation modifiers alter expression of oncogenes and tumor suppressor genes and change methylation states of effector proteins, frequently resulting in aberrant cellular signaling cascades and cellular transformation. In this review, we summarize the role of histone methylation modifiers in regulating the following signaling pathways: NF-κB, RAS/RAF/MEK/MAPK, PI3K/Akt, Wnt/β-catenin, p53, and ERα.

Keywords

Histone methylation; histone methyltransferase; histone demethylase; oncogenic signaling; tumor suppressor pathway

Introduction

Chromatin, a complex of eukaryotic DNA and multiple proteins, serves as the cellular information center, receiving and sending various signals during numerous cellular processes. Cellular signaling events are coupled with covalent and non-covalent modifications of chromatin. Chromatin modifications regulate chromatin architecture and gene expression by affecting multiple interactions among DNA, histones, and chromatin-binding proteins. Of the chromatin modifications, histone methylation has emerged as a key epigenetic mark that regulates gene expression. Histone methylation is modulated by histone methyltransferases and demethylases. Notably, these methylation modifiers also modulate methylation states of many non-histone proteins, including key effectors and components in cellular signaling pathways. In cancer, certain histone methylation modifiers are frequently dysregulated, and this dysregulation is linked to aberrations in gene expression and cellular signaling cascades. This review focuses on how cellular signaling pathways are regulated by histone methylation modifiers.

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Histone methylation and its modifiers

Histone methylation

Histone methylation occurs predominantly on two highly abundant histone residues, lysine (K) and arginine (R), although methylation can take place on other amino acids, including histidine, aspartic acid, and glutamic acid, and on the carboxyl groups of proteins [1–4]. Unlike acetylation and phosphorylation, methylation marks do not alter the charge of histones but serve as docking sites for specific binding proteins called histone readers [5]. Histone methylation, together with other modifications, also can be recognized by combinatory binding modules in histone readers that ultimately affect chromatin architecture and regulate gene expression [6].

Histone lysine methylation occurs at three different levels: mono-, di-, and tri-methylation. This modification is highly conserved across different species, from unicellular organisms to mammals [7], and is linked to either gene activation or repression depending on the target site. For example, methylation at histone H3 lysine 4 (H3K4), H3K36, and H3K79 is generally related to gene activation, whereas that at H3K9, H3K27, and H4K20 is commonly linked to gene silencing. Apart from histones, this modification also occurs in other cellular proteins. Lysine methylation is associated with multiple cellular processes, including cellular signaling pathways, cell fate determination, terminal differentiation, X inactivation, and the spatiotemporal patterning of *Hox* genes [8–15].

Like lysine methylation, arginine methylation occurs on both histones and non-histone proteins. It takes place at a guanidino nitrogen of arginine [16–18]. Arginine residues can be methylated mainly in three different ways: ω-N^G-monomethyl arginine (MMA); ω-N^G, N^G-asymmetric dimethyl arginine (ADMA); and ω-N^G,N'^G-symmetric dimethyl arginine (SDMA). None of these methyl groups, when added to an arginine residue, change its positive charge, but they may affect the protein-protein interaction by eliminating formation of a potential hydrogen bond and changing the bulkiness of arginine side chain [17,19,20]. Arginine methylation regulates a number of different cellular processes, including cellular signaling, transcriptional regulation, RNA metabolism, and DNA damage repair [21].

Histone methylation modifiers

Histone methylation at individual lysine residues is catalyzed by specific lysine methyltransferases (KMTs) and can be removed by specific lysine demethylases (KDMs). SUV39H1 was the first histone KMT identified, and it methylates H3K9 [22]. Since then, numerous KMTs have been identified; they can be divided into two classes on the basis of their conserved catalytic domains. One class contains a highly conserved SET [Su(var)3–9, Enhancer of Zeste, and Trithorax] domain [23]. The other class does not have a SET domain but consists of highly conserved proteins yeast DOT1 (disruptor of telomeric silencing-1; also known as KMT4) and its eukaryotic homologs, such as human and mouse DOT1L (DOT1-Like) [24]. SET-containing KMTs generally methylate lysines within the histone *N*-terminal tails, whereas DOT1 and DOT1L methylate H3K79 within the histone globular core [25,26] (Figure 1).

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The protein arginine N-methyltransferases (PRMTs) catalyze addition of the methyl groups to the arginine residues. PRMTs are classified as type I, type II, type III, or type IV enzymes. Types I, II, and III catalyze methylation on the terminal (i.e., ω) guanidino nitrogen atom. Although types I and II both generate an MMA intermediate, type I PRMTs (PRMT1, 2, 3, 6, and 8 and PRMT4, also known as co-activator–associated arginine methyltransferase 1 [CARM1]) further modify this intermediate to ADMA, whereas type II PRMTs (PRMT5 and 9) catalyze the generation of SDMA [20,27,28]. PRMT7 appears to exhibit type III enzymatic activity to catalyze the formation of MMA. However, PRMT7 was also reported to generate SDMA *in vitro* and in cells, although this activity may be indirect [29–32]. The type IV RMT2 catalyzes monomethylation of the internal (i.e., δ) guanidino nitrogen atom. Most of the PRMTs are known to methylate glycine- and arginine-rich (GAR) motifs in their substrates [33]. In contrast, PRMT4 methylates arginine residues in proline-, glycine-, and methionine-rich (PGM) motifs [34]. Interestingly, PRMT5 can symmetrically dimethylate arginine residues in both GAR and PGM motifs [35]. Like KMTs, PRMTs methylate both histones (Figure 1) and several non-histone proteins [20,36,37].

Histone methylation was once considered a stable and static modification. However, it has been shown that the lysine-specific demethylase 1 (LSD1; also known as KDM1A) removes methyl groups from H3K4me1/2 by utilizing FAD as a co-factor [38]. LSD1 requires Co-REST to demethylate H3K4me1/2 on nucleosomal substrates [39]. Interestingly, it was reported that LSD1 in the presence of androgen receptor may demethylate H3K9me1/2 [40]. Later, JHDM1A, a Jumonji C (JmjC) domain–containing protein, was identified as a demethylase that removes methyl groups from H3K36me1/2 [41,42]. Since then, numerous JmjC-domain-containing histone lysine demethylases, including trimethylated lysine demethylases, have been identified (Figure 1) [40,42–44]. This family of demethylases requires Fe (II) and α-ketoglutarate as cofactors and exhibits a high specificity for target lysine residues. Interestingly, some demethylases demethylate di- and monomethylated but not trimethylated lysines, whereas others preferentially erase methyl groups from tri- and dimethylated lysines or monomethylated lysines [45]. In contrast to lysine demethylases, it remains still unclear whether there is a bona fide arginine demethylase. JMJD6 was reported to have arginine demethylation activity on H4R3 and H3R2 [46,47]. However, JMJD6 was also shown to be rather a hydroxylase that adds a hydroxyl group at the 5-C of a lysine side chain of the splicing factor U2AF65 [48].

It has been shown that histone methylation modifiers control methylation states in non-histone substrates to regulate their activities, as described later in this review. Notably, these non-histone substrates include key components of multiple cellular signaling pathways (e.g., nuclear factor–kappa B [NF-κB], epidermal growth factor receptor [EGFR], RAF1, mitogen-activated protein kinase (MAPK) kinase kinase 2 [MAP3K2], p53, and estrogen receptor [ER],) (Table 1). Aberrant methylation of histones and these non-histone proteins has been linked to various human cancers [49,50].

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Regulation of signaling pathways by histone methylation modifiers

Generally, methylation modifiers can regulate various signaling pathways by I) directly methylating and demethylating their components, including receptors and downstream effectors; II) transcriptionally modulating expression of their components; and III) modulating the activities of their components via physical interaction (Figure 2).

Histone methylation modifiers and NF-κB signaling

NF-κB signaling plays an important role in regulating multiple biological processes, including immune response, cell proliferation, and animal development. The NF-κB family of transcription factors comprises five members: p65 (RelA), RelB, c-Rel, p105/p50, and p100/p52. The precursor subunits p105 and p100 undergo proteolytic processing to become p50 and p52, respectively. Of the five members, the p50-p65 heterodimer is the key contributor to canonical NF-κB signaling. The p50-p65 heterodimer is inactive in a heterotrimeric complex consisting of p50, p65, and IκB in the cytoplasm (Figure 3). In response to a wide variety of cellular stimuli, IκB is phosphorylated by the IκB kinase complex (IKK) and is removed by ubiquitination-mediated degradation. Then, the p50–p65 heterodimer is released to be translocated to the nucleus. The p50-p65 heterodimer binds to the promoters of its target genes and induces gene expression [51–53]. Constitutive activation of NF-κB signaling is linked to numerous pathological states, including tumorigenesis and inflammation [54,55]. NF-κB signaling is controlled by multiple post-translational modifications [56]. Interestingly, NF-κB activities are modified by methylation and demethylation, as described in the subsequent paragraphs.

Lysine methylation of NF-κB

Using genetic and biochemical approaches, Lu *et al.* showed that K218 and K221 of p65 can be methylated by the H3K36 methyltransferase NSD1 and demethylated by the H3K36me1/2 demethylase KDM2A (also known as FBXL11 and JHDM1A). NSD1 activates NF-κB activity, whereas KDM2A reduces it. They showed that the proliferation of HT29 colon cancer cells was promoted by NSD1-mediated methylation of p65 at K218/K221 but was antagonized by KDM2A-catalyzed demethylation of the same sites. Interestingly, NF-κB also increased expression of the *KDM2A* gene to form a negative feedback regulatory loop [57] (Figure 3). Subsequently, Zhang *et al.* documented that the plant homeodomain finger protein 20 (PHF20) promotes NF-κB transcriptional activity by interacting with methylated p65 at K218 and K221 [58]. Specifically, the interaction between PHF20 and methylated p65 blocks the association between p65 and the serine-threonine protein phosphatase 2A (PP2A) and thereby maintains the active, phosphorylated status of p65.

Ea and Baltimore showed that the SET domain-containing protein 9 (SET9) methylates p65 at K37 upon activation of NF-κB by tumor necrosis factor alpha (TNFα) [59]. It should be noted that SET9 was initially shown to be a H3K4 mono-methyltransferase [60,61] but later was reported to be unable to methylate nucleosomal H3K4 [62]. This p65 methylation facilitates the binding of p65 to the promoters of several NF-κB–regulated genes, such as *IKBA*, interferon gamma–induced protein 10, and *TNFA*, during TNFα stimulation. In line

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with this, expression of these genes was significantly reduced in $p65^{-/-}$ mouse embryonic fibroblast cells expressing the K37Q mutant as compared to those that expressed wild type p65 [59]. Lu *et al.* indicated that p65 methylation at K37 regulates genes distinct from those regulated by NSD1-mediated methylation of p65 at K218/K221 [63]. Seemingly contradictory to these studies was the report by Yang *et al.* that SET9-mediated monomethylation of p65 at the K314 and K315 residues brings about proteosomal degradation of p65, leading to decreased NF-κB activity in response to TNF-*a* stimulation [64] (Figure 3).

Levy *et al.* demonstrated that the SET domain–containing protein 6 (SETD6) can monomethylate p65 on K310 [65]. SETD6-mediated methylation of p65 inhibits p65-driven transcriptional programs, including inflammatory responses in primary immune cells. Mechanistically, SETD6-catalyzed methylation of p65 is recognized by the ankyrin repeat of the histone methyltransferase GLP (G9a-like protein), which modulates p65 target genes to be in a repressed chromatin state through H3K9 methylation [65]. Interestingly, phosphorylation of p65 at Ser311 by protein kinase C-ζ inhibits the association of GLP with p65 K310me1 and de-represses p65 target genes [65] (Figure 3).

Arginine methylation of NF-κB

NF-κB also undergoes arginine methylation. Wei *et al.* showed that R30 of p65 can be dimethylated by PRMT5, leading to activation of NF-κB signaling [66]. It was also shown that the expression of most NF-κB–inducible genes (~85%) that are downregulated by the p65-R30A mutant is also reduced by PRMT5 loss, suggesting that PRMT5-mediated R30 methylation of p65 is critical for NF-κB activity (Figure 3). Because PRMT5 expression is frequently elevated in many types of cancer, it is possible that PRMT5 overexpression promotes tumorigenic events by enhancing NF-κB signaling [67].

Physical interaction between histone methyltransferases and NF-κB

The H3K36 methyltransferase NSD2 (also known as MMSET or WHSC1) acts as a coactivator of NF-κB [68]. NSD2 was overexpressed in prostate cancer and was recruited to the promoters of NF-κB target genes, including *IL6*, *IL8*, *VEGFA*, *CCND1*, *BCL2*, and *BIRC5*, in castration-resistant prostate cancer cells. NSD2 then activated NF-κB target genes by increasing H3K36me2 and H3K36me3 levels. In addition, NSD2 interacts with the transcriptional co-activator and acetyltransferase p300 and facilitates cytokine-induced recruitment of NF-κB and p300 to the promoters of NF-κB target genes, resulting in increased levels of p300-catalyzed histone acetylation for gene activation [68].

The H3K27 methyltransferase EZH2 generally plays a critical role in epigenetic gene silencing. However, EZH2 functions differently in ER-negative basal-like breast cancer cells in which it physically interacts with p65 and RelB and constitutively activates NF-κB target genes [69]. This function of EZH2 is not dependent on its catalytic activity. It should be noted that in ER-positive lumina-like breast cancer cells, EZH2 downregulates expression of NF-κB target genes by interacting with ER and depositing H3K27 methylation [69]. Thus, EZH2 may have a dichotomous function in regulating NF-κB signaling in breast cancer cells. The histone H3K9 methyltransferase G9a (also known as EHMT2) was reported to

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interact with RelB and to induce RelB-mediated gene silencing [70]. These studies suggest that NF-κB interacts with different methylation modifiers to have a dual and context-dependent function in regulating expression of its target genes.

Histone methylation modifiers and RAS/RAF/MEK/MAPK signaling

The MAPKs regulate diverse cellular processes, including cell proliferation, cell migration, cellular differentiation, and survival, in response to extracellular signals. The best-studied MAPK family of proteins includes extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun amino-terminal kinases 1 to 3 (JNK1 to 3), p38 (α, β, γ, and δ), and ERK5. Aberrant regulation of these MAPKs is associated with various pathological conditions, including cancer. It has been well documented that the activities of MAPKs are modulated by phosphorylation states that are controlled by multiple kinases and phosphatases [71–75]. Recent studies have shown that the activities of MAPKs are also regulated by arginine and lysine methylation, as described in this section.

Arginine methylation of EGFR and RAF1

PRMT5 regulates MAPK signaling by methylating the upstream activators of MAPKs, such as EGFR and RAF1. PRMT5 monomethylates EGFR receptor at R1175 to increase *trans*-autophosphorylation of EGFR at Tyr (Y) 1173 [76], which in turn provides a docking site for the SH2 domain of the phosphatase SHP1 (also known as PTPN6) (Figure 4). The recruitment of SHP1 downregulates EGFR-ERK signaling. Blocking of R1175 methylation amplifies EGFR signaling and concomitantly increases proliferation, migration, and invasion of breast epithelial cells. Although the mode of action of R1175 methylation is not very well understood, it is possible that R1175 methylation increases the interaction between EGFR and the Y1173 kinase or inhibits the recognition of EGFR by the Y1173 phosphatase [76]. PRMT5 also regulates MAPK signaling by methylating the serine/threonine-protein kinase RAF1 (also known as CRAF) in melanoma cells [77]. PRMT5 enhances RAF1 degradation by methylating RAF1 at R563, reducing the activities of the downstream kinases, such as MEK1/2 and ERK1/2. RNAi-mediated or pharmacological inhibition of PRMT5 activity increases the amplitude and duration of RAS-dependent ERK phosphorylation in response to growth factors. These studies suggest that PRMT5 can have a tumor-suppressive property by downregulating EGFR and RAF1 signaling. However, multiple lines of evidence also indicate that PRMT5 may have an oncogenic function in several tumor types, including leukemia and breast cancer, by activating AKT signaling [78] and repressing tumor suppressor proteins [79].

SMYD3-mediated lysine methylation of MAP3K2

SET and MYND domain containing 3 (SMYD3) preferentially catalyzes H4K5 monomethylation, and to a lesser extent dimethylation and trimethylation at H4K5 [80]. Mazur *et al.* reported that SMYD3 is predominantly localized in cytoplasm and methylates MAP3K2 at K260 [81] (Figure 4). This methylation is dependent on SMYD3's catalytic activity and also correlates with ERK1/2 phosphorylation levels. Mechanistically, SMYD3-mediated K260 methylation of MAP3K2 inhibits its binding to the PP2A complex, a negative regulator of the MAPK pathway [81]. High SMYD3 expression also correlates with

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progression of pancreatic and lung cancer [81]. Consistent with this, pancreas-specific loss of *Smyd3* prevented inflammation-induced neoplastic lesions and formation of metaplastic ducts in pancreas-conditional K-RAS^{G12D} and p53-null mice [81]. Similarly, lung-specific loss of *Smyd3* in lung-conditional K-RAS^{G12D} significantly inhibited K-RAS^{G12D}–driven lung adenocarcinoma formation. The tumor-promoting function of Smyd3 in K-RAS^{G12D}–induced tumorigenesis is dependent on its catalytic activity [81]. This study highlights an important role for SMYD3-mediated methylation of MAP3K2 in enhancing oncogenic K-RAS signaling.

Transcriptional regulation of MAPK signaling by histone methylation modifiers

Histone methylation modifiers transcriptionally regulate MAPK signaling. In particular, we recently showed that KDM2A acted as a critical regulator of ERK signaling in lung cancer cells [82]. KDM2A was frequently upregulated in tumor samples from lung cancer patients, and high KDM2A levels were associated with poor prognosis [82]. Thus, KDM2A may be a prognostic marker for lung cancer and a therapeutic target. KDM2A overexpression repressed expression of the dual-specificity phosphatase-3 (*DUSP3)* gene by demethylating H3K36me2 at the *DUSP3* promoter (Figure 4). Because DUSP3 preferentially dephosphorylates ERK1/2 in lung cancer cells, KDM2A-mediated repression of *DUSP3* amplifies ERK1/2 signaling to increase cell proliferation and invasion [82]. Consistent with this, KDM2A depletion drastically inhibited tumorigenicity and invasion of lung cancer cells in mouse xenograft models. The tumor-promoting function of KDM2A is dependent largely on its enzymatic activity [82]. Interestingly, Chen *et al.* showed that the H3K9me1/2 demethylase JMJD1A (also called JHDM2A) positively regulates expression of Spry2, a key negative regulator of ERK1/2 in human bronchial epithelial BEAS-2B cells [83]. Both hypoxia and nickel (an environmental carcinogen) inhibit JMJD1A's enzymatic activity, resulting in decreased expression of Spry2 and increased ERK1/2 signaling [84].

Interestingly, EZH2 epigenetically represses expression of the *DAB2IP* gene, which encodes a RAS GTPase-activating protein in prostate cancer cells. Because DAB2IP suppresses RAS and NF-κB through distinct domains, EZH2-mediated repression of DAB2IP expression activates RAS and NF-κB signaling to promote tumor growth and metastasis [85]. In addition, EZH2 interacts with phosphorylated p38 and enhances the p38 signaling pathway in breast cancer cells to promote their migration and metastasis [86].

Histone methylation modifiers and PI3K/AKT signaling

Like the RAS/RAF/MEK/MAPK pathway, the phosphatidylinositol 3-kinase (PI3K)/the serine-threonine kinase AKT (also called protein kinase B) /mammalian target of rapamycin (mTOR) pathway is a major signaling pathway that regulates cell proliferation, growth, and survival. It has been shown extensively that this pathway is modulated by several post-translational modifications, including phosphorylation [87–90], ubiquitination [91,92], and sumoylation [93,94]. Arginine and lysine methylation have emerged as an important type of modification that regulates upstream and downstream factors of AKT signaling, as described in this section.

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Regulation of PI3K/AKT signaling pathway by PRMT1

PRMT1 has been shown to regulate the PI3K/AKT signaling pathway through arginine methylation. Downstream of PI3K-AKT signaling, PRMT1 methylates Forkhead box O (FOXO) at conserved R248 and R250 residues within a consensus motif (i.e., R-X-R-X-X-S/T) for AKT-mediated phosphorylation [95] (Figure 5). This methylation blocks AKT-mediated Ser253 phosphorylation of FOXO1, which leads to cytoplasmic localization and ubiquitin-mediated proteosomal degradation of FOXO1. Loss of PRMT1 or its enzymatic activity leads to a decrease in oxidative stress–induced apoptosis dependent on AKT-mediated Ser253 phosphorylation of FOXO1 [95]. In addition, PRMT1 specifically methylates BCL-2 antagonist of cell death (BAD) at the R94 and R96 residues, which reside in an AKT phosphorylation motif (Figure 5). PRMT1-catalyzed methylation of BAD impedes AKT-mediated phosphorylation of BAD at Ser99, blocking the interaction of BAD with phosphoserine binding 14-3-3 proteins. Thus, PRMT1 induces mitochondrial localization of BAD, thereby promoting apoptosis [96].

In contrast, PRMT1 appears to facilitate AKT activation in response to estrogen treatment. PRMT1 methylates ERα at the R260 residue within the DNA-binding domain of ERα [97]. R260 methylation of ERα takes place in the cytoplasm of normal and malignant epithelial breast cells. ERα-R260 is hypermethylated in a subset of breast cancers. This methylation event induces the interaction of ERα with the Src kinase and the p85 subunit of PI3K (a heterodimer composed of a p110 catalytic subunit and a p85 regulatory subunit) (Figure 5). The focal adhesion kinase (FAK), a Src substrate, is also recruited to ERα/PI3K/Src via Src. Such multi-protein interaction induces AKT activation to promote cell proliferation, survival, and migration [97].

Regulation of PI3K/AKT signaling pathway by EZH2

Gonzales *et al.* showed that EZH2 overexpression enhances PI3K/AKT signaling through activation of AKT isoform 1 in breast cancer cells [98]. Interestingly, EZH2 interacts with AKT-1. EZH2-induced phenotypes, such as BRCA1 nuclear export, aneuploidy, and mitotic defects, are dependent on AKT-1 activation. Consistent with these findings, high EZH2 protein levels were associated with increased levels of phospho-AKT-1 (Ser473) and decreased nuclear levels of phospho-BRCA1 (Ser1423) in invasive breast cancer samples [98].

Histone methylation modifiers and Wnt/β-catenin signaling

Wnt/β-catenin signaling is critical for normal development and tissue homeostasis [99,100], and its aberrant regulation is linked to tumorigenesis [101]. Notably, β-catenin is stabilized in response to Wnt activation and binds to the DNA-binding proteins T-cell factor (TCF) and lymphoid enhancing factor-1 (LEF-1) to activate β-catenin target genes [102,103]. The Wnt/β-catenin signaling pathway is also controlled by histone methylation modifiers, as summarized in Figure 6.

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Regulation of Wnt/β-catenin signaling pathway by EZH2

EZH2 enhances RAF1-ERK-β-catenin signaling in breast tumor initiating cells (BTICs) [104]. In BTICs, EZH2 overexpression induces *RAF1* gene amplification by downregulating expression of the DNA damage repair protein RAD51. RAF1 amplification enhances ERK-β-catenin signaling to increase survival and expansion of BTICs [104]. ERK phosphorylates and primes glycogen synthase kinase 3β (GSK-3β) for its subsequent inactivation [105]. Inactivation of GSK-3β stabilizes functional β-catenin, because GSK-3β–catalyzed phosphorylation of β-catenin promotes β-catenin degradation.

EZH2 also activates Wnt/β-catenin signaling by silencing expression of Wnt pathway antagonists. In hepatocellular carcinoma, for instance, ectopic overexpression of EZH2 promoted proliferation of immortalized hepatocytes by concomitantly reducing expression of several Wnt inhibitors, including *AXIN2*, *NKD1*, *PPP2R2B*, *PRICKLE1*, and *SFRP5* [106]. In gastric cancer cells, EZH2 activated Wnt/β-catenin-signaling by epigenetically repressing the Wnt signaling antagonist CXXC4, thereby promoting tumorigenic phenotypes [107]. In addition, EZH2-mediated transcriptional repression of the Wnt signaling antagonist DKK1 contributes to increased tumorigenicity of lung cancer cells that were exposed to tobacco smoke condensate. [108].

Interestingly, EZH2 acts as a transcriptional activator for β-catenin target genes independent of its methyltransferase activity linked to gene repression. EZH2 directly binds to β-catenin and ERα and subsequently enhances transactivation of MYC and CCND1 genes by ERα and β-catenin. This gene activation by EZH2 leads to cell cycle progression in breast cancer cells [109]. Recently, it was also shown that PCNA-associated factor (PAF) is associated with the β-catenin transcriptional complex and upregulates β-catenin target genes by recruiting EZH2 to their promoters [110].

Regulation of Wnt/β-catenin signaling by other histone methylation modifiers

Dot1L was shown to activate *senseless*, a Wnt target gene, by methylating H3K79 at its promoter in Drosophila [111]. Interestingly, Dot1L-mediated H3K79 methylation of the *senseless* promoter requires the monoubiquitination of H2B by the Rad6/Bre1 complex [111]. The SET domain containing lysine methyltransferase-8 (SET8; also known as SETD8, KMT5A) also acts as a mediator of Wnt signaling [112]. Specifically, SET8 directly associates with LEF1/TCF4, and this interaction is controlled by Wnt3a. Thus, SET8 is recruited to Wnt-activatable genes, such as *AXIN2*, and positively regulates them, possibly by monomethylating H4K20 [112]. NSD2 has been found to be overexpressed in several cancer types and to interact with some Wnt-signaling regulators, including β-catenin. NSD2 positively regulates expression of *CCND1*, a target gene of the β-catenin/Tcf-4 complex, via H3K36 trimethylation [113].

It has been shown that PRMT2 is required for Wnt/β-catenin–dependent establishment of the dorsal developmental program in *Xenopus*. Specifically, PRMT2 is recruited by β-catenin to β-catenin target genes and may establish poised chromatin structure by generating asymmetrically dimethylated H3R8 at their promoters [114]. PRMT1 negatively regulates β-catenin–dependent transcription by stabilizing Axin, an inhibitor of Wnt signaling. PRMT1

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physically interacts with and methylates Axin at R378. This PRMT1-mediated methylation stabilizes Axin [115].

Histone methylation modifiers and the p53 pathway

p53 is a well-studied tumor suppressor that is mutated in approximately 50% of human cancers; it is a transcription factor that regulates the cell cycle, apoptosis, and DNA repair in response to a variety of genotoxic stresses. It has been well documented that the activity and stability of p53 are modulated by multiple types of post-translational modifications [116–118], including phosphorylation, acetylation, ubiquitination, and sumoylation [119–121]. Because p53 methylation has already been reviewed elsewhere [122–125], we will only briefly summarize a few selected studies regarding p53 methylation.

p53 methylation was first documented by the Reinberg group, who showed that SET9 (alias SET7) monomethylates p53 at K372 [62]. This methylation increases the stability of nuclear p53, resulting in both enhanced expression of the p53 target gene *p21* and increased levels of p53-mediated apoptosis [62]. p53-K372 monomethylation is important for subsequent acetylation of p53 that is also linked to *p21* gene activation [126].

In contrast to p53-K372 methylation, SMYD2-catalyzed K370 monomethylation (K370me1) of p53 represses gene-activating function of p53 by reducing chromatin-associated p53, suggesting that K370 monomethylation is a repressive mark for p53 activity [127]. Consistent with this, RNAi-mediated depletion of SMYD2 significantly induces p53 target gene expression and enhances p53-mediated apoptosis [127]. SMYD2-mediated methylation of K370 is also inhibited by SET9-directed K372 methylation. Interestingly, p53-K370 methylation can be reversed. Berger and colleagues also reported that K370me2 of p53 is the preferred substrate of LSD1, although LSD1 demethylates both K370me1 and K370me2 [128]. In contrast to K370me1 of p53, K370me2 of p53 enhances p53 transcriptional activity by interacting with 53BP1, a p53 co-activator that plays an important role in DNA damage response [129,130]. Thus, LSD1-mediated demethylation of K370me2 inhibits p53 function by abolishing the interaction of K370me2 with 53BP1 [128].

Shi *et al.* showed that SET8 monomethylates p53 at K382 to inhibit its transcriptional activity and to consequently decrease expression of its target genes [131]. The H3K9 methyltransferases G9a and GLP were shown to dimethylate p53 at K373 [132]. These methyltransferases were suggested to inhibit p53 function, because knockdown of G9a and GLP increases apoptosis [132].

Histone methylation modifiers and estrogen receptor signaling

ER signaling contributes to normal cell growth, development, and tumorigenesis [133–137]. ERα is a ligand-dependent transcription factor that regulates gene expression [138–140]. In response to estrogen, ER's transcriptional activity is modulated by numerous coactivators and corepressors [141–143], including histone-modifying enzymes [144].

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Regulation of ER signaling by lysine methyltransferases and demethylases

Dreijerink *et al.* showed that menin, a tumor suppressor and an important component of MLL1 and MLL2 H3K4 methyltransferase complexes, functions as a transcriptional co-activator of ERα [145]. MLL1–4 are associated with the estrogen-induced activation of *HOXC13* [146]. In addition, the H3K4 methyltransferase MLL4 (also known as MLL2, KMT2D and ALR) was shown to directly interact with ERα and to cooperate with ERα for transcriptional activation of ERα target genes. Consistent with this, MLL4 knockdown inhibited proliferation of ERα-positive MCF-7 cells [147]. Interestingly, MLL4 depletion was also shown to impede proliferation and invasion of ERα-negative cells [148]. Together, MLL1–4 each may play a role in ERα-mediated transcriptional activation.

Kim *et al.* demonstrated that SMYD3 acts as a co-activator of ERα and potentiates ERα-mediated activation of ERα target genes [149]. Recently, Zhang *et al.* reported that SMYD2 directly methylates ERα at K266. This methylation Inhibits acetylation of ERα at K266/268 to decrease ERα's transactivation activity and may be antagonized by LSD1-mediated demethylation [150]. LSD1 is recruited to most ERα target genes and is required for ERα-dependent gene activation [151], although LSD1 is also linked to gene repression. It was reported, similarly, that ERα-mediated chromatin looping requires LSD1-mediated demethylation of the repressive H3K9me2 [152].

Regulation of ER signaling by arginine methyltransferases

Arginine methyltransferases play an important role in ER signaling by methylating arginine residues in both histones and non-histone proteins [141]. Metivier *et al.* showed that activation of ERα by estrogen induces the oscillatory recruitment of ERα coactivators, including PRMT4 and PRMT1, on its target genes [153]. PRMT4 is required for estrogen-induced cell cycle progression of MCF-7 breast cancer cells [154]. It dimethylates H3R17 at the *E2F1* promoter in an ERα-dependent manner, leading to increased expression of the cell cycle transcriptional factor E2F1 [154]. PRMT4-mediated methylation of steroid receptor coactivator 3 (SRC-3) dissociates the interaction between PRMT4 and SRC-3, resulting in decrease of ERα-mediated transcription [155]. Thus, it was proposed that PRMT4 has a dual-function coactivator that activates transcription by histone arginine methylation but terminates ER signaling by SRC-3 methylation [155].

PRMT1 facilitates ERα-induced transcriptional activation by asymmetrically dimethylating H4R3 at ERα target genes [156]. As mentioned above, PRMT1 methylates ERα at R260 within ERα's DNA-binding domain, inducing the association of ERα with PI3K and Src. This association leads to AKT phosphorylation at S473 and subsequent cell cycle progression [97]. In addition to ERα, PRMT1 also methylates ERα cofactors and modulates their activities. For example, PRMT1 methylates receptor-interacting protein 140 (RIP140), a ligand-dependent corepressor for ERα and other nuclear receptors, at the R240, R650, and R948 residues. PRMT1-mediated methylation of RIP140 inhibits the repressive activity of RIP140 [157]. PRMT1 methylates peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a transcriptional coactivator for ERα and other nuclear receptors, at R665, R667, and R669. PRMT1-catalyzed methylation of PGC-1α enhances PGC-1α's coactivator activity [158].

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Future perspectives

Recent systematic sequencing studies of cancer genomes have revealed that multiple histone methylation modifiers are frequently mutated and amplified in diverse cancer types. For instance, the H3K27 demethylase *UTX* and *MLL4* undergo somatic mutations in several tumor types (e.g., renal cancer and medulloblastoma) and thus may act as tumor suppressor genes in such tumors. Indeed, mouse genetic experiments showed that UTX may act as a tumor suppressor in acute lymphoid leukemia (ALL) [159]. EZH2 is often amplified and overexpressed in multiple malignancies, such as prostate and breast cancer, in which EZH2 may be an oncogene. It should be noted that EZH2 may have a tumor suppressive role in the myeloid malignancies and ALL. In fact, EZH2 often undergoes loss-of-function mutations in these malignancies (reviewed in [160]), and its loss in hemopoietic tissues in mice showed the high frequency of spontaneous T-cell ALL [161]. Taken together, these studies highlight the importance of histone methylation modifiers in properly regulating signaling pathways and maintaining cellular homeostasis.

As summarized herein, dysregulated histone methylation modifiers may drive or promote tumorigenesis and metastasis by altering transcriptional programs and cellular signaling pathways. Importantly, aberrantly expressed modifiers are in principle targetable, because they have intrinsic enzymatic activities. In fact, many small molecule inhibitors against specific histone methylation modifiers have been reported. Some specific inhibitors, including inhibitors against LSD1 (ORY-1001 and GSK2879552), DOT1L (EPZ-5676), and EZH2 (E7438, GSK2816126, and CPI-1205), have entered clinical phase 1 trials [162]. Successful results of these trials may provide new avenues for therapeutic interventions. We believe that an exciting era has opened in chromatin and epigenetics research.

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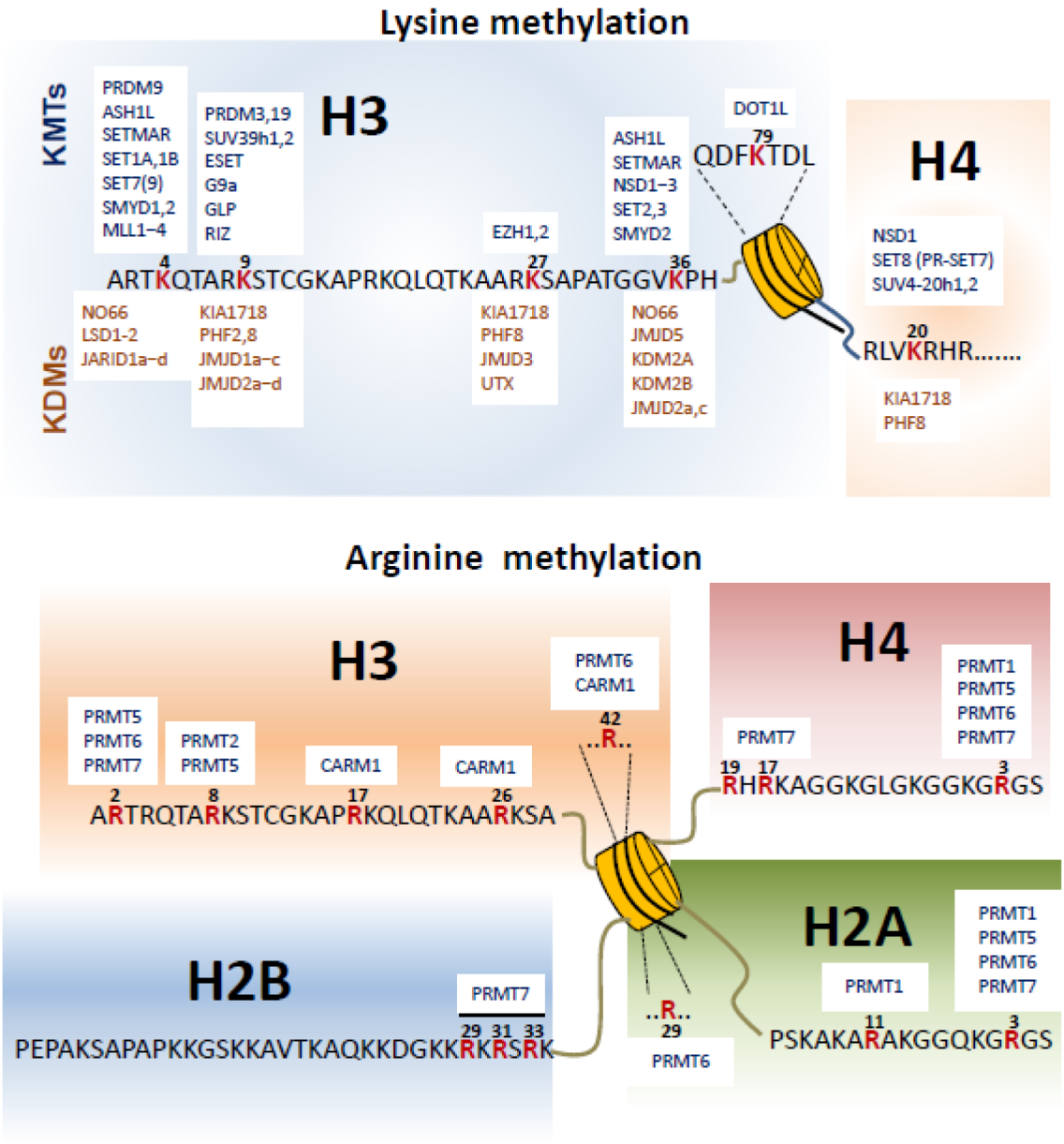

Figure 1. Histone methylation and its modifiers

Histone lysine methyltransferases (KMTs) and lysine demethylases (KDMs) for six major lysine methylation sites in histones are aligned for their cognate sites (top panel). Arginine methylation sites and their corresponding protein arginine methyltransferases (PRMTs) are also depicted (bottom panel).

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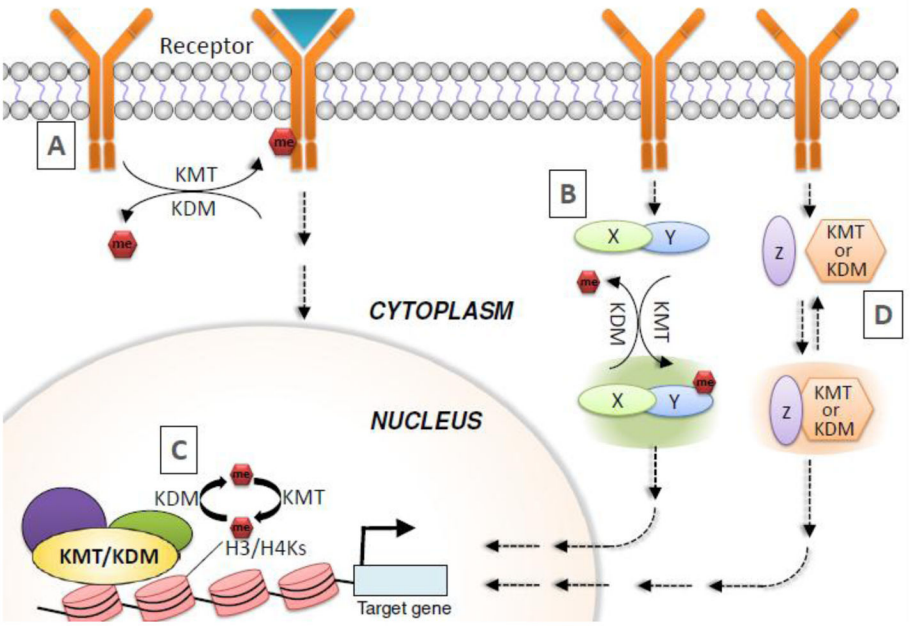

Figure 2. Overview of the molecular mechanisms underlying regulation of signaling pathways by methylation modifiers

Methylation modifiers can modulate signaling pathways by I) methylating and demethylating receptor kinases (A), effectors (e.g., kinases) (B), and activators/repressors of effectors (B); II) transcriptionally regulating expression of components in signaling pathways (C); and III) controlling the activities of signaling components via physical interaction (D). KDM, lysine demethylases; KMT, lysine methyltransferases; Me, methylation.

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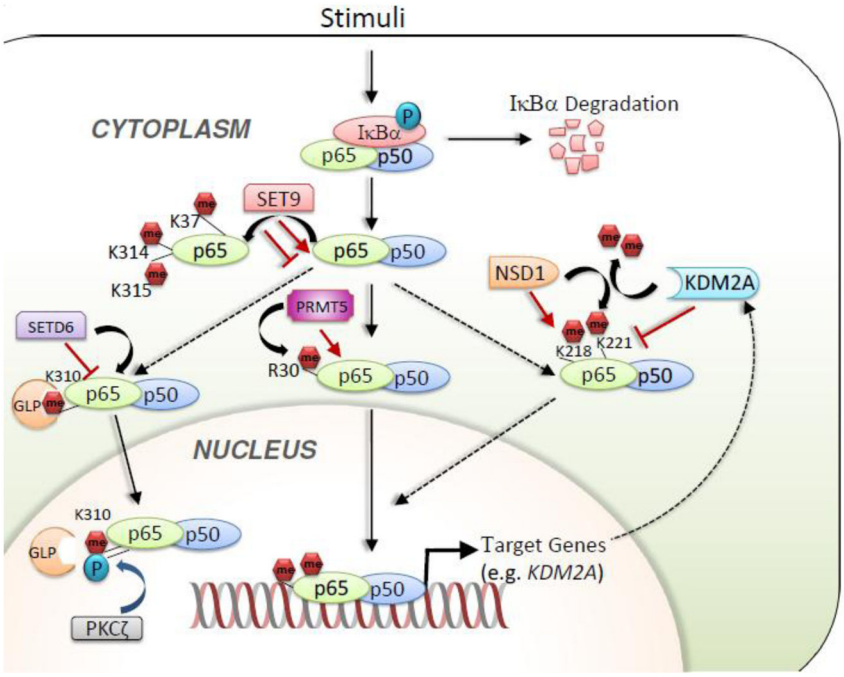

Figure 3. Regulation of NF-κB signaling pathways by methylation modifiers

Components of NF-κB, including p65, can be regulated by multiple methyltransferases and demethylases.

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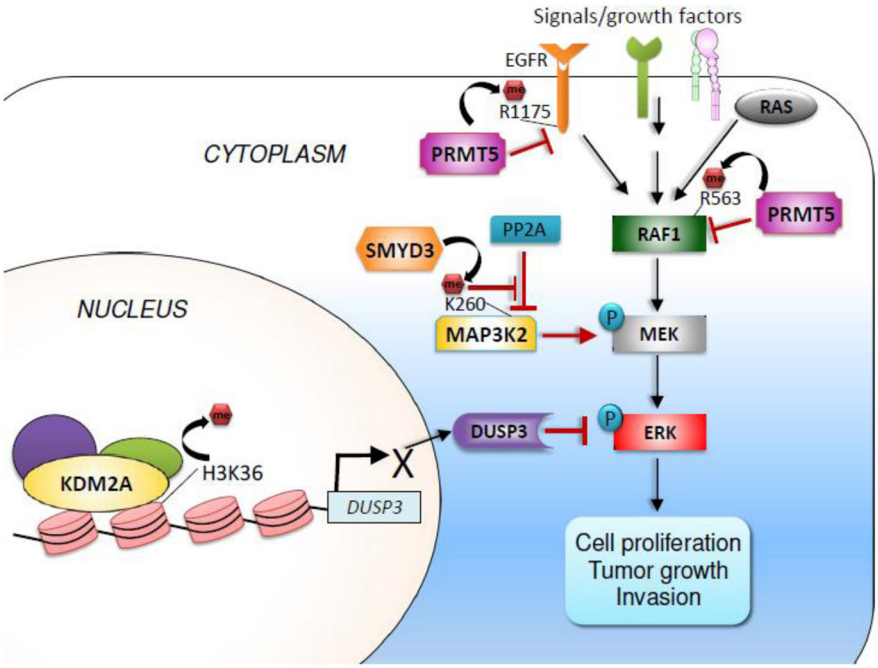

Figure 4. Methylation modifiers and MAPK signaling pathways

The lysine demethylase KDM2A activates ERK signaling by repressing expression of the ERK phosphatase *DUSP3*, while the lysine methyltransferase SMYD3 activates MAPK signaling through methylation of MAP3K2. PRMT5 methylates EGFR and RAF1 to downregulate MAPK signaling.

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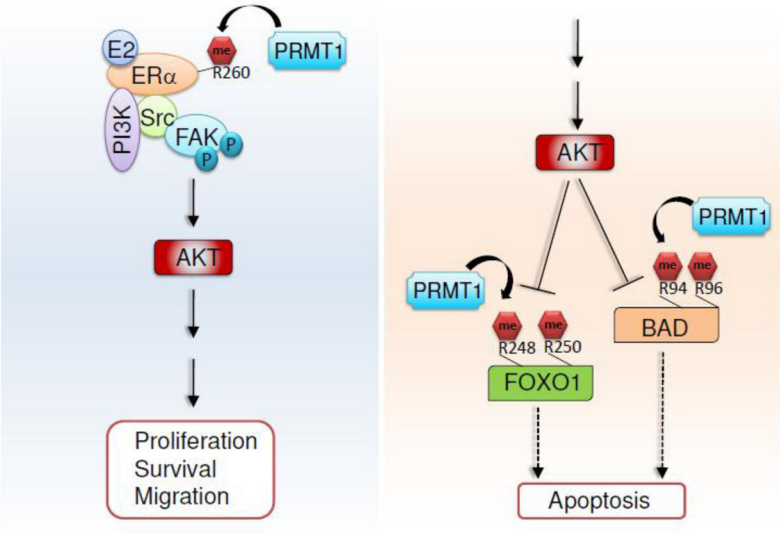

Figure 5. Regulation of AKT signaling pathways by PRMT1

PRMT1 enhances AKT signaling by methylating ERα in response to estrogen. In contrast, PRMT1-mediated methylation of FOXO1 and BAD interferes with AKT-mediated inhibition of apoptosis.

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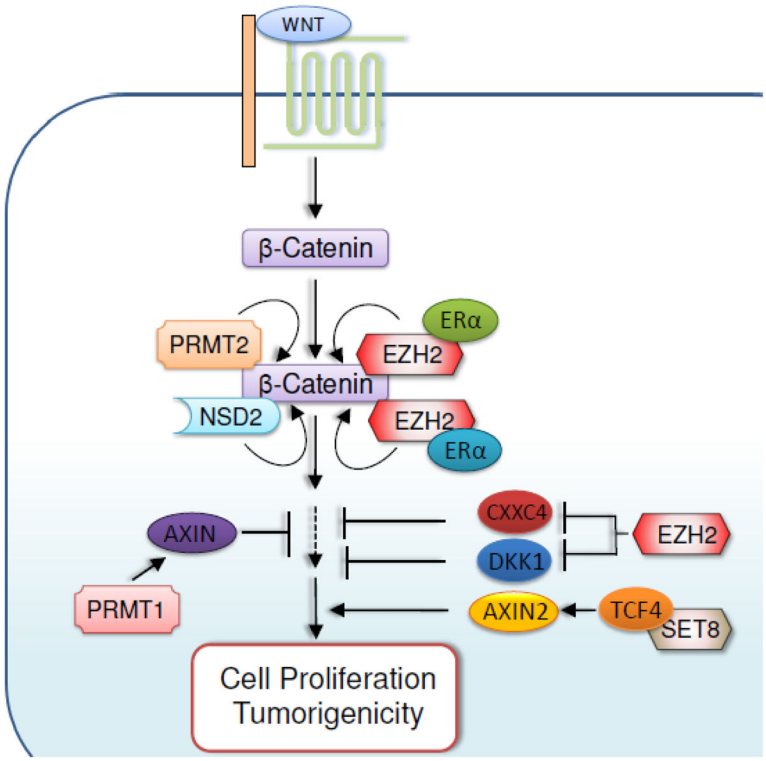

Figure 6. Regulation of Wnt/β-catenin signaling pathways by methylation modifiers

Wnt/β-catenin signaling can be enhanced by the methyltransferases PRMT2, EZH2, SET8, and NSD2, but can be inhibited by PRMT1.

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Table 1

Non-histone targets of histone methylation modifiers

Signaling pathway	Methylation modifiers	Substrate	Residue(s)	Phenotypic changes/functions	Refs
NF-κB signaling	NSD1	p65	K218, K221	Activates NF-κB signaling	[57]
	KDM2A	p65	K218, K221	Inhibits NF-κB signaling	[57]
	SET9	p65	K37, K314, K315	K37 activates NF-κB signaling; K314 and K315 reduce p65 stability and promote degradation	[59] [64]
	SETD6	p65	K310	Inhibits p65-driven transcription	[65]
	PRMT5	p65	R30	Activates NF-κB signaling	[66]
MAPK signaling	SMYD3	MAP3K2	K260	Activates MAP3K2 and thereby activates ERK1/2	[81]
	PRMT5	EGFR	R1175	Inhibits EGFR signaling	[76]
	PRMT5	RAF1	R563	Enhances RAF1 degradation and inhibits ERK1/2	[77]
PI3K/Akt signaling	PRMT1	FOXO	R248, R250	Enhances FOXO degradation and apoptosis	[95]
	PRMT1	BAD	R94, R96	Promotes mitochondrial localization and apoptosis	[96]
Wnt signaling	PRMT1	Axin	R378	Negatively regulates Wnt signaling	[115]
	EZH2	PAF, β-catenin	independent of its catalytic activity	Activates Wnt signaling	[109, 110]
ER signaling	PRMT4	p300	R2142	Disrupts interaction between GRIP1 and p300	[163]
		SRC-3		Inhibits ERα-mediated transcription	[155]
	SMYD2	ERα	K266	Inhibits ERα activity	[150]
	PRMT1	ERα	R260	Activates ER signaling	[97]
		RIP140	R240, R650, R948	Activates ER signaling	[157]
		PGC-1α	R665, R667, R669	Enhances co-activation	[158]
p53 pathway	SET7/SET9	p53	K372	Positive effect on stability, apoptosis	[62]
	SMYD2	p53	K370	Reduces DNA binding ability, p53-mediated apoptosis	[127]
	LSD1	p53	K370me2	Inhibits 53BP1 binding and p53 function	[128]
	SET8	p53	K382	Reduces transcriptional activity, apoptosis	[131]
	G9a/GLP	p53	K373	Inhibits p53 activity and its dependent apoptosis	[132]