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REVIEW

Lysine methylation signaling of non-histone proteins in the nucleus

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Abstract

Lysine methylation, catalyzed by protein lysine methyltransferases (PKMTs), is a central post-translational modification regulating many signaling pathways. It has direct and indirect effects on chromatin structure and transcription. Accumulating evidence suggests that dysregulation of PKMT activity has a fundamental impact on the development of many pathologies. While most of these works involve in-depth analysis of methylation events in the context of histones, in recent years, it has become evident that methylation of non-histone proteins also plays a pivotal role in cell processes. This review highlights the importance of non-histone methylation, with focus on methylation events taking place in the nucleus. Known experimental platforms which were developed to identify new methylation events, as well as examples of specific lysine methylation signaling events which regulate key transcription factors, are presented. In addition, the role of these methylation events in normal and disease states is emphasized.

Keywords Post-translational modifications (PTMs) · Protein lysine methyltransferases (PKMTs) · Lysine methylation · Non-histone methylation · Methylation signaling

Introduction

Reversible, covalent post-translational modifications (PTMs), such as phosphorylation, acetylation and methylation, are critical modulators of many biological processes [1]. While phosphorylation is the most extensively studied PTM to date, lysine methylation is emerging as a key player in the regulation of various cellular processes [2, 3]. Methylation of lysine residues is driven by protein lysine (K) methyltransferases (PKMTs), which catalyze the transfer of a methyl group from the methyl donor, *S*-adenosylmethionine (SAM), to the target lysine [4, 5]. Two groups of PKMTs are known; the first group contains a catalytic SET domain [SU(var), enhancer of Zeste and Trithorax] [4, 6–8], while the second group belongs to the seven-β-strand (7βS) family found in eukaryotes, prokaryotes and archaea [9–11].

A lysine residue can be mono-, di- or tri-methylated (Fig. 1a). Each state of methylation creates a unique signature that can act to recruit specific trans-acting factors ("readers"), then triggering specific downstream signaling pathways [12] (Fig. 1b). Methylation was initially discovered by Richard Abler and Maurice Rees in 1959, on flagellin from the bacterium *Salmonella typhimurium* [13]. Methylation of histones was discovered just 5 years later, by Murray [14]. In recent years, most of the focus of methylation research has been on histone methylation and less on the methylation of non-histone proteins. This is likely due to the relatively slow progress in the development of adequately sensitive quantitative proteomics tools for identification of new methylation events on non-histone proteins, as well as their lower overall methylation abundance compared to histones.

Detection of lysine methylation

"Phosphorylome" and "acetylome" are terms commonly used to define the organism-specific protein repertoire which is subjected to phosphorylation and acetylation, respectively. However, attempts to define the "methylome" associated with different cellular and physiological processes have been

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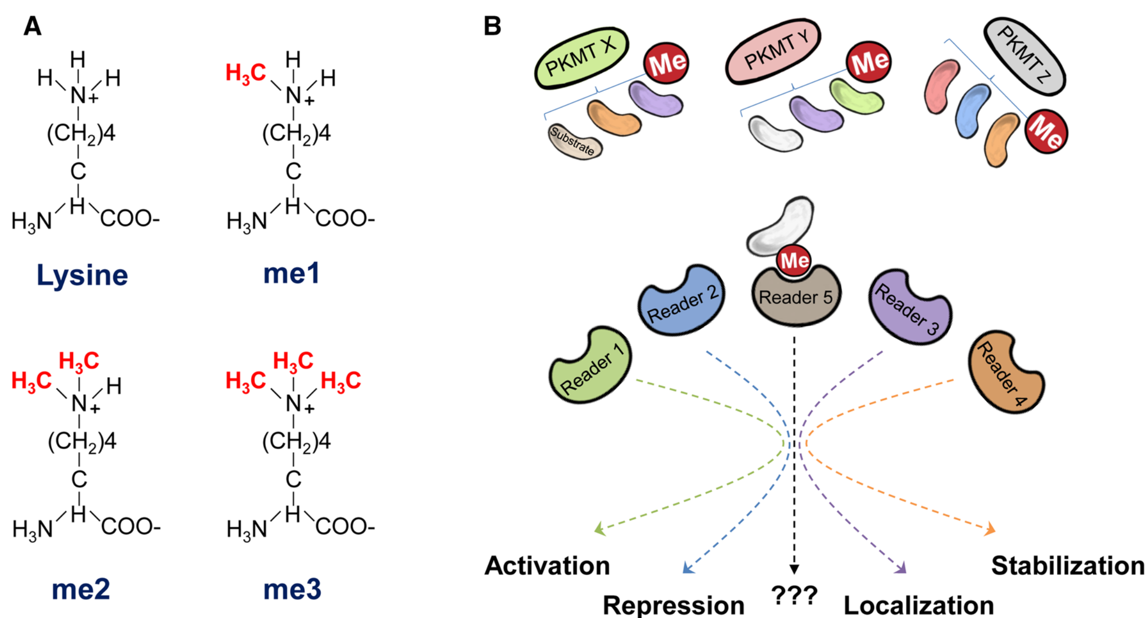

Fig. 1 Lysine methylation-associated signalling. **a** Chemical structure of lysine and its methylated derivatives—a hydrogen moiety on the lysine side chain with one (me1), two (me2) or three (me3) methyl groups (shown in red). **b** The scheme depicts a methylation-associated signalling cascade by which a given PKMT (*x*, *y* and *z*) methylates a repertoire of substrates, which are recognized by different "readers", which then transduce diverse biological signals

limited by both the relatively small molecular mass shift (14 Da) and the lack of a dramatic charge difference between an un-methylated and methylated residue. The importance of lysine methylation in diverse cellular processes has highlighted the need to develop reliable approaches and experimental platforms to identify new methylation events.

Candidate-based approaches

The most straightforward approach uses radioactively labeled SAM, which enables the detection of methylated substrates (peptides and full-length proteins) in in vitro methylation assay (Fig. 2A) [15]. Potential pitfalls of this approach are nonspecific binding of SAM and the fact that this approach does not indicate which amino acid undergoes methylation, as, in addition to lysines, arginine, histidine, aspartate and glutamate as well as the *N* terminus of proteins are potential targets for methylation [10, 16–18]. Pan-methyl antibodies are also widely used to identify new methylation events (Fig. 2B) [19]. However, these antibodies suffer from poor selectivity, resulting in cross-reactivity with other modifications [20], low sensitivity and low reproducibility between different lot numbers. Site-specific antibodies raised against a specific methylation site are essential to decipher the physiological consequences of a given methylation event; however, their specificity remains to be validated and working conditions should be optimized.

SAM serves as a universal methyl donor and leads to the generation of *S*-adenosylhomocysteine (SAH) following methylation. Therefore, many efforts focusing on the development of quantitative assays for PKMT activity rely on the detection of methylated products or on the formation of SAH (Fig. 2C). SAH detection assays, which were summarized by Shechter et al. [21], have the advantage of providing a general methylation detection method, regardless of the nature of the protein or DNA acceptor [21–29]. Most of these assays enable Michaelis–Menten (MM) kinetic analysis of the catalytic activity of PKMTs for mechanistic studies and some can be adapted for high-throughput screening for the discovery of novel PKMT inhibitors.

High-throughput approaches

Usage of peptide and protein arrays (Fig. 2D) provides an efficient means of screening thousands of potential candidate substrates in an un-biased manner. The detection is performed either by antibodies, radiolabeling with tritium-labeled SAM or with methyl-lysine-binding domains. In the peptide array approach, a library of random peptides spanning a fixed consensus sequence or a free lysine is incubated with a given PKMT. Bioinformatics and structural analyses are then performed to predict new substrates, which are further validated at the protein level, both in vitro and in cells [30–36]. We recently developed an in vitro proteomic approach using ProtoArrays, to identify novel substrates of

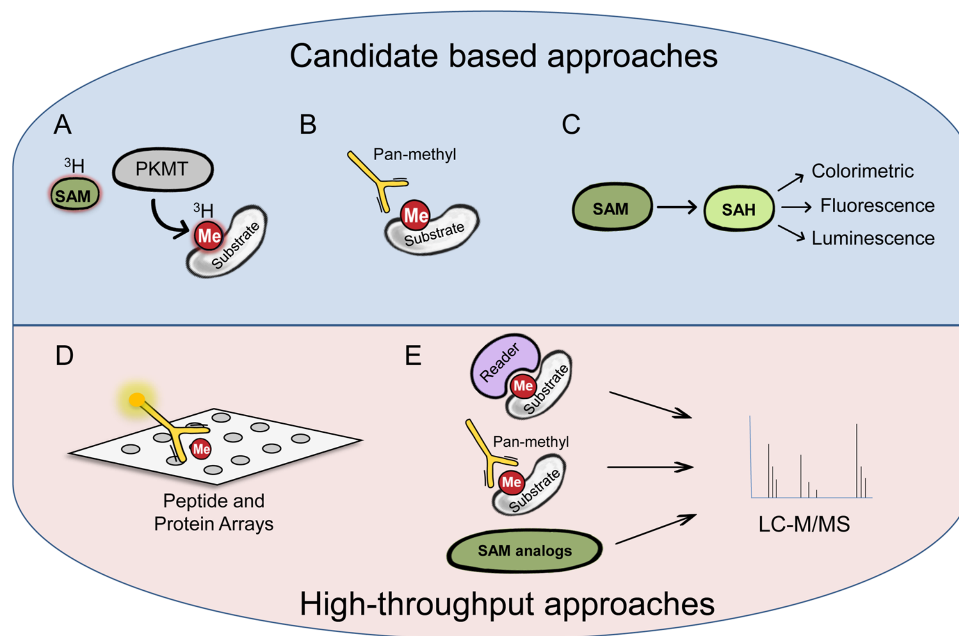

Fig. 2 Detection of lysine methylation. The most common approaches to identify new methylation events are presented. The scheme is divided into candidate-based (top part) and high-throughput (bottom part) experimental platforms (see text for details). A Radioactive in vitro methylation assay in the presence of tritium-labeled SAM (^{3}H), PKMT and a substrate of interest. B Recognition of a methylated substrate with a pan-methyl antibody. C Different assays for the detection of methylated products which rely on the formation of *S*-adenosylhomocysteine (SAH). D Usage of peptide and protein arrays for screening thousands of potential candidate substrates in an un-biased manner. E Few examples of enrichment steps required for mass spectrometry-based approach (see text for details)

the SETD6 and SETD7 PKMTs. The system contains over 9500 highly purified recombinant human proteins, expressed in insect cells as N-terminal GST-fusion proteins, which are immobilized at spatially defined positions on nitrocellulose-coated glass microscope slides. The arrays are subjected to on-chip PKMT reactions, followed by detection either with a pan-methyl antibody that recognizes methylated proteins or with a tritium-radiolabeled SAM [20].

Mass spectrometry is the approach most commonly used to identify new methylations across the proteome, as well as the precise site and state (mono, di or tri) of methylation; yet, the approach requires an enrichment step (Fig. 2E) [37, 38]. Ong and Mann were the first to suggest the usage of commercially available pan-methyl antibodies to enrich samples for methylated proteins for mass spectrometry-based analyses [39, 40]. Later, Garcia et al. developed pan-specific methyl-lysine antibodies that were used for immunoprecipitation experiments followed by mass spectrometry, which initially identified 552 [41] and later 1000 [42] new methylation events across the proteome. At about the same time, a different set of a pan-methyl antibodies was raised against a peptide library containing mono-, di- and tri-methylated peptides. In HCT116 cells, these antibodies enabled identification of more than 150 new methylation sites [43].

Methyl-lysine-binding readers (Fig. 2E) were recently applied to enrich samples for methylated proteins prior to mass spectrometry analysis. The malignant brain tumor (MBT) domain of the protein L3MBTL1, which recognizes mono- and di-methylated proteins, was utilized to enrich methylated proteins in a stable isotope labeling with amino acids in cell culture (SILAC) approach [44] and was used to identify new substrates for G9A, G9a-like protein (GLP) [32] and SETMAR [45] from cell extracts. In a similar approach, the chromodomain of HP1β was used to enrich for methylated proteins and led to the discovery of 29 new methylated proteins [46]. Methylated proteins can also be enriched using chemical methods, which exploit bioorthogonally reactive chemical moieties, such as azide or alkine, which enable the attachment of affinity tags in place of the methyl group [47–51].

Methylation of non-histone substrates in the nucleus

Accumulating evidence has indicated that lysine methylation is essential for the regulation of various signalling pathways. The remaining sections of this review will concentrate on proteins which are methylated in the nucleus, with focus on transcription factors (TFs). When possible, the biological, functional and biochemical consequences of the presented nuclear factor methylation are discussed.

p53

The tumor suppressor p53, an extensively studied TF, with key roles in the transcriptional regulation of many cellular programs in response to a variety of genotoxic stresses, has been shown to be methylated on several lysines, by different PKMTs. p53 mono-methylation by SETD7 at K372 increases p53 stability as well as the expression of p53 target genes and apoptosis [52]. Several years after this discovery, K372me1 (K369 in mouse) was shown to prevent p53 acetylation at K317, K370 and K379 [53], all of which are important sites of p53 regulation [54, 55]. Interestingly, K372 methylation by SETD7 also inhibits SMYD2-mediated mono-methylation of p53 at K370, which was shown to repress p53 transcriptional activity [56]. Lysine-specific histone demethylase 1 (LSD1), which demethylates p53 at K370, displays a strong in vivo preference for p53K370me2 than for K370me1 [57]. Mechanistically, K370me2 methylation promotes the association between p53 and the coactivator p53-binding protein (53BP1) through the tandem Tudor domains in 53BP1, leading to p53 activation. During gene repression, LSD1 prevents the accumulation of K370me2 by demethylating the site, and preventing 53BP1 from binding to methylated p53 [57]. Methylation of p53 at K373 and K382 was also shown to regulate p53 cellular activity. p53 methylation at K373 by G9A and GLP inhibits pro-apoptotic p53 activity [58]. Methylation of p53 at K382 by SETD8 inhibits its transcriptional activity [59] through the recruitment of the transcriptional repressor L3MBTL1 to p53 target gene promoters [60]. In contrast, the Tudor domain of PHF20 was shown to directly bind p53K370me2 and p53K382me2, and to positively regulate p53 stability and activity [61]. In addition, SETD8-mediated di-methylation of NUMB, which associates with p53 at K158 and K163, was suggested to lead to enhanced p53 ubiquitylation and degradation [62]. Several years later, an extensive study by the Jeltsch group showed that NUMB is not a substrate of SETD8 in vitro, but concluded that in vivo SETD8 methylation of NUMB cannot be ruled out [63].

Beta-catenin

β-Catenin is a transcription regulator of a wide range of target genes and plays an important role in cell proliferation, cell fate determination, and tumorigenesis of many cancer types. Methylation of β-catenin at K180 by SETD7/9 interferes with the interaction between β-catenin and GSK3β, and thus decreases β-catenin stability under oxidative stress conditions [64]. SMYD2-mediated K133 methylation of β-catenin promotes its interaction with the transcription factor FOXM1, enhancing its nuclear translocation and facilitating its activation of Wnt downstream genes [65]. In two sequential papers [66, 67], Zaph et al. showed that SETD7 is part of a complex containing YAP, AXIN1 and β-catenin. The authors showed that SETD7-dependent methylation of YAP at K494 facilitates Wnt-induced nuclear accumulation and activation of β-catenin target genes. Furthermore, we have recently shown that SETD6 participates in the canonical Wnt signaling cascade by forming a complex with PAK4 (p21-activated kinase 4) and β-catenin at the chromatin, leading to the activation of β-catenin target gene transcription [68]. Taken together, these observations suggest that β-catenin activity can be mediated by direct and indirect methylation events, leading to the assembly of different protein complexes which govern precise transcriptional programs. Future studies are still required to determine whether functional cross-talk exists between the different PKMTs (SMYD2, SETD6 and SETD7) and whether they affect formation of β-catenin transcriptional complexes.

RelA

RelA, also known as p65, serves as the major subunit of NF-κB, which is a TF that regulates multiple biological functions including inflammation, immunity and cell proliferation. Apoptosis and concomitant deregulation of NF-κB signaling are linked to many human diseases, such as cancer and autoimmune disorders [69–71]. In recent years, several studies have shown that RelA is methylated at different positions by different PKMTs (Fig. 3a). In response to TNF-α induction, SETD7 methylates RelA at K37, increasing its promoter-binding activity, leading to the subsequent activation of a subset of NF-κB target genes [72]. In contrast, monomethylation of RelA by SETD7 at K314 and K315, inhibits its activity by inducing the proteasome-mediated degradation of promoter-associated RelA [73]. These two conflicting studies suggest that additional regulatory mechanisms mediate these opposing biological downstream effects. Stark et al. demonstrated that RelA methylation at K218 and K221 by NSD1 increases RelA transcriptional activity in response to cytokine stimulation [74]. The group identified the F-box and leucine-rich repeat protein 11 (FBXL11) as the demethylase acting on RelA. They demonstrated that FBXL11 and NSD1 function as an enzyme pair regulating RelA transcriptional activity through reversible lysine methylation/demethylation of these residues. We have previously shown that SETD6 mono-methylates RelA at K310, which represses RelA transcriptional activity in the absence of stimulation [75–78]. More specifically, RelAK310me1 leads to the constitutive repression of RelA target genes by recruitment of GLP, which catalyzes H3K9me2 and leads to chromatin silencing and gene repression. In response to stimulation with TNF-alpha and lipopolysaccharide (LPS), RelA is phosphorylated at serine 311 (RelAS311ph) by protein kinase C (PKC)ξ, which physically blocks the interaction between GLP and RelAK310me1, resulting in transcription

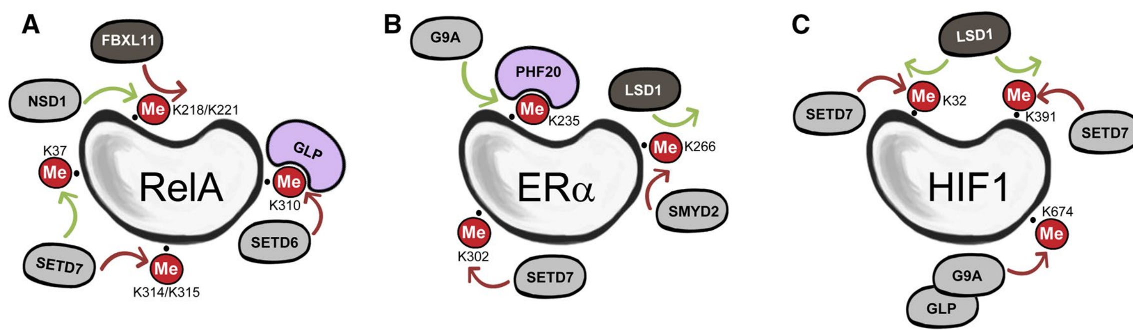

Fig. 3 Lysine methylation signalling mediated by writers, erasers and readers for the transcription factors RelA (**a**), Erα (**b**) and HIF1 (**c**). PKMTs are represented in light gray, erasers are in dark gray and the readers are in purple. The methylated residue numbers are also indicated. Green and red arrows represent a positive or a negative effect on the transcription factor, respectively

activation [78]. Taken together, NF-κB activity is intricately regulated by lysine methylation of RelA, in a positive or negative manner, depending on the methylation site.

RB and E2F1

RB is a chromatin-associated protein that inhibits the transcription of cell cycle genes in the G1–S transition phase, primarily through the repression of E2F1. CDK-mediated phosphorylation of RB relieves its inhibition of E2F1, enabling cell cycle progression [79–81]. Given their substantial role in cell cycle progression, the cellular functions of both RB and E2F1 are tightly regulated by many post-translational modification, such as phosphorylation, acetylation, SUMOylation, ubiquitination, and methylation [82]. SMYD2 methylates RB at K860 during re-entry into the cell cycle, which, in turn, facilitates direct interaction between RB and the methyl-binding protein L3MBTL1 [83]. In contrast, in various cancer cell lines, SMYD2-driven RB methylation at K810 enhances Ser 807/811 phosphorylation of RB, resulting in promotion of cell cycle progression by E2F1 [84]. Interestingly, a year earlier, the same group had shown that MYPT1, a known regulator of RB Ser 807/811 phosphorylation, is regulated by the balance between K442 methylation and demethylation by SETD7 and LSD1, respectively [85]. This observation added another layer to the coordinated and tight regulation of RB activity during the cell cycle.

Kontaki et al. discovered a dynamic interplay between SETD7 and LSD1 in the methylation of E2F1 at K185. After DNA damage, methylated E2F1 is less stable and is prone to ubiquitination and degradation, due to inhibition of E2F1 acetylation and phosphorylation [86]. In contrast, Yuan et al. showed that K185 methylation by SETD7 leads to E2F1 stabilization and upregulation of its pro-apoptotic target genes, p73 and Bim. They suggested that LSD1 is the active K185 demethylase [87]. The precise downstream effect of K185me1 remains an open question and requires further study. A key point in such studies is elucidation of the cross-talk between E2F1 methylation and other modifications regulating E2F1 and perhaps also RB activity.

Nuclear receptors

Nuclear receptors (NRs) are a family of TFs that regulate numerous physiological processes such as cell differentiation, development, inflammation, proliferation and metabolism [88, 89]. In recent years, accumulating evidence has indicated that many NRs are modified by numerous PTMs, including methylation, and have also shown a direct link to the pathophysiological progression of many diseases [90–92]. Oestrogen receptor alpha (ERalpha) was shown to be di-methylated at K235 by G9A. Recognition of the methylated residue by the Tudor domain of PHF20 results in recruitment of the MOF HAT complex to ERalpha target gene promoters, leading to the activation of transcriptional programs which are essential for the proliferation and survival of ER-positive breast cancer cells [93]. ERalpha is also a target for methylation at K302 by SETD7, which leads to its activation [94]. Interestingly, a breast cancer-associated mutation at K303 was shown to increase K302 methylation [94]. In addition, K266 of ERalpha is directly methylated by SMYD2. Upon oestrogen stimulation, the K266 methyl mark is removed by LSD1, resulting in efficient ERalpha activation [95]. The crystal structure of SMYD2 in complex with a target K266 ERalpha peptide was shown in a later work [96]. Taken together, three lysines on ERalpha are targeted for methylation by three different PKMTs (Fig. 3b). Two (K235 and K302) are positive regulators, while K266 is a repressive mark. It would be interesting to test if the two activating marks co-exist or whether sequential methylation is required for fine-tuning of ERalpha activity in response to stimulation.

The androgen receptor (AR) is methylated by SETD7 at K630, which results in enhanced AR transcriptional activity in several kidney and prostate cell lines [97]. At the

same time, another report showed that AR is methylated by SETD7 at K632, which led to receptor activation by facilitating both inter-domain communication between the N- and C-termini and its recruitment to androgen-target genes [98]. While the methylation site in these two reports was different, both were within the KLKK motif, which is similar to other SETD7 target sequences. Thus, it seems that differences between reports may reflect variations in experimental procedures or low specificity of analytical approaches. In any case, the discrepancy between them remains to be resolved.

Oxidative stress-related transcription factors

HIF1 is a key TF, with hundreds of target genes that control many aspects of cell biology, including oxidative stress response, angiogenesis, metabolism and stem cell pluripotency [99]. Like many other TFs, HIF1 activity is modulated by numerous PTMs, including methylation (Fig. 3c). Liu et al. reported that SETD7 methylation of HIF1α at K32 and of HIF-2α at K29 negatively regulates their transcriptional activity [100]. Another group confirmed that HIF1 is methylated by SETD7 at K32 [101], a site which falls within the consensus SETD7 methylation sequence defined earlier by several groups [33, 102]. LSD1 was shown to reverse HIF-1 methylation and increase its stability [101]. These observations were further validated by generation of a HIF-1 methylation-deficient knock-in mouse, which showed enhanced retinal angiogenesis, tumor growth and angiogenesis via HIF-1alpha stabilization [101]. Interestingly, SETD7 was also shown to di-methylate HIF-1 at K391, with hydroxylation at P402/564 being a prerequisite for this methylation. Lee et al. suggested that a conformational change of HIF1α upon hydroxylation might enable SETD7 recognition of this HIF1α methylation site [103]. As in the case of K32 monomethylation [101], LSD1 is the active K391 demethylase. These reports provide further support of a model in which LSD1 also directly suppresses HIF1α hydroxylation which is required for SETD7-dependent methylation [103]. Recently, HIF1 was shown to be mono- and di-methylated at K674 by G9A and GLP, which led to decreased HIF-1 transcriptional activity and to expression of a subset of HIF-1 downstream target genes in glioblastoma multiforme (GBM) cells. This cascade resulted in inhibited GBM cell migration under hypoxia [104]. It seems that the dynamic interplay between the different HIF1 sites targeted for methylation (K32, K391 and K672) by three different PKMTs (SETD7, G9A and GLP) and by demethylating activity of LSD1 plays a pivotal role in the cellular response to hypoxia.

Several reports have highlighted the methylation of additional non-histone substrates which are important for the delicate regulation of hypoxia. For example, under hypoxic conditions, reptin, a chromatin remodelling factor, is methylated by G9A at K67, which leads to downregulation of the transcription of hypoxia-responsive genes via recruitment of a repressive complex including HDAC1, to a subset of hypoxia-responsive target promoters [105]. The methylation of FOXO3 at K270 by SETD7 inhibits the activation of the pro-apoptotic gene Bim, resulting in negative regulation of apoptosis in neurons [106]. Interestingly, K271, which is adjacent to K270 was also shown to be methylated by SETD7. K271me decreases FOXO3 protein stability, while moderately increasing its transcriptional activity [107]. It remains to be determined how K270 and K271 methylation events affect FOXO3 activity and whether it is specific to cell type or to target gene type. We have recently shown that FOXM1, which, like FOXO3, belongs to the Forkhead box superfamily, is targeted for methylation by SETD3, which modulates its regulation of VEGF expression [108]. Under basal conditions, FOXM1 methylation by SETD3 at the chromatin inhibits VEGF activation. In response to hypoxia, SETD3 and FOXM1 levels are reduced, leading to decreased interaction with FOXM1, dissociation of both SETD3 and FOXM1 from the VEGF promoter and de-repression of VEGF transcription [108]. Similar results were observed during hypoxic pulmonary hypertension in rats [109], further supporting the functional interplay between SETD3 and FOXM1 under oxidative stress conditions. The poly (ADP-ribose) polymerase 1 (PARP1—also known as ARTD1), which catalyzes the poly-ADP-ribosylation of proteins using NAD+, was shown to be methylated by two different PKMTs, each targeting a different lysine. More specifically, SMYD2 mono-methylates PARP1 at K528, which is located in the protein's catalytic domain, enhancing PARP1 activity after oxidative stress [110]. Similarly, SETD7-dependent methylation of PARP1 at K508 triggers PARP1 recruitment to DNA damage sites and also enhances its enzymatic activity following oxidative stress [111]. Future research is required to understand if SMYD2 and SETD7 work independently under different cellular conditions or in concert.

Other non-histone nuclear protein methylation substrates

In addition to the major transcription factors discussed above, several other non-histone nuclear proteins, displaying a wide variety of cellular functions, were shown to be methylated. The transcription factor STAT3 was shown to be di-methylated at K140 by SETD7 and demethylated by LSD1. This methylation destabilizes STAT3 tyrosine phosphorylation, thereby negatively regulating STAT3-dependent transcription of certain genes [112]. Phosphorylation of EZH2 at S21 was shown to enhance STAT3 tri-methylation at K180 by EZH2 and its activation in glioblastoma stem cells [113]. K49 di-methylation by EZH2 was shown to activate STAT3

in response to IL6 stimulation [114]. It was also suggested that STAT3 is methylated by SMYD2 at K685 in renal cyst growths; however, it remains to be determined if this is a direct or indirect methylation event, as no in vitro methylation assay with recombinant proteins was performed [115].

The hedgehog signalling pathway regulates the activity of several proteins, including Gli3 [116]. The transcription factor Gli3 is methylated by SETD7 at K436 and K595, leading to its increased stability and resulting in enhanced hedgehog pathway signalling. Furthermore, this methylation event was shown to contribute to non-small cell lung cancer growth and metastasis, both in vitro and in vivo [117]. The transcription factor GATA4, a key regulator of heart development in mice and humans [118], was shown to be methylated at K299 by EZH2, which inhibited its transcriptional activity [119]. The transcription factor interferon regulatory factor 3 (IRF3), essential for innate antiviral immunity, is mono-methylated by NSD3 at K366, which upregulates its transcription activity upon viral infection [120].

The splicing regulator RBM25 is mono-methylated at K77, by a yet unidentified PKMT. K77me1 abrogates its interaction with the splicing factor SRFS2 [121]. Using a quantitative proteomic analysis-based method, Carlson et al. identified that the splicing factor snRNP70 is mono-methylated by SETMAR at K130 [45]. The specific downstream roles of methylated RBM25 and snRNP70 remain elusive, but are likely related to the regulation of alternative splicing.

Methylation of non-histone proteins has also been implicated in the regulation of DNA replication. The replication regulator PCNA [122] is di-methylated by EZH2 at K110, which was shown to stabilize the PCNA trimer complex, increasing its binding affinity to the polymerase POLδ, and leading to efficient replication [123]. PCNA is also methylated by SETD8 at K248 [124], enhancing its interaction with FEN1, which has important functions in Okazaki fragment ligation during replication [125]. FEN1 was shown to be mono-methylated by SETD7 at K377 during S phase. However, this methylation event had neither an impact on the protein's flap activity, nor an impact on its interaction with key partners during replication, such as PCNA [126]. EGFR mono-methylation at K721 by NSD3 was recently shown to enhance its interaction with PCNA, leading to accelerated DNA replication in squamous cell carcinoma of the head and neck (SCCHN) cells [127].

DNMT1 encodes the maintenance DNA methyltransferase (DNMT) and is responsible for the methylation of hemimethylated CpG sites shortly after DNA replication. Regulation of DNMT1 stability was shown to be mediated by several enzymes. Methylation of K142 on DNMT1 by SETD7 leads to DNMT1 degradation [128], which can be blocked by phosphorylation of DNMT1 at Ser143 by AKT1 kinase [129], and can be removed by LSD1 [130, 131]. An elegant interplay between methyl-lysine-binding proteins was shown to regulate the downstream consequences of DNTM1 methylation at K142. The MBT domain of PHF20L1 specifically binds K142me1 and inhibits DNMT1 ubiquitination and degradation [132]. In contrast, the MBT domain of L3MBTL3 binds K142me1 and recruits CRL4 ubiquitin ligase to degrade DNMT1. In fact, L3MBTL3 is required for DNMT1 proteolysis triggered by LSD1 or PHF20L1 deficiency in a cell cycle-dependent manner [130]. DNMT1 was also shown to be methylated in vitro at K70 by G9A; however, the biological significance of this methylation event remains to be determined [35]. UHRF1 interaction with DNMT1, which leads to its recruitment to DNA and stimulation of DNA maintenance activity [133, 134], was suggested to be partly mediated by methylation of the DNA ligase 1, LIG1. LIG1 methylation at K126 by G9A/GLP enables recruitment of UHRF1 via it Tudor domain, to replication foci, leading to efficient methylation maintenance [135]. Interestingly, G9A showed a higher affinity for the tri-methylation of the activating transcriptional factor 7-interacting protein (ATF7IP) at K16 in mouse embryonic stem cells compared to K126 di-methylation of LIG1 [136], which supports the notion that different substrates are methylated in different ways in different biological contexts. In addition to the regulation of DNMT1 by lysine methylation, the de novo methyltransferase DNMT3a is di-methylated by G9A/GLP at K44 in mouse and at K47 in human. DNMT3aK44me2 is specifically recognized by the chromodomain of MPP8, which results in a repressive chromatin state with H3K9me and DNA CpG methylation [137].

There are several examples of lysine methylation of protein members of the basal eukaryotic transcription complex. TAF10, a component of the general transcription factor TFIID complex, is mono-methylated by SETD7 at K189. This event increases the affinity of the interaction of TAF10 with RNA polymerase II, which can partly explain the resultant stimulatory effect on transcription [102, 138]. In addition, TAF7, another subunit of the same complex, was shown to be methylated, in vitro, at K5 by SETD7 [102]; however, additional studies are required to determine whether it is also methylated in cells and if it has any effect on TFIID cellular functions. Elongin A, which is part of the elongation complex that interacts with the phosphorylated form of the RNA polymerase II C-terminal domain (CTD) [139, 140], was identified as a substrate of EZH2 within the PRC2 complex. Elongin A mono-methylation at K754 results in transcriptional downregulation of target genes in mouse embryonic stem (mES) cells [141].

Future perspectives

The growing breadth of the known methylome landscape is expanding our understanding of the significant contribution of methylation events in regulation of the cellular

activity of non-histone proteins involved in a broad spectrum of cellular and developmental processes, with direct and indirect effects on human pathologies. While this review focused on lysine methylation events in the nucleus, this PTM has been observed in other cellular compartments as well, as summarized by others [38, 142–147].

The potential role of lysine methylation as a central PTM underscores the need for the development of technologies to study it. As described in this review, the research community is reaching the point at which advanced experimental platforms will enable identification and precise mapping of thousands of potential new methylation events. One of the main bottlenecks in this field is translation of these data into a practical understanding of the underlying biology. The vast complexity of the biological processes integrating such events requires a synchronized set of signals, which is partly achieved by the individual methylation event and its cross-talk with others. As described in this review, proteins can be targeted for methylation at the same residue by different PKMTs and under distinct physiological settings, all leading to diverse biological effects. Moreover, there are examples of PKMTs which trigger opposite functional outcomes in a single protein. To fully understand the biology behind lysine methylation, methylation events must be viewed in the context of an entire signalling pathway mediated by writers, erasers and readers, with a precise biological end-point. Many challenges remain, including resolution of several important questions: (1) what are the upstream signals (cell stimulation, protein–protein interaction, microRNA, etc.) that control the dynamics and kinetics of a given methylation event and regulate the enzymatic activity of the enzymes that add and remove the methyl mark? (2) Which effector proteins read the methylation marks and transduce them to downstream outputs? (3) How are specific readers recruited to a defined methylated residue in a complex cellular structure in different cells/tissue under dynamic cellular physiological changes? Addressing these critical questions and others will not only be a seminal contribution to our understanding of lysine methylation per se, but will also provide a remarkable opportunity to integrate knowledge on lysine methylation-associated signalling with the accumulated knowledge on other cellular signalling processes in human health and disease.

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References

1. Greer EL, Shi Y (2012) Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 13:343–357
2. Murn J, Shi Y (2017) The winding path of protein methylation research: milestones and new frontiers. Nat Rev Mol Cell Biol 18:517–527
3. Black JC, Van Rechem C, Whetstine JR (2012) Histone lysine methylation dynamics: establishment, regulation, and biological impact. Mol Cell 48:491–507
4. Albert M, Helin K (2010) Histone methyltransferases in cancer. Semin Cell Dev Biol 21:209–220
5. Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705
6. Del Rizzo PA, Trievel RC (2014) Molecular basis for substrate recognition by lysine methyltransferases and demethylases. Biochim Biophys Acta 1839:1404–1415
7. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M (2012) Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov 11:384–400
8. Cheng X, Collins RE, Zhang X (2005) Structural and sequence motifs of protein (histone) methylation enzymes. Annu Rev Biophys Biomol Struct 34:267–294
9. Falnes PO, Jakobsson ME, Davydova E, Ho A, Malecki J (2016) Protein lysine methylation by seven-beta-strand methyltransferases. Biochem J 473:1995–2009
10. Petrossian TC, Clarke SG (2011) Uncovering the human methyltransferasome. Mol Cell Proteom 10(M110):000976
11. Kagan RM, Clarke S (1994) Widespread occurrence of three sequence motifs in diverse *S*-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. Arch Biochem Biophys 310:417–427
12. Peterson CL, Laniel MA (2004) Histones and histone modifications. Curr Biol 14:R546–R551
13. Ambler RP, Rees MW (1959) Epsilon-*N*-methyl-lysine in bacterial flagellar protein. Nature 184:56–57
14. Murray K (1964) The occurrence of epsilon-*N*-methyl lysine in histones. Biochemistry 3:10–15
15. Wang C, Lazarides E, O'Connor CM, Clarke S (1982) Methylation of chicken fibroblast heat shock proteins at lysyl and arginyl residues. J Biol Chem 257:8356–8362
16. Kudithipudi S, Jeltsch A (2016) Approaches and guidelines for the identification of novel substrates of protein lysine methyltransferases. Cell Chem Biol 23:1049–1055
17. Webb KJ, Lipson RS, Al-Hadid Q, Whitelegge JP, Clarke SG (2010) Identification of protein N-terminal methyltransferases in yeast and humans. Biochemistry 49:5225–5235
18. Stock A, Clarke S, Clarke C, Stock J (1987) N-terminal methylation of proteins: structure, function and specificity. FEBS Lett 220:8–14
19. Iwabata H, Yoshida M, Komatsu Y (2005) Proteomic analysis of organ-specific post-translational lysine-acetylation and -methylation in mice by use of anti-acetyllysine and -methyllysine mouse monoclonal antibodies. Proteomics 5:4653–4664
20. Levy D, Liu CL, Yang Z, Newman AM, Alizadeh AA, Utz PJ, Gozani O (2011) A proteomic approach for the identification of novel lysine methyltransferase substrates. Epigenet Chromatin 4:19
21. Burgos ES, Walters RO, Huffman DM, Shechter D (2017) A simplified characterization of *S*-adenosyl-L-methionine-consuming enzymes with 1-step EZ-MTase: a universal and straightforward coupled-assay for in vitro and in vivo setting. Chem Sci 8:6601–6612

22. Duchin S, Vershinin Z, Levy D, Aharoni A (2015) A continuous kinetic assay for protein and DNA methyltransferase enzymatic activities. Epigenet Chromatin 8:56
23. Burgos ES, Gulab SA, Cassera MB, Schramm VL (2012) Luciferase-based assay for adenosine: application to *S*-adenosyl-L-homocysteine hydrolase. Anal Chem 84:3593–3598
24. Hemeon I, Gutierrez JA, Ho MC, Schramm VL (2011) Characterizing DNA methyltransferases with an ultrasensitive luciferase-linked continuous assay. Anal Chem 83:4996–5004
25. Ibanez G, McBean JL, Astudillo YM, Luo M (2010) An enzyme-coupled ultrasensitive luminescence assay for protein methyltransferases. Anal Biochem 401:203–210
26. Dorgan KM, Wooderchak WL, Wynn DP, Karschner EL, Alfaro JF, Cui Y, Zhou ZS, Hevel JM (2006) An enzyme-coupled continuous spectrophotometric assay for *S*-adenosylmethionine-dependent methyltransferases. Anal Biochem 350:249–255
27. Collazo E, Couture JF, Bulfer S, Trievel RC (2005) A coupled fluorescent assay for histone methyltransferases. Anal Biochem 342:86–92
28. Hendricks CL, Ross JR, Pichersky E, Noel JP, Zhou ZS (2004) An enzyme-coupled colorimetric assay for *S*-adenosylmethionine-dependent methyltransferases. Anal Biochem 326:100–105
29. Schaberle TF, Siba C, Hover T, Konig GM (2013) An easy-to-perform photometric assay for methyltransferase activity measurements. Anal Biochem 432:38–40
30. Weirich S, Kudithipudi S, Jeltsch A (2016) Specificity of the SUV4-20H1 and SUV4-20H2 protein lysine methyltransferases and methylation of novel substrates. J Mol Biol 428:2344–2358
31. Lanouette S, Davey JA, Elisma F, Ning Z, Figeys D, Chica RA, Couture JF (2015) Discovery of substrates for a SET domain lysine methyltransferase predicted by multistate computational protein design. Structure 23:206–215
32. Moore KE, Carlson SM, Camp ND, Cheung P, James RG, Chua KF, Wolf-Yadlin A, Gozani O (2013) A general molecular affinity strategy for global detection and proteomic analysis of lysine methylation. Mol Cell 50:444–456
33. Dhayalan A, Kudithipudi S, Rathert P, Jeltsch A (2011) Specificity analysis-based identification of new methylation targets of the SET7/9 protein lysine methyltransferase. Chem Biol 18:111–120
34. Rathert P, Dhayalan A, Ma H, Jeltsch A (2008) Specificity of protein lysine methyltransferases and methods for detection of lysine methylation of non-histone proteins. Mol BioSyst 4:1186–1190
35. Rathert P et al (2008) Protein lysine methyltransferase G9a acts on non-histone targets. Nat Chem Biol 4:344–346
36. Cornett EM et al (2018) A functional proteomics platform to reveal the sequence determinants of lysine methyltransferase substrate selectivity. Sci Adv 4:eaav2623
37. Carlson SM, Gozani O (2014) Emerging technologies to map the protein methylome. J Mol Biol 426:3350–3362
38. Lanouette S, Mongeon V, Figeys D, Couture JF (2014) The functional diversity of protein lysine methylation. Mol Syst Biol 10:724
39. Ong SE, Mann M (2006) Identifying and quantifying sites of protein methylation by heavy methyl SILAC. Curr Protoc Protein Sci Chapter 14:Unit 14 9
40. Ong SE, Mittler G, Mann M (2004) Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Nat Methods 1:119–126
41. Cao XJ, Arnaudo AM, Garcia BA (2013) Large-scale global identification of protein lysine methylation in vivo. Epigenetics 8:477–485
42. Cao XJ, Garcia BA (2016) Global proteomics analysis of protein lysine methylation. Curr Protoc Protein Sci 86:24 8 1–24 8 19
43. Guo A et al (2014) Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. Mol Cell Proteom 13:372–387
44. Carlson SM, Moore KE, Green EM, Martin GM, Gozani O (2014) Proteome-wide enrichment of proteins modified by lysine methylation. Nat Protoc 9:37–50
45. Carlson SM, Moore KE, Sankaran SM, Reynoird N, Elias JE, Gozani O (2015) A proteomic strategy identifies lysine methylation of splicing factor snRNP70 by the SETMAR enzyme. J Biol Chem 290:12040–12047
46. Liu H et al (2013) A method for systematic mapping of protein lysine methylation identifies functions for HP1beta in DNA damage response. Mol Cell 50:723–735
47. Islam K et al (2013) Defining efficient enzyme–cofactor pairs for bioorthogonal profiling of protein methylation. Proc Natl Acad Sci USA 110:16778–16783
48. Blum G, Islam K, Luo M (2013) Bioorthogonal profiling of protein methylation (BPPM) using an azido analog of *S*-adenosyl-L-methionine. Curr Protoc Chem Biol 5:45–66
49. Islam K, Bothwell I, Chen Y, Sengelaub C, Wang R, Deng H, Luo M (2012) Bioorthogonal profiling of protein methylation using azido derivative of *S*-adenosyl-L-methionine. J Am Chem Soc 134:5909–5915
50. Binda O, Boyce M, Rush JS, Palaniappan KK, Bertozzi CR, Gozani O (2011) A chemical method for labeling lysine methyltransferase substrates. ChemBioChem 12:330–334
51. Peters W et al (2010) Enzymatic site-specific functionalization of protein methyltransferase substrates with alkynes for click labeling. Angew Chem Int Ed Engl 49:5170–5173
52. Chuikov S et al (2004) Regulation of p53 activity through lysine methylation. Nature 432:353–360
53. Kurash JK et al (2008) Methylation of p53 by Set7/9 mediates p53 acetylation and activity in vivo. Mol Cell 29:392–400
54. Luo J, Su F, Chen D, Shiloh A, Gu W (2000) Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature 408:377–381
55. Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD, Berger SL (1999) p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. Mol Cell Biol 19:1202–1209
56. Huang J et al (2006) Repression of p53 activity by Smyd2-mediated methylation. Nature 444:629–632
57. Huang J et al (2007) p53 is regulated by the lysine demethylase LSD1. Nature 449:105–108
58. Huang J, Dorsey J, Chuikov S, Perez-Burgos L, Zhang X, Jenuwein T, Reinberg D, Berger SL (2010) G9a and Glp methylate lysine 373 in the tumor suppressor p53. J Biol Chem 285:9636–9641
59. Shi X et al (2007) Modulation of p53 function by SET8-mediated methylation at lysine 382. Mol Cell 27:636–646
60. West LE, Roy S, Lachmi-Weiner K, Hayashi R, Shi X, Appella E, Kutateladze TG, Gozani O (2010) The MBT repeats of L3MBTL1 link SET8-mediated p53 methylation at lysine 382 to target gene repression. J Biol Chem 285:37725–37732
61. Cui G et al (2012) PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53. Nat Struct Mol Biol 19:916–924
62. Dhami GK et al (2013) Dynamic methylation of Numb by Set8 regulates its binding to p53 and apoptosis. Mol Cell 50:565–576
63. Weirich S, Kusevic D, Kudithipudi S, Jeltsch A (2015) Investigation of the methylation of Numb by the SET8 protein lysine methyltransferase. Sci Rep 5:13813
64. Shen C et al (2015) SET7/9 regulates cancer cell proliferation by influencing beta-catenin stability. FASEB J 29:4313–4323
65. Deng X et al (2017) Critical roles of SMYD2-mediated beta-catenin methylation for nuclear translocation and activation of Wnt signaling. Oncotarget 8:55837–55847

66. Oudhoff MJ et al (2016) SETD7 controls intestinal regeneration and tumorigenesis by regulating Wnt/beta-catenin and Hippo/YAP signaling. Dev Cell 37:47–57
67. Oudhoff MJ et al (2013) Control of the hippo pathway by Set7-dependent methylation of Yap. Dev Cell 26:188–194
68. Vershinin Z, Feldman M, Chen A, Levy D (2016) PAK4 methylation by SETD6 promotes the activation of the Wnt/beta-catenin pathway. J Biol Chem 291:6786–6795
69. Natoli G (2009) Control of NF-kappaB-dependent transcriptional responses by chromatin organization. Cold Spring Harb Perspect Biol 1:a000224
70. Ghosh S, Hayden MS (2008) New regulators of NF-kappaB in inflammation. Nat Rev Immunol 8:837–848
71. Hoffmann A, Natoli G, Ghosh G (2006) Transcriptional regulation via the NF-kappaB signaling module. Oncogene 25:6706–6716
72. Ea CK, Baltimore D (2009) Regulation of NF-kappaB activity through lysine monomethylation of p65. Proc Natl Acad Sci USA 106:18972–18977
73. Yang XD, Huang B, Li M, Lamb A, Kelleher NL, Chen LF (2009) Negative regulation of NF-kappaB action by Set9-mediated lysine methylation of the RelA subunit. EMBO J 28:1055–1066
74. Lu T, Jackson MW, Wang B, Yang M, Chance MR, Miyagi M, Gudkov AV, Stark GR (2010) Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. Proc Natl Acad Sci USA 107:46–51
75. Kublanovsky M, Aharoni A, Levy D (2018) Enhanced PKMT-substrate recognition through non active-site interactions. Biochem Biophys Res Commun 501:1029–1033
76. Feldman M, Levy D (2018) Peptide inhibition of the SETD6 methyltransferase catalytic activity. Oncotarget 9:4875–4885
77. Chang Y, Levy D, Horton JR, Peng J, Zhang X, Gozani O, Cheng X (2011) Structural basis of SETD6-mediated regulation of the NF-kB network via methyl-lysine signaling. Nucleic Acids Res 39:6380–6389
78. Levy D et al (2011) Lysine methylation of the NF-kappaB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-kappaB signaling. Nat Immunol 12:29–36
79. Dyson NJ (2016) RB1: a prototype tumor suppressor and an enigma. Genes Dev 30:1492–1502
80. Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A (2015) RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. Oncotarget 6:17873–17890
81. Giacinti C, Giordano A (2006) RB and cell cycle progression. Oncogene 25:5220–5227
82. Munro S, Carr SM, La Thangue NB (2012) Diversity within the pRb pathway: is there a code of conduct? Oncogene 31:4343–4352
83. Saddic LA, West LE, Aslanian A, Yates JR 3rd, Rubin SM, Gozani O, Sage J (2010) Methylation of the retinoblastoma tumor suppressor by SMYD2. J Biol Chem 285:37733–37740
84. Cho HS et al (2012) RB1 methylation by SMYD2 enhances cell cycle progression through an increase of RB1 phosphorylation. Neoplasia 14:476–486
85. Cho HS et al (2011) Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. Cancer Res 71:655–660
86. Kontaki H, Talianidis I (2010) Lysine methylation regulates E2F1-induced cell death. Mol Cell 39:152–160
87. Xie Q, Bai Y, Wu J, Sun Y, Wang Y, Zhang Y, Mei P, Yuan Z (2011) Methylation-mediated regulation of E2F1 in DNA damage-induced cell death. J Recept Signal Transduct Res 31:139–146
88. Tran M, Liu Y, Huang W, Wang L (2018) Nuclear receptors and liver disease: summary of the 2017 basic research symposium. Hepatol Commun 2:765–777
89. Weikum ER, Liu X, Ortlund EA (2018) The nuclear receptor superfamily: a structural perspective. Protein Sci 27(11):1876–1892
90. Brunmeir R, Xu F (2018) Functional regulation of PPARs through post-translational modifications. Int J Mol Sci 19:1738
91. Tecalco-Cruz AC (2018) Molecular pathways involved in the transport of nuclear receptors from the nucleus to cytoplasm. J Steroid Biochem Mol Biol 178:36–44
92. Anbalagan M, Huderson B, Murphy L, Rowan BG (2012) Post-translational modifications of nuclear receptors and human disease. Nucl Recept Signal 10:e001
93. Zhang X et al (2016) G9a-mediated methylation of ERalpha links the PHF20/MOF histone acetyltransferase complex to hormonal gene expression. Nat Commun 7:10810
94. Subramanian K et al (2008) Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Mol Cell 30:336–347
95. Zhang X et al (2013) Regulation of estrogen receptor alpha by histone methyltransferase SMYD2-mediated protein methylation. Proc Natl Acad Sci USA 110:17284–17289
96. Jiang Y, Trescott L, Holcomb J, Zhang X, Brunzelle J, Sirinupong N, Shi X, Yang Z (2014) Structural insights into estrogen receptor alpha methylation by histone methyltransferase SMYD2, a cellular event implicated in estrogen signaling regulation. J Mol Biol 426:3413–3425
97. Ko S, Ahn J, Song CS, Kim S, Knapczyk-Stwora K, Chatterjee B (2011) Lysine methylation and functional modulation of androgen receptor by Set9 methyltransferase. Mol Endocrinol 25:433–444
98. Gaughan L et al (2011) Regulation of the androgen receptor by SET9-mediated methylation. Nucleic Acids Res 39:1266–1279
99. Semenza GL (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 33:207–214
100. Liu X, Chen Z, Xu C, Leng X, Cao H, Ouyang G, Xiao W (2015) Repression of hypoxia-inducible factor alpha signaling by Set7-mediated methylation. Nucleic Acids Res 43:5081–5098
101. Kim Y et al (2016) Methylation-dependent regulation of HIF-1alpha stability restricts retinal and tumour angiogenesis. Nat Commun 7:10347
102. Couture JF, Collazo E, Hauk G, Trievel RC (2006) Structural basis for the methylation site specificity of SET7/9. Nat Struct Mol Biol 13:140–146
103. Lee JY et al (2017) LSD1 demethylates HIF1alpha to inhibit hydroxylation and ubiquitin-mediated degradation in tumor angiogenesis. Oncogene 36:5512–5521
104. Bao L et al (2018) Methylation of hypoxia-inducible factor (HIF)-1alpha by G9a/GLP inhibits HIF-1 transcriptional activity and cell migration. Nucleic Acids Res 46:6576–6591
105. Lee JS et al (2010) Negative regulation of hypoxic responses via induced Reptin methylation. Mol Cell 39:71–85
106. Xie Q et al (2012) Lysine methylation of FOXO3 regulates oxidative stress-induced neuronal cell death. EMBO Rep 13:371–377
107. Calnan DR et al (2012) Methylation by Set9 modulates FoxO3 stability and transcriptional activity. Aging (Albany NY) 4:462–479
108. Cohn O, Feldman M, Weil L, Kublanovsky M, Levy D (2016) Chromatin associated SETD3 negatively regulates VEGF expression. Sci Rep 6:37115
109. Jiang X, Li T, Sun J, Liu J, Wu H (2018) SETD3 negatively regulates VEGF expression during hypoxic pulmonary hypertension in rats. Hypertens Res 41:691–698
110. Piao L, Kang D, Suzuki T, Masuda A, Dohmae N, Nakamura Y, Hamamoto R (2014) The histone methyltransferase SMYD2

methylates PARP1 and promotes poly(ADP-ribosyl)ation activity in cancer cells. Neoplasia 16:257–264, 264 e2
111. Kassner I, Andersson A, Fey M, Tomas M, Ferrando-May E, Hottiger MO (2013) SET7/9-dependent methylation of ARTD1 at K508 stimulates poly-ADP-ribose formation after oxidative stress. Open Biol 3:120173
112. Yang J et al (2010) Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. Proc Natl Acad Sci USA 107:21499–21504
113. Kim E et al (2013) Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell 23:839–852
114. Dasgupta M, Dermawan JK, Willard B, Stark GR (2015) STAT3-driven transcription depends upon the dimethylation of K49 by EZH2. Proc Natl Acad Sci USA 112:3985–3990
115. Li LX, Fan LX, Zhou JX, Grantham JJ, Calvet JP, Sage J, Li X (2017) Lysine methyltransferase SMYD2 promotes cyst growth in autosomal dominant polycystic kidney disease. J Clin Invest 127:2751–2764
116. Dai P et al (2002) Ski is involved in transcriptional regulation by the repressor and full-length forms of Gli3. Genes Dev 16:2843–2848
117. Fu L, Wu H, Cheng SY, Gao D, Zhang L, Zhao Y (2016) Set7 mediated Gli3 methylation plays a positive role in the activation of Sonic Hedgehog pathway in mammals. Elife 5:e15690
118. Pu WT, Ishiwata T, Juraszek AL, Ma Q, Izumo S (2004) GATA4 is a dosage-sensitive regulator of cardiac morphogenesis. Dev Biol 275:235–244
119. He A et al (2012) PRC2 directly methylates GATA4 and represses its transcriptional activity. Genes Dev 26:37–42
120. Wang C, Wang Q, Xu X, Xie B, Zhao Y, Li N, Cao X (2017) The methyltransferase NSD3 promotes antiviral innate immunity via direct lysine methylation of IRF3. J Exp Med 214:3597–3610
121. Carlson SM, Soulette CM, Yang Z, Elias JE, Brooks AN, Gozani O (2017) RBM25 is a global splicing factor promoting inclusion of alternatively spliced exons and is itself regulated by lysine mono-methylation. J Biol Chem 292:13381–13390
122. Stoimenov I, Helleday T (2009) PCNA on the crossroad of cancer. Biochem Soc Trans 37:605–613
123. Peng A, Xu X, Wang C, Yang J, Wang S, Dai J, Ye L (2018) EZH2 promotes DNA replication by stabilizing interaction of POLdelta and PCNA via methylation-mediated PCNA trimerization. Epigenet Chromatin 11:44
124. Takawa M et al (2012) Histone lysine methyltransferase SETD8 promotes carcinogenesis by deregulating PCNA expression. Cancer Res 72:3217–3227
125. Zheng L, Dai H, Qiu J, Huang Q, Shen B (2007) Disruption of the FEN-1/PCNA interaction results in DNA replication defects, pulmonary hypoplasia, pancytopenia, and newborn lethality in mice. Mol Cell Biol 27:3176–3186
126. Thandapani P, Couturier AM, Yu Z, Li X, Couture JF, Li S, Masson JY, Richard S (2017) Lysine methylation of FEN1 by SET7 is essential for its cellular response to replicative stress. Oncotarget 8:64918–64931
127. Saloura V et al (2017) WHSC1L1-mediated EGFR mono-methylation enhances the cytoplasmic and nuclear oncogenic activity of EGFR in head and neck cancer. Sci Rep 7:40664
128. Esteve PO, Chin HG, Benner J, Feehery GR, Samaranayake M, Horwitz GA, Jacobsen SE, Pradhan S (2009) Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. Proc Natl Acad Sci USA 106:5076–5081
129. Esteve PO, Chang Y, Samaranayake M, Upadhyay AK, Horton JR, Feehery GR, Cheng X, Pradhan S (2011) A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. Nat Struct Mol Biol 18:42–48
130. Leng F, Yu J, Zhang C, Alejo S, Hoang N, Sun H, Lu F, Zhang H (2018) Methylated DNMT1 and E2F1 are targeted for proteolysis by L3MBTL3 and CRL4(DCAF5) ubiquitin ligase. Nat Commun 9:1641
131. Wang J et al (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 41:125–129
132. Esteve PO et al (2014) Methyllysine reader plant homeodomain (PHD) finger protein 20-like 1 (PHF20L1) antagonizes DNA (cytosine-5) methyltransferase 1 (DNMT1) proteasomal degradation. J Biol Chem 289:8277–8287
133. Jeltsch A, Jurkowska RZ (2014) New concepts in DNA methylation. Trends Biochem Sci 39:310–318
134. Rothbart SB, Dickson BM, Ong MS, Krajewski K, Houliston S, Kireev DB, Arrowsmith CH, Strahl BD (2013) Multivalent histone engagement by the linked tandem Tudor and PHD domains of UHRF1 is required for the epigenetic inheritance of DNA methylation. Genes Dev 27:1288–1298
135. Ferry L et al (2017) Methylation of DNA ligase 1 by G9a/GLP recruits UHRF1 to replicating DNA and regulates DNA methylation. Mol Cell 67(550–565):e5
136. Tsusaka T et al (2018) Tri-methylation of ATF7IP by G9a/GLP recruits the chromodomain protein MPP8. Epigenet Chromatin 11:56
137. Chang Y et al (2011) MPP8 mediates the interactions between DNA methyltransferase Dnmt3a and H3K9 methyltransferase GLP/G9a. Nat Commun 2:533
138. Kouskouti A, Scheer E, Staub A, Tora L, Talianidis I (2004) Gene-specific modulation of TAF10 function by SET9-mediated methylation. Mol Cell 14:175–182
139. Kawauchi J et al (2013) Transcriptional properties of mammalian elongin A and its role in stress response. J Biol Chem 288:24302–24315
140. Aso T, Haque D, Barstead RJ, Conaway RC, Conaway JW (1996) The inducible elongin A elongation activation domain: structure, function and interaction with the elongin BC complex. EMBO J 15:5557–5566
141. Ardehali MB, Anselmo A, Cochrane JC, Kundu S, Sadreyev RI, Kingston RE (2017) Polycomb repressive complex 2 methylates elongin A to regulate transcription. Mol Cell 68(872–884):e6
142. Carlson SM, Gozani O (2016) Nonhistone lysine methylation in the regulation of cancer pathways. Cold Spring Harb Perspect Med 6:a026435
143. Zhang X, Huang Y, Shi X (2015) Emerging roles of lysine methylation on non-histone proteins. Cell Mol Life Sci 72:4257–4272
144. Hamamoto R, Saloura V, Nakamura Y (2015) Critical roles of non-histone protein lysine methylation in human tumorigenesis. Nat Rev Cancer 15:110–124
145. Biggar KK, Li SS (2015) Non-histone protein methylation as a regulator of cellular signalling and function. Nat Rev Mol Cell Biol 16:5–17
146. Moore KE, Gozani O (2014) An unexpected journey: lysine methylation across the proteome. Biochim Biophys Acta 1839:1395–1403
147. Feldman M, Vershinin Z, Goliand I, Elia N, Levy D (2019) The methyltransferase SETD6 regulates mitotic progression through PLK1 methylation. Proc Natl Acad Sci USA 116:1235–1240

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