PRMT5 in gene regulation and hematologic malignancies

Fen Zhu, Lixin Rui*

Department of Medicine and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53792, USA

Received 15 March 2019; accepted 6 June 2019
Available online 19 June 2019

KeyWords
Gene regulation; Hematologic malignancies; Metabolism; Pathogenesis; PRMT5

Abstract Arginine methylation is a common posttranslational modification that governs important cellular processes and impacts development, cell growth, proliferation, and differentiation. Arginine methylation is catalyzed by protein arginine methyltransferases (PRMTs), which are classified as type I and type II enzymes responsible for the formation of asymmetric and symmetric dimethylarginine, respectively. PRMT5 is the main type II enzyme that catalyzes symmetric dimethylarginine of histone proteins to induce gene silencing by generating repressive histone marks, including H2AR3me2s, H3R8me2s, and H4R3me2s. PRMT5 can also methylate nonhistone proteins such as the transcription factors p53, E2F1 and p65. Modifications of these proteins by PRMT5 are involved in diverse cellular processes, including transcription, translation, DNA repair, RNA processing, and metabolism. A growing literature demonstrates that PRMT5 expression is upregulated in hematologic malignancies, including leukemia and lymphoma, where PRMT5 regulates gene expression to promote cancer cell proliferation. Targeting PRMT5 by specific inhibitors has emerged as a potential therapeutic strategy to treat these diseases.

Introduction

Protein arginine methylation is a type of post-translational modification that occurs as common as phosphorylation and ubiquitination.¹ Arginine side chain has 2 terminal guanidino (NH2) groups that can be subjected to methylation. Depending on where the methyl group is added, there are 3 types of arginine methylation in mammalian cells: ω-NG-monomethylation, ω-NG,N′G-symmetric dimethylation and ω-NG,N′G-asymmetric dimethylation (Fig. 1).

* Corresponding author. 1111 Highland Ave. Madison, WI, 53705, USA. Fax: +608 262 4598.
E-mail address: lrui@medicine.wisc.edu (L. Rui).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2019.06.002
2352-3042/© 2019, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Arginine methylation does not change the charge of proteins but increases hydrophobicity and reduces hydrogen bond donor. Therefore, arginine methylation can significantly alter protein-protein, protein-DNA, and protein-RNA interactions and has profound biological significance. Arginine methylation of histones (H2AR3, H3R8, H3R2 and H4R3) coordinates the formation of a transcriptional complex that regulates gene transcription. Arginine methylation of transcription factors and other signaling molecules alters their transcriptional activities. Arginine methylation of spliceosomal proteins is required for the assembly of spliceosomal complex and proper gene splicing. As such, arginine methylation governs important cellular processes and impacts development, cell growth, proliferation, and differentiation. To date, 9 protein arginine methyltransferases (PRMTs) have been identified, and they are classified as type I and type II enzymes responsible for the formation of asymmetric and symmetric dimethylarginine, respectively. In this review, we will focus on PRMT5, a main type II enzyme, and discuss its role in gene regulation and hematologic malignancies.

Discovery and characterization of PRMT5

PRMT5 is a highly conserved gene from unicellular eukaryotes to higher organisms. In search of second-site mutations that were synthetic lethal in combination with the deletion of the Histone H3 amino terminus in budding yeast Saccharomyces cerevisiae, a new gene product histone synthetic lethal 7 (Hsl7) was identified. Hsl7 was not essential for growth, but it regulated morphology of budding yeast. Deletion of Hsl7 led to elongated buds and undivided nuclei. This may be explained by the activation of a morphogenesis checkpoint that delays nuclear division. Skb1 is a homolog of Saccharomyces cerevisiae Hsl7 in the fission yeast Schizosaccharomyces pombe. Skb1 was identified as an interactor and modulator of shk1 kinase, which was an essential component of the Ras1/Cdc42 signaling cascade in controlling the viability and normal morphology of the fission yeast. Similar to Hsl7, Skb1 was not essential for growth and it participated in controlling cell morphology. Deletion of Skb1 led to premature exit from G2 and less elongated cells, which was opposite to the role of Hsl7 in controlling cell morphology. Xenopus Hsl7 homologue promoted mitotic entry, which is controlled by the DNA replication checkpoint.

The human homologue of skb1 (Skb1Hs) was characterized as a Janus Kinase 2 (JAK2) interacting protein in the yeast two-hybrid system, termed Jak binding protein 1 (JBP1). JBP1 was shown to possess methyltransferase activity towards histone H4 and H2A, but whether the methylation is arginine specific was not clear. Another group independently cloned Skb1Hs in an attempt to identify proteins interacting with nonstructural protein-3 (NS3) of the hepatitis C virus (HCV). They discovered that Skb1Hs is an arginine methyltransferase termed PRMT5 that contains conserved domains of AdoMet-dependent protein arginine methyltransferases, and exerts arginine methyltransferase activity towards myelin basic protein (MBP). Although MBP was the first protein found with arginines symmetrically dimethylated, whether MBP is a direct target of PRMT5 was not determined in this work. Later in the same year, a study using amino acid analysis of the in vitro methylated MBP revealed that PRMT5 catalyzes the formation of symmetric dimethylarginines. Thus, PRMT5 was identified as the first type II arginine methyltransferase. However, the yeast Hsl7 was shown to catalyze only monomethylation of calf histone H2A in vitro, and no Hsl7-dependent methylation of endogenous yeast histones was observed in vivo.

Biochemical, structural and enzymological studies further demonstrated the methylation mechanism of PRMT5. PRMT5 functions in a methylosome complex, and several cofactors are important for PRMT5 activity, stability and substrate specification. Methylsome protein 50 (MEP50) is a key cofactor for PRMT5 activity, and depletion of MEP50 significantly reduces methylsome activity. plCln associates with and assists the methylation of Sm proteins by PRMT5. While Rik41 competes with plCln for binding to PRMT5 and directs the activity of PRMT5 towards the RNA binding protein nucleolin. PRMT5 contains a two domain structure containing an N terminal domain and C terminal catalytic domain. The N terminal domain has a TIM barrel structure which interacts with the C terminal catalytic domain of adjacent dimers and other cofactors that are important for PRMT5 activity. MEP50 forms a

Figure 1  Arginine methylation catalyzed by PRMTs. Whether PRMT7 catalyzes symmetric dimethylation (Type II) or monomethylation of arginines (Type III) is controversial.
hetero-octameric complex with PRMT5. In this complex 4 PRMT5 molecules form a tetramer in a head-to-tail fashion with 4 MEP50 molecules binding to the N terminal domain of PRMT5 molecules. The C terminal catalytic domain of PRMT5 contains a Rossmann fold structure for SAM binding and its gain of asymmetric arginine dimethylase activity. Oncogenic JAK2V617F mutant is shown to inhibit methyltransferase activity of PRMT5. A study in Caenorhabditis elegans found that Phe379 is the key to determine the type II activity of CePRMT5, as F379M mutation not only increases the activity of CePRMT5 but also makes CePRMT5 capable of catalyzing both symmetric and asymmetric dimethylation of arginines. The corresponding F327M mutation in human PRMT5 also results in the gaining of asymmetric arginine dimethylase activity.

PRMT5 methylates substrates in a nonprocessive manner, which allows for the release of the mono-methylated substrate from PRMT5 but facilitates dimethylation when the concentration of monomethylated substrates exceeds that of unmethylated substrates. The domain structure of PRMT5 is illustrated in Fig. 2. CePRMT5 shares high sequence homology with human PRMT5. However, CePRMT5 does not associate with a MEP50 homologue and functions as a homodimer.

### Gene regulation and cellular functions mediated by PRMT5

Since the discovery of PRMT5, researchers have identified its diverse substrates, from histone proteins to non-histone nuclear and cytoplasmic proteins. Methylation of these substrates by PRMT5 is involved in many cellular processes, including transcription, DNA repair, RNA processing, proliferation and metabolism.

### Regulation of gene transcription

In 2002, the first direct experimental evidence demonstrated that PRMT5, as a type II arginine methylase, is involved in controlling gene transcription and cell proliferation. PRMT5 is a component of the cyclin E1 repressive complex (CERC) based on mass spectrometry analysis in NIH3T3 cells. Chromatin immunoprecipitation (ChiP) revealed that PRMT5 and dimethylated H4R3 are present at the transcription start site of cyclin E1, leading to the suppression of cyclin E1 gene transcription and cell proliferation. The first large scale interrogation of gene expression regulated by PRMT5 was done by microarray analysis in PRMT5 knockdown NIH3T3 cells, which revealed 227 up-regulated genes, including tumor suppressors and cell cycle inducers, and 43 down-regulated genes. PRMT5 was shown to directly methylate H3R8 and H4R3 in vitro. The overall effect on the proliferation of NIH3T3 cells was to stimulate cell proliferation and promote anchorage dependent cell growth, which contradicts the previous study. Nevertheless, these two and other studies all suggested that PRMT5 catalyzes histone methylation that suppresses gene transcription. However, genome wide studies have yielded controversial results. ChiP sequencing (ChiP-seq) analysis in CD4+ T cells using antibody recognizing both H2A and H4 (symmetric dimethyl R3) demonstrated no preference of those marks for either active or silent promoters. In mouse embryonic stem cells and mouse embryonic fibroblasts, H4R3me2s is a hallmark of GC rich regions and is generally independent of transcriptional levels or DNA methylation. PRMT5 catalyzed H3R2me2s supports euchromatin maintenance and marks active promoters in B cells. Additionally, gene expression profiling in human embryonic stem cells (hESCs) revealed changes in the expression of only 78 genes after PRMT5 knockdown, 62 of which were downregulated, suggesting a major role for PRMT5 in promoting gene expression.

In addition to histones and chromatin remodeling complexes, PRMT5 controls gene transcription through methylaing transcription factors and other signaling molecules. PRMT5 mediated methylation of Arg 1175 of epidermal growth factor receptor (EGFR) positively modulates EGF-induced EGFR trans-autophosphorylation at Tyr1173, which has suppressive effects on extracellular signal—regulated kinase (ERK) activation and cell proliferation. Methylation of p65 at Arg30 by PRMT5 in response to IL-1β stimulation promotes the ability of NF-κB to bind to kB elements for gene transcription. Methylation of the transcriptional corepressor KAP1 contributes to gene repression. Epstein–Barr virus (EBV) nuclear antigen 2 (EBNA2) has an arginine glycine repeat domain that can be methylated by PRMT5. Methylation of EBNA2 by PRMT5 has been shown to stimulate EBNA2-dependent transcription and may be important for EBV induced transformation of B cells. PRMT5 can also methylate EBNA1, but this methylation appears to regulate the cellular localization of EBNA1 rather than to change the transcriptional activity of EBNA1. Methylation of Kuβ3-like factor 4 (KLF4) (Arg374) by PRMT5 leads to stabilization and accumulation of KLF4 protein, which antagonizes γ–radiation induced apoptosis and promotes tumorigenesis. Methylation of B cell lymphoma 6 (BCL6) at Arg305 by PRMT5 contributes to the suppressive activity of BCL6, which is required for germinal center formation.

In addition to its suppressive or active role in gene transcription, PRMT5 can also regulate transcriptional

![Figure 2](image_url) Functional domains of PRMT5.
specificity of target genes in the context of cellular conditions. During the DNA damaging response, methylation of p53 at Arg333, Arg335, and Arg337 by PRMT5 has an important functional consequence on p53 activity. Arginine methylation promotes the expression of genes such as p21 that induce cell cycle arrest, while PRMT5 depletion increases apoptosis. Arginine methylation affects the promoter specificity based on p53 ChIP analysis of its target genes p21, GADD45 and PUMA. Methylation of RAF proteins by PRMT5 increases their degradation and limits the amplitude and duration of ERK signaling in response to growth factors, affecting the evoked biological response. Inhibition of PRMT5-mediated RAF methylation enhances ERK signaling, redirecting the cellular response from proliferation to differentiation. Methylation of E2F1 at Arg111 and Arg113 by PRMT1 promotes cell apoptosis, while methylation by PRMT5 locks E2F1 into its cell cycle progression mode. Methylation of androgen receptor (AR) at Arg761 by PRMT5 attenuates AR recruitment to its target genes and inhibits transcription of genes expressed in the differentiated prostate epithelium. Methylation of the methyl DNA binding domain protein 2 (MBD2) by PRMT5 reduces the affinity of MBD2 to histone deacetylase (HDAC) silencing complex, thus impairing the transcriptional repression function of MBD2 in cells.

DNA replication and repair

Flap endonuclease 1 (FEN1) is a multifunctional enzyme involved in DNA replication, DNA repair and apoptotic DNA fragmentation. Methylation of FEN1 by PRMT5 promotes its interaction with PCNA and recruitment to DNA replication loci. Rad9 is critical for DNA repair and cell cycle checkpoint control. Methylation of Rad9 by PRMT5 is required for cellular resistance to DNA damage stresses, and for activation of S/M and G2/M checkpoints. TIP60 is a histone acetyltransferase involved in chromatin remodeling and homologous recombination mediated DNA repair. PRMT5 methylates RUVBL1, a cofactor that regulates TIP60 activity. PRMT5 can also directly regulate TIP60 activity through alternative splicing. PRMT5 depletion leads to defects in DNA replication and repair, resulting in DNA damage accumulation.

Transcription elongation and termination

Transcription elongation factors SPT5 and FCP1 are substrates of PRMT5. Methylation of SPT5 by PRMT5 at Arg698 negatively regulates association of SPT5 with RNA polymerase II and gene promoters. Methylation of FCP1 at Arg913 and Arg916 by PRMT5 does not change the affinity between FCP1 and RNA polymerase II, but FCP1-associated PRMT5 can methylate histones. RNA polymerase II subunit POLR2A is subjected to methylation by PRMT5. Symmetric dimethylation of Arg1810 in the POLR2A C terminal domain promotes recruitment of SMN and RNA–DNA helicase senataxin, which are required for resolving RNA-DNA hybrids created by RNA polymerase II and transcription termination.

RNA processing

Methylation of SmD1, SmD3 and SmB by pICln-associated PRMT5 directs the Sm proteins to the survival of motor neuron (SMN) proteins, where they are associated with newly exported snRNA to form spliceosomal snRNPs. In the Eμ-Myc transgenic mouse model, PRMT5 is a key enzymatic component of Myc promoted transcription of core snRNP assembly genes. PRMT5 regulates the splicing of genes involved in DNA repair pathway. Knockout of PRMT5 reduces the productive transcript levels of DNA repair genes, leading to DNA damage accumulation. Cells with wild type p53 is more sensitive to PRMT5 inhibition.

Metabolism

PRMT5 is involved in metabolism of bile acid, glucose and lipid. In response to the bile acid signal, the small heterodimer partner (SHP) is stabilized and activated to suppress the synthesis and production of bile acid, fatty acid and glucose. In this process, PRMT5 has been shown to increase SHP activity by methyllating SHP at Arg57. Upon glucose induction in hepatocellular carcinoma (HCC), PRMT5 competes with cyclin dependent kinase inhibitor 2A (CDKN2A) for binding to cyclin dependent kinase 4 (CDK4) and promotes cell cycle transition from G1 to S phase. Arg 24 of CDK4 is critical for its binding to both CDKN2A and PRMT5, but is not symmetrically dimethylated, suggesting methyltransferase activity independent function of PRMT5.

Sterol regulatory element-binding protein (SREBP) is a transcription factor controlling de novo lipogenesis in tumors. PRMT5 methylates SREBP1α at Arg321, which is required for SREBP1α transcriptional activity. This methylation can also prevent GSK3β mediated phosphorylation of SREBP1α on S430, which promotes proteasomal degradation of SREBP1α. Thus, PRMT5 promotes lipogenesis and tumor growth through both activating and stabilizing SREBP1α. In addition, symmetric dimethylation of SREBP1a at Arg321 is increased in human hepatocellular carcinoma tissue and correlates with poor prognosis. In response to a high fat diet, PRMT5 expression is upregulated by unknown mechanisms. Increased PRMT5 expression promotes activation of the PI3K-akt pathway, which suppresses the expression of master transcription factors of mitochondrial biogenesis and promotes high fat induced steatosis.

Whole transcriptome profiling in human diffuse large B cell lymphoma (DLBCL) cell lines has also revealed that PRMT5 regulates PI3K-AKT activity, glycolysis and cholesterol homeostasis. However, the mechanism by which PRMT5 regulates metabolism in DLBCL is largely unknown.
Current dogma is that PRMTs catalyze methylation of arginine-glycine rich motifs, but proteome-wide analysis of arginine methylation has revealed that methylation is independent of such a sequence context.\(^1\) The methylation sites of known substrates of PRMT5 indicate that arginine methylation can occur without having glycine next to it (Table 1).

| Substrate | Methylation site | Biological function | reference |
|-----------|------------------|---------------------|-----------|
| MBP       | R107: KGRGL      | Myelin integrity    | 10–12, 89–92 |
| SmD1, SmD3, SmB | C terminal arginine- and glycine-rich domains | Gene splicing | 17–19, 55–57 |
| H3R8      | R8: TARKS        | Transcriptional repression | 27, 74, 78 |
| H3R2      | R2: ARTKQ        | Transcriptional activation | 34, 35 |
| H4/H2AR3  | R3: SGRGK        | Transcriptional repression or independent of transcription | 26, 27, 31 |
| p53       | R333/335/337: QIRGKREFE | Apoptosis, cell cycle | 44 |
| FEN1      | R192: LMRHL      | DNA replication, DNA repair | 50 |
| EGFR      | R1175: YLRVA     | Signaling activation | 37 |
| CRAF      | R563: VGRGK      | Signaling activation | 45 |
| SHP       | R57: TCREA       | Bile acid biosynthesis, lipid synthesis, glucose production | 61 |
| E2F1      | R111/113: RGRGKHP | Apoptosis, cell cycle | 46, 47 |
| p65       | R30: KQRGK       | NFκB activation | 38 |
| EBNA1, EBNA2 | arginine- and glycine repeat domains | Gene transcription, cellular localization | 40, 41 |
| KLF4      | R374/376/377: PKKGRRGW | Protein stability | 42 |
| POLR2A    | R1810: SPRYTT    | Transcription termination | 54 |
| AR        | R761: NSRML      | Transcriptional activity | 48 |
| RUVBL1    | R205: QGGRCD     | DNA repair | 52 |
| BCL6      | R305: EERPS      | Transcriptional activity | 43 |
| SPT5      | R698: RGRGR      | Suppress transcriptional elongation | 28 |
| FCP1      | R913/916: GGRGPRGH | unknown | 53 |
| MBD2      | N terminal arginine- and glycine-rich domains | Suppress transcription repression function of MBD2 | 49 |
| KAP1      | R308: NKRGR      | Transcriptional repression | 39 |
| MYCN      | R242: GGRQK      | Protein stability | 94 |
| CF 1 m68  | R202/204/206: GGRGPRGF | Not determined | 95 |
| FGF-2\(^{13}\) | N terminal arginine- and glycine-rich domains | Nuclear shuttling | 96 |
| RPS10     | R158/160: FGRGRGQ | Ribosome assembly | 97 |
| G3BP1     | C terminal arginine- and glycine-rich domains | Repress stress granule assembly | 98 |
| ASK1      | R89: GSRRT       | Inhibits apoptosis | 99 |
| PDCD4     | R110: KGRGKL     | Cell proliferation | 100 |
| srGAPs    | R927: AGRSK      | Cell spreading, migration | 101 |
| PDGFR\(^x\) | R554: KPRYE     | Protein stability | 102 |
| HOXA9     | R140: SARRG      | Induction of endothelial cell leukocyte adhesion molecules | 103 |
| GM130     | R6: ETRQG       | Golgi apparatus ribbon formation | 104 |
| Piwi proteins | N terminal RG/RA motifs | Compartmentalization of small RNA pathway | 105 |
| Rad9      | R172/174/175: IGRGRRV | DNA damage | 51 |
| TDH       | R180: SPRNP      | Somatic cell reprogramming | 106 |
| SREBP1    | R321: QSRGE      | Lipogenesis | 65 |
| GLI1      | R515: GTRGRG     | Protein stability | 107 |
PRMT5 in hematologic malignancies

PRMT5 in normal hematopoiesis

Several mouse models have been established to investigate the importance of PRMT5 in hematopoiesis. Deletion of PRMT5 is embryonic lethal due to the abrogation of pluripotent cells in blastocysts.67 Conditional knockout of PRMT5 in mouse bone marrow hematopoietic stem and progenitor cells (HSPCs) disrupts quiescent cell status and leads to exhaustion of HSPCs.21,60 Consequently, erythroid differentiation, T cell and B cell development are impaired, resulting in severe pancytopenia.21 Deletion of p53 significantly delays PRMT5 knockout induced HSC exhaustion.60 Deletion of PRMT5 just prior to the start of fetal liver hematopoiesis causes severe block in erythroid development and is also embryonic lethal.22 Ablation of PRMT5 in pro-B cells blocks B cell development at the pro-B stage, leading to absence of mature B cells in spleen. Concurrent deletion of p53 fully recovers the number of pro-B cells, but not pre-B cells, suggesting a p53 independent role of PRMT5 in pro-B cell development.68 PRMT5 protects activated B cells from apoptosis and promotes proliferation of activated B cells, which are independent of p53 activation.58 During germinat center (GC) formation, the methylation of BCL6 by PRMT5 is necessary for the full transcriptional repressive effects of BCL6.43 Deletion of PRMT5 in GC B cells impairs GC formation and immunoglobulin affinity maturation.43,68 PRMT5 also prevents premature plasma cell differentiation.68 The role of PRMT5 in mouse B cell development is briefly summarized in Fig. 3. However, it should be kept in mind that mouse models may not recapitulate human biology. PRMT5 has been shown to regulate human embryonic stem cell proliferation but not pluripotency.36 Knockdown of PRMT5 expression in human CD34 + cord blood cells promotes progenitor cell expansion and accelerates erythroid differentiation.23 Knockdown of PRMT5 in a B cell progenitor acute lymphoblastic leukemia cell line Nalm6 induces cell differentiation from the pre-B to immature B stage. While overexpression of PRMT5 in human mature B cells promotes dedifferentiation back to the pre-B/immature B stages.69

PRMT5 in the pathogenesis of hematologic malignancies

Leukemia

After the identification of PRMT5 as a JAK2 binding protein, the functional consequence of this interaction was not reported until 12 years later. Liu F. et al. found that JAK2 harboring activating mutations (V717F, K539L) interacts with PRMT5 more strongly than wild type JAK2, and is able to phosphorylate PRMT5. Phosphorylation of PRMT5 by constitutively active JAK2 disrupts the interaction of PRMT5 with its co-activator MEP50−, thus impairing methyltransferase activity of PRMT5. Downregulation of PRMT5 activity contributes to mutant JAK2 induced myeloproliferative disease.21 PRMT5 is expressed in acute myoid leukemia (AML) cell lines at various levels, and inhibition of PRMT5 leads to DNA damage accumulation and reduces AML cell viability.62 In AML, PRMT5 has been shown to epigenetically silence the expression of miR-29b. As a result, transcription factor Sp1, one of the targets of miR-29, is upregulated by PRMT5 indirectly. Then, PRMT5 and Sp1 participate in a transcriptional complex that activates the transcription of receptor tyrosine kinase FLT3.70 PRMT5 is upregulated in human T-cell leukemia virus type-1 (HTLV-1) mediated T cell transformation as well as in T-cell leukemia/lymphoma cell lines and patient PBMCs.71 Inhibition of PRMT5 decreases cancer cell proliferation.71 In pediatric acute lymphoblastic leukemia (ALL), PRMT5 was found to be expressed at initial diagnosis while likely to be absent at complete remission,69 suggesting an oncogenic role of PRMT5 in ALL development. PRMT5 is highly expressed in CD34 + cells originated from bone marrow of chronic myelogenous leukemia (CML), compared to that from normal bone marrow.72 BCR-ABL upregulates PRMT5 expression in CML CD34 + cells, while PRMT5 in turn upregulates BCR-ABL expression in CML cells. Genetic or pharmacologic inhibition of PRMT5 severely impairs the proliferation of CML CD34 + cells both in vitro and in vivo.72

Lymphoma

PRMT5 expression is upregulated in lymphoma.58,73−76 PRMT5 knockdowm in DLBCL- and MCL- cell lines

Figure 3 Role of PRMT5 in murine B cell development.21,43,60,68 GC: germinat center; LZ: light zone.
...PRMT5 directly silences the expression of axin-related protein (AXIN2) and WNT inhibitory factor 1 (WIF1), which are negative regulators of the pro-survival WNT/\beta-catenin signaling pathway and AKT/GSK3β pathway.\textsuperscript{76} In a transgenic mouse model, PRMT5 cooperates with Cyclin D1 T286A to induce aggressive T cell lymphoma/leukemia.\textsuperscript{77} PRMT5 expression is required for tumor development driven by multiple oncogenes (NOTCH1, c-MYC, and MLL-\textsuperscript{AF9}).\textsuperscript{77} This study also demonstrates that arginine methylation of p53 by PRMT5 reduces expression of proapoptotic p53 transcriptional targets.\textsuperscript{77} PRMT5 is required for tumor maintenance in the Eμ-myC transgenic mouse model since homozygous deletion of PRMT5 results in full disease free survival.\textsuperscript{58} PRMT5 is also important for virus induced lymphomagenesis.\textsuperscript{76} Inhibition of PRMT5 blocks EBV induced B cell transformation. During EBV infection of B cells, PRMT5 is upregulated and contributes to initiation and maintenance of EBV induced B cell transformation.\textsuperscript{68} We also found that PRMT5 is highly expressed in DLBCL, MCL, Hodgkin lymphoma and Burkitt lymphoma cell lines when compared to naïve B cells.\textsuperscript{79} The level of PRMT5 expression is also elevated in DLBCL and MCL patient samples. PRMT5 knockout inhibits the proliferation of DLBCL cell lines both \textit{in vitro} and in xenograft mouse models.\textsuperscript{79} Moreover, inhibition of PRMT5 using a small molecule inhibitor significantly reduces tumor volume and improves animal survival in a DLBCL patient derived xenograft mouse model.\textsuperscript{79}

It is now well accepted that PRMT5 is upregulated in different types of lymphoma, however the mechanism by which it is upregulated remains largely unknown. In MCL, the decreased miR-92b and miR-96 expression accelerates PRMT5 translation.\textsuperscript{75} We discovered that BTK–NF-κB signaling induces PRMT5 transcription in activated B cell-like (ABC) DLBCL cells while BCR downstream PI3K-AKT-MYC signaling upregulates PRMT5 expression in both ABC and germinal center B cell-like (GCB) DLBCL cells.\textsuperscript{79}

Multiple myeloma
In multiple myeloma (MM), high PRMT5 expression is associated with decreased progression-free survival and overall survival.\textsuperscript{80} Inhibition of PRMT5 by a specific inhibitor inhibits proliferation of both MM cell lines and patient MM cells, and decreases tumor growth in a xenograft mouse model.\textsuperscript{80} PRMT5 interacts with Tripartite motif-containing protein 21 (TRIM21), an E3 ubiquitin ligase for IKKβ, to block TRIM21-mediated degradation of IKKβ, allowing activation of NF-κB signaling.\textsuperscript{80}

Targeting PRMT5 in hematologic malignancies

Given the important role PRMT5 plays in cancer development, targeting PRMT5 by specific inhibitors holds promise in clinical application. Methylthioadenosine (MTA) is an analog of the methyl donor S-adenosyl methionine (SAM), and specifically inhibits PRMT5 enzymatic activity.\textsuperscript{81,82} 5-Methylthioadenosine phosphorylase (MTAP) is one of the enzymes involved in the methionine salvage pathway that metabolizes MTA to adenine and methionine. Due to its proximity to the tumor suppressor gene CDKN2A, MTAP was reported to be frequently co-deleted with CDKN2A in many human tumors, which leads to the accumulation of MTA in cells and sensitizes cells to further PRMT5 inhibition.\textsuperscript{81,82}

To date, several small molecule inhibitors targeting PRMT5 have been developed by different groups with various selectivity and potency.\textsuperscript{70,71,73,78,83,84} EPZ015666 (GSK3235025) is an SAM cooperative inhibitor of PRMT5 that binds at the peptide binding site of PRMT5.\textsuperscript{73} In preclinical studies, EPZ015666 efficiently inhibits MCL growth both \textit{in vitro} and in cell line derived xenograft mouse models with IC50 at a low nanomolar range.\textsuperscript{73,83} EPZ015666 also significantly inhibits growth of MM cells.\textsuperscript{80} The similar compound EPZ015866 (GSK3203591) has been investigated in \textit{in vitro} studies.\textsuperscript{43,59,83} A more potent PRMT5 inhibitor, EPZ015938 (GSK3326595), is being used for two phase I trials (NCT02783300, NCT03614728) in solid tumors, non-Hodgkin lymphoma and leukemia.\textsuperscript{84}

Given the importance of PRMT5 for normal physiology, targeting PRMT5 might elicit severe side effects. Combination therapy by lowering the dosage of PRMT5 inhibitors is a way to reduce drug toxicity and avoid drug resistance. In human AML cell lines, deletion of PRMT5 leads to defects in homologous recombination due to aberrant splicing of key factors involved in chromatin modification and DNA repair.\textsuperscript{87} As a result, cells rely more on poly(ADP-ribose) polymerase (PARP) to repair their DNA. Inhibition of both PRMT5 and PARP synergistically inhibits the growth of AML cells while proliferation of normal human CD34 positive cord blood cells is less affected at similar concentrations.\textsuperscript{22} In DLBCL patient derived primary cancer cells, targeting PRMT5 enhances the effectiveness of BCL6 inhibitors in inhibiting cell proliferation.\textsuperscript{43} Our work reveals PI3K-AKT dependent expression of PRMT5 in DLBCL.\textsuperscript{79} Genomic and biochemical analyses demonstrate that PRMT5 promotes cell cycle progression and activates PI3K-AKT signaling, suggesting a feedback regulatory mechanism to enhance cell survival and proliferation.\textsuperscript{79} Co-targeting PRMT5 and AKT by their specific inhibitors synergistically inhibits the proliferation of both DLBCL cell lines and DLBCL primary cancer cells.\textsuperscript{79}

Conclusions and future directions

PRMT5 plays an important role in both the normal physiology and the development of human cancer including hematologic malignancies. Rational design of combination targeted therapies with PRMT5 inhibitors has emerged as an effective cancer treatment strategy. Our current knowledge about the mechanism by which PRMT5 activity or expression is deregulated in cancer cells is still limited. Future work should include identifying PRMT5 substrates and characterizing the pathologic role of PRMT5 in different types of human cancer. Gaining more insights into PRMT5 tumorigenesis will allow for the development of effective targeted therapeutic strategies for cancer treatment.

Conflicts of interest

The authors have no conflicts of interest to disclose.
Acknowledgement

This work was supported by the National Institutes of Health/National Cancer Institute (NIH/NCI) grant R01 CA187299.

Abbreviations

AR androgen receptor
ASK1 apoptosis signal-regulating kinase 1
ALL acute lymphoblastic leukemia
AML acute myeloid leukemia
AXIN2 axin related protein
BCL6 B cell lymphoma 6
CDK4 cyclin dependent kinase 4
CDKN2A cyclin dependent kinase inhibitor 2A
CERC cyclin E1 repressive complex
CF I m68 mammalian cleavage factor I 68kDa
ChIP chromatin immunoprecipitation
CML chronic myelogenous leukemia
CREB cAMP response element binding
CRTC2 CREB regulated transcriptional coactivator 2
DLBCL diffuse large B cell lymphoma
ABC DLBCL activated B cell like DLBCL
GCB DLBCL germinal center B cell like DLBCL
EBNA Epstein–Barr virus (EBV) nuclear antigen
EGFR epidermal growth factor receptor
ERK extracellular signal–regulated kinases
ERK extracellular signal–regulated kinases
FEN1 flap endonuclease 1
FGF-2 fibroblast growth factor 2 23kDa
G3BP1 Ras GTPase-activating protein-binding protein 1
GADD45 growth arrest and DNA damage 45
GC germinal center
GL1 Glial-Associated Oncogene 1
GM130 130 kDa cis-Golgi matrix protein
GSK3β glycogen synthase kinase 3 beta
H2AR3 histone H2A arginine 3
H3R8 histone H3 arginine 8
H3R8 histone H3 arginine 8
H4R3 histone H4 arginine 3
HCC hepatocellular carcinoma
HCV hepatitis C virus
HDAC histone deacetylase
hESC human embryonic stem cells
HOXA9 homeobox protein Hox-A3
HSPCs hematopoietic stem and progenitor cells
HTLV-1 human T-cell leukemia virus type-1
JAK2 janus kinase 2
JBP1 jak binding protein 1
KAP1 KRAB-associated protein 1
KLFL4 Krüppel-like factor 4
KRAB Krüppel associated box
MBD2 methyl DNA binding domain protein 2
MBP myelin basic protein
MCL mantle cell lymphoma
MEP50 methylsomes protein 50
MMP multiple myeloma
MTA methylthioadenosine
MTAMP methylthioadenosine phosphorylase
PARP poly(ADP-ribose) polymerase
PDCD4 programmed cell death 4
PDGFRα platelet-derived growth factor receptor alpha
PI3K phosphoinositide 3-kinases
PKA protein kinase A
PRC2 polycomb repressor complex 2
PRMT protein arginine methyltransferase
PUMA p53 upregulated modulator of apoptosis
RAF rapidly accelerated fibrosarcoma
RPS10 ribosomal protein s10
RUVBL1 RuvB-like 1
SAM S-adenosylmethionine
SHP small heterodimer partner
SNM survival of motor neurons
SREBP sterol regulatory element-binding protein
srGAPs slit-Robo GTPase-activating proteins
TDH L-threonine dehydrogenase
TRIM21 tripartite motif-containing protein 21
WIF1 WNT inhibitory factor 1

References

1. Larsen SC, Sylvestersen KB, Lund A, et al. Proteome-wide analysis of arginine monomethylation reveals widespread occurrence in human cells. Sci Signal. 2016;9(443):ra9.
2. Ma XJ, Lu Q, Grunstein M. A search for proteins that interact genetically with histone H3 and H4 amino termini uncovers novel regulators of the Swe1 kinase in Saccharomyces cerevisiae. Genes Dev. 1996;10(11):1327–1340.
3. Lew DJ, Reed SI. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. J Cell Biol. 1995;129(3):739–749.
4. McMillan JN, Longtime MS, Sia RA, et al. The morphogenesis checkpoint in Saccharomyces cerevisiae: cell cycle control of Swe1p degradation by Hsl1p and Hsl7p. Mol Cell Biol. 1999;19(10):6929–6939.
5. Gilbreth M, Yang P, Wang D, et al. The highly conserved skb1 gene encodes a protein that interacts with Shk1, a fission yeast Ste20/PAK homolog. Proc Natl Acad Sci U S A. 1996;93(24):13802–13807.
6. Gilbreth M, Yang P, Barholomew G, et al. Negative regulation of mitosis in fission yeast by the shk1 interacting protein skb1 and its human homolog, Skb1Hs. Proc Natl Acad Sci U S A. 1998;95(17):14386–14876.
7. Yamada A, Duffy B, Perry JA, Kornbluth S. DNA replication checkpoint control of Wee1 stability by vertebrate Hsl7. J Cell Biol. 2004;167(5):841–849.
8. Pollack BP, Kotenkov SV, He W, Izotova LS, Barnoski BL, Pestka S. The human homologue of the yeast proteins Skb1 and Hsl7p interacts with Jak kinases and contains protein methyltransferase activity. J Biol Chem. 1999;274(44):31531–31542.
9. Rho J, Choi S, Seong YR, Cho WK, Kim SH, Im DS. Prmt5, which forms distinct homo-oligomers, is a member of the protein-arginine methyltransferase family. J Biol Chem. 2001;276(14):11393–11401.
10. Baldwin GS, Carnegie PR. Isolation and partial characterization of methylated arginines from the encephalitogenic basic protein of myelin. Biochem J. 1971;123(1):69–74.
11. Baldwin GS, Carnegie PR. Specific enzymic methylation of an arginine in the experimental allergic encephalomyelitis protein from human myelin. Science. 1971;171(3971):579–581.
12. Eylar EH, Brostoff S, Hashim G, Caccam J, Burnett P. Basic A1 protein of the myelin membrane. The complete amino acid sequence. J Biol Chem. 1971;246(18):5770–5784.
13. Branscombe TL, Frankel A, Lee JH, et al. PRMT5 (Janus kinase-binding protein 1) catalyzes the formation of
symmetric dimethylarginine residues in proteins. J Biol Chem. 2001;276(35):32971–32976.

14. Miranda TB, Sayegh J, Frankele A, Katz JE, Miranda M, Clarke S. Yeast Hs7 (histone synthetic lethal 7) catalyses the in vitro formation of omega-N-(G)-monomethylarginine in calf thymus histone H2A. Biochim J. 2006;395(3):563–570.

15. Friesen WJ, Wyce A, Paushkin S, et al. A novel WD repeat protein component of the methylosome binds Sm proteins. J Biol Chem. 2002;277(10):8243–8247.

16. Burgos ES, Wiliczek C, Onikubo T, et al. Histone H2A and H4 N-terminal tails are positioned by the MEG50 WD repeat protein for efficient methylation by the PRMT5 arginine methyltransferase. J Biol Chem. 2015;290(15):9674–9689.

17. Friesen WJ, Paushkin S, Wyce A, et al. The methylosome, a 205 complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. Mol Cell Biol. 2001;21(24):8289–8300.

18. Prusty AB, Meduri R, Prusty BK, Vanselow J, Schlosser A, Fischer U. Impaired spliceosomal UsnRNP assembly leads to dimethylarginine-modified Sm proteins. Proc Natl Acad Sci U S A. 2011;109(44):17960–17965.

19. Antonysamy S, Bonday Z, Campbell RM, et al. Crystal structure of the human PRMT5:MEP50 complex. Proc Natl Acad Sci Unit States Am. 2012;109(21):8258–8263.

20. Liu F, Cheng G, Hamard P-J, et al. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine symmetric dimethylation by PRMT5. J Biol Chem. 2011;286(3):1976–1986.

21. Liu F, Cheng G, Hamard P-J, et al. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine symmetric dimethylation by PRMT5. Mol Cell Biol. 2011;31(2):283–294.

22. Sun L, Wang M, Lv Z, et al. Structural insights into protein arginine symmetric dimethylation by PRMT5. Mol Cell Biol. 2013;33(12):4603–4616.

23. Wang M, Fuhrmann J, Thompson PR. Protein arginine methyltransferase 5 catalyzes substrate dimethylation in a distributive fashion. Biochemistry (Mosc). 2014;53(50):7884–7892.

24. Fabbrizio E, Messaoudi SE, Polanowska J, et al. Negative regulation of transcription by the type II arginine methyltransferase PRMT5. EMBO Rep. 2002;3(7):641–645.

25. Pal S, Vishwanath SN, Ejdjumet-Bromage H, Tempst P, Sif S. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol. 2004;24(21):9630–9645.

26. Kwak YT, Guo J, Prajapati S, et al. Methylation of SPT5 regulates its interaction with RNA polymerase II and transcriptional elongation properties. Mol Cell. 2003;11(4):1055–1066.

27. Pal S, Yun R, Datta A, et al. mSin3A/Histone deacetylase 2- and PRMT5-containing Brg1 complex is involved in transcriptional repression of the myc target gene cad. Mol Cell Biol. 2003;23(21):7475–7487.

28. Tae S, Karkhanis V, Velasco K, et al. Bromodomain protein 7 interacts with PRMT5 and PRC2, and is involved in transcriptional repression of their target genes. Nucleic Acids Res. 2011;39(13):5424–5438.

29. Zhao Q, Rank G, Tan YT, et al. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. Nat Struct Mol Biol. 2009;16(3):304–311.

30. Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007;129(4):823–837.

31. Girardot M, Hirasawa R, Kacem S, et al. PRMT5-mediated histone H4 arginine-3 symmetrical dimethylation marks chromatin at G + C-rich regions of the mouse genome. Nucleic Acids Res. 2014;42(1):235–248.

32. Migliori V, Müller J, Phalke S, et al. Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Nat Struct Mol Biol. 2012;19(2):136–144.

33. Yuan C-C, Matthews AGW, Jin Y, et al. Histone H3R2 symmetric dimethylation and histone H3K4 trimethylation are tightly correlated in eukaryotic genomes. Cell Rep. 2012;1(2):83–90.

34. Gkountela S, Li Z, Chin CJ, Lee SA, Clark AT. PRMT5 is required for human embryonic stem cell proliferation but not pluripotency. Stem Cell Rev Rep. 2014;10(2):230–239.

35. Liu C-D, Cheng C-P, Fang J-S, et al. Regulation of the EBNA1 transduction amplitude and cell fate through CRAF. Nat Cell Biol. 2013;15(2):174–181.

36. Hsu J-M, Chen C-T, Chou C-K, et al. Crosstalk between Arg 1175 methylation and Tyr 1173 phosphorylation negatively modulates EGFR-mediated ERK activation. Nat Cell Biol. 2011;13(2):1–18.

37. Wei H, Wang B, Miyagi M, et al. PRMT5 dimethylates R30 of the p65 subunit to activate NF-kB. Proc Natl Acad Sci U S A. 2011;108(33):13516–13521.

38. di Caprio R, Ciano M, Montano G, Costanzo P, Cesaro E. KAP1 is a novel substrate for the arginine methyltransferase PRMT5. Biology. 2015;4(1):41–49.

39. Liu C-D, Cheng C-P, Fang J-S, et al. Modulation of Epstein–Barr virus nuclear antigen 2-dependent transcription by protein arginine methyltransferase 5. Biochem Biophys Res Commun. 2013;430(3):1097–1102.

40. Shir K, Kapoor P, Jiang K, et al. Regulation of the EBNA1 Epstein-Barr virus protein by serine phosphorylation and arginine methylation. J Virol. 2006;80(11):5261–5272.

41. Hu D, Gur M, Zhou Z, et al. Interplay between arginine methylation and ubiquitylation regulates KLF4-mediated genome stability and carcinogenesis. Nat Commun. 2015;6:8419.

42. Lu X, Fernando TM, Lossos C, et al. PRMT5 interacts with the BCL6 oncoprotein and is required for germinal center formation and lymphoma cell survival. Blood. 2013;122(13):2026–2039.

43. Jansson M, Durant ST, Cho E-C, et al. Arginine methylation regulates the p53 response. Nat Cell Biol. 2008;10(12):1431–1439.

44. Andreu-Pérez P, Esteve-Puig R, de Torre-Minguela C, et al. Protein arginine methyltransferase 5 regulates ERK1/2 signal transduction amplitude and cell fate through CRAF. Sci Signal. 2011;4(190):ra58.

45. Cho E-C, Zheng S, Munro S, et al. Arginine methylation controls growth regulation by EZF1. EMBO J. 2012;31(7):1785–1797.

46. Zheng S, Moehlenbrink J, Lu Y-C, et al. Arginine methylation-dependent reader-writer interplay governs growth control by EZF1. Mol Cell. 2013;52(1):37–51.

47. Mounir Z, Korn JM, Westerling T, et al. ERG signaling in prostate cancer is driven through PRMT5-dependent methylation of the Androgen Receptor. eLife. 2016:5.

48. Tan CP, Nakielny S. Control of the DNA methylation system component MBD2 by protein arginine methylation. Mol Cell Biol. 2006;26(19):7224–7235.

49. Guo Z, Zheng L, Xu H, et al. Methylation of FEN1 suppresses nearby phosphorylation and facilitates PCNA binding. Nat Chem Biol. 2010;6(10):766–773.
51. He W, Ma X, Yang X, Zhao Y, Qiu J, Hang H. A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage. *Nucleic Acids Res.* 2011; 39(11):4719–4727.

52. Clarke TL, Sanchez-Bailon MP, Chiang K, et al. PRMT5-Dependent methylation of the Tip60 coactivator RUVBL1 is a key regulator of homologous recombination. *Mol Cell.* 2017; 65(5):900–916, e7.

53. Amante S, Napolitano G, Licciardo P, et al. Identification of proteins interacting with the RNApol FCP1 phosphatase: FCP1 forms a complex with arginine methyltransferase PRMT5 and it is a substrate for PRMT5-mediated methylation. *FEBS Lett.* 2005;579(3):683–689.

54. Zhao DY, Gish G, Braunschweig U, et al. SMN and symmetric arginine dimethylation of RNA polymerase II C-terminal domain control termination. *Nature.* 2016;529(7584):48–53.

55. Massenet S, Pellizzoni L, Paushkin S, Mattaj IW, Dreyfuss G. The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. *Mol Cell Biol.* 2002;22(18):6533–6541.

56. Neuenkirchen N, Chari A, Fischer U. Deciphering the assembly pathway of Sm-class U snRNPs. *FEBS Lett.* 2008;582(14):2997–3002.

57. Pesiridis GS, Diamond E, Van Duyne GD. Role of pICln in methylation of Sm proteins by PRMT5. *J Biol Chem.* 2009; 284(32):21347–21359.

58. Koh CM, Bezzi M, Low DHP, et al. MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphogenesis. *Nature.* 2015;523(7583):96–100.

59. Gerhart SV, Kellner WA, Thompson C, et al. Activation of the p53-MDM4 regulatory axis defines the anti-tumour response to PRMT5 inhibition through its role in regulating cellular splicing. *Sci Rep.* 2018;8(1):9711.

60. Tan DQ, Li Y, Yang C, et al. PRMT5 modulates splicing for the liver. *Proc Natl Acad Sci.* 2007;104(22):9158–9163.

61. Kanamaluru D, Xiao Z, Fang S, et al. Arginine methylation by PRMT5 at a naturally occurring mutation site is critical for liver metabolic regulation by small heterodimer partner. *Mol Cell Biol.* 2011;31(7):1540–1550.

62. Tsai W-W, Niessen S, Goebel N, Wu Z, Tan DQ, Li Y, Yang C, et al. PRMT5 modulates splicing for the liver. *Proc Natl Acad Sci.* 2013;110(22):8870–8875.

63. Yang H, Zhao X, Zhao L, et al. PRMT5 competitively binds to CDK4 to promote G1-S transition upon glucose induction in hepatocellular carcinoma. *OncoTarget.* 2016;7(44):72131–72147.

64. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest.* 2002;109(9):1125–1131.

65. Liu L, Zhao X, Zhao L, et al. Arginine methylation of SREBP1a via PRMT5 promotes de novo lipogenesis and tumor growth. *Cancer Res.* 2016;75(6):1260–1272.

66. Huang L, Liu J, Zhang X-Q, et al. Inhibition of protein arginine methyltransferase 5 enhances hepatic mitochondrial biogenesis. *J Biol Chem.* 2018;293(28):10884–10894.

67. Tee W-W, Pardo M, Theunissen TW, et al. Prmt5 is essential for early mouse development and acts in the cytoplasm to maintain ES cell pluripotency. *Genes Dev.* 2010;24(24):2772–2777.

68. Litzler LC, Zahn A, Meli AP, et al. PRMT5 is essential for B cell development and germinal center dynamics. *Nat Commun.* 2019;10(1):22.

69. Mei M, Zhao X, Zhou J-W, et al. PRMT5-mediated H4R3me2 confers cell differentiation in pediatric B-cell precursor acute lymphoblastic leukemia. *Clin Cancer Res Off J Am Assoc Cancer Res.* 2019;25(8):2633–2643.

70. Tarighat SS, Santanham R, Frankhouser D, et al. The dual epigenetic role of PRMT5 in acute myeloid leukemia: gene activation and repression via histone arginine methylation. *Leukemia.* 2016;30(4):789.

71. Panfil AR, Al-Saleem J, Howard CM, et al. PRMT5 is upregulated in HTLV-1-mediated T-cell transformation and selective inhibition alters viral gene expression and infected cell survival. *Viruses.* 2015;8(11).

72. Jin Y, Zhou J, Xu F, et al. Targeting methyltransferase PRMT5 eliminates leukemia stem cells in chronic myelogenous leukemia. *J Clin Investig.* 2016;126(10):3961–3980.

73. Chan-Pereyre E, Kuplast KG, Majer CR, et al. A selective inhibitor of PRMT5 with in vivo and in vitro potency in MCL models. *Nat Chem Biol.* 2015;11(6):432–437.

74. Chung J, Karkhanis V, Tae S, et al. Protein arginine methyltransferase 5 (PRMT5) inhibition induces lymphoma cell death through reactivation of the retinoblastoma tumor suppressor pathway and polycomb repressor complex 2 (PRC2) silencing. *J Biol Chem.* 2013;288(49):35534–35547.

75. Pal S, Baiocchi RA, Byrd JC, Grever MR, Jacob ST, Sif S. Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma. *EMBO J.* 2017;36(15):3558–3569.

76. Chung J, Karkhanis V, Baiocchi RA, Sif S. Protein arginine methyltransferase 5 (PRMT5) promotes survival of lymphoma cells via activation of WNT/β-CATENIN and AKT/GSK3β proliferative signaling. *J Biol Chem.* 2019; 249(14):7692–7710.

77. Li Y, Chitnis N, Nakagawa H, et al. PRMT5 is required for lymphogenesis triggered by multiple oncogenic drivers. *Cancer Discov.* 2015;5(3):288–303.

78. Alniri L, Mahesanen KV, Yan F, et al. Selective inhibition of protein arginine methyltransferase 5 blocks initiation and maintenance of B-cell transformation. *Blood.* 2015;125(16):2530–2543.

79. Zhu F, Guo H, Bates PD, et al. PRMT5 is Upregulated by B-Cell Receptor Signaling and Forms a Positive-Feedback Loop with PI3K/AKT in Lymphoma Cells. *Leukemia.* 2019;23(4):996–1002.

80. Gullà A, Hideshima T, Bianchi G, et al. Protein arginine methyltransferase 5 has prognostic relevance and is a drug-gable target in multiple myeloma. *Leukemia.* 2018;32(4):996–1002.

81. Kryukov GV, Wilson FH, Ruth JR, et al. MTAP deletion confers cellular sensitivity to DNA damage. *Science.* 2016;351(6278):1214–1218.

82. Mavrakis KJ, McDonald ER, Schlabach MR, et al. Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. *Science.* 2016;351(6278):1208–1213.

83. Duncan KW, Rioux N, Boriack-Sjodin PA, et al. Structure and function guided design in the identification of PRMT5 tool compound EPZ015666. *ACs Med Chem Lett.* 2016;7(2):162–166.
86. Lee J-H, Cook JR, Yang Z-H, et al. PRMT7, a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. *J Biol Chem*. 2005;280(5):3656–3664.

87. Zurita-Lopez CI, Sandberg T, Kelly R, Clarke SG. Human protein arginine methyltransferase 7 (PRMT7): a type III enzyme forming ω-NG-monomethylated arginine residues. *J Biol Chem*. 2012;287(11):7859–7870.

88. Feng Y, Hadjikyriacou A, Clarke SG. Substrate specificity of human protein arginine methyltransferase 7 (PRMT7): the importance of acidic residues in the double E loop. *J Biol Chem*. 2014;289(47):32604–32616.

89. Chanderkar LP, Paik WK, Kim S. Studies on myelin-basic-protein methylation during mouse brain development. *Biochem J*. 1986;240(2):471–479.

90. Crang AJ, Jacobson W. The relationship of myelin basic protein (arginine) methyltransferase to myelination in mouse spinal cord. *J Neurochem*. 1982;39(1):244–247.

91. DesJardins KC, Morell P. Phosphate groups modifying myelin basic proteins are metabolically labile; methyl groups are stable. *J Cell Biol*. 1983;97(2):438–446.

92. Ghosh SK, Rawal N, Syed SK, Paik WK, Kim SD. Enzymic methylation of myelin basic protein in myelin. *Biochem J*. 1991;275(Pt 2):381–387.

93. Ancelin K, Lange UC, Hajkova P, et al. Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat Cell Biol*. 2006;8(6):623–630.

94. Park JH, Szemes M, Vieira GC, et al. Protein arginine methyltransferase 5 is a key regulator of the MYCN oncprotein in neuroblastoma cells. *Mol Oncol*. 2015;9(3):617–627.

95. Martin G, Ostareck-Lederer A, Chari A, et al. Arginine methylation in subunits of mammalian pre-mRNA cleavage factor I. *RNA*. 2010;16(8):1646–1659.

96. Bruns A-F, Grothe C, Claus P. Fibroblast growth factor 2 (FGF-2) is a novel substrate for arginine methylation by PRMT5. *Biol Chem*. 2009;390(1):59–65.

97. Ren J, Wang Y, Liang Y, Zhang Y, Bao S, Xu Z. Methylation of ribosomal protein S10 by protein-arginine methyltransferase 5 regulates ribosome biogenesis. *J Biol Chem*. 2010;285(17):12695–12705.