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Versatility of PRMT5-induced methylation in growth control and development

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Abstract

Arginine methylation governs important cellular processes that impact growth and proliferation, as well as differentiation and development. Through their ability to catalyze symmetric or asymmetric methylation of histones and non-histone proteins, members of the protein arginine methyltransferase (PRMT) family regulate chromatin structure and expression of a wide spectrum of target genes. Unlike other PRMTs, PRMT5 works in concert with a variety of cellular proteins including ATP-dependent chromatin remodelers and co-repressors to induce epigenetic silencing. Recent work also implicates PRMT5 in the control of growth-promoting and pro-survival pathways, demonstrating its versatility as an enzyme involved in both epigenetic regulation of anti-cancer target genes and organelle biogenesis. These studies not only provide insight into the molecular mechanisms by which PRMT5 contributes to growth control, but also justify targeting PRMT5 therapeutically.

PRMT5-mediated arginine methylation extends beyond epigenetics

Modulation of chromatin structure involves many chromatin-remodeling factors, including enzymes that can post-translationally modify histones, and it is well established that histone modification represents a fundamental step in regulating DNA accessibility during various cellular processes such as transcription, replication and DNA repair [1, 2]. Conversion of chromatin from a repressed form to a more open and active state and *vice versa* relies on the coordinated interplay between histone-modifying enzymes, ATP-dependent chromatin remodelers, and DNA-modifying enzymes. The importance of chromatin modification by these enzymes is reflected by their critical roles in control of gene expression, cell proliferation and organismal development.

Histones undergo a variety of post-translational modifications in their globular domains and N-terminal tails, and among the known histone modifications, lysine acetylation and methylation have been the most studied [3]. A distinct group of enzymes termed protein arginine methyltransferases (PRMTs) specializes in arginine methylation of both histones and other cellular proteins. PRMT family members play a pivotal role in the regulation of diverse cellular processes that range from transcription and RNA processing to signaling, differentiation, apoptosis and tumorigenesis [4, 5]. PRMT enzymes are classified into two

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subgroups depending on the type of modification they catalyze. Type I PRMT enzymes (PRMT1-4, PRMT6 and PRMT8) generate ω -N^G-monomethyl and ω -N^G, N^G-asymmetric dimethyl arginines, whereas type II PRMT enzymes (PRMT5, PRMT7 and PRMT9) catalyze the formation of ω -N^G-monomethyl and ω -N^G, N^G-symmetric dimethyl arginine residues [6]. Previous reviews have summarized what is known about most PRMTs [4, 5]; however, a significant amount of work has recently been published describing the role of PRMT5 in a variety of cellular processes including ribosome biogenesis [7], assembly of the Golgi apparatus [8], cellular differentiation [9-11] and germ cell specification [12, 13]. The relevance of PRMT5 in cancer biology has also become evident, as more studies have shown that PRMT5 is involved in the regulation of major signaling pathways that impact cell death and malignant transformation [14-24]. In this review, we will highlight some of the recent work that further characterizes the biological function of PRMT5 and that implicates it in the control of cell proliferation and development.

PRMT5 modulates cell growth and transformation

PRMT5 is a type II methyltransferase that is known to be associated with multiple protein complexes including human SWI/SNF chromatin remodelers (Figure 1a) [25]. As part of the SWI/SNF complex, PRMT5 hypermethylates promoter histones H3R8 and H4R3, and triggers transcriptional silencing of cell cycle regulatory and tumor suppressor genes (Figure 1b) [25-27]. Knockdown of PRMT5 expression is associated with slow growth, whereas PRMT5 overexpression causes cellular hyperproliferation. PRMT5 levels are elevated in a variety of transformed cells, and it appears that this is a direct result of increased translation [26, 27]. MicroRNA (miRNA) profiling in chronic lymphocytic leukemia, mantle cell lymphoma cell lines and clinical samples indicates that aberrant PRMT5 expression inversely correlates with the levels of PRMT5-specific miRNAs. Overexpression of any of these miRNAs restored PRMT5 to its basal levels, supporting the notion that they are directly involved in translational regulation of PRMT5. Elevated expression of PRMT5 in transformed B cells correlates with hypermethylation of H3R8 and H4R3 [26, 27], and global gene profiling combined with chromatin immunoprecipitation (ChIP) studies revealed that SWI/SNF-associated PRMT5 negatively controls transcription of growth inhibitory genes [26, 27]. The fact that PRMT5 is overexpressed in cancer cells and that it epigenetically silences specific tumor suppressors shows that PRMT5 controls the growth of transformed B cells, and suggests that therapeutically targeting PRMT5 might help restore expression of anti-cancer genes and inhibit growth of cancerous cells.

PRMT5 also participates in the transcriptional silencing of the cullins, CUL4A and CUL4B, which encode scaffolding proteins for the E3 ligase that targets chromatin licensing and DNA replication factor 1 (CDT1) for degradation during S phase. Recent work demonstrated that PRMT5 mediates the ability of mutant cyclin D1–cyclin-dependent kinase 4 (CDK4) to repress CUL4A/B transcription and thereby stabilize CDT1 (Figure 1a and b) [16]. As cells traverse the G1 to S phase, cyclin D1 becomes phosphorylated on T286 and is exported to the cytoplasm, where it undergoes ubiquitin-dependent proteolysis [15]; substitutions to T286 render it resistant to phosphorylation and subsequent degradation. The consequence is increased cyclin D1–CDK4 activity, enhanced CUL4A/B repression, and stabilization of CDT1, which leads to DNA re-replication and transformation [16]. These conclusions were supported by affinity purification of flag-tagged cyclin D1 T286A, which associated with PRMT5, its binding partner, methylome protein 50 (MEP50), and the BRG1 SWI/SNF ATPase during the G1 to S transition. Blocking PRMT5/MEP50 methyltransferase activity by knocking down *PRMT5* expression or by using global PRMT inhibitors resulted in *CUL4A/B* transcriptional derepression and CDT1 degradation during S phase, indicating that PRMT5 is involved in the epigenetic silencing of key target genes that regulate levels of the replication-licensing factor CDT1 [16].

Regulation of cell cycle control genes can also be mediated by association of PRMT5 with other ATP-dependent chromatin remodelers. Affinity purification of NuRD remodeling enzymes showed that NuRD complexes containing the methyl-CpG binding domain protein 2 (MBD2) subunit also contain PRMT5 and its binding partner MEP50 (Figure 1a) [28]. The presence of PRMT5/MEP50 in MBD2-NuRD requires the MBD2 N-terminal region, which contains 14 RG repeats and is methylated by PRMT5. ChIP studies showed that PRMT5 co-localized with MBD2 and other NuRD subunits on the promoters of several target genes, including *CDKN2A* (which encodes p14^{ARF} and p16^{INK4a}) (Figure 1b). Furthermore, enrichment of PRMT5-MBD2-NuRD depends on DNA methylation, because treatment with the hypomethylating agent 5-aza-2'-deoxycytidine (5-azadC) reduced its association with target promoters [28]. In a separate study, the PRMT5-NuRD interaction was confirmed, and PRMT5 knockdown had a positive effect on the interaction between MBD2 and histone deacetylase (HDAC) complexes. Moreover, biochemical characterization of PRMT5-methylated MBD2-NuRD indicated that it had lower affinity for methylated DNA. These studies suggest that methylation of MBD2 has an inhibitory effect on its interaction with HDAC repressor complexes and alters its binding properties to the methylated DNA, thereby triggering derepression of MBD2-NuRD target genes [29].

A more recent report showed that the proto-oncogene *SKI*, which encodes a nuclear protein that function through interaction with SMAD2/3/4, N-CoR/SMRT, and HDAC3, also interacts with PRMT5 (Figure 1a). Biochemical characterization of the SKI complex revealed that it can deacetylate and methylate histones *in vitro*, and recruitment studies demonstrated that the SKI complex binds the promoter region of the growth inhibitor target gene, *SMAD7* (Figure 1b). Moreover, siRNA-mediated knockdown studies showed that PRMT5, HDAC3 and SKI mediate transcriptional repression of *SMAD7* in the absence of transforming growth factor (TGF)- β . These studies demonstrate that PRMT5 can act in concert with other histone-modifying enzymes to regulate expression of target genes that control cell growth and proliferation [17].

Among non-histone proteins targeted by PRMT5 is the p53 tumor suppressor protein, which is involved in the regulation of cell proliferation, cell cycle progression and cell death [18]. Under normal conditions, p53 protein expression is maintained at low levels via E3 ubiquitin ligase MDM2-mediated proteolysis. Upon DNA damage, p53 becomes activated, dissociates from MDM2, and its levels increase due to enhanced protein stability [30]. Numerous post-translational modifications to p53 that either enhance or inhibit its tumor suppressor activity have also been reported [31-33]. In particular, PRMT5 interacts with and methylates p53 at R333, R335 and R337 upon DNA damage (Figure 1a). The consequence is altered recruitment to target genes in a promoter-specific manner and inhibition of p53 oligomerization (Figure 1b) [19]. Although their occurrence is rare, mutations that alter p53 R333 and R337 have been detected; indeed, substitution of p53 R337 to either a histidine or cysteine is known to destabilize p53 and is associated with human cancer. Moreover, p53 R337C substitution occurs in individuals with Li Fraumeni syndrome, a disorder known to increase the risk of developing cancer [34-37]. Additional alterations to this residue have also been found in other tumors, highlighting the importance of arginine methylation in the control of p53-mediated events [38].

A more recent study in *Caenorhabditis elegans* showed that PRMT-5 negatively regulates DNA damage-induced apoptosis as evidenced by the increase of germ cell death upon gamma-ray irradiation in worms lacking PRMT-5 [21]. When PRMT-5 was reintroduced, there was a clear decrease in germ cell apoptosis, indicating that PRMT-5 antagonizes DNA-damage induced apoptosis in *C. elegans*. Genetic inactivation of components of the core cell death pathway indicated that the mechanism by which PRMT-5 inhibits DNA damage-induced apoptosis is through transcriptional suppression of the CEP-1 (the p53

ortholog) target gene *egl-1*, an early driver of cell death (Figure 1b). Association of PRMT-5 with CEP-1 and the co-activator CBP-1 resulted in CBP-1 R234 methylation, a site also conserved in human p300 and CBP. Methylation of CBP-1 inhibited its ability to acetylate CEP-1/p53 and probably histones, thus causing transcriptional repression of *egl-1* and promoting germ cell survival [21]. Collectively, these findings demonstrate that PRMT-5 can target p53-regulated pathways by directly modifying p53 and altering its biochemical properties or by post-translationally modifying its associated co-activators and affecting their catalytic activity.

Further support for a role of PRMT5 in cell cycle control and p53 tumor suppressor function came from work demonstrating that reducing expression of PRMT5 inhibits MCF-7 breast cancer cell proliferation and the transition from G1 to S. Treatment of MCF-7 cells with doxorubicin enhanced the stability of p53 and increased expression of p53 target genes including *MDM2* and *CDKN1A* (which encodes p21). However, PRMT5 knockdown resulted in decreased p53 stability and target gene expression, and ChIP assays indicated that decreased expression of *CDKN1A* was due to reduced p53 recruitment to its promoter, indicating that PRMT5 is required for multiple aspects of p53 function in response to genotoxic stress [18].

The concept of functional interactions between PRMT5 and known tumor suppressors is also demonstrated by work showing that programmed cell death (PDCD) 4, a prognostic indicator for several tumor types [39] and a regulator of translation [40], associates with and is methylated by PRMT5. In an orthotopic breast tumor model, overexpression of both PRMT5 and PDCD4 enhanced tumor growth in a manner dependent upon both PRMT5 catalytic activity and the PDCD4 residue targeted by PRMT5. Examination of patient samples showed a correlation between elevated *PDCD4* and *PRMT5* expression and poor outcome [41], reflecting the relevance of PRMT5 level and function in patient survival.

Another important pathway regulated by PRMT5 involves the SNAIL transcription factor, which is involved in the regulation of embryonic development and metastasis. SNAIL regulates epithelial to mesenchymal transition by altering cell adhesion through transcriptional repression of E-cadherin (*CDH1*). Several transcriptional co-repressors interact with SNAIL including AJUBA, which can bridge multiple interactions with several chromatin-modifying enzymes. Recent work showed that AJUBA forms a complex with PRMT5 and recruits it to SNAIL-repressed *CDH1* (Figure 1a and b) [20]. Knockdown of either AJUBA or PRMT5 abrogated their association with the *CDH1* promoter and resulted in transcriptional derepression of *CDH1*. These studies broaden our view of the impact that PRMT5 has on cell growth and tumorigenesis by indicating a role for PRMT5 in cell adhesion.

Association of PRMT5 with various binding partners appears to influence its substrate specificity. A yeast two-hybrid assay identified a nuclear protein dubbed cooperator of PRMT5 (COPR5) as a PRMT5 interacting partner (Figure 1a). COPR5-associated PRMT5 preferentially methylates histone H4R3 *in vitro*; however, when PRMT5 is purified from cells in which COPR5 is knocked down, methylation of histone H3 is more prevalent, suggesting that the COPR5–PRMT5 complex methylates H4 more efficiently than H3. *In vivo* both COPR5 and PRMT5 are enriched at the cyclin E (*CCNE*) promoter where the PRMT5-induced epigenetic mark H4R3 is hypermethylated; however, COPR5 knockdown leads to transcriptional derepression of *CCNE* (Figure 1b) [42]. These experiments show that COPR5 is required for PRMT5 recruitment to the *CCNE* promoter, and indicate that COPR5 directs PRMT5 methyltransferase activity toward H4R3.

PRMT5 functions as a co-repressor as well as a co-activator during development and differentiation

A role for PRMT5 during embryonic development was recently demonstrated through studies that showed that *Prmt5*-null mice are early embryonic lethal between E3.5 to E6.5 [43]. In addition, the failure to derive embryonic stem (ES) cells from *Prmt5*-null blastocysts and the fact that pluripotency genes were down-regulated as differentiation genes became up-regulated in PRMT5 knockdown ES cells strongly suggests that PRMT5 is required for stem cell pluripotency. Biochemical analysis indicated that PRMT5 methylated histone H2A prior to its incorporation into chromatin, an event that correlated with repression of differentiation genes. Furthermore, the physical interaction between PRMT5 and signal transducer and activator of transcription (STAT)3 suggested that PRMT5 might also repress differentiation genes in ES cells through the leukemia inhibitory factor (LIF)-STAT3 signaling pathway.

PRMT5 has also been implicated in epigenetic regulation during mouse germ cell specification by directly interacting with the transcriptional repressor B-lymphocyte-induced maturation protein (BLIMP)1 (Figure 1a) [12]. Translocation of the BLIMP1-PRMT5 complex from the nucleus to the cytoplasm during embryogenesis results in H2AR3/H4R3 hypomethylation and derepression of genes involved in germ cell specification (Figure 1b). Moreover, BLIMP1 expression in embryonic P19 carcinoma cells results in repression of germ cell specification genes and hypermethylation of histones H2AR3 and H4R3 at these loci. The role of BLIMP1, PRMT5 and H2A/H4R3 dimethylation in human fetal germ cell development has also been demonstrated, highlighting the relevance of this mechanism in maintenance of the undifferentiated and pluripotent state [12, 13, 43]. Studies in *Drosophila* also reveal that PRMT5 is crucial for germ cell formation. Loss of *dart5* (also called *Capsuleen*), the fly ortholog of *PRMT5*, causes a defect in spermatocyte maturation and infertility. By contrast, female *dart5* mutants are fertile; however, their embryos fail to form the pole cells that give rise to germ cells in adults [44]. The enzymatic activity of Dart5 is required for the methylation of spliceosomal proteins in oocytes and controls the localization of proteins in the pole plasm that are essential for germ cell formation [45]. These findings suggest that PRMT5 might regulate germ cell formation by epigenetic and nonepigenetic mechanisms.

PRMT5 can also promote somatic cell reprogramming [46], consistent with the findings from studies of early development, ES cells and germ cells. Knocking down PRMT5 reduced the reprogramming efficiency with classical Yamanaka factors (OCT3/4, SOX2, KLF4, MYC); however, the mechanism remains undetermined. Intriguingly, a type I PRMT inhibitor that enabled OCT4-induced mouse embryonic fibroblast (MEF) reprogramming in combination with a TGF- β inhibitor was recently identified [47], suggesting that type I PRMTs might negatively regulate the reprogramming process. Thus, it seems that PRMT5 and one or more type I PRMTs might have opposing functions in somatic cell reprogramming.

Tissue-specific gene expression is also regulated by PRMT5. Using the human β -globin locus as a model, a link has been established between PRMT5-mediated histone methylation, DNA methylation, and gene repression. Expression of globin genes undergoes developmental stage-specific regulation during which expression of fetal specific γ -globin gene precedes that of the adult specific β -globin gene. In adult bone marrow erythroid cells, transcriptional silencing of the genes encoding fetal (γ) globin is mediated by the transcriptional factor NF-E4, which can interact with PRMT5 (Figure 1a) [48]. Recruitment of PRMT5 results in H4R3 methylation, which in turn serves as a direct binding site for DNA methyltransferase DNMT3A, and subsequent DNA methylation of CpG dinucleotides

flanking the γ -globin gene transcriptional start site (Figure 1b) [49]. PRMT5 binding and enzymatic activity are crucial for the recruitment of a repressive multisubunit complex containing DNMT3A, HDAC1, SUV4-20h1, and casein kinase (CK)2 α to the γ -globin promoter. Remarkably, PRMT5 also influenced other epigenetic marks including H4S1ph, H4K20me3, and to some extent H3K9me3 and H3K27me3, which were reduced in cells in which PRMT5 function was abrogated [49, 50]. These experiments show that recruitment of PRMT5 is crucial for coordinated binding of repressive chromatin-modifying enzymes to the developmentally regulated γ -globin gene.

In contrast to the roles of PRMT5 in developmental programs discussed thus far, analysis of skeletal muscle differentiation indicates that PRMT5 acts as a transcriptional co-activator. Knockdown of PRMT5 inhibited myogenesis in culture, and ChIP analysis indicated that PRMT5 binding and PRMT5-mediated H3R8 methylation inducibly occurred at myogenic promoters shortly after the onset of differentiation [9, 10]. Analysis of the myogenin promoter revealed that the absence of PRMT5 prevented binding and chromatin remodeling by the SWI/SNF enzyme, an obligatory step for myogenin expression, indicating that the co-regulatory function of PRMT5 and SWI/SNF is context-dependent. Interestingly, this work indicated a temporal requirement for PRMT5 in differentiation, given that gene activation events subsequent to myogenin expression did not directly require PRMT5. Instead, a type I PRMT, PRMT4, was required for these gene activation events [10, 11]. Unlike the case in somatic cell reprogramming, where the different PRMTs might work in opposition, these data indicate apparently independent roles (PRMT5 is required for early myogenic gene activation, whereas PRMT4 is not. By contrast, PRMT5 is not required for late myogenic gene expression while PRMT4 is) for PRMT4 and PRMT5 in the same biological process.

PRMT5 methylates multiple targets in the cytoplasm, modulating growth-promoting and cell death-inducing pathways

The cellular localization of PRMT5 differs between non-transformed and transformed cells. In most primary and immortalized cells, PRMT5 is primarily located in the cytosol with a small amount in the nucleus; however, this distribution is reversed in transformed cells [25, 27]. Therefore, it appears that PRMT5 localization and the substrates it targets in each cellular compartment might play a critical role in the control of cell growth and proliferation. Epidermal growth factor receptor (EGFR) is a transmembrane receptor that undergoes several post-translational modifications that influence its biological activity. Activation of EGFR induces its kinase activity and autophosphorylation, which in turn initiates downstream signaling events associated with cellular proliferation and tumorigenesis. EGFR interacts and co-localizes with PRMT5 (Figure 2a), and PRMT5 knockdown reduces monomethylation of EGFR R1175, suggesting that PRMT5 is directly involved in EGFR methylation. Furthermore, comparison of breast cancer cell lines expressing either wild type or mutant EGFR/R1175K indicated that EGFR methylation suppressed its biological function as demonstrated by increased growth, efficient tumor formation and greater migration and invasion capabilities of cells that express mutant EGFR (Figure 2b). Methylation of R1175 down-regulates EGFR function by promoting phosphorylation of Tyr1173, a binding site for SHP1, a protein tyrosine phosphatase that inhibits ERK signaling. Thus, it is conceivable that PRMT5-mediated EGFR methylation leads to reduced ERK signaling, whereas demethylation or substitution of R1175 could have the opposite effect. It is also possible that in transformed cells, relocalization of PRMT5 from the cytosol to the nucleus could result in reduced EGFR methylation and potentiate ERK signaling, which in turn could enhance the growth of cancer cells [22].

PRMT5 can also antagonize pro-apoptotic signaling pathways. The tumor necrosis factors (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily that

has anti-tumor activity and immense therapeutic potential [51]. Binding of TRAIL to death receptors (DR4 and DR5) assembles the death-inducing signaling complex (DISC), which induces apoptosis [52, 53]. Recent work showed that PRMT5 physically interacts with DR4 and DR5 (Figure 2a and b), and that knockdown of PRMT5 potentiates TRAIL-induced tumor cell death, indicating that PRMT5 inhibits the killing effect of TRAIL [24]. The molecular mechanism is independent of the PRMT5 methyltransferase activity and involves nuclear factor (NF)- κ B, because TRAIL-induced IKK activation and I κ B degradation were reduced, as was expression of NF- κ B target genes, in PRMT5 knockdown cells. PRMT5 knockdown sensitizes cancer, but not normal, cells to TRAIL-induced death, and PRMT5 overexpression endows a cancer cell line with increased resistance to TRAIL-induced death, suggesting that targeting PRMT5 in TRAIL-based therapies might increase the efficiency of tumor cell targeting [24].

Cross-talk between PRMT5 and signaling molecules has been demonstrated, and oncogenic mutations in PRMT5-interacting kinases have been implicated in tumorigenesis. The PRMT5–MEP50 complex can methylate members of the Janus kinase (JAK) family (Figure 2a). Although the physical interaction between PRMT5 and JAK2 was identified more than a decade ago [54], the functional significance of this interaction was only recently unraveled. JAK2 is a tyrosine kinase that binds to the cytoplasmic domain of hematopoietic cytokine receptors, which are activated by ligands such as erythropoietin and granulocyte colony stimulating factor, and transduces signaling by phosphorylating downstream targets (Figure 2b). Activation or oncogenic mutation impairs negative regulation of JAK2 and results in constitutive activation of its tyrosine kinase activity, which in turn enhances downstream signaling cascades and promotes cellular proliferation [55]. JAK2 mutations have been detected in the majority of patients with myeloproliferative neoplasms, a type of stem cell disorder characterized by enhanced proliferation of hematopoietic stem cells [56–58]. Interestingly, constitutively active JAK2 V617F and JAK2 K539L mutant kinases bound more avidly to and phosphorylated PRMT5 both *in vitro* as well as *in vivo*, and impaired its methyltransferase activity by disrupting its interaction with MEP50 and affecting global H2AR3 and H4R3 methylation [23]. By contrast, wild type JAK2, even when activated, did not phosphorylate PRMT5. These studies demonstrate that oncogenic JAK2 kinases promote myeloproliferative disease and erythroid differentiation, at least in part, by inactivating PRMT5, and suggest that in the context of hematopoietic cells, inhibition of PRMT5 activity might impinge on EGFR signaling, thereby inducing cell proliferation and tumorigenesis.

PRMT5–MEP50-mediated arginine methylation has been implicated in a variety of cytosolic processes, including biogenesis of Sm-class ribonucleoproteins. Sm proteins are components of the spliceosomal U1, U2, U4 and U5 small nuclear ribonucleoproteins (snRNPs). PRMT5, as well as PRMT7, another type II PRMT, methylate three of the seven Sm proteins and enhance their binding to the survival motor neuron (SMN) complex (Figure 2a and b) [59]. Interaction of methylated Sm proteins with the SMN complex facilitates their loading onto the Sm site of small nuclear RNA (snRNA), thereby giving rise to U-snRNPs. Knockdown of either PRMT5 or PRMT7 reduces symmetric methylation of Sm proteins and disrupts snRNP assembly. In a separate investigation designed to discover proteins affected by lack of methylation, two *Arabidopsis thaliana* glycine-rich RNA-binding proteins (AtGRP7 and 8), which are homologs of the human heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and hnRNP A2/B1 that are known to inhibit pre-mRNA splicing, were identified as PRMT5 substrates, thus providing confirmation for the role played by PRMT5 in RNA processing [60]. In addition to its association with MEP50, PRMT5 also forms a complex with either pICln (Chloride channel, nucleotide sensitive 1A), which recruits Sm proteins, or the Rio domain-containing protein kinase (RIOK)1, which competes with pICln for PRMT5 binding (Figure 2a) [61, 62]. Just as interaction of PRMT5

with COPR5 or BLIMP1 restricts its methyltransferase activity to specific histones, association of PRMT5 with either pICln or R1OK1 directs its catalytic activity toward Sm proteins or nucleolin, respectively. In addition to its involvement in regulating rDNA transcription and pre-ribosome packaging, nucleolin also functions as a shuttle protein that transports viral and cytoplasmic proteins between the cytoplasm and nucleus. The relevance of the PRMT5–nucleolin complex became apparent when the cancer cell growth inhibitor AS1411, a G-rich quadruplex forming oligonucleotide, was shown to alter the activity of PRMT5 in prostate cancer cells as demonstrated by transcriptional derepression of its target tumor suppressor genes. Furthermore, the interaction between PRMT5 and nucleolin was reduced in the nucleus and increased in the cytosol in cells treated with AS1411, indicating that AS1411 alters the localization of nucleolin-associated PRMT5 [63]. Thus, it will be important to test the efficacy of AS1411 in other cancer cell types known to overexpress PRMT5, and to determine if it has similar growth inhibitory effects that can be exploited clinically.

PRMT5-induced arginine methylation has been implicated in other cellular processes, including assembly of the Golgi apparatus, ribosome biogenesis, and RNA-mediated gene silencing (Figure 2a and b). In an attempt to better understand the cytoplasmic function of PRMT5, interacting partners were identified by biochemical purification [8]. In addition to MEP50, GM130, which is a Golgi matrix protein involved in reassembly of the Golgi complex after cell division, was identified as a PRMT5-associated protein and shown to co-localize with PRMT5 as well as other Golgi markers. PRMT5 methylated GM130 at R6, R18 and R23, and substitution of these sites to lysines affected Golgi apparatus ribbon formation. When PRMT5 was knocked down, the fraction of fragmented Golgi apparatus increased, suggesting that PRMT5 impacts fusion of Golgi membranes by methylating GM130 and influencing its association with other structural proteins of the Golgi apparatus [8]. PRMT5 has also been shown to interact with and methylate ribosomal protein S10 (RPS10), a member of the ribosome 40S subunit [7]. Methylation of RPS10 appears to be critical for ribosome assembly, protein synthesis and cell proliferation. Mutations that affect methylated arginine residues result in an unstable protein that is inefficiently incorporated into ribosomes. Moreover, RPS10 knockdown cells grow slower than control cells, and re-expression of wild type, but not mutant, RPS10 restores cell proliferation, thus emphasizing the importance of PRMT5-induced methylation in ribosome biogenesis and growth control.

PRMT5 methylates another group of small RNA-binding proteins called Piwi, which are exclusively present in germ cells and interact with a specific class of small non-coding RNAs called piwi-interacting RNAs (piRNAs). In mouse embryonic germ cells, members of the Piwi family repress transcription of transposable elements by inducing *de novo* methylation of their DNA sequences. Biochemical purification of Piwi interacting proteins led to the identification of several Tudor domain-containing proteins as well as PRMT5 and MEP50. Some of the Piwi proteins were shown to be PRMT5 substrates, and alterations to specific arginine residues within the N-terminal region of the Piwi protein, MILI, resulted in loss of interaction and colocalization with Tudor proteins, suggesting that PRMT5-induced methylation of Piwi proteins is critical for their recognition by the Tudor family of proteins and proper function of the piRNA pathway [64].

Concluding remarks

Biochemical and physiological characterization has demonstrated that PRMT5 is crucial for maintenance of normal cell growth, differentiation and development. Several reports have shown that PRMT5 overexpression is associated with hyperproliferation of cancer cells, and that inhibition of its activity leads to derepression of anti-cancer genes and slow growth. The function of PRMT5-induced methylation is manifested through a variety of cellular proteins

including cell surface receptors, signaling molecules, structural components of various organelles and chromatin binding proteins. Of particular interest is the association of PRMT5 with ATP-dependent chromatin remodeling enzymes. The outcome of these interactions appears to be different in that association of PRMT5 with SWI/SNF does not result in modification of its subunits or biochemical activity; however, interaction of PRMT5 with NuRD leads to MBD2 methylation and decreases its affinity for methylated DNA. By interacting with two distinct chromatin remodeling complexes, PRMT5 can have a broader effect on target gene regulation, and in the case of SWI/SNF, PRMT5 epigenetically modifies promoter histones at target tumor suppressor and cell cycle regulatory genes, hence causing their transcriptional silencing. Association of PRMT5 with NuRD has also been shown to have a silencing effect on a *CDKN2A*; however, biochemical characterization of methylated NuRD indicates that the complex does not bind methylated DNA very efficiently, raising the possibility that *in vivo* certain silenced target genes might become transcriptionally derepressed. Direct involvement of PRMT5 in epigenetic silencing of E-cadherin provides additional evidence that altered expression of PRMT5 impacts cancer cell proliferation and metastasis. Therefore, these studies clearly demonstrate that the association of PRMT5 with ATP-dependent remodeling complexes promotes cell survival.

PRMT5 plays additional, essential roles in growth control via participation in growth and death-inducing signals involving EGFR signaling and TRAIL-induced cell death. Based on these findings, altering PRMT5 levels or catalytic activity might allow these important cellular circuits to be therapeutically manipulated. Although high-throughput screens have identified PRMT inhibitors, as yet there is no evidence that these compounds specifically target individual PRMTs [16, 65]. Therefore, there is a great need for better and more specific PRMT inhibitors. Another area that remains under-explored is the characterization of enzymes that remove arginine methylation marks. Since the discovery of peptidyl arginine deiminase, type IV (PADI4), an enzyme capable of converting methylarginine to citrulline [66, 67], there has been little progress in understanding how methylarginine marks are erased. Several proteins that contain the Jumonji C domain, which is predicted to erase mono- and di-methyl arginine marks without altering the protein sequence, exist. However, of these proteins, only JMJD6 has been shown to possess demethylation activity [68], and this finding has recently been questioned [69, 70]. Better understanding of how PRMT5-induced arginine methylation controls cell proliferation will therefore require characterization of its antagonistic arginine demethylases. Furthermore, conducting additional high-throughput screens to identify specific modulators of both PRMTs and arginine demethylases will likely be invaluable in the race to find more effective drugs that can stop cancer cell growth.

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Figure 1.

PRMT5-induced epigenetic and post-translational changes have a significant impact on cell growth and proliferation. Through multiple interactions with specific transcription factors and chromatin-modifying enzymes, PRMT5 is capable of controlling critical cellular pathways. (a) PRMT5 associates with specific ATP-dependent chromatin remodelers, co-repressors as well as co-activators, and controls expression of key target genes involved in growth control, metastasis, differentiation and development. (b) PRMT5 alone or in combination with MEP50 interacts with a variety of transcriptional regulators to epigenetically modify specific histone arginine residues including H3R8 and H4R3, and to induce gene silencing. PRMT5-induced H4R3 methylation orchestrates recruitment of repressor DNA methyltransferase DNMT3A. PRMT5 also promotes transcriptional silencing by post-translationally modifying chromatin-binding proteins such as MBD2, p53 and CBP, and altering their biochemical properties.

Figure 2.

Role of PRMT5 in cell signaling and organelle biogenesis. Association of PRMT5 with various receptors, signaling molecules and organelle subunits allows it to impact cell proliferation, tumorigenesis and cell death. (a) PRMT5 targets multiple proteins in the cytoplasm and modulates signaling pathways, RNA processing, and organelle assembly. (b) Methylation of EGFR by PRMT5 has an inhibitory effect on ERK signaling and affects cellular proliferation, migration and invasion, while interaction of PRMT5 with death receptors DR4 and DR5 leads to inhibition of TRAIL-mediated apoptosis and induction of pro-survival gene expression. In the context of pluripotent stem cells, phosphorylation of PRMT5 by oncogenic JAK kinase mutants results in loss of its methyltransferase activity, and hyperproliferation of hematopoietic stem cells. Through its ability to interact with and methylate various cytoplasmic proteins, PRMT5 is also involved in assembly of spliceosomal proteins, Golgi apparatus, and the 40S ribosomal subunit.