Versatility of PRMT5-induced methylation in growth control and development

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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 histone 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 prosurvival pathways, which demonstrates 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 therapeutic targeting of PRMT5.

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 the regulation of 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 coordinated interplay between histone-modifying enzymes, ATP-dependent chromatin remodelers and DNA-modifying enzymes. The importance of chromatin modification by these enzymes is reflected in 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. 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 subgroups depending on the type of modification they catalyze. Type I PRMT enzymes (PRMT1–4, PRMT6 and PRMT8) generate $\omega$-$N^\text{G}_{\text{dim}}$-monomethyl and $\omega$-$N^\text{G}_{\text{sym}}$-asymmetric dimethyl arginines, whereas type II PRMT enzymes (PRMT5, PRMT7 and PRMT9) catalyze the formation of $\omega$-$N^\text{G}_{\text{sym}}$-monomethyl and $\omega$-$N^\text{G}_{\text{sym}}$-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 that describes 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, because 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 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 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 seems that this is a direct result of increased translation [26,27]. miRNA profiling in chronic lymphocytic leukemia, mantle cell lymphoma cell lines and clinical samples has revealed that aberrant PRMT5 expression is inversely
correlated with levels of PRMT5-specific miRNAs. Overexpression of any of these miRNAs restored PRMT5 to its basal levels, which supports 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 therapeutic targeting of PRMT5 might help to restore expression of anti-cancer genes and inhibit growth of cancerous cells.

PRMT5 also participates in transcriptional silencing of the cullins CUL4A and CUL4B, which encode scaffolding proteins for the E3 liga 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,b) [16]. As cells traverse the G1 to S phase, cyclin D1 is phosphorylated on T286 and is exported to the cytoplasm, where it undergoes ubiquitin-dependent proteolysis [15]; substitutions of T286 render the protein 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, methylsone protein 50 (MEP50) and the BRG1 SWI/SNF ATPase during the G1 to S transition. Blocking of 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, which indicates 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 p14ARF and p16INK4a) (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 functions 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. On DNA damage, p53 is activated and 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, R355 and R337 on 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, which highlights 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 an increase in germ cell death on gamma-ray irradiation in worms lacking PRMT-5 [21]. When PRMT-5 was reintroduced, there was a clear decrease in germ cell apoptosis, which indicates 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 methylation of CBP-1 R234, a site also conserved in human p300 and CBP. Methylation of CBP-1 inhibited its ability to acetylate CEP-1/p53 and probably histones, which thus caused transcriptional repression of egl-1 and promoted 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 reduced PRMT5 expression 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 caused by reduced p53 recruitment to its promoter, which indicates 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 protein 4 (PDCD4), 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 on 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], which reflects 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 the E-cadherin gene (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,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 of PRMT5 on cell growth and tumorigenesis by indicating a role for PRMT5 in cell adhesion.

Association of PRMT5 with various binding partners seems 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, which suggests 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 towards 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 suffer from early embryonic lethality between embryonic days 3.5 and 6.5 [43]. In addition, the failure to derive embryonic stem (ES) cells from Prmt5-null blastocysts and the downregulation of pluripotency genes as differentiation genes were upregulated in PRMT5 knockdown ES cells strongly suggest that PRMT5 is required for stem cell pluripotency. Biochemical analysis indicated that PRMT5 methylated histone H2A before 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 (STAT3) 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 resulted in repression of germ cell specification genes and hypermethylation of histones H2AR3 and H4R3 at these loci. A role for BLIMP1, PRMT5 and H2A/H4R3 dimethylation in human fetal germ cell development has also been demonstrated, which highlights the relevance of this mechanism in maintenance of the undifferentiated and pluripotent state [12,13,43]. Studies in Drosophila also revealed 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]. Dart5 enzyme activity is required for 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 non-epigenetic mechanisms.

PRMT5 can also promote somatic cell reprogramming [46], consistent with findings from studies of early development, ES cells and germ cells. Knockdown of 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], which suggests that type I PRMTs might negatively regulate the reprogramming process. Thus, it seems that PRMT5 and one or more type I PRMT5 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 the 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 transcriptional start site of the γ-globin gene (Figure 1b) [49]. PRMT5 binding and enzyme activity are crucial for the recruitment of a repressive multisubunit complex containing DNMT3A, HDAC1, SUV4-20h1 and casein kinase (CK)2α to the γ-globin promoter. Remarkably, PRMT5 also influenced other epigenetic marks including H4S1 ph, 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,
which indicates 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 of somatic cell reprogramming, in which the different PRMTs might work in opposition, these data indicate apparently independent roles for PRMT4 and PRMT5 in the same biological process (PRMT5 is required for early myogenic gene activation, whereas PRMT4 is not; by contrast, PRMT5 is not required for late myogenic gene expression whereas PRMT4 is).

PRMT5 methylates multiple targets in the cytoplasm and thus modulates 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 seems that PRMT5 localization and the substrates it targets in each cellular compartment might play a crucial 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, which suggests 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 expressing mutant EGFR (Figure 2b). Methylation of R1175 downregulates 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 factor (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,b) and that knockdown of PRMT5 potentiates TRAIL-induced tumor cell death, which indicates that PRMT5 inhibits the killing effect of TRAIL [24]. The molecular mechanism is independent of the PRMT5 methyltransferase activity and involves nuclear factor (NF)-κB, because TRAIL-induced IKK activation and IκB degradation were reduced, as was expression of NF-κB target genes, in PRMT5 knockdown cells. PRMT5 knockdown sensitizes tumor, but not normal, cells to TRAIL-induced death, and PRMT5 overexpression endows a cancer cell line with increased resistance to TRAIL-induced death, which suggests that targeting of 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 K539L mutant kinases bound more avidly to and phosphorylated PRMT5 both in vitro and 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 splicosomal U1, U2, U4 and U5 small nuclear ribonucleoproteins (snRNPs). PRMT5, as well as PRMT7, another type II PRMT, methylates three of the seven Sm proteins and enhances their binding to the survival motor neuron (SMN) complex (Figure 2a,b) [59]. Interaction of methylated Sm proteins with the SMN complex facilitates their loading onto the Sm site of small nuclear RNA (snRNA), which thereby gives 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, which thus provided confirmation of 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 2. Role of PRMT5 in cell signaling and organelle biogenesis. Association of PRMT5 with various receptors, signaling molecules and organelle subunits enables 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) Methyltransferase of EGFR by PRMT5 has an inhibitory effect on ERK signaling and affects cellular proliferation, migration and invasion, whereas 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.
complex to indicate involvement was as PRMT5, with either pICln or RIOK1 directing 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 it was shown that the cancer cell growth inhibitor AS1411, a G-rich quadruplex-forming oligonucleotide, alters 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, which indicates 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,b). In an attempt to provide a better understanding of 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, which suggests that PRMT5 impacts fusion of Golgi membranes by methylating GM130 and influencing its association with other structural proteins of the Golgi apparatus [8]. It has also been shown that PRMT5 interacts with and methylates ribosomal protein S10 (RPS10), a component of the ribosome 40S subunit [7]. Methylation of RPS10 seems to be crucial 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 more slowly than control cells, and re-expression of wild-type, but not mutant, RPS10 restores cell proliferation, which thus emphasizes the importance of PRMT5-induced methylation in ribosome biogenesis and growth control.

PRMT5 methylates another group of small RNA-binding proteins called Piwi that 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, which suggests that PRMT5-induced methylation of Piwi proteins is crucial 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 studies 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 manifest 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 seems 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, and hence causes 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, which raises 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 of PRMT5 levels or catalytic activity might facilitate therapeutic manipulation of these important cellular circuits. 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 underexplored is the characterization of enzymes that remove arginine methylation marks. Since the discovery of peptidy1 arginine deiminase type IV (PAD4), 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 demethylatation activity [68], and this finding has recently been questioned [69,70]. A better understanding of how PRMT5-induced arginine methylation controls cell proliferation will therefore require characterization of its antagonistic arginine demethylases. Furthermore, additional high-throughput screens to identify specific modulators of both PRMTs and arginine demethylases will probably be invaluable in the race to find more effective drugs that can stop cancer cell growth.

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