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Protein arginine methylation of non-histone proteins and its role in diseases

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Abbreviations: For list of abbreviations, see page 38.

Protein arginine methyltransferases (PRMTs) are a family of enzymes that can methylate arginine residues on histones and other proteins. PRMTs play a crucial role in influencing various cellular functions, including cellular development and tumorigenesis. Arginine methylation by PRMTs is found on both nuclear and cytoplasmic proteins. Recently, there is increasing evidence regarding post-translational modifications of non-histone proteins by PRMTs, illustrating the previously unknown importance of PRMTs in the regulation of various cellular functions by post-translational modifications. In this review, we present the recent developments in the regulation of non-histone proteins by PRMTs.

The Protein Arginine Methyltransferase Family

Ten mammalian PRMTs have been identified in humans so far.[1] The PRMTs vary in length, but all contain a conserved core region that includes a methyltransferase (MTase) domain, a β-barrel, and a dimerization arm. PRMTs are AdoMet-dependent methyltransferases, which means they catalyze highly specific methyl group transfers from the ubiquitous cofactor S-adenosyl-l-methionine (AdoMet) to a multitude of biological targets in the cell. Besides PRMTs, there are many other types of AdoMet-dependent methyltransferases as well. While the MTase binding domain is highly conserved in other AdoMet-dependent methyltransferases, the 7 β-barrel domains are quite unique to the PRMT family.[2] As shown in **Figure 1**, PRMTs have 1–2 highly conserved MTase domains, which include motif I (VLD/EVGXGXG), post I (V/IXG/AXD/E), motif II (F/I/VDI/L/K), motif III (LR/KXXG), and THW loop.[3]

Although PRMT family members share many common features, they also have their own unique features and different distribution patterns. As summarized in **Table 1**, PRMT1 is the predominant PRMT in mammalian cells, and performs over 80% of PRMT activity in cell. It was the first human PRMT isolated, cloned, purified, and characterized regarding its substrates and its pattern of methylation,[4] and it is found throughout the cell. The sequence is highly conserved in all eukaryotes. PRMT1 is the smallest in the PRMT family, containing 361 amino acids. PRMT1 in humans has at least 3 major transcript variants.[4] The only differences in the polypeptide sequences of these isoforms are alternative translation initiation sites at the N termini. It has been suggested that there may be differences in substrate preference between the 3 isoforms.[4] PRMT2 is primarily situated within the nucleus (**Table 1**) and contains an N-terminal SH3 domain. It contains 433 amino acids. The putative AdoMet binding site cap is located about 104 amino acids upstream of the YFGSY sequence.[4] PRMT3 predominantly exists in the cytoplasm and nucleus (**Table 1**) and contains a C2H2 zinc-finger domain at its N terminus.[5] PRMT3 has 531 amino acids. Its N-terminal portion, between residues 230 and 367, has the most highly conserved sequences; the C-terminal half is less conserved, except residues 466 and 479.[6] Co-activator-associated methyltransferase CARM1/PRMT4 mainly exists in the nucleus as a transcriptional co-activator.[7] The core domain starts from the conserved $Y^{149}FQFY^{153}$ motif.[4] It contains 608 amino acids. PRMT5 was originally named Janus kinase-binding protein 1 (JBP1), located in both the nucleus and cytoplasm,[8] but predominately in the cytoplasm. In humans, PRMT5 has 637 amino acids. The sequences near the beginning and end of the conserved domain are SYLQY and FSWFP, respectively.[4] PRMT6 has a very similar structure to PRMT1. It is the second smallest member in PRMTs family, containing 375 amino acids. PRMT6 is exclusively located in the nucleus.[9,10] Its well-conserved sequence includes the YYECY motif.[4] Another member of PRMTs family is PRMT7, a 692-amino acids-long protein. PRMT7 is very unique, because it contains 2 MTase domains that are located at the N and C termini: the N-terminal MTase domain is highly conserved.[11,12] PRMT7 is found both in cytoplasm and nucleus. PRMT8 is a plasma membrane-associated protein mainly expressed in brain tissue.[13] The structure is extremely similar to PRMT1 (over 80%), but has a unique N-terminal myristoylation motif that helps PRMT8 localize on the plasma membrane. This unique N-terminal domain of PRMT8 makes its methylation ability 10-fold lower than PRMT1, suggesting that the unique N-terminal domain of PRMT8 can inhibit its catalytic activity. PRMT8 automethylates at R58 and R73 in the N-terminal region.[14] So far, only PRMT6 (at R35 site) and PRMT8 are known to have automethylation capability.[15] PRMT8, the third smallest member in the PRMT

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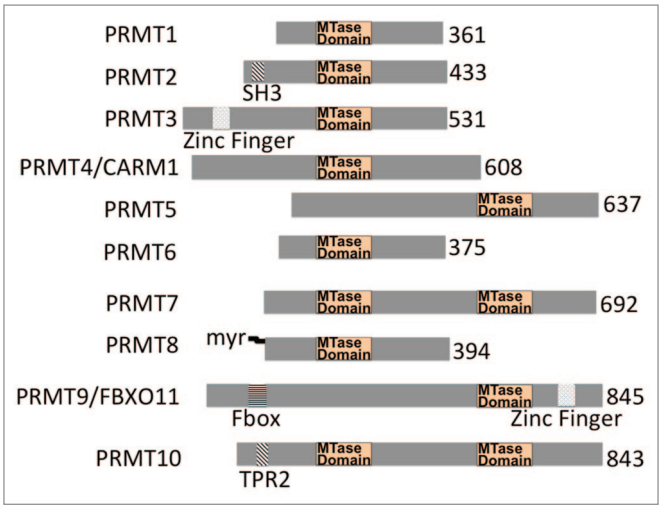

Figure 1. The structure of PRMTs (adapted from ref. 1). All PRMTs universally have 1–2 methyltransferase (MTase) domains. Some have other particular domains. For example, PRMT2 has a SRC homology 3 (SH3) domain. PRMT3 and 9 have zinc finger domains. In addition, PRMT9 also has an F-box domain. PRMT10 has a tetratricopeptide repeat 2 (TPR2) domain. PRMT8 has a unique N-terminal myristoylation (myr) motif.

family, has 394 amino acids. PRMT9, also called F-box only protein 11 (FBXO11), has been found throughout the cell, including the nucleus and the cytoplasm.[16] It has a unique F-box domain on the N terminus and zinc finger domain on the C terminus. It is the largest in the PRMT family, containing 845 amino acids. The last PRMT family member currently known is PRMT10. It is a putative protein that also has 2 MTase motifs and a TPR2 (tetratricopeptide repeats) motif on its N terminus. It is located exclusively in the cytoplasm and has 843 amino acids, making it the second largest protein among PRMT family members.

Besides the classification above, PRMTs can be further classified as type I–IV according to the methylarginine products. As shown in **Figure 2**, type I enzymes catalyze the formation of ω- N^G- monomethylarginine (ωMMA) and asymmetric ω-N^G, N^G- dimethylarginines (ω-aDMA); Type-II enzymes catalyze the formation of ωMMA and symmetric ω-N^G, N^G- dimethylarginines (ω-sDMA); type-III enzymes catalyze the formation of ωMMA only,[3] and type IV enzymes uniquely catalyze the formation of δ-N^G-MMA.[17] Type I–III exist in mammalian cells; type IV has only been described in yeast and possibly in plants due to its high sequence similarities.[17] PRMT1, 2, 3, 4, 6, and 8 display type-I activity;[18] PRMT5, 7 and 9 display type-II activity; in addition to type-II activity, PRMT7 also displays type-III activity. The activity of PRMT10 has not yet been characterized.[3]

Methylation of Histone Proteins by PRMTs

PRMTs were initially found to directly methylate various histone proteins (**Table 2**) to regulate different cellular functions. For example, post-translational modification of histones is known to occur on the tail region and contributes to the histone code. PRMT1 and 2 are known to methylate histone H4R3;[19,20] histone H3 is methylated by PRMT4 on R2, R17, and R26.[21] PRMT5 methylates histone H2AR3, H3R8, and H4R3, negatively regulating the expression of tumor-suppressor genes and thus acting as an oncogene. PRMT5 protein levels are increased in leukemia and lymphoma cells.[22] The overexpressed PRMT5 elevates symmetric methylation of H3R8 and H4R3, located in the promoter regions of target genes (such as Retinoblastoma

Table 1. Distribution of PRMTs

Name	Primary localization	Alternate localization	Tissue	Enzyme classification
PRMT1	Nucleus	Cytoplasm	Adrenal, Brain, Cerebellum, Ciliary body, Colon, Esophagus, Frontal cortex, Frontal lobe, Heart, Immature Dendritic cell, Kidney, Liver, Lung, Mature Dendritic cell, Ovary, Pancreas, Placenta, Prostate, Retina, Stomach, Testis, Thyroid, Urinary bladder	Type I
PRMT2	Nucleus		Serum, Testis	Type I
PRMT3	Cytoplasm	Nucleus, Ribosome	Adrenal, Heart, Liver, Ovary	Type I
PRMT4	Nucleus		Adrenal, Cerebellum, Ciliary body, Colon, Esophagus, Frontal cortex, Frontal lobe, Gall bladder, Kidney, Liver, Lung, Ovary, Pancreas, Placenta, Prostate, Stomach, Testis, Thyroid, Urinary bladder	Type I
PRMT5	Cytoplasm	Nucleus	Adrenal, Bile, Brain, Cerebellum, Ciliary body, Colon, Esophagus, Frontal cortex, Frontal lobe, Gall bladder, Heart, Immature Dendritic cell, Kidney, Liver, Lung, Optic nerve, Ovary, Pancreas, Prostate, Retina, Stomach, Testis, Urinary bladder, Urine	Type II
PRMT6	Nucleus		Adrenal, Liver, Ovary	Type I
PRMT7	Cytoplasm	Nucleus	Brain, Lung, Testis	Type II and III
PRMT8	Plasma Membrane		Brain	Type I
PRMT9	Nucleus	Cytoplasm	Liver	Type II
PRMT10	Cytoplasm		Esophagus, Ovary, Stomach	N/A

protein [RB1] [p105], Retinoblastoma-like 1 [RBL1] [p107], and Retinoblastoma-like 2 [RBL2] [p130]), in turn silencing the RB family of tumor suppressor genes.[23] In addition, PRMT5, in association with methylosome protein 50 (Mep50), methylates cytosolic histone H2A (H2AR3me2) to repress differentiation genes in ES cells.[24] Most recently, H3R2 was shown to be symmetrically dimethylated by PRMT5. H3R2me2 binds to WD repeat domain 5 (WDR5) and helps to recruit WDR5 to euchromatin for transcriptional activation in P493–6 human B cells and human promyelocytic leukemia cell line HL60.[25] Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Additionally, PRMT5 negatively regulates some tumor suppressors, including ST7 and NM23, whose decreased expression correlates with H3R8 methylation in vitro.[22] Moreover, PRMT5 protein levels are high in transformed chronic lymphocytic leukemia (B-CLL) cell lines.

PRMT6 has been shown to methylate histones H2AR29 and H3R2, decreasing transcription of homeobox (Hox) genes and myelocytomatosis oncogene-dependent genes.[9,26] Additionally, knockdown of PRMT6 leads to delay of cell cycle progression and defect of G_2 checkpoint in human osteosarcoma cell line U2OS. Overexpression of PRMT6 leads to increased H3R2 dimethylation at the transcriptional start sites of p21 and p27 promoters and decreased expression of p21 and p27.[27]

PRMT7 plays a role in male germ-line imprinted gene methylation through interaction with testis-specific factor CCCTC-binding factor (zinc finger protein)-like (CTCFL) and H4R3me2s accumulation.[28] Like PRMT5, PRMT7 symmetrically dimethylates H3R2. H3R2me2 enhances binding of WDR5, correlating with changes in chromatin markers and expression of genes belonging to fundamental pathways of transcription regulation and protein post-translational modification.[25] PRMT7 also dimethylates H2AR3 and H4R3 in the promoter region of polymerase (DNA directed) δ1, catalytic subunit (POLD1), negatively regulating cellular response to DNA damage.[29] PRMT8 is able to methylate H2A (no specific site) and H4 (no specific site) via N-terminal myristoylation.[14] No histone substrates for PRMT3, 9, or 10 have been found so far.

Methylation of Non-Histone Proteins By PRMTs

Since arginine methylation of non-histone proteins is an emerging area for which few reviews are available,[30,31] we will mainly focus on recent discoveries of arginine methylations of non-histone proteins and their important roles in various cellular functions.

RNA processing and transcriptional elongation

RNA binding proteins (RBPs) often contain glycine–arginine-rich (GAR) motifs and are considered major substrates for PRMTs. Methylation may either negatively or positively influence RNA–protein interactions by preventing formation of hydrogen bonds by steric hindrance and causing arginine residues to become more hydrophobic.[32] It has been reported that arginine methylation by PRMT5 helps small nuclear ribonucleoprotein-associated protein B/B' (Sm protein B/B'), like Sm4 (LSm4), small ribonucleoprotein particle protein SmD1 and 3 (SmD1 and 3) assemble into mature small nuclear ribonucleoproteins (snRNPs).[33] All 25 occurrences of the triplet GAR in these 4 proteins are symmetrically dimethylated, with the exception of 3 arginines in LSm4 and one in SmD3 for which no data are available. Further use of methylated peptides or arginine methylation inhibitors can block the survival motor neuron (SMN) and Sm interactions so that Sm proteins cannot translocate into the nucleus to facilitate snRNP assembly.[33] Furthermore, PRMT5 methylates transcription elongation factor SPT5 at R698, which decreases SPT5 association with RNA polymerase II, suggesting that PRMT5 might be involved in regulating transcriptional elongation.[34]

Cell cycle and DNA repair

During mitosis, the 68-kDa Src-associated substrate in mitosis (Sam68) was bound and methylated by PRMT1 on the proline motif P3 at amino acids 280–339, which promoted the Sam68 nuclear localization and facilitated the export of unspliced HIV RNA into the cytoplasm. In PRMT1-deficient embryonic stem cells, Sam68 was localized to the cytoplasm. Additionally, deletion of the PRMT1 methylation sites and the use of methylase inhibitors resulted in Sam68 accumulation in the cytoplasm.[35] PRMT1 is also required for proper cell cycle progression, such as G_2/M and G_1/S checkpoint activations.[36]

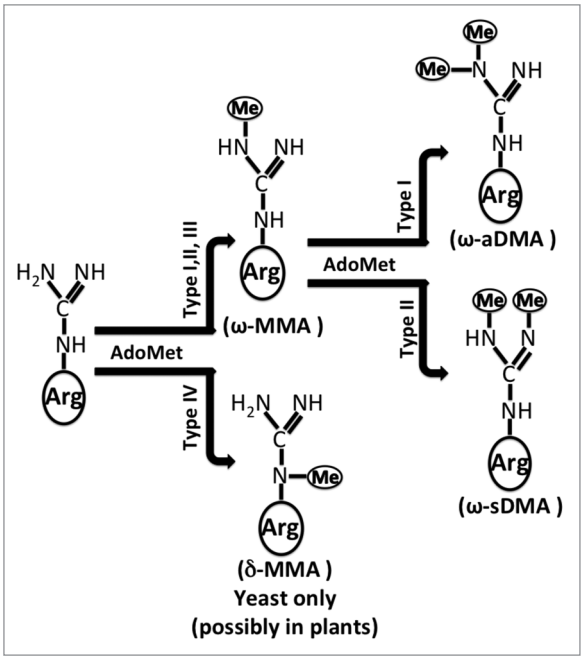

Figure 2. Methylation of arginine residues (adapted from ref. 17). Three types of methylated arginine residue products have been proven in mammalian cells. They are ωMMA, ω-aDMA, and ω-sDMA. ωMMA refers to a single methyl group placed on the terminal nitrogen atom. Its production is catalyzed by type I, II, or III PRMTs. ω-aDMA refers to 2 methyl groups placed on the same terminal nitrogen atom. It is catalyzed by type I PRMTs. ω-sDMA refers to 2 methyl groups placed on each of the terminal nitrogens. It is catalyzed by type II PRMTs. δ-MMA so far only has been described in yeast (possibly in plants), and refers to a single methyl group placed on the internal guanidino nitrogen atom. Abbreviations: ω-aDMA, asymmetric ω- N^G, N^G- dimethylarginines; ωMMA, ω - N^G-monomethylarginine; ω-sDMA, symmetric ω - N^G, N^G- dimethylarginines; δ-MMA, δ-monomethylarginine

Meiotic recombination 11 (MRE11) contains a nuclease domain which is active in homologous recombination repair (HRR). In addition to the nuclease domain, MRE11 harbors a GAR motif on amino acids 554–680 that can be methylated by PRMT1. The data show cells containing hypomethylated MRE11 displayed inefficient DNA damage repair that was significantly rescued by induction of the MRE11-RAD50-NBS1 complex (MRN).[37] Tumor suppressor 53-binding protein 1 (53BP1) is another critical protein involved in DNA repair due to the high affinity with both single- and double-stranded DNA.[38] PRMT1 methylates the GAR motif of 53BP1 on amino acids 1319–1480 and helps 53BP1 localize to DNA damage sites in fibroblast cells.[39] Transfected 53BP1 with a mutated GAR motif would no longer function as a methyl-acceptor.[39] Additionally, PRMT1-deficient mouse embryonic fibroblasts (MEFs) are hypersensitive to the DNA damaging agent etoposide and fail to recruit homologous recombination RAD51 recombinase to DNA damage foci in vitro.[36]

Table 2. Specific examples of arginine methylations on histone and non-histone proteins

Family member	Known histone substrate	Known non-histone protein substrates	Year published
PRMT1	H4R3[19]	hnRNP A1 (R194)[81]	1994
		BTG1 (Non specific sites)[82]	1996
		TIS2 (Non specific sites)[82]	1996
		IFNα/β(interferon-α receptors (IFNAR1)-intracytoplasmic domain (IC) domain)[83]	1997
		ILF3 (COOH-terminal region)[84]	2000
		SPT5 (R681, 696, and 698)[34]	2003
		Scaffold attachment factor A (SAF-A) (from amino acid 778 to 793)[85]	2004
		53BP1(GAR motif between amino acids 1319–1480)[39]	2005
		MRE11 (GAR domain)[37]	2005
		FMRP (R544 and 546)[86]	2006
		Sam68 (proline motif P3, R280–339)[87]	2007
		SLM (GAR)[87]	2007
		ERα (R260)[44]	2008
		RUNX1 (R206 and R210)[88]	2008
		TAF15 (Arg-Gly-Gly (RGG) repeats)[89]	2009
		BCR (R198)[40]	2010
		CF Im59 (C termini) and CF Im68 (C termini and GAR motif)[72]	2010
		Ash2L (R296)[64]	2011
PRMT2	H4R3[19,20]	ERα (AF-1, DNA binding domain and hormone binding domain)[90]	2002
		Glutathione transferase (GAR motif)[91]	2009
PRMT3	N/A	FMRP (R544 and R546)[86]	2006
		rpS2 (GAR motif)[92]	2007
PRMT4/CARM1	H3R2[21]	PABP1 (R455 and 460)[93]	2002
	H3R17[21]	(CBP)/p300 (R2142)[94]	2005
	H3R26[21]	FMRP (R544 and R546)[86]	2006
		Sox9 (high-mobility group (HMG) domain)[95]	2009
		CA150 (proline-, glycine-, methionine-rich (PGM) motif)[96]	2009
		SmB (PGM motif)[96]	2009
		U1C (PGM motif)[96]	2009
		SF3b4 (PGM motif)[96]	2009

PRMT5 also plays important roles in cell cycle and DNA repair. PRMT5 knockout triggers cell cycle arrest in G_1 by inducing p53 expression upon recognition of DNA damage, eventually leading to p53-dependent apoptosis.[40] PRMT5 has been shown to affect DNA repair by methylating p53 at selected arginine residues R333, R335, and R337 in the p53 oligomerization domain, increasing the response to DNA repair.[40] A study demonstrated that in *C. elegans* PRMT5 downregulated DNA damage induced apoptosis by methylating CBP-1 at R234.[41] When DNA damage-induced apoptosis is negatively regulated, the risk of genomic instability and tumor progression is augmented.

In addition to PRMT1 and 5, PRMT6 has also been shown to methylate Polβ on R83 and R152, thus enhancing DNA binding ability and stimulating DNA polymerase activity, eventually triggering base excision repair.[42]

Regulation of signaling downstream of receptors

Recent studies showed that PRMTs could mediate signal transduction downstream of the B-cell antigen receptor (BCR)[43] and estrogen receptor α (ERα).[44] PRMT1 methylated the Igα subunit of the BCR at R198, negatively regulating the phosphoinositide-3 kinase (PI3K) pathways and promoting B cell differentiation.[43] Moreover, PRMT1 can methylate ERα at R260 in its DNA-binding domain. This is required for mediating the extranuclear function of the receptor by triggering its interaction with the p85 subunit of PI3K and Rous sarcoma oncogene (Src) and recruitment of focal adhesion kinase (FAK).[44]

Regulation of transcription factors (e.g., nuclear factor κB)

PRMTs can also regulate the function of transcription factors. As we mentioned above, p53 is regulated by PRMT5 through direct arginine methylation.[40] There are several studies showing that PRMT1, 2, and 4 can regulate the activity of the ubiquitous inducible transcription factor, nuclear factor κB (NFκB). NFκB plays an important role in the transcriptional regulation of genes involved in inflammation, cell survival, and tumorigenesis. Hottiger's group showed that PRMT4 is a novel transcriptional coactivator of NFκB and functions as a promoter-specific regulator of NFκB recruitment to chromatin. PRMT4 acts in a gene-specific manner, mainly by enhancing NFκB recruitment to cognate sites. Moreover, PRMT4 synergistically coactivates NFκB-mediated transactivation in concert with the transcriptional coactivators, p300/CREB-binding protein, and the p160 family of steroid receptor coactivators. These results suggest that the cooperative action between protein arginine methyltransferase PRMT4 and protein lysine acetyltransferases p300 regulates NFκB-dependent gene activation.[45]

In another report, the same research group further demonstrated that PRMT1 synergistically coactivates NFκB-dependent gene expression at the promoters of macrophage inflammatory protein 2 and human immunodeficiency virus 1 long-terminal repeat in concert with the transcriptional coactivators p300/CREB binding protein, PRMT4, and poly (ADP-ribose) polymerase 1(PARP1).[46,47]

Ganesh et al. reported that PRMT2 inhibits NFκB-dependent transcription and promotes apoptosis. PRMT2 exerted this effect by blocking nuclear export of the inhibitor of NFκB (IκBα) through a leptomycin-sensitive pathway, increasing nuclear IκBα and decreasing NFκB DNA binding mediated by the highly conserved S-adenosylmethionine-binding domain of PRMT2.[48]

Although the above reports suggest the correlation between NFκB and PRMT1, 2, and 4, none of the reports had shown the regulation of NFκB is through direct arginine methylation. Recently, our lab discovered that PRMT5 dimethylates the subunit p65 at R30 to activate NFκB.[49] Complex post-translational modifications of the p65 subunit of NFκB are a major aspect of the extremely flexible regulation of NFκB activity. Although phosphorylation, acetylation, ubiquitilation, and lysine methylation of NFκB have been well described,[46,50-55] direct arginine methylation on NFκB has not previously been found. We discovered that expression of the R30A mutant of p65 substantially decreased the ability of NFκB to bind to κB elements and to drive gene expression. A model in which dimethyl R30 is placed into the crystal structure of p65 predicts new Van der Waals contacts that stabilize intraprotein interactions and indirectly increase the affinity of p65 for DNA.[49] PRMT5 was the only arginine methyltransferase that co-precipitated with p65,

Table 2. Specific examples of arginine methylations on histone and non-histone proteins (continued)

Family member	Known histone substrate	Known non-histone protein substrates	Year published
PRMT5	H2AR3[22]	MBP (R107)[97]	1971
	H3R2[25]	LSm4 (No specific sites)[98]	2000
	H3R8[22]	Sm D1 (GAR motif)[98]	2000
	H4R3[22]	Sm D3 (GAR motif)[98]	2000
		EBNA-2 (R325–376)[99]	2003
		SPT5 (R698)[34]	2003
		EBNA-1 (R325–376)[69]	2006
		RBL2 (H3R8 and H4R3)[23]	2008
		p53 (R333, 335, 337)[40]	2008
		CBP-1 (R234)[41]	2009
		CF Im68 (GAR motif)[72]	2010
		Ash2L (R296)[64]	2011
		PDCD4(R110)[61]	2011
		HoxA (R140)[71]	2012
		NFkB (R30)[49]	2013
PRMT6	H2AR29[9]	GAR motif[100]	2002
	H3R2[10]	PRMT6[100]	2002
		HIV Tat (from amino acid 49 to 63)[101]	2005
		HMGA1a (R57 and 59)[102]	2006
		DNA polymerase β (R83 and 152)[42]	2006
		PRMT6 (R35)[15]	2013
PRMT7	H2AR3[103]	GAR motif[11]	2004
	H3R2[25]		
	H4R3[103]		
PRMT8	H2A (No specific sites)[14]	GAR motif[14]	2002
	H4 (No specific sites)[14]	Myelin basic protein (MBP) (No specific sites)[14]	2002
		PRMT8 (R58and 73)[14]	2007
		Ewing sarcoma (EWS) (arginine-glycine-glycine repeats (RGG))[104]	2008
PRMT9/FBXO11	N/A	P-SmD1 (No specific sites)[105]	2006
PRMT10	N/A	N/A	

Note: Exact R residues are noted in **Table 2** only if it was clearly stated in the original report.

and its overexpression increased NFκB activity, while PRMT5 knockdown had the opposite effect. Microarray analysis revealed that most of the NFκB-inducible genes downregulated by the R30A mutation are similarly downregulated by knocking down PRMT5. Many cytokine and chemokine genes are among these, and conditioned media from cells expressing the R30A mutant of p65 had much less NFκB-inducing activity than media from cells expressing the wild-type protein. A model in **Figure 3** summarizes this novel finding. Furthermore, PRMT5 is frequently found overexpressed in many types of cancer, often to a striking degree, indicating that high levels of this enzyme may promote tumorigenesis, at least in part by facilitating NFκB-induced gene expression.

PRMTs as Therapeutic Targets for Diseases

Cancer

PRMTs play an important role in carcinogenesis. For example, dysregulation of the PRMT1 and PRMT6 genes promote growth of bladder and lung cancer cells, and abrogation of the expression of these genes significantly suppresses growth of bladder and lung cancer cells.[56] This phenomenon appears to be attributable to increased serum aDMA levels during carcinogenesis.[56] Knockdown of the expression of PRMT1 and PRMT6 by siRNA transfection can suppress breast cancer cell proliferation.[56] The methylation status of H4R3 that is methylated by PRMT1 is highly relevant to tumor grade and helps to predict cancer recurrence.[3] Besides PRMT1 and 6, PRMT4 has also shown to be overexpressed in human grade-III breast tumors, colorectal cancer, and prostrate adenocarcinomas; knockdown of PRMT4 inhibits proliferation of breast, colorectal, and prostate cancer cell lines.[57]

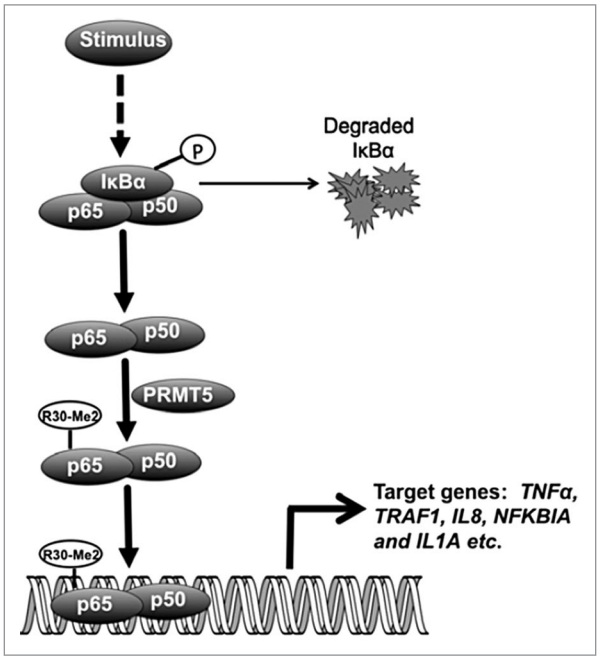

Figure 3. A model of regulation of NFκB by PRMT5 (adopted from ref. 49). In addition to previously known regulatory pathways, NFκB is regulated by PRMT5-mediated methylation of p65 on R30, which affects the expression of many NFκB-induced genes.

The above examples, such as PRMT1, 4 and 6 are all from type-I PRMTs. PRMT5, a typical enzyme of type-II PRMTs, has been shown to promote cell growth and transformation by different mechanisms. Aggarwal et al. suggested that the nuclear cyclin D1/CDK4 kinase regulates cullin4 (CUL4) expression and triggers neoplastic growth via activation of PRMT5.[58] Gu et al. suggested that PRMT5 is essential for growth of lung adenocarcinoma A549 cells. Knockdown of PRMT5 in A549 cells suppresses cell growth and tumor xenografts in nude mice partially through downregulation of the fibroblast growth factor receptor (FGFR) signaling pathway.[59] Oncomine data also suggest that among colon cancer patients, most have elevated PRMT5 expression levels, strongly indicating that PRMT5 promotes colon tumor growth. In fact, PRMT5 is overexpressed in a variety of cancers, such as colon, ovary, kidney, lung, bladder, liver, pancreas, breast, prostate, cervix, and skin, with especially high expression in colon cancer.[49] PRMT5 was also called JAK-binding protein 1, because it interacts with JAK. It has been shown that myeloproliferative leukemia neoplasm patients have the JAK2 mutant JAK2V617F, which binds to PRMT5 more strongly than wild-type JAK2, leading to the neoplastic phenotype.[60] Furthermore, PRMT5 can accelerate tumor growth by methylating R110 on tumor suppressor programmed cell death 4 (PDCD4) in an orthotopic model of breast cancer,[61] leading to the utility of PDCD4/PRMT5 as both a prognostic biomarker and a potential target for chemotherapy.[61] It has also been shown that PRMT5 is involved in tumor formation by upregulating G_1 cyclins/cyclin-dependent kinases and the PI3K/AKT signaling cascade in lung cancer cell lines A549 and H1299.[62] In tumor cells, E2F-1 is methylated on R111 and R113 by PRMT5, reducing its stability. Analysis of a subgroup of colorectal cancer cells indicates that high levels of PRMT5 frequently coincide with low levels of E2F-1 and reflect a poor clinical outcome.[63] In addition, PRMT5 has also been shown to methylate Ash2L (absent, small, or homeotic)-like (*Drosophila*) at R296, which is associated with transformation of human tumors.[64]

Cardiovascular and pulmonary disease

Type-I PRMTs catalyze ω-MMA and ω-aDMA synthesis. Studies showed that elevated ω-aDMA plasma levels are associated with endothelial dysfunction-related cardiovascular and pulmonary diseases, including coronary heart disease (CHD)[65] and pulmonary hypertension.[66] Dimethylarginine dimethylaminohydrolase-1 and -2 (DDAH) regulate endothelial nitric oxide (NO) production through interactions with ω-aDMA that may increase susceptibility to cardiovascular diseases.[67]

Other diseases

In addition to the diseases mentioned above, PRMTs are also found to be involved in virus-related diseases. For example, PRMT4 increases trans-activator X (Tax) transactivation of the human T-cell lymphotropic virus type 1 (HTLV-1) long-terminal repeat (LTR) through direct binding to Tax. This binding promotes methylation of histone H3 (R2, R17, and R26).[68] PRMT5 is also known to methylate EBNA1, an Epstein–Barr virus protein in a stretch of multiple regions from R325 to R376,

helping the localization of EBNA1 and consequently inducing the formation of EBNA1 rings around the nucleoli.[69] In addition to PRMT4 and 5, PRMT6 has been shown to decrease RNA pol II transcription of viral genes by negatively regulating the trans-activator of transcription (Tat) expression. Overexpression of PRMT6 reduced Tat transactivation of HIV-1 production and viral replication.[70]

In addition to the above examples regarding virus-related diseases, PRMTs also have been shown to be involved in the endothelial cell (EC) inflammatory response. One report suggested that PRMT5-induced methylation of HOXA9 (Homeobox protein Hox-A9) on R140 is required for EC-leukocyte adhesion molecule (ELAM) expression during the EC inflammatory response.[71]

Moreover, PRMT5, together with its cofactor WD45, methylates the mammalian cleavage factor I (CF I [m]) subunit of CF I(m)68 at its GAR motif, stimulating methylosome subunit pICln (pICln, also named CLNS1A). pICln induces an outwardly rectifying, nucleotide-sensitive chloride current when expressed in *Xenopus* oocytes.[72] pICln prevents the interaction of Sm proteins with the SMN protein, inhibiting snRNP biogenesis.[73] pICln may act as a PRMT5 co-substrate when bound to Sm proteins, by stimulating Sm protein methylation at its RG repeats.[73]

Perspectives and Future Directions

There are several known PRMT inhibitors, such as S-adenosyl homocysteine (AdoHcy), which compete with products of the methyltransferase reaction to bind the active site of the enzyme without specificity for the PRMT isotype.[74] Recently, several small-molecule PRMT inhibitors have been synthesized. C21, a chloroacetamidine-bearing histone H4 tail analog, acts as an irreversible PRMT1 inhibitor.[75] AMI-1 (protein arginine N-methyltransferases inhibitor 1) specifically inhibits arginine, but not lysine, methyltransferase activity in vitro and does not compete for the AdoMet binding site.[76] Also, an inhibitor named compound 2 was reported to be able to inhibit co-activator-associated PRMT1 with an IC_{50} of 30 nM.[77] Recently, 2 potent and selective inhibitors of PRMT4 were reported. One was methylgene compound 7a,[78] the other was Bristol-Myers Squibb compound 7f.[79] Both were pyrazole-based inhibitors, and functioned at nanomolar concentrations, displaying >100-fold selectivity for the PRMT4 over other related enzymes. The structure of PRMTs and existing PRMT inhibitors provide a guide for the synthesis of new inhibitors.

As we discussed in this review, PRMTs play a crucial role in various biological processes, including RNA processing, DNA repair, signal transduction, and transcriptional regulation. To fully understand these processes, several progressions are required. First, the site of histone methylation needs to be further assessed. PRMTs are extremely promising therapeutic targets for various types of cancer. Discovery of additional methylation sites may lead to the development of more specific inhibitors in the near future. Second, due to the wide range of non-histone substrates compared with histone substrates and the cellular functions mediated by these non-histone substrates, it is important to understand the interaction mechanisms between PRMTs and non-histone substrates. Third, PRMT5 is notable in that it was the first enzyme known to synthesize sDMA on arginine residues in proteins[8] and is known to have important roles in transcriptional modulation, such as the dimethylation of R30 of the p65 subunit of NFκB, leading to its activation. In the future, chemical inhibition of PRMT5 methyltransferase activity could be used to affect function of PRMT5 and its factors. Recently, the crystal structure of the human PRMT5:MEP50 complex has been reported.[80] The structure of the surprising hetero-octametric complex reveals the close interaction between the 7-bladed β-propeller MEP50 and the N-terminal domain of PRMT5, and it delineates the structural elements of substrate recognition. This information would shed light on the synthesis of new inhibitors of PRMT5, and facilitate the development of novel treatments for human diseases.

Abbreviations

ADMA, asymmetric dimethylarginines; AdoHcy, S-adenosyl homocysteine; AdoMet, S-adenosyl methionine; AMI-1, protein arginine N-methyltransferase inhibitor 1; AshL, absent, small, or homeotic-like (*Drosophila*); B-CLL, B-cell chronic lymphocytic leukemia; BCR, B-cell antigen receptor; CARM1, coactivator-associated arginine methyltransferase; CBP, CREB binding protein; CDK, cyclin-dependent kinase; CFI, cleavage factor I; CHD, coronary heart disease; CIP, CDK interacting protein; CREB, cAMP response element-binding protein; CTCFL, CCCTC-binding factor (zinc finger protein)-like; CUL, cullin; DDAH, dimethylarginine dimethylaminohydrolase; DNA, deoxyribonucleic acid; EBNA, Epstein–Barr virus nuclear antigen; EC, endothelial cell; ELAM, EC-leukocyte adhesion molecules; ER, estrogen receptor; ES, embryonic stem cell; FAK, focal adhesion kinase; FBXO, F-box only protein; FGFR, fibroblast growth factor receptor; 53BP1, 53-binding protein1; G, glycine; GAR, glycine arginine rich; HIV, human immunodeficiency virus; Hox, homeobox protein; HRR, homologous recombination repair; HTLV, human T-cell lymphotropic virus; IκBα, nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α; IC50, half maximal inhibitory concentration; JBP, Janus kinase-binding protein; K, Lysine; KIP, kinase inhibitory protein; LSm, like Sm; LTR, long terminal repeat; MEFs, mouse embryonic fibroblast cells; Mep, methylosome protein; MMA, monomethylarginine; MMP, matrix metalloproteinase; Mre, meiotic recombination; MRN, MRE11-RAD50-NBS1 complex; MTase, methyltransferase; NBS, Nijmegen breakage syndrome; NFκB, nuclear factor-κB; NM, nonmetastatic protein; NO, nitric oxide; PARP, Poly ADP-ribose polymerase; PDCD, programmed cell death protein; p53, tumor suppressor 53; PI3K, phosphoinositide 3-kinase; POLD, DNA polymerase delta catalytic subunit; PRMTs, protein arginine methyltransferases; R, arginine; Rb, retinoblastoma protein; RBL, retinoblastoma-like; RBPs, RNA binding proteins; RNA, ribonucleic acid; Sam, Scr-associated substrate in mitosis; sDMA, symmetric dimethyarginines; Sm, small nuclear ribonucleoprotein-associated protein; SMN, survival of motor neuron; snRNPs, small nuclear ribonucleoproteins; Src, Rous sarcoma oncogene; ST, suppressor of tumorigenicity protein; Tat, trans-activator of transcription;

Tax, trans-activator X; TPR, tetratricopeptide repeats; TSP, thrombospondin; TNFα, tumor necrosis factor α; WDR5, WD repeat domain 5

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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