

Insights into the methyltransferase activity and methylation status of PRDM16 in malignant tumors

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Abstract

The PRDM16 (PRDI-BF1-interacting and RIZ1-homologous domain 16) participates in a range of biological processes including tumorigenesis. The protein regulates gene transcription through intrinsic chromatin-modifying enzymatic activity or by forming complexes with histone modifications or other nuclear proteins. Accumulating evidence indicates that the methyltransferase activity and its methylation status of *PRDM16* had been further studied. Significant advances have been made in elucidating the role and underlying molecular mechanisms of methyltransferase activity and its methylation status of *PRDM16* in hematologic tumors and solid tumors. This review focus on the advances of PRDM16 in terms of its methyltransferase activity and methylation status in tumors. Meanwhile, we also discuss the future perspectives of *PRDM16* methylation.

Keywords: *PRDM16*, Methyltransferase activity, Methylation status, Malignant tumor

Introduction

The PRDM protein family consists of 17 members that are structurally defined by the combination of a conserved N-terminal PR (PRDI-BF1 and RIZ1 homology) domain and a variable number of zinc fingers. The PR domain is similar to the SET (suppressor of variegation 3–9, enhancer of zeste, and trithorax) domain found in many histone lysine methyltransferases (HMTs) that function in chromatin-mediated transcriptional regulation [1,2].

These genes regulate a diverse array of biological processes, such as cell proliferation and differentiation, cell cycle progression, and the maintenance of immune cell homeostasis, through epigenetic modulation of gene expression. This regulation occurs via their intrinsic histone methyltransferase (HMTase) activity or through interactions with other chromatin-modifying enzymes. In cancer, tumor-specific dysregulation of *PRDM* genes results in altered expression patterns, driven by either genetic or epigenetic alterations. Most *PRDM* genes commonly generate two major molecular isoforms—one containing the PR domain and one lacking it—through alternative splicing or the differential usage of promoters. These isoforms often exert opposing biological functions, particularly in oncogenesis, where an imbalance between them is frequently observed. PRDM proteins are therefore implicated in tumor initiation, progression, and metastasis, and aberrant expression levels are associated with adverse clinical outcomes and poor prognosis. These characteristics underscore their potential as diagnostic and prognostic biomarkers in cancer, as well as promising targets for novel therapeutic strategies.

Compared with other members of the PRDM family, the role of *PRDM16* in tumorigenesis remains a subject of debate. To date, varying methylation levels of *PRDM16* and differential methylase activities have been observed not only in hematopoietic malignancies but also in several types of solid tumors though with different and/or conflicting results, which collectively suggest that this gene may possess dual functionalities, acting as both an oncogene and a tumor suppressor gene. Therefore, this review aims to elucidate its involvement in tumor development by integrating an analysis of its functional characteristics, such as its methyltransferase activity and its methylation status.

Molecular Structure of PRDM16

PRDM16 (PRDI-BF1-interacting and RIZ1-homologous domain 16) is a zinc finger protein first identified in 2000 and exhibits significant homology to the *MDS1/EVI1* gene (myelodysplastic syndrome 1/ecotropic virus integration site 1) [3,4]. The zinc finger structure that binds specifically to DNA for transcriptional activity and can recognize and bind RNA and protein, which are of the C₂H₂ type. As a member of PRDM proteins [5,6], the human *PRDM16* gene, located on chromosome 11p36.32, contains 17 exons and encodes a zinc finger protein with a positive regulatory (PR) domain, which also contains a proline rich domain (PRR), inhibitory domain (RD), and acidic domain (AD). The *PRDM16* gene encodes a protein consisting of 1,276 amino acid residues and shares 64% nucleotide and 63% amino acid sequence similarity with *MDS1/EVI1* [7]. Furthermore, it possesses an identical domain structure to that of *MDS1/EVI1*. Nishikata, *et al.* isolated a splicing variant of PRDM16 that encodes a shorter isoform, termed sPRDM16. Besides, three transcription start sites were revealed: one located in exon 1 and two within exon 2. Two distinct translation

products were detected: a 170 kDa protein corresponding to full-length PRDM16 (fPRDM16) and a 150 kDa protein representing short form of PRDM16 (sPRDM16) (**Figure 1A**). Additionally, there are four known PRDM16 protein isoforms, among which fPRDM16 and sPRDM16 have been most extensively studied [8]. These two isomers differ in the presence or absence of the PR domain. The longer isoform, fPRDM16, contains the PR domain, whereas the shorter variant, sPRDM16, lacks this domain and acts as a dominant-negative subtype in cancer [9,10]. Importantly, the presence or absence of the PR domain in PRDM16 has been shown to influence its functional roles in disease initiation and progression.

Methyltransferase Activity of the PRDM16 in Hematologic Tumors and Solid Tumors

Recently, PRDMs potentially function as methyltransferases through their PR domain [4,11], given its high homology with the SET domain. The methyltransferase activities of PRDM16 containing the PR domain need to be further studied, with current studies suggesting either H3K4me1 [12] or H3K9me1 [13] specificity (**Figure 1B**). Zhou *et al.* firstly discovered that PRDM16 is a highly specific H3 K4 methyltransferase on chromatin and its intrinsic activity is essential for suppressing mixed-lineage-leukemia (MLL1)-rearranged acute leukemia. To determine the substrate specificity of PRDM16 on chromatin, they purified the PR/SET domain of PRDM16 and the PRDM16 mutant (PRDM16mut) carrying two amino acid mutations (C113F and V115G) using an insect cell expression system. To explore substrate specificity, they generated histone mutants in which lysine at positions 4, 9, 27, 36 and 79 was mutated to glutamine (Q), respectively. They found that wild-type PRDM16 cannot methylate H3K4Q *in vitro*, but can methylate H3 containing other lysine mutations (i.e.K9Q, K27Q,

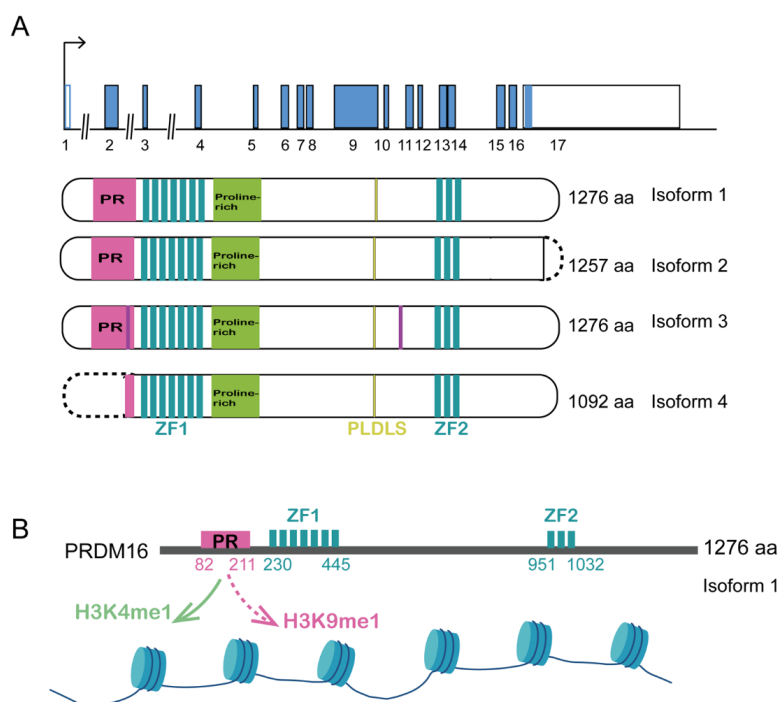


Figure 1. Structure of PRDM16.

K36Q and K79Q). As a control, PRDM16mut did not show HKMT activity under the same conditions, confirming that H3K4 methylation is caused by the intrinsic activity of the PR domain. To detect the specificity of PRDM16 on nucleosomes, they used recombinant nucleosomes containing wild-type H3, H3K4Q, H3K9Q or H3K27Q mutants as substrates. PRDM16 cannot methylate nucleosomes containing H3K4Q, but has strong activity against nucleosomes composed of H3K9Q or H3K27Q. The PR domain of PRDM16 has intrinsic HKMT activity and is specific to nucleosome H3K4 *in vitro*. Collectively, PRDM16 is a histone H3K4 methyltransferase on chromatin. Mutation in the N-terminal PR domain of PRDM16 abolishes the intrinsic enzymatic activity of PRDM16 [12]. Prostate cancer is one of the most common malignant tumors in men, and histone methylation has been implicated in its development. Zhu *et al.* identified PRDM16 as a histone methyltransferase that influences the survival of prostate cancer cells. Their findings indicated that PRDM16 suppresses apoptosis by down-regulating BCL-2 expression and up-regulating BAK expression [14]. To sum up, these findings suggest that methyltransferase activity of PRDM16 plays significant and diverse roles in the pathogenesis of hematologic tumors and solid tumors.

Roles of Hypomethylated PRDM16 in Hematologic Tumors

PRDM16 was first reported to be expressed in t(1;3) (p36;q21)-positive leukemia cells and in the normal uterus and fetal kidney but not in the bone marrow or other tissues, indicating that ectopic expression of PRDM16 was specific to t(1;3)(p36;q21)-positive

myelodysplastic syndromes/acute myeloid leukemia (MDS/AML) in 2000 [3]. Following the discovery of PRDM16 in AML, researchers have not only focused on its physiological role in lipid metabolism [15–17], but have also increasingly concentrated on elucidating the molecular mechanisms underlying PRDM16 methylation status and their association with hematologic tumors development [18,19]. Genome-wide DNA methylation analyses of 64 pediatric AML patients demonstrated that significant methylation alterations at 9 CpG sites are associated with PRDM16 expression levels and methyl group abnormalities in AML. These findings suggest that PRDM16 DNA methylation may serve as a promising novel biomarker for pediatric AML [20,21]. Sodium bisulfite sequencing studies have confirmed that PRDM16 exhibits hypomethylation and is frequently expressed during the development of adult T-cell leukemia (ATL), indicating that the aberrant transcription of PRDM16 may be linked to DNA hypomethylation. Further investigations reveal that, the expression of PRDM16 in ATL cells is suppressed through DNA hypermethylation [22]. Collectively, these findings suggest that PRDM16 exhibits hypomethylation and plays significant and diverse roles in the pathogenesis of hematologic tumors (**Figure 2A**).

Roles of Hypomethylated PRDM16 in Solid Tumors

Evidence indicates that PRDM16 functions as a tumor suppressor based on hypomethylation status in various human malignancies, including lung cancer [23–25], renal cell carcinoma [26], colorectal cancer [27] and pancreatic cancer [28]. For example, studies have demonstrated that the promoter region of PRDM16 undergoes methylation, leading to its downregulation in lung cancer

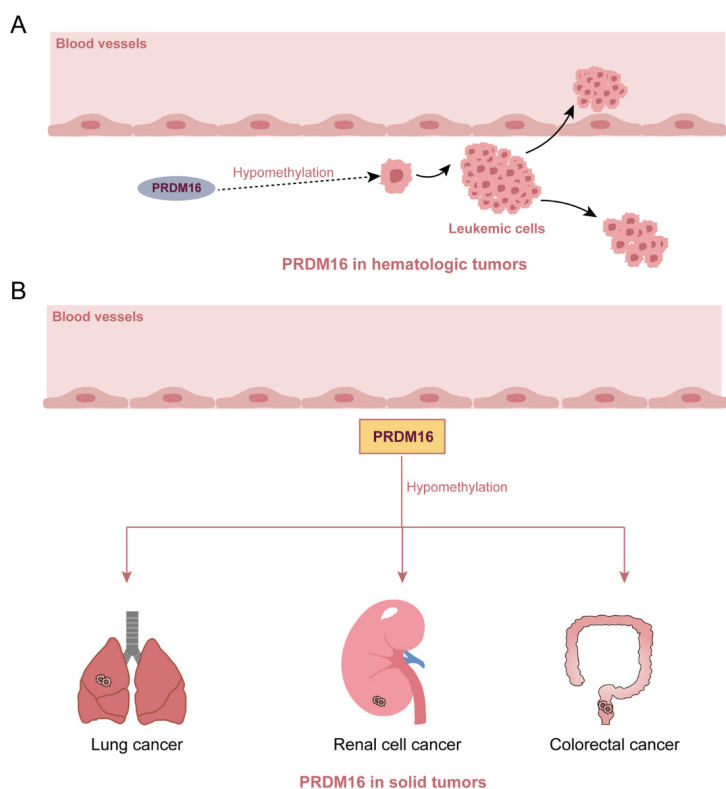


Figure 2. Roles of PRDM16 in hematologic tumors and solid tumors.

cells. Treatment with demethylating agents has been shown to restore *PRDM16* expression and suppress tumor cell proliferation [29]. Reduced or absent *PRDM16* mRNA expression has been observed in non-small cell lung cancer (NSCLC) tissues, correlating with higher methylation levels in tumor tissues compared to adjacent normal lung tissues [30]. Furthermore, in patients with lung adenocarcinoma (LUAD), *PRDM16* expression is frequently decreased due to copy number loss and extensive methylation. Patients with low *PRDM16* expression exhibit poorer overall survival compared to those with high expression level [31]. A separate study revealed that *PRDM16* is epigenetically silenced in renal cell carcinoma (RCC) through promoter hypermethylation. In this study, *PRDM16* has been shown to inhibit RCC cell growth both *in vitro* and in mouse xenograft models by suppressing Semaphorin 5B (*SEMA5B*) expression, highlighting its role as a tumor suppressor in RCC. This inhibitory function is mediated through PRDM16's interaction with the transcriptional co-repressor C-terminal binding protein (CTBP1/2) [32], which is essential for the downregulation of *SEMA5B* and tumor growth [33]. Our study found that methylation level of *PRDM16* was associated with CRC and lung metastasis of CRC by DNA methylation sequencing. Furthermore, we identified methylation sites within the promoter region of *PRDM16*. *PRDM16* expression was significantly lower in human CRC tissue samples and dramatically associated with tumor size, T stage, overall survival rates and disease-free survival rates of CRC patients. Down-regulation of PRDM16 significantly promoted proliferation, migration, and invasion of CRC cells by regulating epithelial-to-mesenchymal transition (EMT) pathway *in vitro* and *in vivo*. Decitabine which was a methylase inhibitor increased *PRDM16* expression and inhibited CRC progression *in vitro* and *in vivo*. Further study showed that PRDM16 interacted with peroxisome proliferator-activated receptor gamma (PPAR γ) in nucleus and upregulated its expression in CRC. Collectively, our study reported that DNA methylation-induced suppression of *PRDM16* in CRC metastasis through the PPAR γ /EMT pathway [34]. Furthermore, Izquierdo, *et al.* suggested that the methylation status of *PRDM16* may contribute to the initiation and progression of CRC through its involvement in visceral adipose tissue dysfunction [35]. Moreover, *PRDM16* has been identified as a candidate gene for pancreatic cancer liquid biopsy methylation markers [36], and may be the most likely candidate methylation marker for pancreatic cancer liquid biopsy in the future. The tumor-suppressive function of PRDM16 provides new insights into the molecular etiology of pancreatic ductal adenocarcinoma (PDAC) [37]. Collectively, these findings underscore that PRDM16 exhibits hypomethylation and plays significant and diverse roles in the pathogenesis of solid tumors (**Figure 2B**).

Future Perspectives

Studies utilizing cellular and animal models have demonstrated that PRDM16 may exert methyltransferase activity. As a member of the PRDM family, PRDM16 has received relatively limited attention regarding its methyltransferase function given the high homology between the PR domain of PRDM proteins and the SET domain. Further investigation is warranted to confirm its methyltransferase capabilities and to identify potential methylation sites. Furthermore, at present, only a few articles have identified the activity of PRDM16 as a methyltransferase, but there are no reports on the mechanism by which PRDM16 acts as a methyltransferase to affect tumor progression. In addition, research on the methylation

status of *PRDM16* has remained at the stage of identifying the DNA methylation status of *PRDM16*, and there are few articles exploring the mechanism of DNA methylation of *PRDM16*. Additionally, studies examining how methylation status influence PRDM16 functions, such as its roles in adipocyte differentiation, immune regulation, and tumor proliferation-are currently scarce and merit further exploration.

Furthermore, it has been reported that the tumor suppressor miR-101 could reverse the PRDM16 hypomethylation status thus suppressing its expression through direct epigenetic regulation, finally leading to mitochondrial disruption and apoptosis. After that, few non-coding RNAs have been reported to be involved in the DNA methylation process of *PRDM16* [38,39]. Additional research is necessary to determine whether the methylation status of *PRDM16* is predominantly regulated at the transcriptional, translational, or post-translational level.

Moreover, it was analyzed systematically that *PRDM16* was the m6A RNA methylation-related gene in liver hepatocellular carcinoma, including the expression, interaction, function, and prognostic values, which provided an important theoretical basis for *PRDM16* m6A RNA methylation in liver cancer [40]. Subsequently, additional research is necessary to determine whether the RNA methylation level of *PRDM16* is related to the tumorigenesis of solid tumor.

Credit Author Statement

WY, WYT drafted the manuscript. HA revised the manuscript.

Conflict of Interest Statements

None declared.

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