The Functions of PRDM3 in Different Cell Behaviors

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ABSTRACT

PRDM3 is a unique protein with an N-terminal PR domain and zinc finger motifs in the PRDM family and is very important in acute leukemia. PRDM3 has a special structural function with the particular PR domain that plays an essential role in acute leukemia and hematopoiesis. PRDM3 has unique mechanisms for different cell behaviors, including apoptosis, cell differentiation, and proliferation. These mechanisms are associated with leukemias. PRDM3 is also a protein that may have an enzyme function to modify chromatin and govern gene expression. Finally, these functions of PRDM3 in different cell behaviors showed that PRDM3 is a promising therapeutic target for leukemia.

Introduction

PRDM3 is a transcription factor in the family of PRDM (PR domain-containing) proteins, including an N-terminal PR-SET domain and an array of C2H2 zinc finger motifs [15] (Figure 1). PRDM3 is encoded by the MECOM locus (MDS1 and EVI1 complex locus). This locus is found in chromosomal band 3q26.2 [5]. It comprises the fusion of two coding genes, myelodysplasia syndrome 1 (MDS1) and ecotropic virus integration site 1 (EVI1), that have different transcription starting sites and isoform subgroups created by alternative splicing events [5] (Figure 1). As a result, PRDM3 is also known as MDS1-EVI1, and it is made as a naturally occurring splice variant in which the splicing event of MDS1 to EVI1 generates a novel protein containing a PR domain [12]. This PR domain possesses an N-terminal domain that neither MDS1 nor EVI1 has and has been determined to share homology with the PR domain and made MDS1-EVI1 become part of the PR domain protein (PRDM) family, termed PRDM3 [10]. Several studies suggested that PRDM3 plays a critical role in acute leukemia. Up to 10% of acute myeloid leukemia (AML) cases with poor survival outcomes have chromosomal rearrangements or proviral insertions at the PRDM3 locus gene [12]. The PRDM3 is a promising target for acute leukemia treatment.

Figure 1. Schematic for PRDM3 (or MDS1-EVI1), MDS1, and EVI1 proteins, indicating the location of the specific domain. The 110 amino acid PR/SET methyltransferase domain is shown in pink. Green denotes C2H2-zinc finger

motifs. The N- and C-terminal zinc finger domains are formed by zinc finger motifs 1 through 7 and 8 through 10. Yellow indicates the C-terminal binding protein (CtBP). EVI1 lacks the PR-SET domain (PR), is N-terminally truncated, and has zinc finger domains.

In terms of developing new therapeutic targets for acute infant leukemia, MECOM-PRDM3 is more promising compared with other signaling pathways. Acute leukemia is a kind of malignant clonal hematopoietic stem cell disease. It can occur in all ages and has the highest mortality rate of all malignant tumors under 35 years old [39]. In acute leukemias, most cases are linked with rearrangements of the MLL gene at chr11q23, up to 80% in acute lymphocytic leukemia (ALL) and 50% in acute myeloid leukemia (AML) cases, respectively [29]. These cases are often resistant to chemotherapy [34]. The 5' portion of the MLL gene joined with some leukemia associated Chr11q23 translocations [8]. These translocations cause the formation of MLL fusion proteins (MFP), whose leukemogenic activity is brought on by the abnormal activation of downstream genes, including DOT1L, HOX genes, and MECOM [19, 3]. The activation of these downstream genes promotes leukemogenic activation. Currently, the therapeutics targeted on the DOT1L gene and HOX gene failed since the activation of the HOX gene most likely results in the expansion of progenitor cells with the maintenance of immature phenotype. The exact role of DOT1L in leukemia is not clear [11], and a targeted inhibitor of DOT1L (EPZ5676) has revealed toxicity [6].

Therefore, many researchers started to focus on developing new therapeutics based on the axis of MECOM-PRDM3. MFP can bind to and upregulate the MECOM promoter, which causes overexpression of PRDM3 [3, 1, 37], and this overexpression is correlated with poor prognosis and resistance to chemotherapy of AML [3, 2]. Knocking down PRDM3 can increase the sensitivity to chemotherapy and suppresses the growth of AML cells [3, 1, 4]. Since PRDM3 is a non-essential gene for cellular and organismal survival [38], treatments that block PRDM3 activity are probably safe and well-tolerated. Furthermore, the function of the PR domain of PRDM3 in acute leukemia is very crucial to future targeted therapeutics development. The PR domain of PRDM3 is unique, which has H3K9 monomethyltransferase activity [27] and is critical for MFP transformation [37]. Therefore, investigating the function of the PR domain in PRDM3 is significant to acute leukemia, and Targeting the PR domain of PRDM3 can be an innovative therapeutic approach.

Moreover, PRDM3 can also work with PRDM16 and is essential in translocation-induced human acute leukemia. PRDM16 (also termed MEL1) resembles PRDM3 and shares 53% sequence identity with the N-terminus of PRDM3 [35]. The PRDM3 and PRDM6, via canonical PLDLS C-terminal binding protein (CtBP) -binding sites located between the two zinc finger motifs, bind to the CtBP (Figure 1) [15], which can lead to tumor cellular growth by suppressing transcription downstream of transforming growth factor-β signaling [15, 16, 17]. This part will primarily be discussed in the section on the function of PRDM 3 protein in cell differentiation. In 11q23-rearranged induced acute leukemias, PRDM3 and PRDM16 have been discovered to be crucial downstream elements of MLL/KMT2A translocation proteins [24]. However, the relationship between PRDM3 and PRDM6 in acute leukemia is unclear. Therefore, future studies should address whether PRDM3 or PRDM16 redundancy plays a role in leukemia problems.

Overall, PRDM3 may become a promising target for treating leukemia. Therefore, this review primarily discusses the functions of PRDM3 in different cell behaviors, including apoptosis, cell differentiation, and proliferation. Then this review also reveals that PRDM3 has an enzyme function to modify chromatin and govern gene expression. Finally, it highlights the importance of PRDM3 and its PR domain in medical development, points out future directions for developing PRDM3-based therapeutics for leukemia and gives the potential possibility of PRDM3 targeting medical applications and suggestions.

The Function of PRDM 3 Protein in Cell Apoptosis

PRDM3 has been found to lead to increased tumor cell proliferation and suppress cell apoptosis through multiple mechanisms. Since the PRDM3 is formed by combining the two genes: MDS1 and EVI1, through a naturally occurring

splice variant, the EVI1 is a PR-isoform that lacks the N-terminal PR domain, also named sPRDM3 (short PRDM3) and plays a vital role in cell apoptosis [5]. Apoptosis is the mechanism of programmed cell death and is regulated by many signaling pathways and genes[39]. C-Jun N-terminal kinase (JNK) is one of the signalings involved in the modulation of cell apoptosis, proliferation, and differentiation [7]. JNK signaling pathway can be triggered by extracellular stress stimuli such as UV light, tumor necrosis factor-α (TNF-α), γ-radiation, interleukin-1, and so on [20]. These extracellular stimuli can cause a group of JNK kinases to become active, which phosphorylates and activates JNK [20]. The role of sPRDM3 in the JNK pathway is to prevent the interaction between JNK and its substrate, such as c-Jun, by association with JNK via the first zinc finger domain of sPRDM3 [5, 20](Figure 2A). The PRDM3 can suppress the JNK signaling pathway to widely prevent cells from stress-induced cell death [20] (Figure 2). In the Kurokawa et al. (2011) research, they find sPRDM3 (EVi1) can suppress JNK to protect cells from UV light-induced apoptosis and can also effectively hinder the TNF-α-induced JNK1activation [20] (Figure 2B). Another mechanism of apoptosis is caused by the upregulation of Fas ligand (FasL) expression in T cells induced by stress activation of JNK [9]. The sPRDM3 can block the upregulation of FasL expression in T cells by inhibiting the JNK signaling pathway [20] (Figure 2C). Inhibition of JNK activity enhances the oncogenic potential of PRDM3 by preventing cells from apoptosis [5].

Since apoptosis is crucial in maintaining normal development and homeostasis, many anticancer drugs work by inducing apoptosis, which causes cancer cells to die [39]. Therefore, the key to developing a therapeutic drug target for leukemia is to induce apoptosis in the cancer cells. One of the drugs that have been found to treat cancer by exerting this mechanism is Arsenic trioxide (ATO). ATO is a long history of traditional Chinese drugs, which has been proven to be a successful treatment for acute promyelocytic leukemia (APL), particularly for newly diagnosed and relapsed patients [39, 22]. APL is the M3 subtype of acute myeloid leukemia (AML). The ATO can induce apoptosis in the cancer cell by downregulating the sPRDM3 expression and oncoprotein levels and blocking the suppressed effects of sPRDM3 on the JNK to activate the JNK signaling pathway [22] (Figure 3A). The ATO induces apoptosis in acute leukemia and provides the principle of medicine strategy for acute leukemia via targeting the PRDM3 [39]. Since the sPRDM3 lacks the N-terminal PR domain, the other isoform PRDM3 with the PR domain may be a potential target for other AML and acute leukemia types.

Additionally, Hypermethylated cancer one protein (HIC1) can induce leukemia cell apoptosis by repressing the PRDM3. PRDM3 suppressed apoptosis by modulating the transcriptional activity of the Bcl-xL, which is a multifunctional anti-apoptotic gene [28]. The sPRDM3 can target the Bcl-xL gene via the first zinc fingers, which inhibit apoptosis by upregulating Bcl-xL activity [28]. However, HIC1 can interact with the first zinc finger domain of sPRDM3 by its N-terminal domain, and this interaction deregulates sPRDM3's ability to the DNA binding and transcriptional activity in the BCL-XL promoter, which reduces sPRDM3 ability to inhibit apoptosis [5, 28, 23] (Figure 3B). Therefore, the PRDM3-mediated hindrance in apoptosis can be inhibited by HIC1 [28]. This implies that the innovative therapeutic approach to treat leukemia can be upregulating HIC1to target the PRDM3 and deregulate its activity in apoptosis.

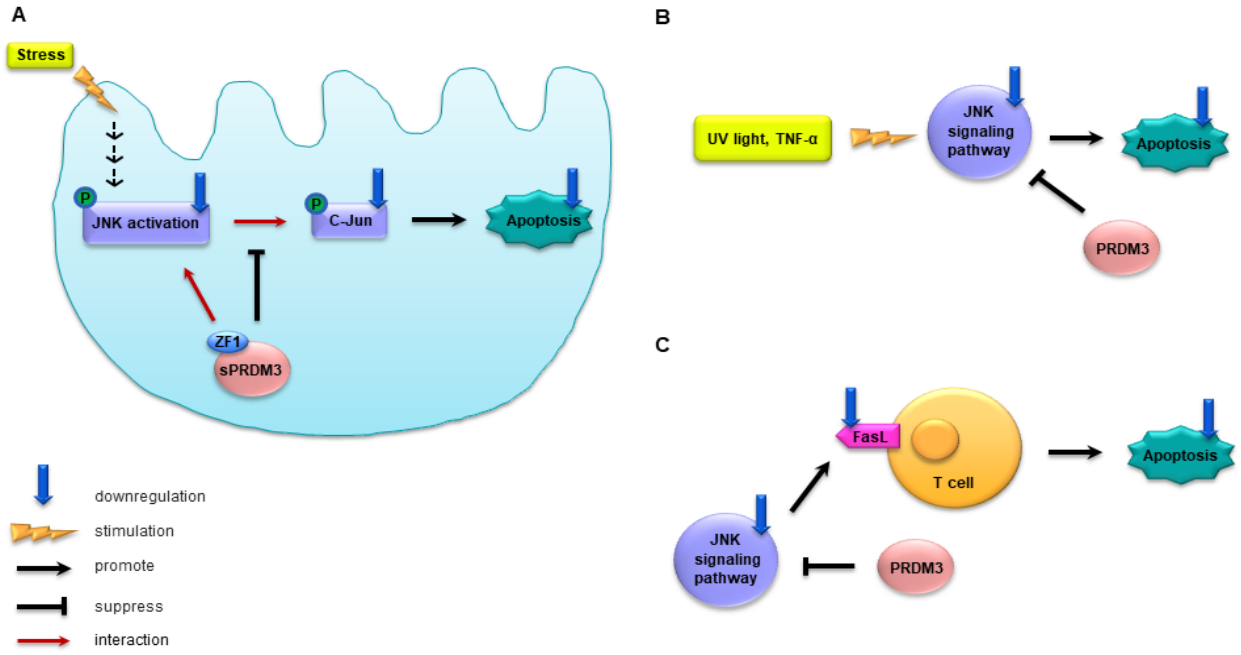

Figure 2. **(A)** The mechanism of sPRDM3 suppression of the stress activation of the JNK signaling pathway. sPRDM3 can bind with JNK via the first zinc finger domain. This interaction is necessary for efficient JNK inhibition, which can block the interaction between JNK and its substrate to prevent apoptosis. **(B)** PRDM3 protects cells from UV light-induced and TNF-α-induced apoptosis by inhibiting the JNK activation. **(C)** The PRDM3 can, by suppressing the JNK signaling pathway, block the apoptosis caused by the upregulation of FasL expression in T cells.

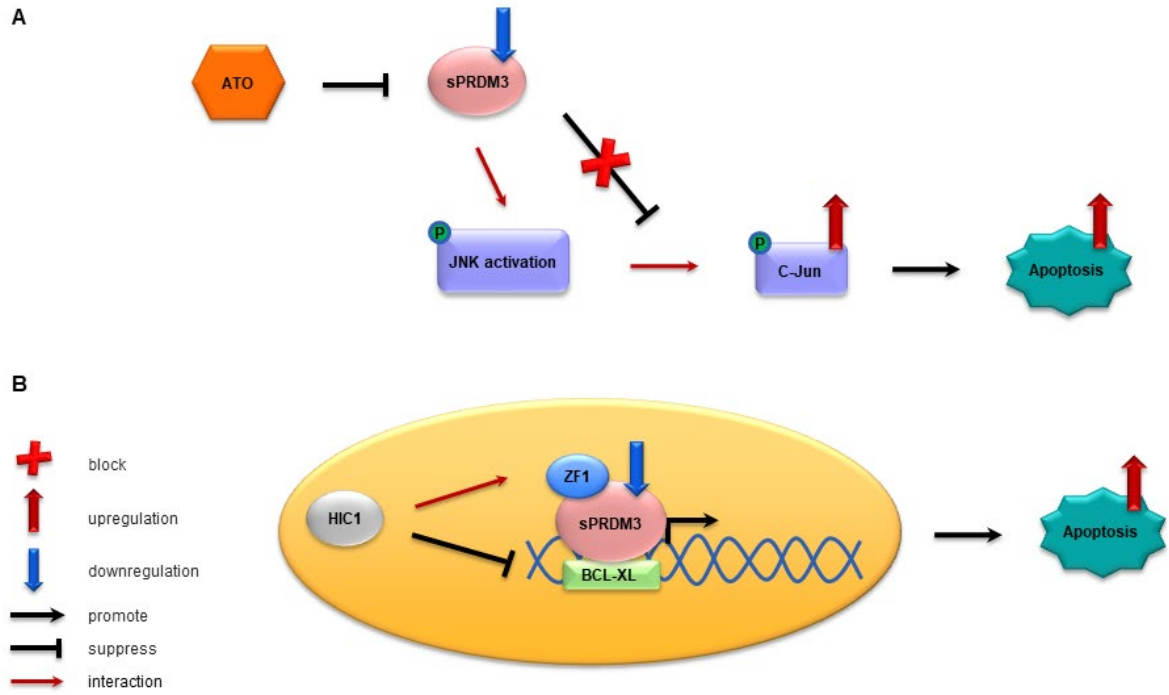

Figure 3. (A) ATO downregulates the sPRDM3 expression and blocks the suppressed effects of sPRDM3 on the JNK to activate the JNK signaling pathway and induce apoptosis in the cancer cell. **(B)** The HIC1 can interact with the first zinc finger domain of PRDM3 to deregulate PRDM3's ability to the DNA binding and transcriptional activity in the BCL-XL promoter, which reduces sPRDM3 ability to suppress apoptosis.

The Function of PRDM 3 Protein in Cell Differentiation

The PRDM3 can negatively modulate the TGF-β signaling, consequently controlling cell differentiation. The TGF-β belongs to the family of secreted cytokines and is not only crucial in normal epithelium differentiation and cell proliferation [5, 25] but also inhibits the proliferation of a wide range of cell types since the TGF-β is also a growth-regulatory factor [21]. In many malignancies, the TGF-β signaling is altered [5]. The PRDM3 can work with PRDM16 to suppress the TGF-β signaling. The PRDM16 interacts with SKI by stabilizing the inactive Smad3-SKI complex on the promoter of TGF-β target genes to suppress the TGF-β signaling [5]. The PRDM3, through its first zinc-finger domain motif, interacts with Smad3, thereby inhibiting the transcriptional activity of Smad3 [21] (Figure 4A). The Smad3 is an intracellular mediator of TGF-β signaling [21]. Therefore, the PRDM3 can negatively regulate TGFβ-signaling by suppressing the DNA binding activity of the Smad3 complex [5]. TGF-β signaling suppresses tumor growth in normal cells by causing apoptosis [5]. Therefore, the therapeutic mechanism of targeting PRDM3 can be the inhibition of PRDM3 activity, which can help regulate the TGF-β signaling, maintain normal cell differentiation, and hinder tumor cell growth.

The PRDM3 can interact with the RUNX1 and inhibit myeloid cell differentiation leading to apoptosis. The RUNX1 is a member of the RUNX family and plays an essential role in the modulating development and maintenance of mammalian hematopoiesis[32, 14] and regulates the expression of several genes involved in the execution of myeloid differentiation programs [32]. Thus, aberration of RUNX1 can cause gene deregulation and leads to the deregulation of hematopoietic programs that result in leukemia[32]. The PRDM3 can, through its 8th zinc finger motif, interact with RUNX1, and this interaction can lead to a reduction of DNA-bound RUNX1 and the inhibition of the gene regulated by RUNX1 [32] (Figure 4B). In the myeloid cell line, it has been found that many genes necessary for myelopoiesis are activated by RUNX1, but PRDM3 blocks the differentiation of myeloid cells and leads to apoptosis [32, 26]. However, the mechanism of this inhibition is still unknown, but the PRDM3 may disrupt DNA-binding transcription factors of RUNX1 that regulate myelopoiesis [32, 26]. Overall, PRDM3 can interact with RUNX1to weakens RUNX1's DNA-binding activity, impairs RUNX1's function, disrupts the myeloid programs, and deregulates hematopoietic programs, ultimately leading to myeloid leukemia [32]. These discoveries contribute to a deeper comprehension of acute leukemias and point to potential new molecular targeted treatments in the future.

The PRDM3 can interact with chromatin modulators NuRD complex to form the protein complexes that cofactor-dependent regulate the stem cell differentiation, development, and neuronal system [23]. The NuRD is the chromatin remodeling complex that can modulate the transcription of genes related to pluripotency and governs the cellular response to differentiation signals in mouse embryonic stem cells (ESCs) [23]. Additionally, the FOG 1/2 proteins are a PRDM factor and can apply their function in interacting with the NuRD complex [23]. PRDM3 can work with PRDM16 proteins to interact with the NuRD complex via the RB binding protein 4 (RBBP4) [15, 23]. The RBBP4 is a chromatin remodeling factor and a mediator that can help chromatin bind the NuRD complex by attaching to histone H3 tails [23, 31]. The FOG1 and FOG2 also, through their N-terminal amino acid sequence, interact with the NuRD complex [23]. Thus, PRDM3/16 and FOG1/2 form a cofactor-dependent regulation mechanism to control gene expression (Figure 4C). PRDM3 and PRDM16 can interact with RBBP4 and the NuRD complex, and these interactions may have a regulatory effect on how neurons are identified and where they are located in different brain structures [23]. FOG1/NuRD complex can inhibit the GATA1 and GATA2 expression, and this suppression can induce hematopoiesis and pause stem cell differentiation and development [23]. Yet, the function of the FOG2/NuRD complex still needs to be discovered [23]. Therefore, the cofactor-dependent regulation of PRDM3/16 and FOG1/2 plays a vital role in stem cell differentiation, development, and neuronal system. These findings will help us better

understand the function of PRDM3 in directing gene expression and identify how PRDM3 interacts with other proteins and molecules.

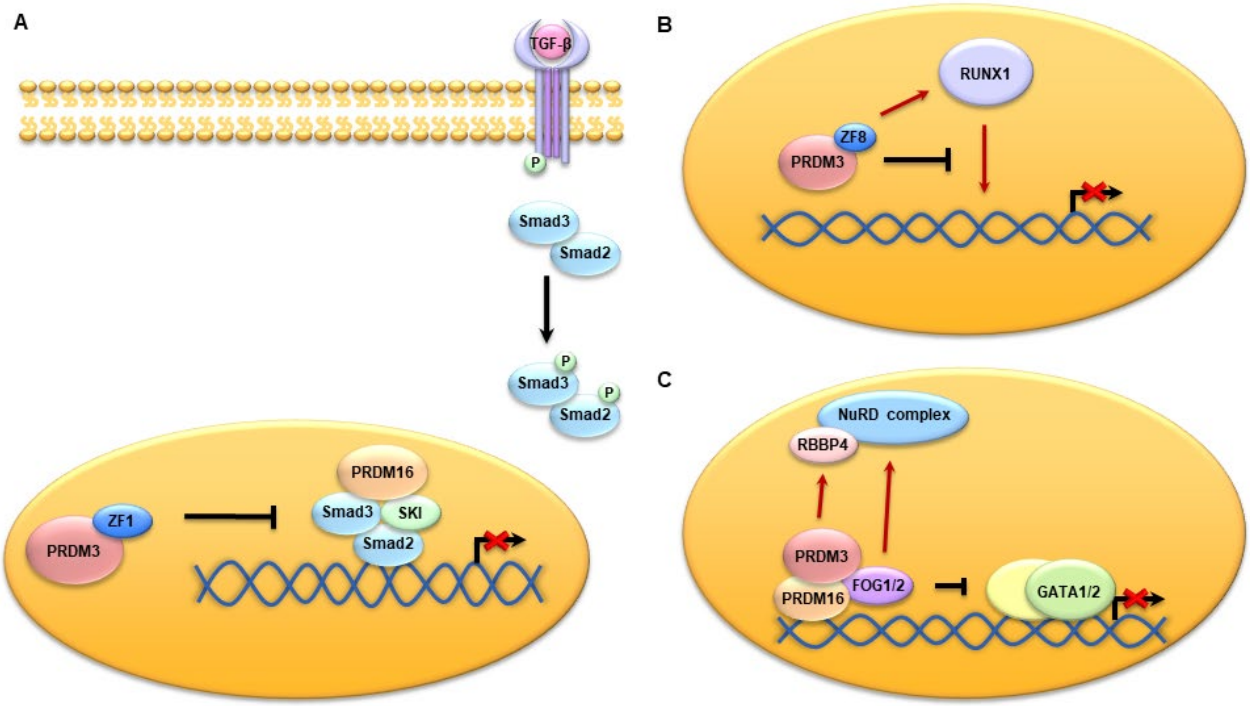

Figure 4. **(A)** The modal of the PRDM3 negatively regulates TGF-β signaling. The PRDM16 associates with SKI and inhibits TGF-β signaling by stabilizing the inactive Smad3-SKI complex. PRDM3 negatively regulates TGF-β signaling through binding and inactivating SMAD3 proteins. **(B)** The PRDM3, through its 8th zinc finger motif, interacts with RUNX1. This interaction can reduce DNA-bound RUNX1 and suppress the gene regulated by RUNX1, which finally inhibits myeloid cell differentiation leading to apoptosis. **(C)** The mechanism of cofactor-dependent regulation of PRDM3/16 and FOG1/2. PRDM3/16 and FOG1/2 can NuRD complex negatively regulate GATA1 and GATA2 gene expression to rest cellular differentiation.

The Function of PRDM 3 Protein in Stem Cell Proliferation

During fetal development, PRDM3 plays a critical regulatory function during fetal development in maintaining hematopoietic stem cells and progenitor cells (HSPC) [23]. GATA2 is a transcription factor that plays an essential role in HSC development and proliferation, and the GATA2 mutations are linked to AML. PRDM3 can promote HSPC proliferation through the activation of GATA2 transcription [36], and the mutation of GATA2 also contributes to the acceleration of sPRDM3-driven leukemogenesis [18]. In early hematopoiesis, PRDM3 can promote HSPC proliferation via the upregulation of GATA-2 and suppress TGF-β signaling [30] (Figure 5A). There may be interactions between these two pathways. Sato et al. (2008) find that GATA-2 may suppress TGF-β- signaling, and TGF-β may change GATA-2 activity at a post-transcriptional level [30]. Therefore, the PRDM3 is the critical target in regulating HSPC proliferation which is very important for treating leukemias.

PRDM3 can also directly regulate the GATA2 by interacting with histone acetyltransferase p300/CBP association factor (PCAF) to increase HSPC proliferation. In leukemia cells and HSPC, the PRDM3 can be acetylated by interaction with PCAF at Lys564 [33] (Figure 5B). PRDM3's capacity to bind the GATA2 promoter region is markedly improved by the acetylation of Lys564 [33]. Therefore, through interaction with PCAF, the acetylation of PRDM3

at Lys564 increases the PRDM3's DNA binding ability and thereby contributes to the activation of GATA2 transcription [33]. These findings indicate that PRDM3 is a vital regulator of the GATA2 gene expression and plays a crucial role in hematopoietic stem cell proliferation.

Another mechanism of PRDM3 to promote stem cell proliferation is to interact with BRG1 in an embryonic fibroblast cell line (NIH 3T3) [23]. BRG1 is a part of the SWI/SNF complex, and by remodeling the chromatin structure and making it more accessible to transcription factors, SWI/SNF primarily promotes gene expression [23]. The E2F1 is a transcription factor that can promote cell proliferation. BRG1 can drastically suppress the activity of the E2F1 promoter, which lowers the stem cell proliferation rate [23] (Figure 5C). However, PRDM3 can prevent this suppression by interacting with BRG1 to promote stem cell proliferation [23]. Repressing the PRDM3 would inhibit stem cell proliferation, a new direction for therapeutics to treat leukemia. Consequently, the inhibition of PRDM3 may be an efficient therapeutic approach to inhibit the growth of leukemias.

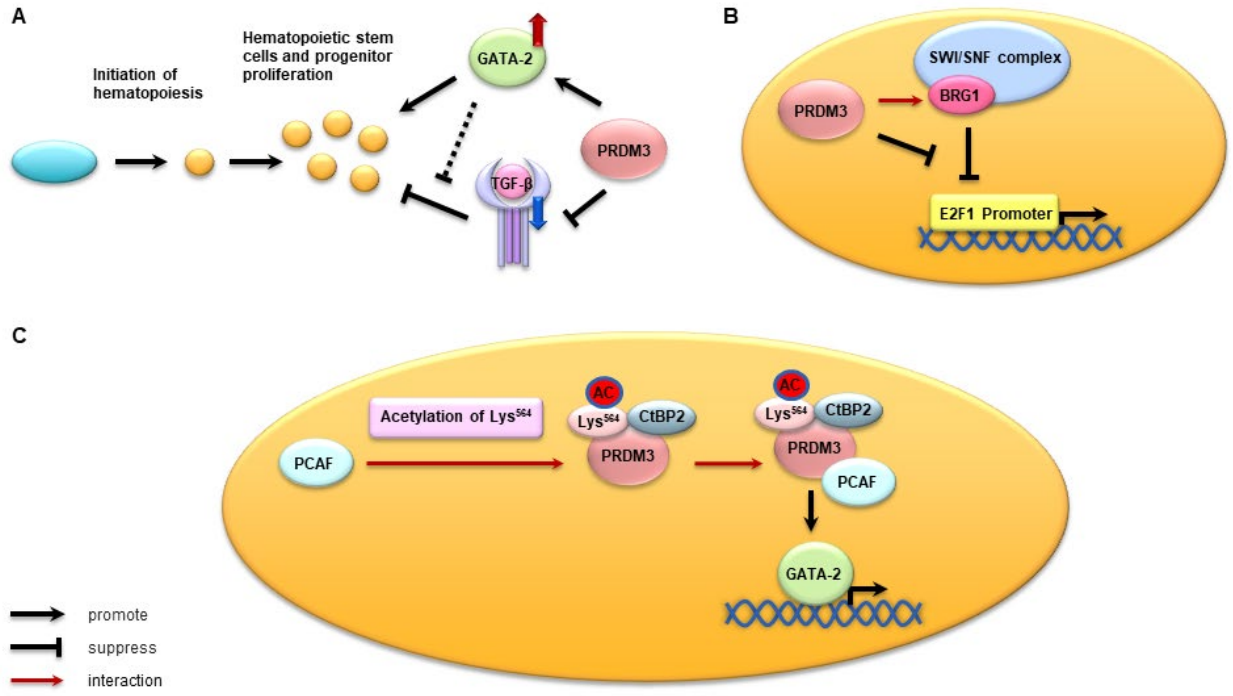

Figure 5. **(A)** The mechanism of the PRDM3 regulation of the hematopoietic stem cells and progenitor cell proliferation. PRDM3 can upregulate the GATA-2 transcription and inhibit the TGF-β signaling from modulating the HSC progenitor cell proliferation. **(B)** The PRDM3 can be acetylated by interaction with PCAF at Lys^{56}. By association with PCAF, the acetylation of PRDM3 at Lys^{564} increases the PRDM3's DNA binding ability and thereby contributes to the activation of GATA2 transcription. **(C)** BRG1 can primarily suppress the activity of the E2F1 promoter, which reduces the rate of stem cell proliferation. PRDM3 can interact with BRG1 to block this inhibition and promote stem cell proliferation.

The Function of PRDM3 as a Chromatin-Modifying Enzyme

It is well known that PRDM3 primarily contains an N-terminal PR-SET domain and an array of zinc finger motifs. The N-terminal PR-SET domain (PR-domain) is classified as a type of SET domain, and it's unique and has biology activity [15]. Some research finds that the PR-domain of PRDM3 has the function of an H3K9me1 methyltransferase

which was able to modify chromatin by adding a mono-methyl group to H3K9 [15, 27, 13]. In Mammalian, heterochromatin plays a vital role in stabilizing gene expression and maintaining genome integrity. The PRDM3 can help to initiate and sustain heterochromatin organization with PRDM16 [27]. The PRDM3 can catalyze H3K9me1 on free histone H3 in the cytoplasm with PRDM16, then transport them to the nucleus and integrated into nucleosomes, where it is converted to H3K9me3 by the Suv39h enzymes at heterochromatic foci [27] (Figure 6). PRDM3 is a transcription factor that highly specifically binds sequence-specific DNA through the first zinc finger and second zinc finger binding domains [13]. Since the PR domain of PRDM3 possesses HMT activity and transcription factor functions, PRDM3 may be able to choose which parts of the genome to bind and how and when to regulate gene transcription through histone modification [13]. Additionally, the HMT activity of PRDM3 is necessary for MLL-AF9-induced leukemogenesis [37, 13]. These findings are significant for PRDM3 and its PR domain in medical development and provide the possibility for suppressing the HMT activity of the PR domain of PRDM3 to treat MLL fusion protein leukemias.

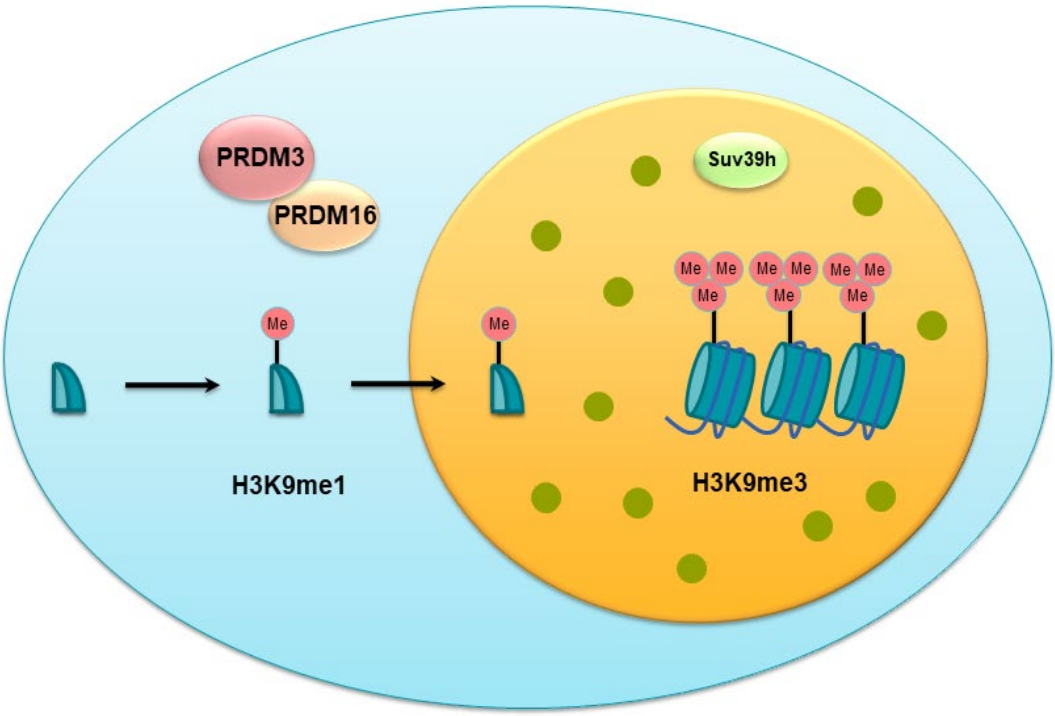

Figure 6. The mechanism of the PRDM3 initiating and maintaining heterochromatin organization with PRDM16. The PRDM3 works with PRDM16 to modify chromatin by adding a mono-methyl group to H3K9, then transports them to the nucleus and integrated into nucleosomes, where it is converted to H3K9me3 by the Suv39h enzymes at heterochromatic foci (green dots).

Discussion

At the beginning of this article, we emphasized that PRDM3 and its PR domain can be a prospected potential drug target. The most significant advantage of PRDM3 as a drug target is that PRDM3 is a non-essential gene for cellular and organismal survival, and as a treatment that blocks its activity is less toxic and well-tolerated. PRDM3 can be a therapeutic intervention target that focuses on developing PRDM3 inhibitors in acute leukemias since the PRDM3 inhibitors can be applied to induce cancer leukemia cell apoptosis, inhibit excessive proliferation of stem cells, block tumor growth differentiation, and promote stem cell normal differentiation.

The PRDM3 can inhibit the stress activation of the JNK signaling pathway and the transcriptional activity of the Bcl-xL gene. This suppression enhances the oncogenic potential of PRDM3 by preventing cells from apoptosis [5]. Therefore, the therapeutic mechanism of targeting PRDM3 can be inhibited the activation of PRDM3 in the apoptosis cell and induce apoptosis to lead the cancer cell to die. The typical drug that has been found to treat cancer by exerting this mechanism is Arsenic trioxide (ATO). The ATO can induce apoptosis in acute leukemia by targeting sPRDM3, providing the principle of medicine strategy for acute leukemia via targeting the PRDM3. The HIC1 can also target and inhibit PRDM3 to induce leukemia cell apoptosis. This provides an innovative therapeutic approach to treat leukemia by upregulating HIC1to target the PRDM3 and deregulate its activity in apoptosis.

Moreover, the inhibition of PRDM3 activity can help to regulate the TGF-β signaling, maintain normal cell differentiation, and hinder tumor cell growth. It can also help interrupt the association between PRDM3 and RUNX1 to maintain hematopoiesis and promote myeloid cell differentiation. The suppression of PRDM3 can also break up its interaction with chromatin modulators NuRD complex, which contribute to regulating stem cell differentiation and maintaining the neuronal system. Consequently, the inhibition of PRDM3 may be an efficient therapeutic approach to block tumor growth differentiation and promote myeloid cell and stem cell normal differentiation.

Furthermore, PRDM3 can promote stem cell proliferation through many different mechanisms, including upregulation of GATA-2 transcription and inhibition of the TGF-β signaling, and interaction with PCAF to direct activation of GATA2, association with BRG1 to activate of E2F1 promoter. Therefore, repressing the PRDM3 would inhibit stem cell proliferation, a new direction for therapeutics to treat leukemia.

However, for the PR domain, the drug should focus on the characterization of the PR domain structure and preventing its HMT activity because the PR-domain of PRDM3 has the function of an H3K9me1 methyltransferase which was able to modify chromatin by the addition of a mono-methyl group to H3K9 to regulate the gene expression [15, 27, 13]. Thus, investigating the enzyme function of the PR domain of PRDM3 and identifying how the PRDM3 interacts with other proteins is also essential for future therapeutic development.

Overall, this review revealed the functions of PRDM3 in different cell behaviors, including apoptosis, cell differentiation, and proliferation. Additionally, it pointed out that PRDM3 has an enzyme function to modify chromatin and direct gene expression. All these mechanisms suggested that PRDM3 and its PR domain are prospective therapeutic targets for acute leukemia. However, the most significant challenge in drug development for PRDM3 is that there are still many unclear functions and mechanisms, such as protein interaction, cell migration, etc. There are a lot of crucial questions we are still unknown such as which specific function PRDM3 has. So in the future, the direction of PRDM3 research can focus on investigating the function and behaviors of PRDM3, particularly in protein interaction, cell apoptosis, and migration.

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