The Multifaceted Roles of PRDM16: Adipose Biology and Beyond

Jingyi Chi¹ and Paul Cohen¹,*

The PRDM [PRDI-BF1 (positive regulatory domain I-binding factor 1) and RIZ1 (retinoblastoma protein-interacting zinc finger gene 1) homologous domain containing] protein family is involved in a spectrum of biological processes including cell fate determination and development. These proteins regulate transcription through intrinsic chromatin-modifying activity or by complexing with histone-modifying or other nuclear proteins. Studies have indicated crucial roles for PRDM16 in the determination and function of brown and beige fat as well as in hematopoiesis and cardiac development, highlighting the importance of PRDM16 in developmental processes in different tissues. More recently, PRDM16 mutations were also identified in humans. The substantial progress in understanding the mechanism underlying the action of PRDM16 in adipose biology may have relevance to other PRDM family members, and this new knowledge has the potential to be exploited for therapeutic benefit.

The PRDM Protein Family

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 (see Glossary). 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]. PRDM proteins modulate crucial cellular processes including cell fate decision, and aberrant function of some members may lead to malignant transformation [1,2]. PRDM proteins play important roles in the development of various cell types (Table 1). For example, PRDM1/BLIMP1 is involved in lymphocyte and germ cell development [3–5], whereas PRDM4, PRDM8, PRDM12, and PRDM13 have important roles in the development of the nervous system [6–15]. In addition, dysregulation of specific members is associated with cancer; PRDM2/RIZ and PRDM5 are tumor suppressors that can induce growth arrest and apoptosis, and are inactivated in several different human cancers, either genetically or epigenetically [16–18].

The mechanisms of action for several PRDM family members have been delineated over the past few decades. Experimental evidence has shown that the PRDM proteins act as transcriptional regulators either through intrinsic chromatin-modifying activity or through recruitment of cofactors and chromatin modifiers in a cell- and promoter-specific context to regulate cellular proliferation and differentiation [1,2]. So far, PRDM2, -3, -6, -8, -9, -13, -16 have been demonstrated to possess intrinsic histone methyltransferase activity [1,14,19]. Specifically, PRDM3 and PRDM16 were found to catalyze mono-methylation of lysine 9 of histone 3 (H3K9me1) [19], while PRDM2 and PRDM8 possess HMT activity to dimethylate H3K9 (H3K9me2) [20,21]. Moreover, PRDM9 catalyzes mono-, di-, and trimethylation of lysine 4 of histone 3 (H3K4me1, H3K4me2, and H3K4me3) [22,23].

PRDM16 plays a key role in adipose biology, regulating the determination and function of brown and beige fat. PRDM16 represents a promising therapeutic target for the treatment of obesity and obesity-related diseases.

PRDM16 is a master transcriptional coregulator in brown adipocytes. It forms complexes with various transcriptional cofactors in a promoter-dependent context, acting bifunctionally to promote expression of brown fat-selective genes and repress white-selective genes.

PRDM16 also regulates hematopoietic and neural stem cell maintenance, patalogensis, and cardiac development. Disruption in PRDM16 function affects cardiac development and leukemogenesis in humans.

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## Table 1. An Overview of the Biological Functions of PRDM Family Proteins

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<tr>
<th>Gene Name</th>
<th>Function</th>
<th>Related Diseases</th>
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<tr>
<td></td>
<td>Master regulator of plasma cell differentiation [3]</td>
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<tr>
<td></td>
<td>Regulates T cell and natural killer cell homeostasis [3,4]</td>
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<td></td>
<td>Controls primordial germ cell fate [5]</td>
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<td>PRDM2 (RIZ, RIZ1, RIZ2)</td>
<td>Coactivator of estrogen-related gene transcription [78].</td>
<td>Retinoblastoma, diffuse large B cell lymphoma (DLBCL)</td>
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<td></td>
<td>Has tumor-suppressive function; overexpression induces apoptosis and differentiation while repressing proliferation in chronic myelogenous leukemia (CML) [16,17]</td>
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<td>PRDM3 (MECOM, EVI-1, MDS1/EVI-1)</td>
<td>Positively regulates proliferation and maintenance of hematopoietic stem cells in mice [79,80]</td>
<td>Acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML)</td>
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<td></td>
<td>Regulates TGFβ signaling pathway [71,72]</td>
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<td></td>
<td>Inhibits c-Jun N-terminal kinase (JNK) activity, preventing stress-induced apoptosis [81]</td>
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<td></td>
<td>Maintains integrity of mammalian heterochromatin [19]</td>
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<tr>
<td>PRDM4 (SC1)</td>
<td>Binds p75 neurotrophin receptor and transduces NGF signaling [82]</td>
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<td></td>
<td>Interacts with PRMT5 to maintain proliferative capacity of neural stem cells [6]</td>
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<tr>
<td>PRDM5</td>
<td>Putative tumor suppressor; expression is reduced in multiple cancer cell lines [18]</td>
<td>Brittle cornea syndrome 2, neupenia</td>
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<td></td>
<td>Regulates genes involved in hematopoiesis, development [83], cell adhesion, and extracellular matrix formation [84]</td>
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<td></td>
<td>Antagonizes Wnt signaling pathway in normal and cancer cells [18,85]</td>
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<tr>
<td>PRDM6 (PRISM)</td>
<td>Regulates vascular smooth muscle differentiation [86]</td>
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<td></td>
<td>A separate isoform, with no role in regulating smooth muscle fate, induces cell cycle arrest and apoptosis in endothelial cells [23]</td>
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<tr>
<td>PRDM7</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>PRDM8</td>
<td>Negatively controls testis steroidogenesis in mice [21]</td>
<td>Early-onset Lafora body disease</td>
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<td></td>
<td>Regulates neocortical development [7] and retina development and maintenance [87]</td>
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<td>PRDM9 (MESETZ)</td>
<td>Controls the initiation and progression of meiosis [88]</td>
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<td></td>
<td>Major regulator of chromosomal recombination hotspots in humans and mice [89]</td>
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<tr>
<td>PRDM10 (TRISTANIN)</td>
<td>Expressed in mesoderm-derived tissues and may function in tissue differentiation [90]</td>
<td>Undifferentiated pleomorphic sarcoma</td>
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<td>PRDM11</td>
<td>Tumor suppressor of Myc-driven lymphoma that regulates the transcription of certain oncogenes [91]</td>
<td>Diffuse large B cell lymphoma (DLBCL)</td>
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<td>PRDM12</td>
<td>Regulates sensory neuronal specification and plays an important role in nociception [10,11]</td>
<td>Congenital insensitivity to pain, hereditary sensory and autonomic neuropathy</td>
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Table 1. (continued)

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<thead>
<tr>
<th>Gene name</th>
<th>Function</th>
<th>Related Diseases</th>
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<tr>
<td>PRDM13</td>
<td>Controls the balance between inhibitory and excitatory neurogenesis during development [13,14]</td>
<td>Medulloblastoma</td>
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<td></td>
<td>Maintains sleep quality, body weight and adiposity when expressed in the dorsomedial hypothalamus [92]</td>
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<td></td>
<td>Regulates amacrine subtype specification in the mouse retina and modulates visual acuity [15]</td>
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<tr>
<td>PRDM14</td>
<td>Regulates embryonic stem cell pluripotency [93]</td>
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<td></td>
<td>Induces the generation of mouse primordial germ cells [5]</td>
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<tr>
<td>PRDM15</td>
<td>Unknown</td>
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<tr>
<td>PRDM16</td>
<td>Regulates brown/beige fat identity and function [27,36]</td>
<td>Cardiomyopathy</td>
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<tr>
<td>MEL1</td>
<td>Regulates oxidative stress in hematopoietic stem cells and neural stem cells [51,52]</td>
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<td></td>
<td>Plays a role in palatogenesis [53]</td>
<td></td>
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<tr>
<td>PRDM17</td>
<td>Unknown</td>
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histone 3 (H3K4me1, -2, and -3) [22]. Most PRDM proteins have HMT activity towards histone 3; however, PRDM6, is an exception because it is an H4K20-specific HMT [23]. Notably, H3K9 methylation is associated with transcriptional repression, whereas H3K4me3 correlates strongly with transcriptional activation. The intrinsic HMT activity of these PRDM proteins allows them to control gene expression through direct modification of chromatin state.

PRDM proteins can also interact with a variety of histone-modifying proteins such as histone deacetylases (HDACs), histone acetyltransferases (HATs) and histone lysine methyltransferases (HMTs), as well as other transcriptional activators or repressors, to coordinately regulate gene expression. Commonly shared interacting chromatin-modifying enzymes among the PRDM family members are euchromatic histone-lysine N-methyltransferase 2 (EHMT2, also known as G9a), HDAC1–3, and p300 [1]. Interestingly, several PRDM proteins associate with protein partners in a cell- and promoter-specific manner. For example, PRDM1/BLIMP silences interferon (IFN)-β by recruiting EHMT2 to the promoter to repress transcription [24], while in primordial germ cells, PRDM1/BLIMP complexes with protein arginine methyltransferase 5 (PRMT5), an arginine HMT that catalyzes symmetrical dimethylation of arginine 3 on H2A and H4 [25].

The PRDM gene family is also characterized by the presence of multiple isoforms generated by alternative splicing or by alternative promoter usage. For example, PRDM1, -2, -3, and -16 have multiple isoforms, and two well-described isoforms are the PR-containing and the PR-lacking isoforms generated from alternative promoter usage [2]. Typically, the PR-lacking isoforms of these PRDM members are often found to be present or upregulated while the PR-containing isoforms are repressed in multiple cancer types, suggesting that the PR-lacking isoform functions as an oncogene whereas the PR-containing isoform behaves as a tumor suppressor [2]. It is possible that the balance of the variants regulates normal cellular homeostasis by chromatin-mediated control of gene expression.

Recent reviews have presented comprehensive descriptions of the PRDM protein family [1,2]. This review focuses on and discusses the significant recent advances that have been made
PRDM16 determines brown fat identity [27].

Figure 1. An Overview of the Role of PRDM16 in Adipose Tissue. Major findings on the role of PRDM16 in adipose biology during the past 8 years are highlighted chronologically. Abbreviations: C/EBPβ, CCAAT/enhancer binding protein β; CTBP1, C-terminal binding protein 1; EHMT1 euchromatic histone-lysine N-methyltransferase 1; MED1, Mediator 1 subunit; miR, microRNA; PPARγ, peroxisome proliferator activated receptor; PRDM16, PR domain containing 16.

regarding the physiological role of PRDM16 and its mechanism of action (Figure 1). Collectively, these emerging findings have broad implications that may be instructive to further understanding the biology of PRDM proteins as a class.

Molecular Structure of PRDM16

Human PRDM16 is on chromosome 1p36, and encodes a protein with four isoforms, as described on UniProtKB/Swiss-Prot (Figure 2A). Among them, two isoforms have been most extensively studied: a full-length 170 kDa PRDM16/MEL1 and a short-form 150 kDa PRDM16/MEL1S, lacking the PR domain at the N terminus, which is produced by an alternative transcript derived from an internal promoter [26]. However, the other two isoforms that result from alternative splicing of the full-length PRDM16/MEL1 transcript have no defined biological roles.

Mouse Prdm16, located on chromosome 4qE2, is expressed in a similar fashion (Figure 2B). However, a mouse isoform of the PR-lacking PRDM16/MEL1S has not been described. Although PRDM16 is expressed in various tissues in mice, such as brown adipose tissue, brain, heart, kidney, and lung, and multiple transcripts of different sizes are detected by Northern blot [27], it remains unknown whether one of these transcripts produces the mouse homolog of human PRDM16/MEL1S. The full-length PRDM16 consists of an N-terminal PR domain and two clusters of C2H2-type zinc fingers: zinc finger 1 (ZF1) containing seven zinc fingers at the N-terminal region and ZF2 with three zinc fingers at the C-terminal region. In addition, it contains a proline-rich domain, a repressor domain, and a C-terminal acidic domain, although the function of these domains has not been elucidated [26].
Physiological and Molecular Roles of PRDM16 in Adipose Tissue

PRDM16 plays a key role in adipose biology, with implications for several physiological processes including energy homeostasis, glucose and lipid metabolism, and body weight regulation.

White, Brown, and Beige Adipose Tissues

Mammals have white and brown adipocytes [28]. White fat cells store energy as triacylglycerol and can become inflamed in the setting of obesity. Brown fat cells, by contrast, dissipate energy as heat, with anti-obesity and favorable metabolic effects. The beneficial actions of brown fat rely on a thermogenic program that uncouples oxidative phosphorylation from ATP synthesis, via the action of the brown fat enriched protein, uncoupling protein 1 (UCP1), that helps to convert chemical energy into heat.

Both white and brown adipocytes require the master regulator peroxisome proliferator-activated receptor γ (PPARγ) for their differentiation and maintenance [29], suggesting that other factors...
are responsible for endowing white and brown fat cells with their unique properties. PPARγ coactivator 1α (PGC-1α), a key regulator of energy metabolism and of mitochondrial biogenesis and function, was identified as a cold-induced, brown fat enriched coactivator of PPARγ [30]. While deletion of PGC-1α was shown to reduce thermogenesis in brown adipocytes, the cells maintained their brown fat identity [31]. This prompted a search for a *bona fide* brown fat identity factor and led to the discovery of PRDM16 in a screen of transcriptional components enriched in brown relative to visceral white fat [27].

In addition to classical brown fat, which forms early during embryogenesis from a precursor cell line that is shared with skeletal muscle [32], mice and humans also have brown-like adipocytes, termed beige adipocytes, that are embedded within white fat, particularly in the subcutaneous depots [33]. Beige cells have similar thermogenic properties as classical brown fat, and they can be induced to express UCP1, but come from a different developmental lineage [33]. Beige fat activity (referred to as ‘*browning*’ or ‘*beiging*’) is highly inducible in response to cold, β-adrenergic agonists, thiazolidinediones (TZDs), and exercise [34]. PRDM16 is also expressed in beige adipocytes, and genetic studies indicate that it is required for beige cell function [35,36].

**PRDM16 Actions in Adipose Tissue *In Vivo***

PRDM16 was identified as a master transcriptional coregulator that determines brown fat differentiation and identity [27]. Forced expression of PRDM16 in white fat cells was able to induce the entire molecular program of brown fat cells. Moreover, transgenic mice that over-express PRDM16 in all fat cells exhibit clusters of multicellular brown-like fat cells within their white fat [27,35], as well as increased energy expenditure and marked improvements in insulin sensitivity while on a high-fat diet, despite only modest protection from dietary obesity [35].

Deletion of PRDM16 in all fat cells, using an adiponectin–Cre line that is expressed late during development of adipose tissue and after the classical brown fat has formed, causes complete ablation of beige fat function, severe insulin resistance, and hepatic steatosis, despite only mild obesity [36]. In the absence of PRDM16, the adipose tissue inflammation was prominent in high-fat fed mice, with larger fat cells and increased macrophage accumulation. Moreover, subcutaneous fat cells began to strongly express visceral fat-selective genes, indicating that PRDM16 is a key regulator of the subcutaneous versus visceral fat phenotype. Classical brown fat is relatively unaffected in this model, likely because it forms early during embryogenesis, before the adiponectin–Cre-mediated deletion of PRDM16. In addition, deletion of PRDM16 using the myogenic factor 5 (Myf5)–Cre line that drives expression of Cre in myoblast progenitors, during the formation of brown fat, results in compensation by the closely related protein PRDM3 [37], which is also expressed in developing brown fat. When both PRDM16 and PRDM3 were deleted in developing brown fat, animals developed an early and severe defect in brown fat formation.

**Molecular Basis of PRDM16 Action in Adipose Tissue**

**Interaction with Other Transcriptional Regulators**

PRDM16 binds directly to DNA through its two zinc-finger domains (ZF1 and ZF2) [26]. However, PRDM16 promotes the brown fat-selective gene program mainly through protein–protein interactions with various transcription factors rather than via sequence-specific DNA binding [27,32,38-40] because PRDM16 with a point mutation in the consensus zinc-finger DNA-binding motif mostly retains its ability to induce brown fat differentiation [27]. A lineage-tracing study by Seale *et al.* demonstrated that brown adipocytes originate from Myf5+ myoblastic precursors [32]. Further mechanistic studies indicate that this differentiation process requires PRDM16 to form a transcriptional complex with CCAAT/enhancer binding protein β (C/EBPβ) to initiate brown fat development [39]. Specifically, binding to PRDM16 enhances C/EBPβ function as a transcriptional activator, inducing the expression of other transcription factors and coactivators, such as PPARγ and PGC1α, both crucial for brown fat maturation. Subsequently,
PRDM16 coactivates PPARγ, PGC1α, and zinc-finger protein 516 (ZFP516) via direct binding, again stimulating their transcriptional activity in adipogenesis and thermogenesis to drive a complete brown fat differentiation program [27,32,41].

PRDM16 can also suppress gene expression through recruiting repressive complexes. It interacts with the histone methyltransferase EHMT1, which functions as a repressor of skeletal muscle- and white-selective genes by epigenetic modifications (Figure 3B, Key Figure) and as a PRDM16 stabilizer to facilitate activation of thermogenesis [37,40]. Deleting EHMT1 in murine adipose tissue results in defective brown and beige fat mediated adaptive thermogenesis [40]. In addition, PRDM16 also complexes with C-terminal binding proteins 1,2 (CTBP1,2) to repress particular white-specific genes, such as resistin and angiotensinogen, by acting directly on their promoters (Figure 3C) [38]. An interesting modulator of PRDM16 action is transducing-like enhancer protein 3 (TLE3), which competes with PRDM16 to complex with PPARγ in adipocytes, and promotes white fat-specific gene expression (Figure 3D) [42]. Mice overexpressing TLE3 have impaired brown fat that favors lipid storage over thermogenesis; conversely, TLE3-deficient mice show increased expression of thermogenic genes.
Recent studies have further identified a role of PRDM16 in promoting higher-order chromatin structure by interacting with the MED1 subunit of the Mediator, a multiprotein complex that plays a key role in connecting transcriptional activators to the general transcriptional machinery at gene promoters [43,44]. PRDM16 facilitates the recruitment of MED1/Mediator to the PPARγ or thyroid receptor-bound enhancer regions of specific brown-specific thermogenic genes, resulting in a bridge between the enhancer and the promoter region, facilitating the assembly of the preinitiation complex and promoting gene expression (Figure 3A). Whether the PRDM16–MED1/Mediator complex is involved in transcriptional repression of white- or muscle-selective genes remains to be established.

The PRDM16 zinc-finger domains physically interact with transcriptional regulators including PPARγ [32], C/EBPβ [39], PGC-1α [27], EHMT1 [40], and MED1 [43,44]. Deletion of the SET domain-related N-terminal PR domain does not affect these interactions or subsequent activity [32,38–40], suggesting that the PR domain may not be involved in brown and beige adipose tissue development. The PR domain is related to the classical SET domain, and thus PR domain-containing proteins are predicted to function as histone-modifying enzymes. In fact, PRDM16, together with PRDM3, are redundant H3K9me1 methyltransferases, initiating the establishment of H3K9me3 and heterochromatin formation, and are required for maintaining mammalian heterochromatin structural integrity [19], indicating that the PR domain of PRDM16 has intrinsic methyltransferase activity and is an important factor in fundamental cellular processes.

Interaction with miRNAs
The action of PRDM16 is subject to another important layer of regulation by a class of small non-coding RNAs termed miRNAs. PRDM16 has been found to be under the control of several miRNAs including miR-133, miR-27, and miR-150, which directly target the 3’ untranslated region (3’UTR) of Prdm16 and negatively regulate its expression [45–49]. Overexpression of these miRNAs disrupted adipogenesis from brown and beige precursor cells, while inhibition markedly promoted this process. Moreover, cold exposure strongly repressed the expression of miR-133 and miR-27, thereby removing their inhibition on Prdm16 and other important transcriptional components required for browning, and leading to brown and beige fat differentiation [45,49]. Reducing miR-133 globally in mice led to browning of white fat and improved insulin sensitivity and glucose metabolism [46]. Interestingly, local inhibition of miR-133 in skeletal muscle resulted in brown adipogenesis from skeletal muscle satellite cells and a similar improvement of whole-body metabolism [47]. By contrast, PRDM16 is found to promote the expression of a downstream miRNA cluster, miR-193b-365, which is an important regulatory mechanism that represses myogenesis [50]. Taken together, PRDM16 plays a central role in brown and beige fat biogenesis by interacting with a complex network of transcriptional regulators and miRNAs to promote the expression of brown-selective genes and suppress muscle- and white-selective genes at the same time.

Other Roles of PRDM16 Beyond Adipose Tissue
PRDM16 in Stem Cells and Development
PRDM16 is also expressed in several stem cell compartments, including hematopoietic stem cells (HSCs) and neuronal stem cells, where it regulates oxidative stress. In the absence of PRDM16, an increase in reactive oxygen species is observed, as well as depletion of stem cells and increased cell death [51]. Another study also identified a role for PRDM16 in HSC establishment and maintenance, with deletion of PRDM16 resulting in increased apoptosis and cycling of HSCs [52]. The wide spectrum of developmental defects in whole-body PRDM16 mutants include cleft palate, altered craniofacial development, and impaired cardiac development [53]. This suggests that PRDM16 is likely to be involved in the differentiation of a host of tissues, which has been confirmed by human disease states involving mutations in PRDM16.
PRDM16 Mutations in Human Pathology

In recent years, several studies have identified humans with mutations in PRDM16, which can result in a variety of phenotypic consequences. Alterations in the protein coding sequence of PRDM16 may lead to the development of cardiomyopathy [54,55]. PRDM16 mutations have been demonstrated as a cause for cardiomyopathy seen in nonsyndromic human myocardial abnormalities as well as in individuals with chromosome 1p36 deletion syndrome, the most common large-scale terminal deletion syndrome in humans that causes severe intellectual disability, and that affects approximately one in 5000 births [54]. More specifically, by aligning regions of chromosomal loss in individuals suffering from terminal 1p36 deletions and cardiomyopathy, a minimal common interval of loss has been identified which affects only the terminal exons 4–17 of PRDM16. Heterozygous PRDM16 point mutations that result in truncation, frameshift, and nonsynonymous substitutions at evolutionarily conserved sequences have also been found to be enriched in individuals with nonsyndromic forms of left ventricular noncompaction or dilated cardiomyopathy [54].

In addition to human studies, animal models were used to dissect the mechanisms by which mutations in PRDM16 contribute to cardiomyopathy. It has been reported that PRDM16 is expressed in the nuclei of cardiomyocytes in both embryonic and adult murine and human hearts [54]. Interestingly, zebrafish models with PRDM16 knockdown and a human truncation transgene showed reduced heart rate and cardiac output, and impaired rates of cardiomyocyte proliferation and coupling [54]. These results support a causal role of PRDM16 mutations in cardiomyopathy, and reveal that aberrant cardiomyocyte proliferation during cardiogenesis may be an important mechanism underlying these abnormalities. Future work on the function of PRDM16 in cardiomyocytes may provide further insights into molecular mechanisms, which could be relevant to the development of preventive and therapeutic candidates for cardiomyopathies.

Apart from deletions and point mutations in the PRDM16 gene, which may lead to cardiomyopathy, chromosomal rearrangements involving PRDM16 may contribute to hematological malignancies [56–59]. Chromosome 1p36 is prone to translocations. Among the many loci within this region, the PRDM16 locus is particularly susceptible to translocations in myeloid and lymphoid malignancies. Reciprocal chromosome translocations occurring at the PRDM16 locus result in either a promoter switch or generation of chimeric genes [56]. Individuals with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) that involve 1p36 rearrangement are frequently reported to have the PRDM16 locus rearranged with the RPN1 (ribophorin I) locus on 3q21, which encodes a housekeeping gene, resulting in translocation of the enhancer element of RPN1 to the S′ region of the PRDM16 gene, thereby leading to constitutive transcriptional activation of PRDM16 in the bone marrow and leukemia cells [56,60]. By contrast, PRDM16 can also form chimeras, either out-of-frame or in-frame, by translocating with partner genes such as RUNX1 (runt-related transcription factor 1) on 21q22 [56,58,59], ETV6 (ets variant 6) on 12p13 [56], and SKI (ski oncogene) on 1p36 [57]. Interestingly, translocations involving PRDM16 through a chimeric fusion can also cause upregulation of PRDM16 expression [56]. However, the functions of the chimeric proteins remain unknown.

Aberrant expression of the short variant of PRDM16 (PRDM16/MEL1S) has been particularly associated with a variety of hematological malignancies including AML, MDS, and T cell leukemia [26,61,62]. Overexpression of the PR domain-lacking PRDM16/MEL1S in the absence of the p53 pathway in bone marrow cells promotes myeloid leukemia in mice [63]. Mechanistically, transgenic experiments with PRDM16/MEL1S showed repressed transforming growth factor β (TGF-β) signaling-mediated growth-inhibitory effects in adult T cell leukemia cells [62], and blocked granulocyte differentiation in interleukin (IL)-3-dependent murine myeloid L-G3 cells [26], which involves small ubiquitin-like modifier (SUMO)-dependent interaction with the
transcriptional repressor CTBP [64]. Furthermore, repression of PRDM16/MEL1S is an important mechanism for preventing myeloid leukemia in homeobox B4 (HOXB4)-mediated hematopoietic stem cell expansion [65]. Taken together, PRDM16/MEL1S, but not PRDM16/MEL1, is likely to induce leukemia through blocking differentiation of progenitor cells and promoting growth of leukemia cells, suggesting that the PR domain might play a role in suppressing tumor initiation and growth. It is possible that an imbalance in the expression of the PR-containing and PR-lacking products, resulting from either genetic or epigenetic disruption, leads to malignancies. Because hematological malignancies involving PRDM16 translocations are also associated with poor prognosis [56,68], a better understanding of the consequences of these translocations may aid in clinical management.

PRDM16 is closely related to PRDM3, sharing 56% amino acid identity and a similar domain structure [60]. Translocations and inversions occurring at chromosome 3q26 that involves the PRDM3-encoding locus, leading to transcriptional activation of EVI1 (ectropic virus integration site 1), a PR-lacking isoform, have also been described in human AML and MDS, and are associated with poor prognosis. EVI1 plays a role in leukemia by blocking myeloid differentiation and apoptosis [67,68].

**PRDM16 Modulates TGFβ Signaling**

PRDM16 plays a role in regulating TGFβ signaling through interaction with SMADs and other transcriptional regulators such as SKI [69,70]. TGFβ ligands signal through dual serine/threonine kinase receptors to activate transcription factor SMADs (Smad- and Mad-related proteins), which bind to other transcription factors and cofactors to modulate cell- and tissue-specific gene expression. Previously, different variants of PRDM3 were found to regulate TGFβ signaling. EVI1 binds to SMAD3 and recruits the transcriptional repressor CTBP to inhibit the transcriptional activity of SMAD3, and thus block TGFβ signaling [71]. By contrast, the PR domain-containing MDS1/EVI1 variant regulates TGFβ signaling in the opposite direction – it enhances cellular responses to extracellular TGFβ [72]. A yeast two-hybrid screen using the MH2 (MAD homology 2) domain of SMADs as bait also identified PRDM16, like EVI1, as a SMAD-binding protein [70]. Interestingly, knockdown of the PR domain-containing PRDM16/MEL1 with siRNA resulted in slightly enhanced TGFβ signaling activity [70], whereas overexpression of PRDM16/MEL1 and PRDM16/MEL1S inhibited TGFβ reporter activity [53], together supporting that PRDM16 can antagonize TGFβ signaling. Furthermore, SKI, a known repressor of TGFβ signaling, and PRDM16/MEL1 act synergistically to inhibit TGFβ signaling by stabilizing inactive SMAD3–HDAC complexes on the promoter of TGFβ target genes in gastric cancer cells [69]. It is interesting to note that data with PRDM3 show that PR-containing and PR-lacking isoforms exert opposing effects on TGFβ signaling, indicating that the PR domain may mediate this pathway; however, this is not the case for PRDM16, where both long and short isoforms repress TGFβ signaling, suggesting that other factors may be involved, such as coregulatory proteins.

**Concluding Remarks and Future Perspectives**

Studies in cellular and animal models indicate that augmenting PRDM16 should increase beige fat function with associated metabolic benefits. However, a deeper understanding is required of how PRDM16 is physiologically regulated. The anti-diabetic drug rosiglitazone can induce beige fat activity, and appears to do so by regulating PRDM16 protein stability [73]. Moreover, miRNAs have been identified that target PRDM16 and the developmental and thermogenic program it regulates [45–50]. Additional work should help to clarify whether PRDM16 is predominantly regulated transcriptionally, translationally, or post-translationally. PRDM16 is also expressed at very low levels in visceral adipocytes [27]. It is not yet clear if there is a pathway in visceral fat that might inhibit PRDM16 gene expression or destabilize the protein. Identification of such a pathway, if present, could be a powerful means to change the phenotype of pathogenic visceral adipose tissue.
There could be great promise in approaches to modulate PRDM16 as a means to engineer healthier adipose tissue. Specifically, this should protect against obesity, insulin resistance, hepatic steatosis, and adipose inflammation. Future studies will be needed to determine the full spectrum of biological effects modulated by PRDM16 in adipocytes.

Moreover, it is not yet known whether increased PRDM16 and beige fat will affect susceptibility to other obesity-associated diseases such as hypertension, cardiovascular disease, and cancer. Further research is also needed to assess whether activation of PRDM16 and beige fat can result in any adverse effects. Excessive activation of beige and brown fat can be seen in the setting of cancer cachexia [74,75], but to date it is unclear what role brown and beige fat may play in either the initiation or progression of cancer. The role of thermogenic fat in atherosclerosis also needs further clarification, with one study suggesting a protective role and another pointing to a deleterious role [76,77]. Finally, investigators will need to be mindful of the broad biology regulated by PRDM16 in diverse cell types and pathways. The current state of knowledge suggests cautious optimism, and further elucidation of the mechanisms underlying the regulation of PRDM16 is expected to provide a clearer view of the therapeutic potential and possible risks of targeting this important regulatory protein.

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