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The duality of PRDM proteins: epigenetic and structural perspectives

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Abstract

PRDF1 and RIZ1 homology domain containing (PRDMs) are a subfamily of Krüppel-like zinc finger proteins controlling key processes in metazoan development and in cancer. PRDMs exhibit unique dualities: (a) PR domain/ZNF arrays—their structure combines a SET-like domain known as a *PR domain*, typically found in methyltransferases, with a variable array of *C2H2 zinc fingers* (*ZNF*) characteristic of DNA-binding transcription factors; (b) transcriptional activators/repressors—their physiological function is context- and cell-dependent; mechanistically, some PRDMs have a PKMT activity and directly catalyze histone lysine methylation, while others are rather pseudomethyltransferases and act by recruiting transcriptional cofactors; (c) oncogenes/tumor suppressors—their pathological function depends on the specific PRDM isoform expressed during tumorigenesis. This duality is well known as the 'Yin and Yang' of PRDMs and involves a complex regulation of alternative splicing or alternative promoter usage, to generate full-length or PR-deficient isoforms with opposing functions in cancer. In conclusion, once their dualities are fully appreciated, PRDMs represent a promising class of targets in oncology by virtue of their widespread upregulation across multiple tumor types and their somatic dispensability, conferring a broad therapeutic window and limited toxic side effects. The recent discovery of a first-in-class compound able to inhibit PRDM9 activity has paved the way for the identification of further small molecular inhibitors able to counteract PRDM oncogenic activity.

Keywords

epigenetic regulation; methyltransferase; PRDM; pseudomethyltransferase; transcription factor; *yin-yang*

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Conflict of interest

EG is cofounders and scientific advisors of IMMU-NOA Pte. Ltd. EG has served on advisory board for Lion TCR Pte. Ltd. The rest of the authors declare no competing financial interests.

Introduction—PRDM proteins: protein domains, origin, and evolution

PRDF1 and RIZ1 homology domain containing (PRDM) proteins represent an evolutionary recent branch of the methyltransferase superfamily. This subfamily contains 19 members (in humans) that control key biological processes, ranging from lineage specification to carcinogenesis [1–5]. All PRDM proteins share a characteristic structure that brings together a SET [Su(var)3–9, Enhancer-of-zeste and Trithorax]-like domain called a PR [PRDF1 (positive regulatory domain I-binding factor 1) domain, a RIZ1 (retinoblastoma protein-interacting zinc finger gene 1)] domain, and a variable array of Cys2-His2 (C2H2) zinc fingers (ZNF). Notable exceptions to this general structure include rare splice variants of PRDM7 and PRDM11 that are C2H2 zinc finger-deficient [3] and several PR-less isoforms, containing only the C-terminal zinc finger domains [2]. A Q-rich, unstructured domain is uniquely present at the C terminus of PRDM10. Consistent with the conserved role of this domain in transcriptional activation [6], its deletion leads to downregulation of PRDM10-regulated genes [7] (Fig. 1).

PRDMs first appeared in nematodes (two PRDMs) and insects (three PRDMs) and expanded across vertebrates. Given the high sequence homology between PR and SET domains, it is likely that PRDMs have evolved from the more ancient SET-containing protein lysine methyltransferases (PKMTs) (e.g., KMT2A/MLL1, SUV39H1) [3]. Intriguingly, despite the similarity between these two domains, only few PRDMs are endowed with an intrinsic methyltransferase activity. Flaws in experimental strategies/design, lack of knowledge of the actual cofactors or substrates, and evolutionary constraints are all plausible explanations for the enzymatically inactive PRDMs. The clear correlation between the distance to the root and methyltransferase activity, with fast-evolving PRDMs being more likely to lose activity, is nonetheless in favor of the latter explanation [8]. In the remainder of this review, we refer to these as pseudomethyltransferases in analogy with the more well-characterized pseudokinases [9].

It is also conceivable that the acquisition of stretches of zinc fingers, often involved in protein–protein and protein–nucleic acid interactions, during the evolutionary process allowed PRDMs to acquire novel functions essential for adaptation [3]. Indeed, despite the variability associated with enzymatic activity in PRDMs, their new DNA-binding capabilities, mediated by the ZNF domains, allowed them to bind to chromatin in a sequence-specific manner and to regulate transcription of target genes. Such regulation can be direct or indirect through tethering of other epigenetic factors. Indirect histone methylation, among other post-translational modifications, has been reported extensively for most PRDM proteins through recruitment of active enzymes to specific target sites [1–3].

In the following section, we examined the primary and tertiary structures of several PKMTs and PRDMs to highlight potentially critical amino acids for the conservation vs. loss of enzymatic activity during the SET-to-PR domain evolution. A special note should be made for *Friend of GATA* proteins, or FOGs, which are a group of proteins sharing structural homology with PRDMs (namely the PR domain and zinc finger array) [10]. FOGs are known to interact with the GATA transcription factor family and play important roles in development and disease [11]. In addition to their noncanonical C2HC ZNFs, similar

to PRDMs, FOGs contain C2H2 ZNFs. In particular, ZNF1, ZNF5, ZNF6, and ZNF9 of FOG-1, and ZNF1, ZNF5, ZNF6, and ZNF8 of FOG-2 physically interact with the GATA-1N-finger, but not the GATA C-finger [12–14]. It is nonetheless unclear whether the remaining ZNFs can directly bind DNA or whether FOGs' functions are entirely mediated by protein–protein interactions with different GATA family members [13,15]. We here propose to call the human FOGs, PRDM18 (FOG-1), and PRDM19 (FOG-2), respectively (Fig. 1).

PR domain: Structural aspects

PRDMs potentially function as methyltransferases through their PR domain, given its high homology with the SET domain [1]. However, experimental evidence of direct methyltransferase activity has been demonstrated for only a few members of the family that show stereoselectivity for the universal methyl donor SAM. Direct enzymatic activity has been demonstrated only for PRDM2 (H3K9me) [16], PRDM7 (H3K4me) [17], and *PRDM9* (H3K4me3, H3K9me1/3, H3K18me1, H3K36me3, and H4K20me1/2) [18–22]. Controversial studies about PRDM3/PRDM16 methyltransferase activity have been reported, and it is not clear whether they act as H3K4me1 [23] or H3K9me1 methyltransferases [24]. Besides, an isolated study reported PRDM8 enzymatic activity (H3K9me), but further confirmations of the results are necessary [25].

On the other hand, indirect activity has been reported for *PRDM1* (G9a/H3K9me2, PRMT5/H4R3me2s) [26,27], *PRDM3* (EZH2/H3K27me3) [28], *PRDM5* (G9a/H3K9me2) [29], *PRDM6* (H4K20me3, G9a/H3K9me2) [30,31], PRDM12 [32–35], and *PRDM14* (EZH2/K27me3) [36,37], suggesting that PRDMs are able to recruit histone 'writers' through protein–protein interactions. Furthermore, PRDMs are able to recruit histone 'erasers' (i.e., demethylases and deacetylases). Examples of the latter include PRDM1 recruitment of the histone demethylase KDM4A to remove repressive H3K9me3 marks in neural development [38] or HDACs to deacetylate histone H3 and repress *C-MYC* transcription during B-cell differentiation [39]. The indirect and direct methyltransferase evidence is summarized in Fig. 2.

Following the crystallization of SET domain proteins, extensive efforts have been made in the last decade to crystalize PRDM family members. Nonetheless, the presence of tandem ZNF arrays, technically difficult to purify and crystalize, has limited the success to crystallization of the PR domain only. More importantly, lack of knowledge regarding the substrates, if any, of different PR domains has further restricted all available structures to the inactive/unbound state, lacking both SAM and peptides/substrates, in their respective pockets (Fig. 3). It was not until 2013 that the first X-ray structure of PRDM9 in complex with a histone H3 peptide and SAH was reported by the Structural Genomics Consortium (PDB code 4C1Q) [18]. This was a seminal paper, highlighting how the PR/SET domain topology is similar to other SET domains, with the conserved PR domain fold surrounded by the pre- and post-SET regions. The construct used in this structure has a truncated post-SET domain fragment that was folded in the presence of SAH and a histone H3 peptide (H3K4me2).

This pioneer work later led to the identification of the first-in-class PRDM inhibitor, MRK-740 [40]. This small molecule is selective for PRDM9 over other SET/PR domain proteins and binds to PRDM9 through a large SAM interaction interface. This mode of binding is based on disrupting the active conformation of the post-SET region, exposing SAM to interact with the inhibitor. Furthermore, MRK-740 is cell-active and was shown to inhibit PRDM9 activity on chromatin, as measured by the ability of the compound to reduce the levels of H3K4me3 at PRDM9 binding regions [40].

Solving the crystal structure of additional PR domains is key to understanding the main differences between active and pseudomethyltransferases. Notably, inclusion of the post-SET region might be critical in such crystallography experiments. Indeed, the transition between the PRDM9 active and inactive states is followed by clear conformational changes in the structure and more precisely in the post-SET domain. The unbound PRDM9 structure shows a closed conformation in which the post-SET region binds across the PR/SET domain, blocking both the peptide and SAH binding cleft [18]. This autoinhibitory conformation was further confirmed with the recently published PRDM9-MRK-740-SAM structure [40]. Superposition of both unbound and inhibitor-bound PRDM9 structures shows no difference in the global conformation; however, the SAH-bound structure adopts a different conformation with a fully folded post-SET domain and a clear translocation of the helix α2 allowing the transition to the active state (Fig. 3A). A closer examination of the PR domain in PRDM9 shows that tyrosine 357 (Y357), glutamate 360 (E360), and tyrosine 361 (Y361) residues are directly involved in the substrate recognition site. E360 stabilizes the closed conformation by forming a salt bridge with arginine 342 (R342), whereas Y361 blocks the binding cleft by forming a hydrogen bond with leucine 294 (L294). Both residues rotate in the SAM/substrate-bound conformation, with Y361 making direct contacts to stabilize SAM. On the other hand, asparagine 320 (N320) and cysteine 321 (C321) residues at position N320 and C321 are crucial for SAM binding (Figs 3B and 4). Interestingly, Y357 is mutated to a serine in PRMD7. This mutation negatively impacts the catalytic efficiency, as demonstrated by the fact that a simple mutation back to tyrosine restores catalytic levels observed for PRDM9 [17] (Fig. 4).

Based on strict sequence alignment, and on the confirmed catalytic activity of PRDM9 [18–22], one would predict that only PRDM2, PRDM7, and potentially PRDM14 are active KMTs (Fig. 4). In Fig. 3, we superimposed publicly available structures of other PRDM proteins to that of PRDM9's PR domain to infer potential catalytic activity. The caveat is that none of these structures contain either SAM/SAH or peptides/substrates, making it difficult to predict subsequent conformational changes. Nonetheless, we do note that N320, which is conserved in the SET domain of active PKMTs (SUV39H1, EZH2, G9a, and KMT2A) and in the active PRDM2, is substituted by either arginine or lysine in PRDM4, PRDM10, and PRDM12. (Figs 3D–F and 4). We predict that such changes would prevent efficient SAM binding. In PRDM2, for which a methyltransferase activity was reported, the corresponding asparagine is conserved, as well as the tyrosine at position 291, forming a hydrogen bond with SAM (Fig. 3C and 4). Comparing the PRDM9 structure with that of PRDM14 is interesting, as most residues interacting with SAM are conserved, but no catalytic activity has been reported to date (Figs 3G and 4). Finally, C321 is substituted by a

proline (P164) in PRDM1 and PRDM10 resulting in loss of the hydrogen bonds observed in the PRDM9 structure and may consequently disrupt SAM binding (Figs 3E,H and 4).

In summary, specific members of the PRDM family are active KMTs, while others are devoid of direct methyltransferase activity (i.e., *pseudomethyltransferases*) and act as docking proteins for enzymatically active epigenetic modifiers. As such, defining the PRDM interactome is paramount to comprehensively understand their role as epigenetic modifiers. As a caveat to our analysis, most PRDM structures published so far lack the post-SET domain, which has a great impact on the enzymatic conformational changes. Thus, it will be important for the field to obtain extended structures that include this region, in order to gain deeper insights on the mechanistic aspects of PRDMs' activation. Last, understanding how certain PR domains bind SAM, while others do not will be important in order to develop PRDM-binding small molecules inhibitors or degraders for therapeutic applications.

ZNF arrays and DNA-binding selectivity

C2H2-type ZNF motifs are among the most prominent structural domains present in chromatin-bound proteins, enabling protein–protein and protein–nucleic acid interactions. Their structure is defined by spacing constraints between cysteines and histidines (X_2-C-$X_{2,4}$-C-X_{12}-H-$X_{3-,5}$-H), which coordinate a centrally located zinc ion and adopt a two-stranded antiparallel β-sheet and an α-helix [41]. The α-helix of each ZNF, often dubbed the recognition helix, is able to engage in sequence-specific contacts with DNA, with each ZNF recognizing approximately three bases. Several groups have attempted to map the cistrome for PRDMs in different cell lines; however, the low expression levels of most PRDMs, their high cell-specific expression, and the lack of specific ChIP-grade antibodies have often impaired these efforts.

As a general rule, most PRDMs are direct sequence-specific chromatin regulators, which utilize between three and four ZNFs to make direct contact with DNA motifs spanning 7 to 15 bases, with the exception of PRDM9 isoforms, which recognize very long motifs using the entirety of their ZNF arrays [42]. We have summarized the experimental evidence of PRDMs' DNA-binding motifs in Fig. 5. In addition, we have used an online prediction tool (http://zf.princeton.edu/ [43,44] to calculate the DNA bases potentially recognized by each ZNF array present at the C terminus of PRDM family members.

PRDM1, also known as BLIMP-1, was originally discovered for reducing beta-interferon expression in the context of viral infection [45] and for its role in B-cell maturation [46]. Later, it was identified as a tumor suppressor in B-cell lymphomas [47–51], where its silencing alone is insufficient to induce tumorigenesis, since it requires additional mutations for lymphoma to develop [52]. The *PRDM1* gene has two alternative promoters, transcribing two isoforms (i.e., PRDM1α and PRDM1β) with opposite functions, but both still able to bind to DNA (a phenomenon described as 'Yin and Yang') [53]. The PRDM1 cistrome has been well characterized in multiple cell lines by ChIP-sequencing [54–57], and a C/T-rich consensus characterized by two consecutive [ACTTTC] repeats is consistent with the predicted consensus of ZNF1–4.

PRDM2, also known as RIZ, was discovered in the mid-90s as a result of its ability to bind retinoblastoma (Rb) and was hypothesized to play a role in neuronal development [58]. It was first reported to have tumor suppressive properties in breast cancer [59], lymphoma [60], and leukemia [61,62]. A PR-less oncogenic isoform has also been identified. Similar to other PRDMs (i.e., PRDM1α/β, PRDM3/EVI1, and PRDM16/MEL1), these two isoforms (named RIZ1 and RIZ2) have opposing functions in cancer and are expressed in different contexts [63–68]. While the predicted ZNF recognition motif is [TCxTCxGAGGxACT], whether PRDM2 binds directly to DNA remains to be explored.

The short isoform of PRDM3, known as EVI1, is a potent oncogene in several leukemia subtypes [69]. PRDM3/EVI1 has two sets of ZNFs, one at the N- and one at the C terminus of the protein, comprised of seven and three ZNF tandem repeats, respectively (Fig. 1). Previous studies have demonstrated that the PRDM3/EVI1 N-terminal ZNF domain recognizes a GATA-like motif [GAC/TA] $X_{0–6}$ [GAT/CA], while the C terminus binds to an ETS-like motif [GAA/TGAT/G], respectively [70–75]. The predicted consensus is consistent with the experimental data.

PRDM4 is a nonessential transcriptional regulator during development, as *Prdm4* null mice are born healthy and in a Mendelian ratio [76]. Using a combination of SELEX (systematic evolution of ligands by exponential enrichment) and ChIP-seq, the binding site for PRDM4 has been determined to be [CTGTTTC] [76], consistent with the predicted consensus of ZNF2–4.

PRDM5 is a well-established tumor suppressor gene in a multitude of cancer types, whose expression is epigenetically silenced through CpG methylation [77,78]. The proposed mechanism for PRDM5's tumor suppressor function involves its ability to negatively regulate WNT signaling through transcriptional regulation of WNT reporters and WNT-responsive genes. This was initially reported in Zebrafish development [79] and has since been replicated in the context of tumorigenesis [77,80,81]. Besides being often deregulated in cancer, PRDM5 is also highly expressed in mouse ES cells (mESCs). While PRDM5 is dispensable for the self-renewal potential of mESCs, it transcriptionally regulates genes important for cell differentiation and development. PRDM5 binds to a [GGxGCxCCxG] consensus [82], consistent with the predicted consensus at the extreme C terminus of the protein (ZNF14–16).

PRDM6 is a poorly characterized member of the family; a study on the role of PRDM6 in vascular-associated smooth muscle cells reported physical interactions with the histone methyltransferase EHMT2/G9a and with class I histone deacetylases, mediated through the PR domain [31]. There is no evidence of direct DNA binding by PRDM6, but it is predicted to recognize a G/T-rich motif with its four ZNFs [TGTxGTTxC].

PRDM8 is known to play a role in the development of the central nervous system, testes, and hematopoietic cells [25,83–85]. Its role in cancer appears to be context-dependent, with some studies describing PRDM8 as a tumor suppressor [86], while others reporting an oncogenic function [87]. Direct DNA binding has not been tested, but is predicted to recognize a short [AxTxGxACC] motif with its three ZNFs.

PRDM9 has been extensively studied for its role in meiotic recombination. PRDM9 binds first to chromatin and labels specific target sites, through deposition of the H3K4me3 and H3K36me3 histone marks [18,19,21,22,88]. Subsequently, proteins responsible for the formation of double-stranded breaks and for meiotic recombination are recruited to these sites [89–91]. Overall, the PRDM9-binding motif identified *in vivo* matches very well with those predicted *in vitro*. However, PRDM9 is unique among other PRDMs as several alleles are present within the same species. These alleles bind to different consensus sites in the genome and drive meiotic recombination at different hot spots [42].

The function of PRDM10 has recently been characterized in early embryonic development [7]. Specifically, PRDM10 mediates its function through direct binding to a [A/GA/GTGGT/AxxG/CxxCC] motif, weakly consistent with the predicted consensus of ZNF4–8. Importantly, its ZNFs are essential to PRDM10's transcriptional activity, which is mediated by the recruitment of coactivators by both the PR- and Q-rich domains [7] (Fig. 1).

Mutations in *PRDM12* have been linked to congenital insensitivity to pain. These mutations occur in a homozygous fashion and affect the development of sensory neurons destined to differentiate into nociceptors [92,93]. Patient mutations span the entire protein, including the PR domain and the ZNF array [92]. No direct evidence has demonstrated PRDM12-DNA binding; however, the three ZNFs are predicted to bind a G/T-rich motif [GTGTGTT].

PRDM13 is expressed in the dorsal neural tube [94] and retina [95] where it plays a role in lineage specification. There is no evidence of direct DNA binding, but rather, PRDM13 has been shown to interact with basic helix–loop–helix transcriptional activators to repress transcription of ventral neural tube specification genes (e.g., *Olig1* and *Olig2*) [96] and *Tlx1/3*. Since TLX1/3 expression would specify the excitatory neuronal lineage, PRDM13-mediated repression promotes differentiation toward the inhibitory lineage. The four ZNFs are predicted to bind a [CxAGTCCGC] motif, but this has not been validated experimentally.

PRDM14 plays a key role in germ cell specification and in the maintenance of embryonic stem cells' naïve pluripotency [97–101]. PRDM14's function is mediated by direct transcriptional regulation of downstream effectors. These have been determined by ChIP-sequencing in relevant cell types (i.e., mouse and human ES and germ cells). The data collected by multiple groups have identified a conserved motif [GGTTAGA/GGACCC]. Unfortunately, *in silico* prediction tools are not able to consistently corroborate the experimental data, suggesting that PRDM14 might use noncanonical modalities to bind DNA or use the ZNFs in a nonsequential order [102–104].

The first functional studies characterized PRDM15 as gatekeeper of naïve pluripotency in mESCs [105]. In mESCs, PRDM15 operates upstream of the WNT and MAPK signaling pathways via transcriptional regulation of critical genes in both pathways, namely *Rspo1* and *Spry1* among others. This role seems to be bypassed *in vivo*, at least partially, as *PRDM15KO* mice implant normally and die at mid-gestation due to growth delay and forebrain malformations reminiscent of holoprosencephaly (HPE) and microcephaly [106]. Indeed, loss-of-function mutations affecting the ZNF domains of *PRDM15* have been recently reported in patients with a syndromic from of HPE [106,107]. One particular

mutation (C844Y) in ZNF15 appears to abolish PRDM15 binding to its target genes, many of which are important for anterior/posterior patterning and forebrain development, and its ability to regulate their expression [106]. The predicted consensus is recognized by ZNF10–15 and matches the experimentally validated binding motif [105,106].

In a 'Yin and Yang' fashion, PRDM16/MEL1 expresses two isoforms, one longer (PRDM16) comprising the PR domain and one shorter (MEL1), which lacks the PR domain and acts as a dominant-negative isoform in cancer [108]. MEL1 binding to chromatin has been mapped by multiple experimental methods. Earlier reports identified two motifs, similar to those of PRDM3/EVI1, by DNA-binding activity by the cyclic amplification and selection of target (CASTing) technique [108]. Later attempts, however, did not confirm these findings by ChIP-seq, and rather identified binding sites consistent with those of EBF and C/EBP at regulatory regions of brown adipose tissue genes (e.g., *Ucp1*, *Ppara*) in brown adipocytes [109] and of LHX2, SOX2/3, NEUROD1, TBR2, and MEIS1 at regulatory regions of cell migration and progenitor amplification genes (e.g., *Nrg1*/*Itga6* and *Mycn*/*Jag1*, respectively) in mouse E15.5 cortex [110]. Similar to PRDM14, PRDM16 might also bind DNA in a noncanonical function, utilizing a combination of the two ZNF arrays (one more centrally located and the second at the C terminus) in a nonconsecutive fashion, or rely on other DNA-binding factors to engage with chromatin. Interestingly, the *in silico* prediction of the binding preference for the three ZNF at the C terminus of PRDM16/MEL1 is consistent with a [CATCc/tTC] motif identified by the earlier study [108].

A last note of interest for FOG-1: Although FOG proteins are known to mediate their transcriptional effects by protein–protein interaction with GATA proteins, it is intriguing to note that our *in silico* analysis (Fig. 5, bottom) predicts the first three ZNFs of FOG-1 to bind to palindromic [GATA] motifs. Whether FOG-1 is able to directly contact DNA, or whether its ZNFs are able in any way to interfere with GATA binding to chromatin, remains to be investigated.

PRDMs in Cancer

Historically, PRDM proteins have been associated with tumor suppressor functions. Most *PRDMs* are either silenced or deleted in cancer through an array of genetic (i.e., polymorphisms, frameshift/inactivating mutations, and chromosomal deletion) and epigenetic (i.e., DNA methylation and transcriptional silencing) mechanisms. For instance, genomic regions containing *PRDM1* (6q21-q22.1) [49], *PRDM2* (1p36) [111], and *PRDM4* (12q23-q24.1) [112] are frequently deleted in cancer. Frameshift and inactivating mutations identified in *PRDM1, PRDM2, PRDM3, PRDM8*, and *PRDM11* have been linked to transformation [1]. In addition, an increasing number of studies have reported downregulation of several *PRDMs* at the transcriptional level via a variety of epigenetic mechanisms involving DNA methylation, histone post-translational modifications, and micro-RNAs, leading to deregulation of the local chromatin structure at PRDM-associated regulatory regions. The underlying molecular mechanisms have been extensively characterized elsewhere [1,4,113].

From a therapeutic perspective, PRDMs have been hard to drug: (a) First, the majority are associated with a tumor suppressive activity (i.e., deleted, mutated, or not expressed in cancer); and (b) their 'Yin and Yang' regulation entails that the oncogenic isoforms (i.e., PRDM1β, EVI1, MEL1) are deprived of the druggable PR domain. Nonetheless, more recent studies have uncovered another category of oncogenic PRDMs in cancer, which are expressed as full length, hence retaining the druggable PR domain. Among the PRDMs showing an oncogenic function, extensive molecular characterization has been provided only for PRDM9, for PRDM14, and recently for PRDM15, while PRDM6 and PRDM10 still lack proper functional validation and demonstration of their pivotal role in carcinogenesis.

We dedicate the next section to discussing these maverick PRDMs and the associated molecular mechanisms linked to cancer.

PRDM9: from meiosis to tumorigenesis

PRDM9 contains an N-terminal Krüppel-associated box (KRAB) domain involved in protein–protein interactions, an SSXRD nuclear localization signal, a PR domain with intrinsic methyltransferase activity, and a set of zinc fingers at the C terminus providing direct site-specific DNA binding [114].

PRDM9 was first characterized as a methyltransferase that catalyzes H3K4me3, followed by additional evidence of its expanded substrate specificity toward other histone lysines [18–22] and potentially nonhistone lysines (i.e., automethylation activity) [115].

As discussed in a previous paragraph, over the last decade, PRDM9 has been established as a major epigenetic regulator of meiotic progression. Typically, PRDM9 is expressed in germ cells. However, a number of recent studies have reported its re-expression in cancer. This is not uncommon, as hundreds of cancer/testis (C/T) genes have been documented to be normally expressed in germ cells and aberrantly reactivated in a variety of tumor types [116]. In a pan-cancer analysis of The Cancer Genome Atlas (TCGA), comprised of data from 32 different cancer types, the upregulation of *PRDM9*, as opposed to neighboring healthy tissues, was reported in about 20% of cases [117,118]. Another meta-analysis of clinical datasets from different tumor types revealed the increased expression of a number of meiotic cancer genes, including PRDM9, in lymphoma and leukemia cell lines [119]. In addition, a recent analysis of a TCGA exome sequencing datasets identified PRDM9 as one of the most mutated genes in the PRDM family. A high mutation rate not localized in any specific hot spot exceeds 10% in uterine corpus endometrial carcinoma, 14% in lung adenocarcinoma, and 15% in skin cutaneous melanoma [2].

Although these studies do not characterize the mutations nor provide any functional evidence, other reports have associated *PRDM9* genetic variants with cancer. For instance, exome sequencing data from families with children affected by B-cell precursor acute lymphoblastic leukemia (B-ALL) revealed a significant excess of rare allelic forms of *PRDM9* [120]. This observation was confirmed in an independent cohort of children with B-ALL, where the excess was found in aneuploid and infant B-ALL patients. The latter findings suggest the involvement of PRDM9 in genomic instability and aneuploidy in cancer. Indeed, PRDM9 expression levels correlate with the abundance of somatic structural

variants (SVs) in several tumor types, with PRDM9-expressing cancers exhibiting a higher number of such breakpoints. Importantly, these SVs are enriched within known PRDM9 binding sites, reinforcing the potential link between PRDM9 expression and genomic instability in cancer [118].

Altogether, while mounting evidence places PRDM9 as a new oncogenic member of the family, in-depth functional studies are needed to characterize its involvement in tumorigenesis. Notably, the recent development of the first-in-class inhibitor for PRDM9 [40] makes the validation of an oncogenic function for this enzyme a high-priority task. Indeed, as a C/T gene, PRDM9 would be an ideal therapeutic target: Not only do C/T genes' germ line-restricted expression profiles make them outstandingly safe targets, but also they are additionally good biomarkers and targets for immunotherapy given their documented immunogenicity in cancer.

PRDM14: an ideal target in oncology with links to stem cell biology

Mechanistic studies have elucidated PRDM14's association with EZH2/H3K27me3, a key repressive histone mark associated with gene silencing. While this association is essential to maintain ESC pluripotency in a naïve state, this association remains to be demonstrated in cancer [37]. The full-length (PR-plus) isoform of PRDM14 is oncogenic, and its genetic locus is often amplified in several tumor types. In particular, PRDM14 expression is upregulated in 25% of lymphoid neoplasms and increased copy-number variants have been identified in breast cancer [121–123]. Ectopic expression of PRDM14 in breast cancer cell lines increases their proliferation and resistance to standard chemotherapeutic combinations, while siRNA knockdown has the opposite effect [123]. Nandi *et al.*, based on the statistic that women with diabetes have a 20% higher risk of developing breast cancer, identified MiR-424 as a key regulator of cancer cell stemness as its overexpression under hyperglycemic conditions resulted in reduced stem cell activity. Particularly, miR-424 regulates cdc42, which, in turn, activates *PRDM14* via PAK1 and STAT4, thus highlighting a role for the miR-424–cdc42–prdm14 axis in breast cancer progression in diabetic patients [124]. This study hints at a crucial role of PRDM14 in integrating extracellular signals with epigenetic modifiers.

PRDM14 function has been particularly well characterized in lymphoid leukemias and T-ALL mouse models, demonstrating full penetrance and accelerated disease onset due to expansion of hematopoietic stem cells, lymphoid progenitors, and blockade of pro-differentiating cellular programs [125]. Specifically, in a PRDM14-induced T-ALL model, NOTCH1 is activated through a RAG-recombinase complex, enabled by a PRDM14-induced permissive chromatin state [125,126]. In addition, upregulation of critical genes in pluripotency, tumor initiation, epithelial-to-mesenchymal transition, and other onco-pathways such as WNT and Ras/MAPK was noted in lymphoblastic leukemia. These latter findings have been validated in other cancers including non-small-cell lung carcinoma [127].

Currently, no studies have identified a PR-less isoform or an isoform with tumor suppressor function.

PRDM15: a context-dependent regulator of multiple oncogenic signaling pathway

PRDM15 is one of the least characterized members of the PRDM family. It was first identified as a candidate gene for a particular phenotype of Down syndrome known as bipolar affective disorder, which maps to human chromosome 21q22.3 [128]. Similar to PRDM9, although to a lesser extent, PRDM15 expression in adult somatic cells is rather low, albeit ubiquitous. This is consistent with its essential role during embryogenesis [106], while being dispensable in adult mice [129]. Intriguingly, PRDM15 expression is upregulated in several subtypes of B-cell lymphomas, as opposed to healthy tissues [129]. Such a selective expression profile, characteristic of most PRDM proteins, is well suited for designing safe therapeutic strategies against oncogenic members of this family. Our studies provide extensive functional and mechanistic characterization of the oncogenic role of PRDM15 in B-cell lymphomas. We provide evidence that perturbation of PRDM15 expression, either genetically using knockout models or pharmacologically using antisense oligonucleotides (AONs), delays tumorigenesis and selectively kills established B-cell lymphomas, both *in vitro* and *in vivo* [129]. Mechanistically, depletion of PRDM15 blocks metabolic processes, mainly glycolysis and mTOR signaling in tumor cells, inducing a 'metabolic crisis' that is detrimental to their survival, while sparing normal tissues that rely less on these pathways. In addition to its expression profile, the role of PRDM15 as a transcription factor able to orchestrate a broad transcriptional program to sustain tumor survival makes it a unique and promising therapeutic target. Indeed, our data show that, as opposed to the widely reported resistance to mTOR or AKT inhibitors, due to rapid signaling rewiring, PRDM15 depletion has long-lasting effects on tumor growth inhibition. Eμ-myc mice lacking the *Prdm15* gene live twice as long as their *Prdm15* WT counterparts. Intriguingly, the KO mice do not develop B-cell lymphomas, but some of them tend to eventually develop either T-cell or less-characterized non-B/non-T-cell malignancies. This is consistent with the fact that B-cell lymphoma cells *in vitro* fail to rewire their signaling or survive PRDM15 depletion.

While the crystal structure of PRDM15 has yet to be solved and potential PRDM15-specific inhibitors may take a while to develop, the use of AONs or PRDM15 degraders may represent interesting alternatives. As an alternative consideration, our studies analyzing the therapeutic potential of drugging the PRDM15 transcriptional network suggest that combination therapy targeting glycolysis and the PI3K/AKT/mTOR signaling axis, both of which have FDA-approved drugs used in the clinic, is an exciting therapeutic strategy for B-cell lymphomas.

PRDM6 and PRDM10: unexplored oncogenes

Unlike the previously reported oncogenic PRDMs, more work needs to be done to better appreciate the significance of those that are less well characterized, including PRDM6 and PRDM10. PRDM6 has been recently identified as a novel driver gene in class 4 medulloblastoma, a class that still lacks a comprehensive analysis of its driver genes. A novel oncogenic function of PRDM6 has been identified thanks to cis expression structural alteration mapping, a method able to infer *enhancer hijacking* events. In particular, *PRDM6* maps 600 kb downstream of the recurrently mutated gene *SNCAIP* and its expression was 20-fold higher, much more than any other gene within the proximal topologically

associated domains (TADs) (*SNCAIP* included). By integrating NGS and topological data, the structural variants that disrupt the *SNCAIP* locus led to novel super-enhancer interactions with neighboring TADs, most notably with the *PRDM6* promoter [130]. These data suggest that there is more to be discovered about PRDM6 in cancer, considering that it appeared to be fundamental in very specific tumor types, thus strengthening the idea that PRDMs function in an inherently context-dependent manner. Broad bioinformatic analyses of publicly available datasets might corroborate this hypothesis of context specificity.

PRDM10 is a sequence-specific transcription factor essential for preimplantation embryos and mouse embryonic stem cell homeostasis as loss of *Prdm10* results in dramatic cell growth inhibition. Indeed, Prdm10 regulates global translation in early embryonic development and mESC homeostasis, acting as the transcription factor of the core component of the eukaryotic translation initiation factor 3 [7]. Aside from basic studies in mESCs, the oncogenic role of PRDM10 has been identified in undifferentiated pleomorphic sarcoma as a consistent proportion of patients are characterized by novel gene fusions involving PRDM10 as the 3′ partner and either MED12 or CITED2 at the 5′ [131]. CADM3 was found to be consistently upregulated both in PRDM10-rearranged soft tumors and in fibroblasts overexpressing a CITED2-PRDM10 fusion protein. ATAC-seq data show enrichment at PRDM10 binding sites of the CITED2-PRDM10 fusion protein, suggesting that the N terminus partner might increase chromatin accessibility to PRDM10 [132]. Hitherto, little is known about the mechanisms by which PRDM10 contributes to cancer evolution, the affected signaling pathways, and whether it has direct or indirect methyltransferase activity.

PRDMs interactome: a missing link

A systematic identification of the interactome for each PRDM in different cell types and organs is still missing and would certainly shed light on the function of specific family members, in particular those lacking a catalytic activity. A key example of the utility of such an approach comes from PRDM9, where the KRAB domain is able to establish protein–protein interactions with multiple proteins important to further modify chromatin and advance toward the phases of meiotic recombination. In particular, CXXC1, EWSR1, EHMT2, and CDYL are all direct PRDM9-KRAB interactors. In addition, PRDM9 is able to interact with the meiotic cohesin REC8 protein and the SYCP1/ SYCP3 synaptonemal complex subunits. Such interactions are partially mediated by DNA looping and, in addition to PRDM9 catalytic activity, are important to ensure meiosis [133].

A second example of how mass spectrometry approaches have been instrumental in characterizing the repressive function of a PRDM protein is the characterization of the PRDM14 interactome. Two groups reported the interaction between PRDM14 and components of the polycomb complex, PRC2 (e.g., EZH2). This interaction is important for PRDM14 to repress genes critical to the activity of the fibroblast growth factor receptor signaling. It also maintains hypomethylated chromatin by preventing the expression of DNA methyltransferases (i.e., *Dnmt3a*, *Dnmt3b*, and *Dnmt3l*) that would lead to a primed epiblast-like state if expressed [97] and, finally, represses essential developmental targets during iPSC reprogramming [37]. A later report characterized the interaction between

PRDM14 and the Mtgr1 transcription corepressor [134]. Mtgr1 is a member of the ETO family of transcriptional corepressors [135] that binds tightly to the pre-SET region and SET domain of PRDM14, occupying overlapping genomic loci.

An additional effort toward identifying the PRDM interactome has recently characterized the relevant interactions that distinguishes between the long and short isoforms of PRDM3 and PRDM16 [136]. The authors focused on the duality of PRDMs and asked what features enable the tumor suppressive functions of the long isoforms. To address this question, they performed co-immunoprecipitation followed by mass spectrometry of GFP-tagged long or short (PR-less) isoforms of both PRDM3 and PRDM16. They found that the long isoforms had significantly enriched interactions with proteins associated with the nucleosome remodeling and deacetylase (NuRD) complex (including Rbbp4, Mta1, Mta2, and Chd4) compared with the truncated proteins. Immunoprecipitation of endogenous human RBBP4 pulled down the full-length, but not the short, isoforms of both proteins, highlighting that they are indeed binding partners. Specifically, the first 12 amino acids of the N termini of the full-length isoforms bind to RBBP4 in the same pocket where histone H3 can also bind. In fact, all three proteins contain conserved lysine and arginine residues that are likely key to their interactions with RBBP4. More intriguingly, related proteins, including PRDM18/FOG-1 and PRDM19/FOG-2, maintain these residues and have been reported to bind to RBBP4 too [137,138]. Overall, Ivanochko *et al.* provide a mechanism by which the long isoforms of PRDM3 and PRDM16 are specifically able to recruit transcriptional corepressor complexes, a function that is lost in their short isoforms. The group proposes that these PRDMs provide a ‘tether’ for the complex—with their ZNF arrays bound to DNA, the PRDMs can bring the NuRD complex to chromatin by interacting with RBBP4 and other protein members. When the truncated isoforms are expressed, this interaction is lost or significantly weakened and transcriptional repression is released, allowing expression of potential oncogenes. It would be worthwhile exploring the transcriptomes of cells with these alternative isoforms to discern the downstream targets of the NuRD/PRDM interaction. Similarly, ChIP-sequencing of both PRDM isoforms in different cellular contexts would also provide insight into their altered capacity to bind to target genes and potentially regulate their transcription [136]. The methodology used by the Arrowsmith laboratory could be used as a framework for future studies of other PRDM interactomes. While the path to targeting PRDMs may take a while, it would be particularly advantageous to identify physical interactors of the oncogenic PRDMs that could be easier to target or which selective inhibitors are already available.

Beyond the structure of PRDMs: therapeutic perspectives and final remarks

We here propose that PRDMs play a fundamental role integrating the upstream signals from oncodrivers or signaling pathways, by either establishing oncogenic transcriptional programs or supporting chromatin remodeling toward a pro-oncogenic phenotype. For instance, the long latency time associated with PRDM1/Blimp1 LOF-induced tumorigenesis [52] supports the hypothesis that PRDM1/Blimp-1 acts as a gatekeeper for oncogenesis. Developmental studies have uncovered the highly tissue-specific expression of PRDM proteins and their involvement in lineage specification and tumorigenesis. The key to understanding their involvement would require a better characterization of the upstream

regulatory mechanisms, currently representing one of the biggest gaps in the literature. In particular, promoter hypermethylation, noncoding RNAs, and other epigenetic mechanisms have been postulated to be pivotal for upstream PRDM regulation. This is, indeed, concordant with the current context-dependent hypothesis of PRDMs' function.

From a functional perspective, PRDMs can be inhibited through different strategies, by tackling either the putative catalytic PR domain or the zinc fingers. ZNF inhibitors have not been translated to the clinic given the lack of protein specificity as this domain is present in multiple proteins mediating DNA-binding interactions [139]. Moreover, there is a lack of understanding on whether it would be more efficient to target ZNFs responsible for protein–nucleic acid interactions or those mediating protein–protein binding.

Recently, MRK-740, a potent, selective, and cell-active probe targeting PRDM9, has been identified as the first-in-class PRDM inhibitor [40]. Yet, there is a pressing need to expand pharmacological research on small molecules targeting other PRDMs, considering their noncatalytic domains involved in noncanonical signaling. By drawing similarities from *pseudokinases*, several members of the PRDM family of proteins may be classified as *pseudomethyltransferases* as they are not able to exert primary methyltransferase activity. Paradoxically, the PR domain lacks the most conserved motif H/RxxNHxC that has been repeatedly demonstrated to be essential for methyltransferase activity in SET domain containing proteins [140]. A recent and promising idea that is developing traction in the field is that of designing proteolysis targeting chimera, hetero-bifunctional molecules linking a compound to an E3 ubiquitin ligase to promote proteasomal degradation via formation of E3 tertiary structures. Identifying small molecule binders for PRDMs would open up new possibilities to develop tool compounds and future clinical molecules [141].

Alternatively, AONs can be a valuable strategy to tackle oncogenic PRDMs by directly downregulating the *PRDM* mRNA levels or by causing the skipping of essential domains. AONs, also known as antisense oligonucleotides (ASOs) or splice-switching antisense oligonucleotides, are short, single-stranded oligonucleotides able to modify RNA expression by several mechanisms such as splice-switching and modulation of protein expression. Thanks to improvements in backbone composition and pharmacokinetic properties, several AONs have been translated to the clinic to treat neurological diseases such as Duchenne muscular dystrophy and spinal muscular atrophy [142]. However, their role in cancer therapy has been hampered by several issues, including selective delivery to tumor cells, plasticity, and clonal heterogeneity of cancer. A new generation of delivery tools including nanoparticles, lipid micelles, and direct chemical conjugation will hopefully solve some of these issues in the near future.

Altogether, PRDMs represent a family of proteins with invaluable therapeutic potential, as several members show oncogenic functions either by gain-of-function mutation, upregulation, or isoform switching. Data on targeting PRDM15 in B-cell lymphoma [129] provide hope that targeting oncogenic PRDMs, and thus entire transcriptional programs, is a promising strategy to overcome resistance and selectively target cancerous cells. Furthermore, genetic studies on PRDMs have shown that they are crucial for embryo development, but become partially or virtually dispensable for somatic homeostasis

during adulthood. Their expression is, however, reactivated during oncogenesis, which makes them safe therapeutic targets in adults. Oncogenesis is a selfish development: It reinstates developmental programs that were meant to be permanently turned off in adulthood, giving cells a growth advantage that paradoxically kills its host. Such processes lead to dedifferentiation and acquisition of aberrant stem cell-like properties. A notable example of how this can be exploited is the treatment of acute promyelocytic leukemia, where pro-differentiating agents that promote terminally differentiation of cancer cells have revolutionized treatment options [143]. Similarly, inducing differentiation through manipulation of master transcriptional regulators, such as PRDMs, is an exciting strategy for cancer therapy.

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Abbreviations

AON	antisense oligonucleotides
B-ALL	B-cell acute lymphoblastic leukemia
C2H2	Cys2-His2
FOG	Friends Of GATA
KO	knockout
KRAB	Krüppel-associated box
mESC	mouse embryonic stem cell
miR	MicroRNA
ncRNA	noncoding RNA
NuRD	nucleosome remodeling and deacetylase
Pak1	p-21-activated kinase
PKMT	protein lysine methyltransferases
PR	PRDF1-RIZ
PRDM	PRDF1 and RIZ1 homology domain containing
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SELEX	systematic evolution of ligands by exponential enrichment

SET	Su(var)3–9, Enhancer-of-zeste and Trithorax
shRNA	short hairpin RNA
siRNA	short interference RNA
SV	somatic structural variants
TAD	topologically associated domain
T-ALL	T-cell acute lymphoblastic leukemia
ZNF	zinc finger

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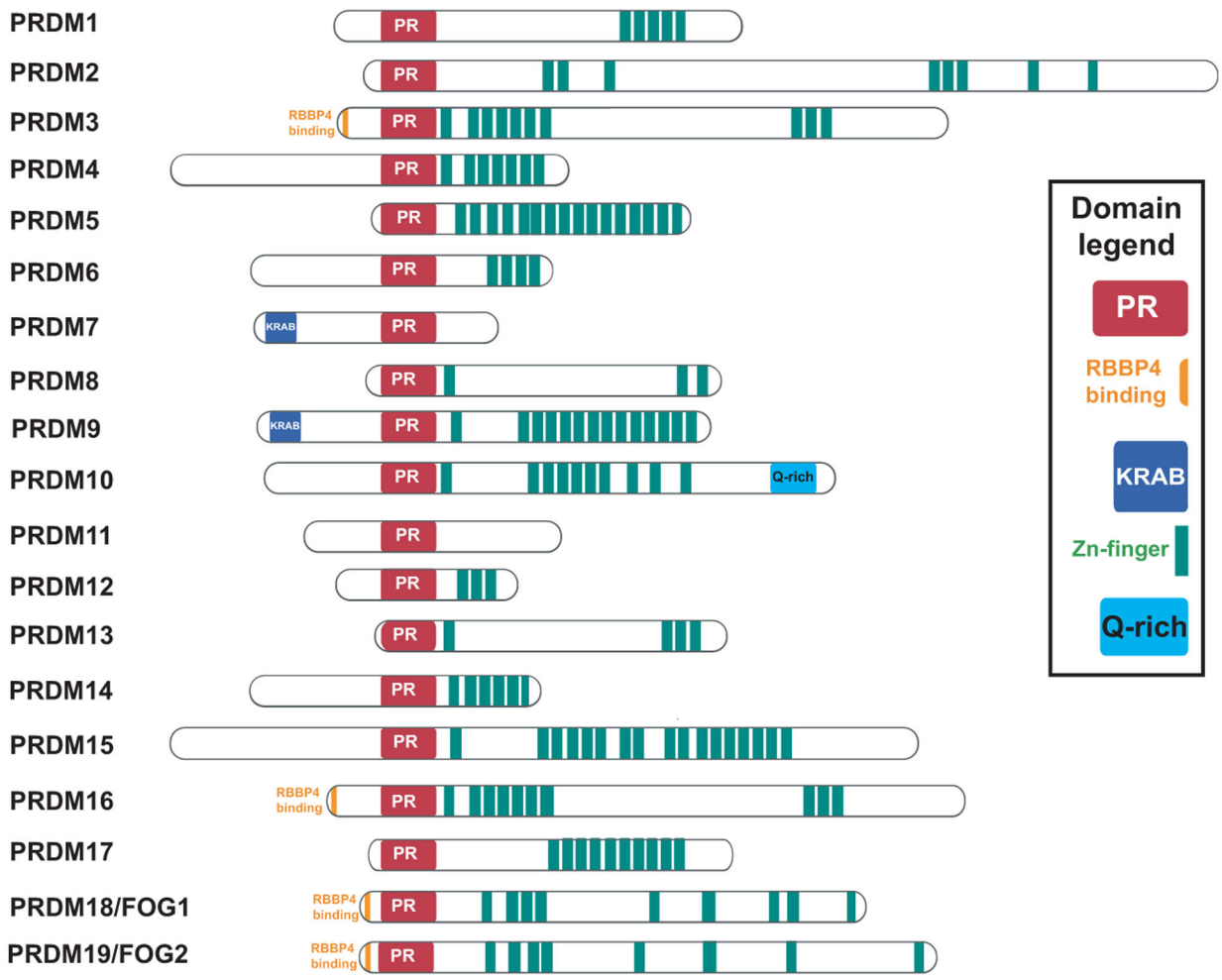

Fig. 1.

Domain structure of human PRDMs. PR domains are highlighted in red and C2H2 zinc fingers in green. Additional relevant domains are depicted including KRAB domains in blue, Q-rich regions in purple, and RBBP4 binding domain in orange. FOG-1 and FOG-2 proteins have been renamed respectively PRDM18 and PRDM19.

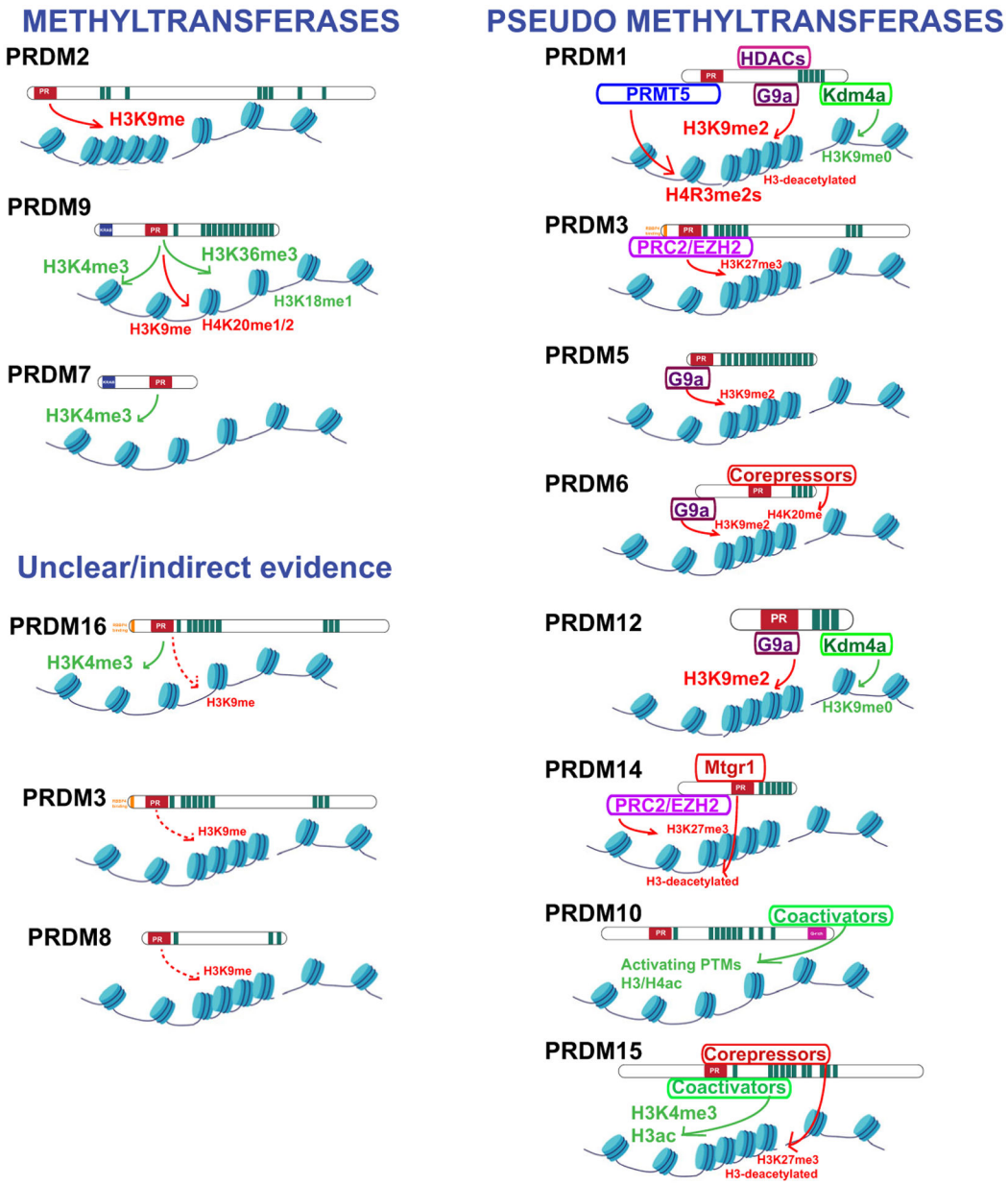

Fig. 2.

Summary of the experimental evidence of PRDM's methyltransferase activity. PRDMs have been classified into three categories: (a) methyltransferases (PRDM2, PRDM7, PRDM9, and PRDM16); (b) methyltransferases with unclear/indirect evidence (PRDM3, PRDM8); and (c) pseudomethyltransferases (PRDM1, PRDM3, PRDM5, PRDM6, PRDM10, PRDM14, PRDM15).

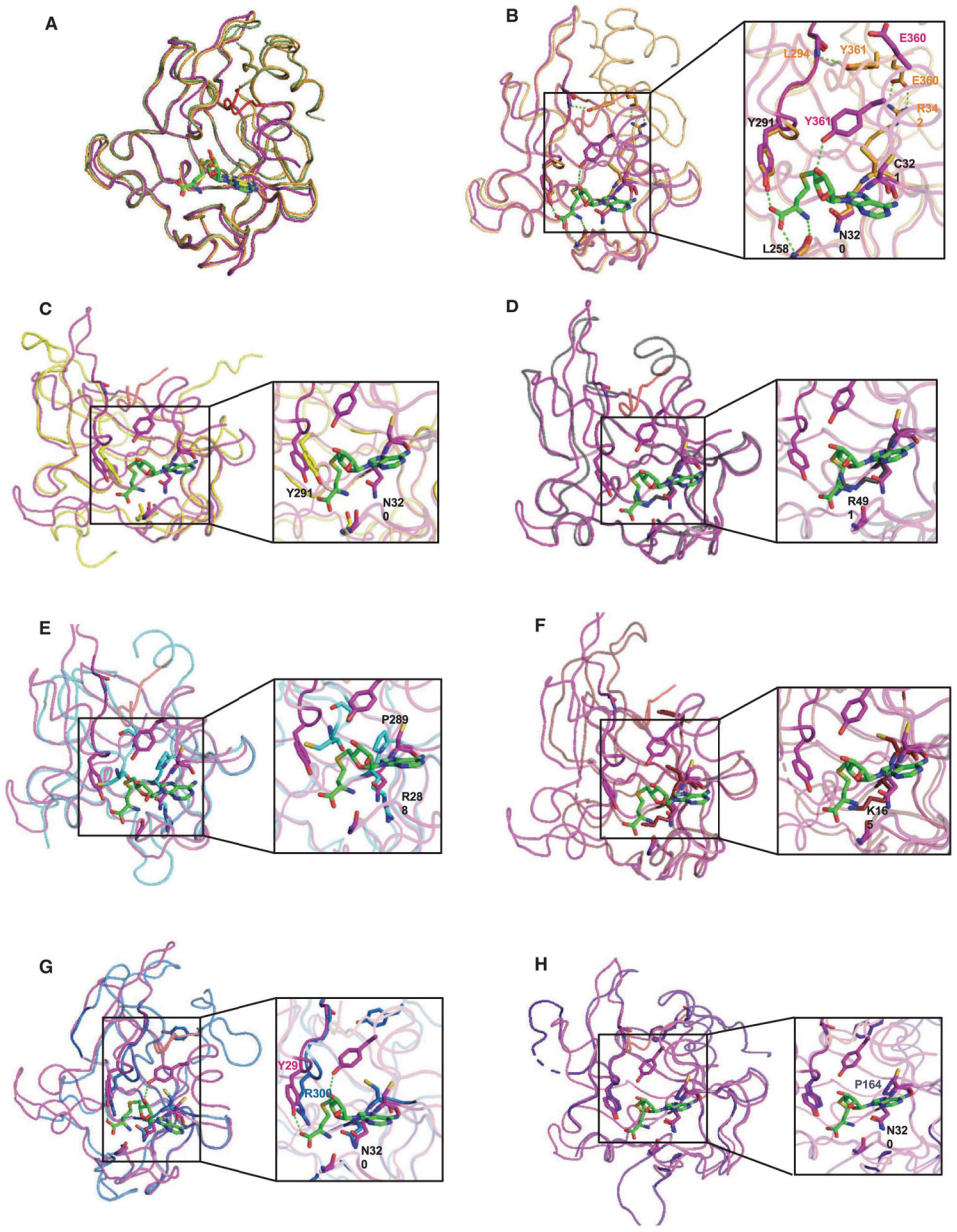

Fig. 3.

3D molecular models highlighting PR/SET domain interactions. (A) Comparison of the PR/SET domain of mPRDM9 (magenta) in complex with H3K4me2 peptide (red) and SAH (green) (PDB code 4C1Q), unbound hPRDM9 (orange) (PDB code 4IJD), and SAM (yellow) and bound hPRDM9 (green) (PDB code 6NM4). (B) Superposition of the PR/SET domain of mPRDM9-SAH (magenta) onto unbound hPRDM9 (orange). (C) PRDM2 (yellow) (PDB code 2QPW). (D) PRDM4 (gray) (PDB code 3DB5). (E) PRDM10 (cyan) (PDB code 3IHX). (F) PRDM12 (red) (PDB code 3EP0). (G) PRDM14 (blue) (PDB code 5ECJ). (H) PRDM1 (purple) (PDB code 3DAL). Residues interacting with SAH are highlighted. PYMOL (https://pymol.org/2/), an open-source molecular visualization system, has been used to generate the reported structures.

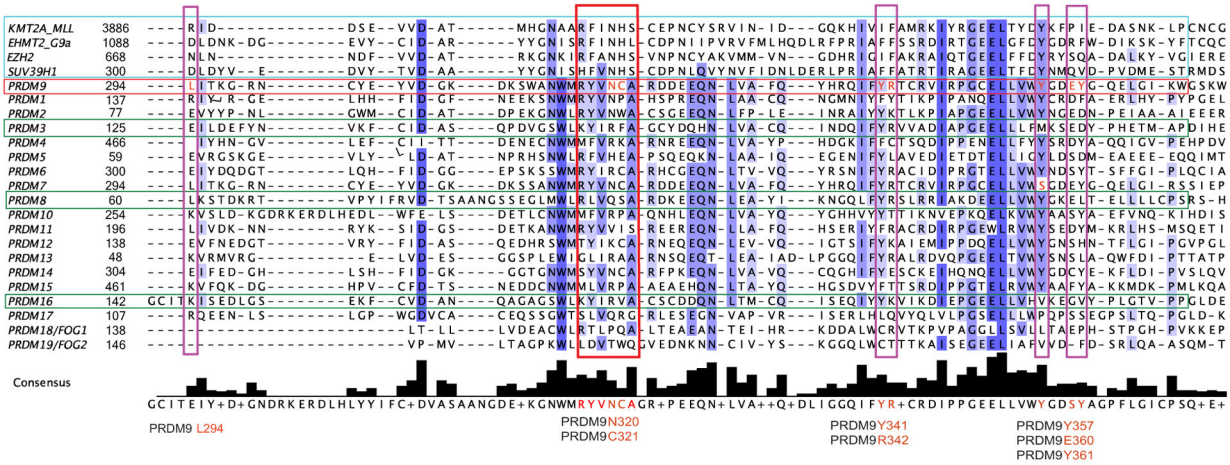

Fig. 4.

Sequence alignment of PRDMs' PR domains and representative human canonical SET domain containing methyltransferases (KMT2/MLL, EHMT2/G9a, Su(var)3–9H1, and EZH2 highlighted in the turquoise box). The green boxes refer to the PRDMs with demonstrated methyltransferase activity (PRDM9, PRDM3, and PRDM16). The red box highlights the exclusive PR domain motif that is associated with putative methyltransferase activity. The purple boxes denote key amino acidic residues critical for methyltransferase activity (identified in Fig. 3). The alignment has been generated by using the T-Coffee multiple sequence alignment algorithm (https://www.ebi.ac.uk/Tools/msa/tcoffee/) accessible through Jalview open-source software (http://www.jalview.org/). The amino acid sequences were downloaded from the publicly accessible database UniProt (https://www.uniprot.org/); their accession numbers are as follows: Q03164 (KMT2A_MLL), Q96KQ7 (EHMT2_G9A), Q15910 (EZH2), O43463 (SUV39H1), O75626 (PRDM1), Q13029 (PRDM2), Q03112 (PRDM3), Q9UKN5 (PRDM4), Q9NQX1 (PRDM5), Q9NQX0 (PRDM6), Q9NQW5 (PRDM7), Q9NQV8 (PRDM8), Q9NQV7 (PRDM9), Q9NQV6 (PRDM10), Q9NQV5 (PRDM11), Q9H4Q4 (PRDM12), Q9H4Q3 (PRDM13), Q9GZV8 (PRDM14), P57071 (PRDM15), Q9HAZ2 (PRDM16), Q9H9D4 (PRDM17), Q8IX07 (PRDM18/FOG1), and Q8WW38 (PRDM19/FOG2).

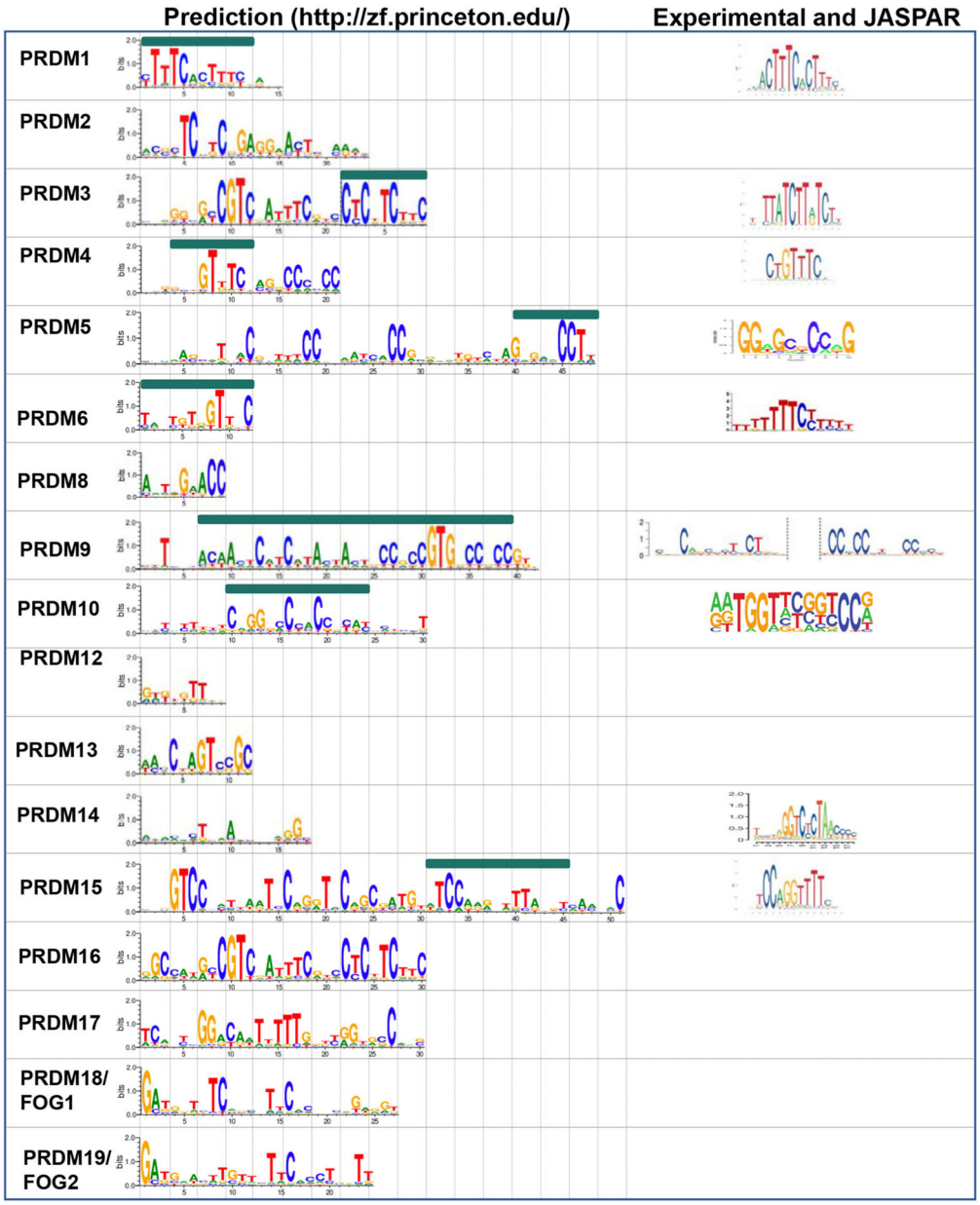

Fig. 5.

Summary of predicted and experimentally demonstrated PRDM zinc finger binding sites. A comparative analysis has been performed between predicted binding sites by using Princeton online tool (http://zf.princeton.edu/) and either experimentally demonstrated binding sites or binding sites deposited in JASPAR database.