

# The Genetic and Epigenetic Role of PRDM16 in Chondrogenesis

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**INTRODUCTION:** Osteoarthritis (OA) is a progressive degenerative disease characterized by degradation of articular cartilage. Medical treatment available for OA is limited and joint replacement is the only option at the later stages<sup>1</sup>. Cell-based therapy using human induced pluripotent stem cells (hiPSCs) can be a promising therapeutic strategy for OA<sup>1</sup>. Recently, our preliminary work showed that PRDM16 is up-regulated at the chondroprogenitor (CP) stage and remained elevated in hiPSC-derived chondrocytes, suggesting its potential regulatory role in chondrogenesis. PRDM16 has been reported to be a transcription and/or epigenetic regulator (i.e., methyltransferase) in various types of cells including adipocytes<sup>2</sup>. However, the detailed genetic and epigenetic mechanisms by which PRDM16 regulates chondrogenesis remains largely unknown. Here, we identified putative PRDM16 DNA binding regions, histone modifications, and its interacting partners (co-factors) at various stages of chondrogenesis using PRDM16 knockout (KO) hiPSCs, CUT&RUN sequencing, co-immunoprecipitation (CoIP), and mass spectrometry.

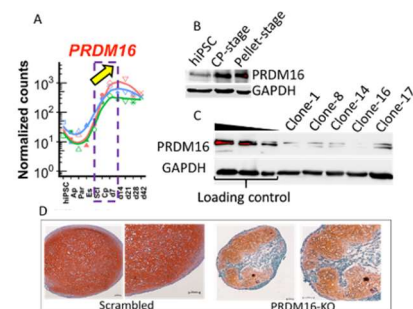
**METHODS:** PRDM16-KO hiPSCs were generated using CRISPR/Cas9 approach followed by single cell cloning. hiPSC chondrogenesis was conducted based on our previously published protocol<sup>3</sup>. CUT&RUN-seq was used to identify the DNA binding positions of PRDM16 and histone modifications (H3K4me3, H3K9me1) in scrambled and PRDM16-KO hiPSCs at CP and day 28 chondrogenic pellet stages. CoIP of PRDM16 followed by Liquid chromatography-mass spectrometry (LC-MS) was performed to identify the PRDM16 interacting proteins. MS-based proteomics was used to investigate the changes in protein expression during chondrogenesis.

**RESULTS:** Bulk RNA-seq and Western blot analyses showed that PRDM16 was up-regulated at the CP stage and remained elevated at pellet stage both at genetic and protein levels (Fig. 1A-B; 3 independent hiPSC lines; X-axis represents each stage of hiPSC chondrogenesis based on our established protocol<sup>3</sup>; AP: anterior primitive streak, Par: paraxial mesoderm, Es: early somite, Scl: sclerotome). Clone16 was used for the following studies based on the results of single cell clonal selection of PRDM16-KO lines (Fig. 1C). D28 PRDM16-KO pellets had significantly decreased Saf-O staining but increased off-target differentiation vs. scrambled pellets (Fig. 1D). CUT&RUN-seq revealed that *SOX8*, *PARPB*, *UPF2*, and *CNTN5* are modulated by PRDM16, H3K4me3, and H3K9me1 (Fig. 2A; the center intersection). Importantly, chondrogenic markers such as *COL2A1*, *COL10A1*, *SOX8*, etc. were targeted by PRDM16. Gene ontology (GO) enrichment analysis of PRDM16 targeted genes indicates that their involvement in histone methylation and demethylation, cell cycle regulation, transcriptional regulation, and extracellular matrix organization (Fig. 2B). Next, DNA binding motif of PRDM16 for several chondrogenic markers were identified by MEME motif Suite (Fig. 2C). Proteomics analysis indicates that critical proteins involved in chondrogenesis including MATN1, COMP, and COL2A1 were down-regulated in PRDM16-KO chondrocytes, while RAB15, ALDH1L1, UTF1, etc. were up-regulated (Fig. 3A). Integrated CUT&RUN-seq and proteomic datasets showed that COMP, COL2A1, COL10A1, and MAFB were down-regulated in PRDM16-KO chondrocytes (Fig. 3B). In addition to MAFB, several transcription factors (TFs) modulating chondrogenesis were also found to be targeted by PRDM16 (Fig. 3C). Using CoIP of PRDM16 followed by LC-MS, we identified that DNMT1, DNMT3B, and EHMT2 are putative binding partners of PRDM16 during chondrogenesis (Fig. 3D-E)

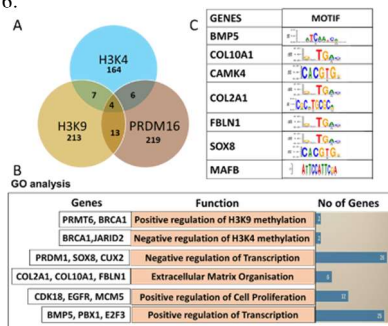
**DISCUSSION:** Since PRDM16 protein has ZFN and SET domains, it is predicted to be involved in DNA binding, protein-protein binding, and epigenetic regulation<sup>2</sup>. The observations that PRDM16 is up-regulated in chondrogenesis and that KO PRDM16 in hiPSCs resulted into decreased chondrogenic potential indicate that PRDM16 is a positive regulator for chondrogenic differentiation. Furthermore, we identified that PRDM16 may regulate expression of *SOX8*, *COL2A1*, and *COL10A1* via its DNA binding capacity. This result is further supported by the evidence that these proteins were down-regulated PRDM16-KO chondrocytes. Additionally, we observed that PRDM16 interacted with histone methylases such as DNMT1 and EHMT2 as well as demethylases like KDM1A. This result is consistent with previous findings showing that the interactions of PRDM16 with these histone modifiers are involved in adipogenesis and myogenic remodeling<sup>4,5,6</sup>. Here, we further identified that DNMT3B, SHMT2, METTL13, etc. are novel possible co-factors of PRDM16 modulating chondrogenic differentiation. We are currently investigating the link of PRDM16 with these histone methylases and demethylases in the context of chondrogenesis using gene knockout strategies.

**SIGNIFICANCE/ CLINICAL RELEVANCE:** A mechanistic understanding of the epigenetic role of PRDM16 in chondrogenesis will provide important insights into the development of therapeutic interventions for OA by targeting PRDM16 signaling pathway.

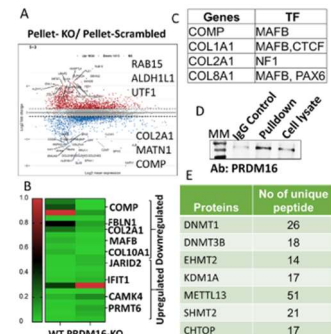
**REFERENCES:** 1. Jang+, *Int J Mol Sci*, 2021; 2. Jiang+, *Front Pharmacol*, 2022; 3. Wu+, *Nat Commun*, 2021; 4. Biferali+, *Sci Adv.*, 2021; 5. Jing+, *Nat Commun*, 2021; 6. Zen+, *Genes Dev*, 2016.



**Fig. 1.** (A, B) Upregulation of PRDM16 at CP and chondrogenic pellet stages. (C) Single cell clonal selection of PRDM16 KO lines. (D) PRDM16-KO pellets had decreased Saf-O staining.



**Fig. 2.** (A) Four genes were targeted by PRDM16 H3K4me3, and H3K9me1. (B) GO analysis of PRDM16 targeted genes. (C) Selected PRDM16 targeted genes involved in chondrogenesis and their DNA binding motif.



**Fig. 3.** (A, B) Selected genes/proteins regulated by PRDM16. (C) TFs targeted by PRDM16 (D, E) Selected PRDM16 interacting partners during chondrogenesis.