PRDM16 positively regulates chondrogenesis and knee joint homeostasis

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INTRODUCTION: Cartilage development is a complex process regulated by tightly coordinated transcription and epigenetic networks. Our preliminary work showed that PRDM16 is upregulated during the course of chondrogenesis of human induced pluripotent stem cells (hiPSCs). PRDM16 possesses PR domains with capacity for histone modification, and zinc finger domains to enable protein-DNA or protein-protein interactions[1]. Microdeletion of the 1p36 locus which includes the PRDM16 region is associated with severe limb defects in humans[2]. PRDM16 is also one of the top down-regulated genes in subchondral bone of patients with osteoarthritis (OA)[3]. It is reported that global knockout (gKO) of Prdm16 in mice results in abnormal osteogenic and chondrogenic differentiation, while heterozygous gKO of Prdm16 inhibits endochondral ossification in the mouse femoral head[4]. However, the detailed molecular mechanisms by which PRDM16 regulates chondrogenesis and joint homeostasis in a cartilage-specific manner remain largely unknown. Here, we hypothesize that PRDM16 is a positive regulator in chondrocyte specification and postnatal knee cartilage homeostasis. We aim to elucidate the regulatory mechanisms of PRDM16 by using our cartilage-specific, conditional knockout (KO) mouse strain and hiPSCs models.

METHODS: All animal procedures were in compliance with UR IACUC. Surgery to destabilize the medial meniscus (DMM) was performed on the left knees of 16-wk-old cartilage-specific Prdm16 KO mice (Col2a1-Cre;Prdm16$^{flox/flox}$; Prdm16 KO). Right knees were used as non-surgery control. Littermates without Prdm16 KO were used as WT control. Both hind limbs were harvested 12-wk-post surgery for microCT (μCT; n=5) and histological analysis (i.e., Safranin O (Saf-O)/Fast green; n=3). Female and male mice were investigated independently. hiPSCs with inducible knockdown (KD) or overexpression (OE) of PRDM16 were generated and differentiated into chondrocytes based on our established protocol[5]. A non-edited cell line was used as control. Chondrogenic pellets (n=4) were harvest at day 28. Data were analyzed with either one-way or two-way ANOVA, with Fisher's LSD post-hoc analysis, as appropriate.

RESULTS: μCT analysis showed increased osteophyte formation in DMM joints as compared to non-surgery joints (**Fig. 1A;** only male mice shown due to space**)**. For both WT female and male mice, DMM joints exhibited a trend of decreasing bone mineralization density (BMD) in the medial meniscus compared to non-surgery joints (**Fig. 1B**). However, BMD of the medial meniscus was comparable in both male and female KO mice following injury. Loss of Saf-O staining and osteophyte formation (green arrows and white dashed lines) were observed in DMM joints (**Fig. 2A;** only male mice shown as female mice are currently being analyzed). In the WT mice, a trend of increased OA severity was observed in the DMM joints compared to the non-surgery joints, but OA severity was similar between operated and non-surgery joints in KO mice (**Fig. 2B**). Non-surgery joints in the KO mice had more severe OA versus those in the WT mice. Western blot validated PRDM16 KO and OE were successfully induced in hiPSC-derived chondrocytes (**Fig. 3A**). PRDM16 KD chondrocytes exhibited severe loss of Saf-O staining, while OE of PRDM16 maintained high staining intensity (**Fig. 3B**). Furthermore, decreased DNA concentration was observed in the pellets of both edited lines versus control, but only OE hiPSCs produced significant amount of GAG compared to KD line (**Fig. 3C**).

DISCUSSION: Previous studies have shown that KO PRDM16 in mice and zebrafish[7] interferes with palatogenesis and craniofacial development through Meckel's cartilage of the madible[8,9]. However, the regulatory role of PRDM16 in limb development is largely unknown. Both male and female KO mice had lower BMD of the medial meniscus in their non-surgery joints versus WT mice, suggesting a critical role of PRDM16 in knee homeostasis. Moreover, Prdm16 KO mice also had a different response to injury as they exhibited comparable BMD in their medial menisci between DMM and non-surgery joints, while medial meniscus BMD of the DMM joints in the WT mice showed a decreasing trend post-injury. Thus, future studies of the role of PRDM16 in osteogenesis or chondrocytes transitioning into osteoblasts are warranted. In the current work, DMM did not lead to higher OA severity in the male KO mice; however, we are increasing our sample size in order to make a definitive conclusion. Most interestingly, non-surgery joints in the KO mice demonstrated more severe OA compared to those in the WT mice, suggesting PRDM16 is necessary to maintain cartilage homeostasis postnatally. In the *in vitro* study, both OE and KD PRDM16 led to decreased DNA content in the pellets, implying a link between PRDM16 and cell viability. Indeed, this result is consistent with previous findings showing that methyltransferase activity of PRDM16 is required for heterochromatin integrity and cell survival[10]. Nevertheless, OE of PRDM16 results in high GAG production that is comparable to control pellets but with fewer cells (lower DNA content), suggesting that these OE cells are highly chondrogenic.

SIGNIFICANCE/CLINICAL RELEVANCE: OA is the most prevalent degenerative joint disorder. Our findings will elucidate the regulatory role of PRDM16 in chondrogenesis and facilitate the development of tissue engineered cartilage for future therapeutic applications.

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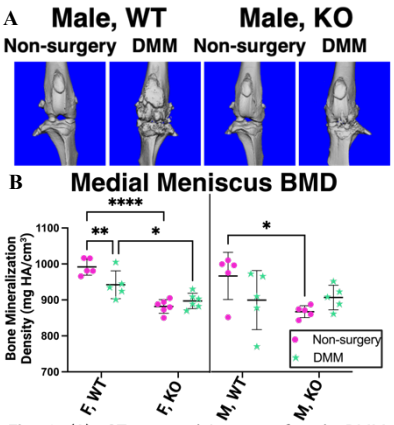

Fig. 1: (**A**) μCT scanned images of male DMM and non-surgery control hind limbs. Female images not included due to space. (**B**) Average mineralization density of medial meniscus (* p <.05, ** p <.01, **** p<.0001).

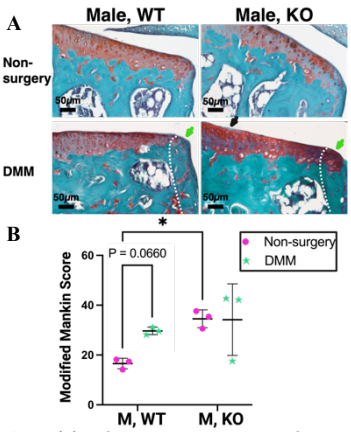

Fig. 2: (**A**) Saf-O/Fast green staining of DMM and non-surgery control medial joints. Arrows indicate cleft of cartilage (black) and osteophyte (green). (**B**) Modified Mankin grading of DMM and non-injury control joints (* p<.05).

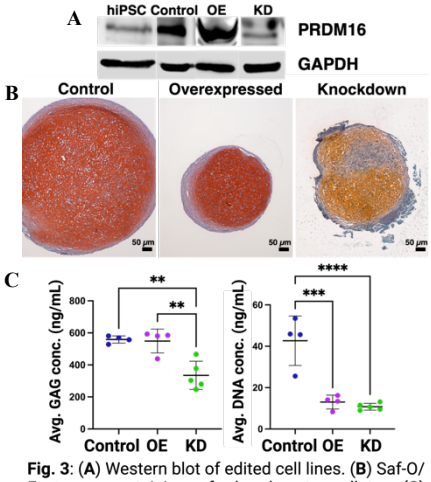

Fig. 3: (**A**) Western blot of edited cell lines. (**B**) Saf-O/ Fast green staining of chondrocyte pellets. (**C**) Average GAG & DNA concentration of chondrocyte pellets (** p <.01, *** p <.001, **** p <.0001).