Applying A PROTAC Drug SHP2D26 to Promote Cartilage Anabolism and Chondrogenesis

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INTRODUCTION: Osteoarthritis (OA) is a leading cause of disability and healthcare costs in the United States, ranking among the top ten diseases for years lost to disability. Current therapies—such as NSAIDs and corticosteroids—are limited to symptomatic relief, while joint replacement remains the only option for advanced disease. Cartilage degradation in OA arises from an imbalance between anabolic repair and catabolic breakdown, a process regulated in part by intracellular signaling pathways governed by reversible protein phosphorylation. SHP2, encoded by *PTPN11*, is a tyrosine phosphatase that negatively regulates chondrogenesis (1) by controlling chondroprogenitor differentiation and restricting new cartilage formation (2). Genetic inhibition of SHP2 enhances the reparative capacity of chondrocytes, leading to thicker articular cartilage and increased expression of anabolic genes (3). These findings highlight SHP2 as a promising therapeutic target for cartilage repair and disease modification. Proteolysis-targeting chimeras (PROTACs) are heterobifunctional small molecules capable of degrading traditionally "undruggable" proteins and have emerged as a powerful platform for novel drug development. SHP2D26 is a new and highly potent SHP2-targeting PROTAC that offers an innovative approach to modulating SHP2 activity. In this study, we investigate the efficacy of SHP2D26 in promoting chondrogenesis *in vitro* and *in vivo* and evaluate its potential for clinical translation.

METHODS: Mice carrying the $Ptpn11^{fx}$ and $Prg4^{CreER}$ alleles (C57BL/6 background) were bred to generate $Tg(Prg4^{CreER};Ptpn11^{fx/+})$ (SHP2CT) and $Tg(Prg4^{CreER};Ptpn11^{fx/fx})$ (SHP2KO) mice. In some studies, $Rosa26^{ZSG}$ or $Rosa26^{Ai9}$ were bred in as a Cre reporter. *Prg4+* articular cartilage cells were isolated from the knee epiphyseal cartilage of two-week-old mice, expanded, and sorted by FACS based on the reporter. Articular cartilage morphology and composition were examined using micro-CT and Alcian Blue/Alizarin Red staining. Dr. Shaomeng Wang (Univ. of Michigan) provided SHP2D26. To trace the fate of *Prg4+* cells, knee joints were collected from P6- and P12-week-old SHP2CT;$R26^{ZSG}$ and SHP2KO;$R26^{ZSG}$ mice, and Prg4+ cells were visualized microscopically on frozen sections, with and without immunostaining using antibodies against chondrogenic and osteogenic markers. To examine the impact of SHP2D26 on articular cartilage, we conducted functional studies, including IL-1 challenge, destabilization of the medial meniscus (DMM) surgery, and systemic administration of SHP2D26 in mice. Cartilage catabolic and anabolic gene expression in response to SHP2D26 treatment was evaluated using RNAScope and qRT-PCR. Group differences were analyzed using Student's t-tests or two-way ANOVA.

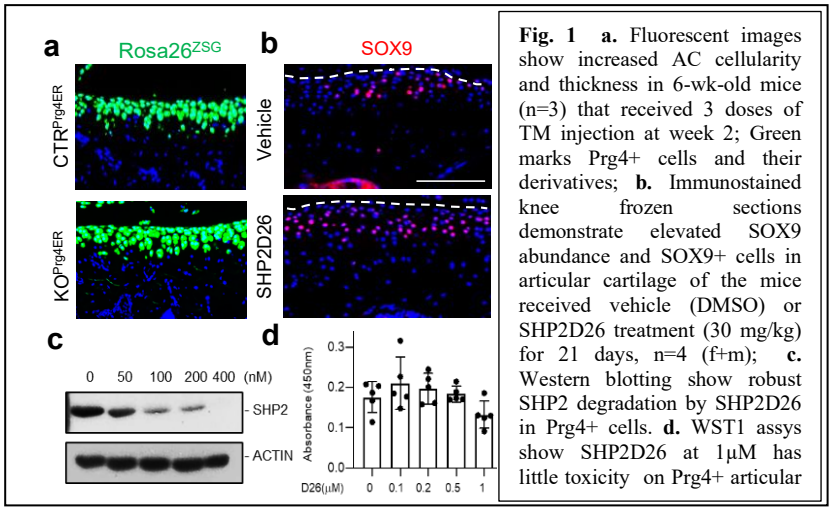

Fig. 1 **a.** Fluorescent images show increased AC cellularity and thickness in 6-wk-old mice (n=3) that received 3 doses of TM injection at week 2; Green marks Prg4+ cells and their derivatives; **b.** Immunostained knee frozen sections demonstrate elevated SOX9 abundance and SOX9+ cells in articular cartilage of the mice received vehicle (DMSO) or SHP2D26 treatment (30 mg/kg) for 21 days, n=4 (f+m); **c.** Western blotting show robust SHP2 degradation by SHP2D26 in Prg4+ cells. **d.** WST1 assys show SHP2D26 at 1μM has little toxicity on Prg4+ articular

RESULTS: SHP2 deletion in Prg4+ cells was found to increase articular cartilage cellularity and thickness at six and twelve weeks of age without apparent adverse effects on the joints. The loss of SHP2 also increased SOX9 protein abundance, boosting SOX9-driven transcriptional activity and upregulating the expression of anabolic genes (*Col2a1, Agc1*). SHP2-deficient chondrocytes were found to be protected from IL-1–induced SOX9 degradation, and SHP2KO mice demonstrated resistance to DMM-induced cartilage degeneration. Importantly, systemic administration of SHP2D26 in adult mice resulted in a modest but significant increase in cartilage thickness and the number of SOX9+ cells, without impacting growth plate cartilage.

DISCUSSION: Our findings demonstrate that SHP2 acts as a negative regulator of cartilage anabolism by modulating SOX9 stability and transcriptional activity. The genetic depletion of SHP2 enhances the anabolic potential of *Prg4+* chondrocytes and provides protection against catabolic insults, while pharmacological degradation using SHP2D26 replicates these anabolic effects in adult cartilage. These results highlight SHP2 as a promising therapeutic target for promoting cartilage regeneration and mitigating OA progression. PROTAC-based protein degradation technologies, such as SHP2D26, offer rapid, reversible, and efficient control of protein abundance, positioning them as an attractive strategy for translational development. The ability to modulate SHP2 signaling without permanently altering the genome may provide a safer and more controllable approach for clinical applications.

SIGNIFICANCE / CLINICAL RELEVANCE: Targeting SHP2 signaling—especially with degraders like SHP2D26—offers a novel therapeutic avenue for enhancing cartilage regeneration and protecting against OA-related cartilage degeneration. By using either genetic ablation or pharmacological degradation of SHP2, we can improve cartilage repair and protect against degeneration, presenting a novel disease-modifying approach for OA.

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