

SHP2 Depletion Promotes Cartilage Anabolic Gene Expression by Increasing SOX9 Protein Stability

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INTRODUCTION Cartilage diseases remain among the costliest and most clinically burdensome musculoskeletal disorders. Therefore, understanding of articular cartilage (AC) and growth plate cartilage (GPC) development and homeostatic mechanism(s) could lead to novel therapeutics for sustaining AC homeostasis, preventing cartilage degeneration, and promoting the repair of injured cartilage. Reversible protein phosphorylation is one of many post-translational protein modifications (PTMs) that are crucial for regulating tissue development and homeostasis. To date, most of the work on PTMs in cartilage has focused on protein phosphorylation by tyrosine kinases (PTKs). However, dephosphorylation by protein tyrosine phosphatases (PTPs) has equally profound functional consequences but is understudied. SHP2, encoded by PTPN11, is a cytoplasmic PTP. Somatic SHP2 loss-of-function mutations in human cause a benign cartilage tumor syndrome, and conditional SHP2 deletion in chondrogenic cells in mice provokes the formation of osteochondromas and thicken GPC, though the cellular and molecular mechanisms remain incompletely understood. Here, we interrogate the impact of SHP2 depletion in Prg4+ cells on AC in mice. Investigating the impact of SHP2 depletion in AC should provide important insights into fundamental processes of cartilage biology and the potential translational application of SHP2 agonists and antagonists.

RESULTS SHP2 expression and SHP2KO mice were born at the expected Mendelian ratios and exhibited similar body weight and length, growth plate thickness and overall joint structure over postnatal life. However, AC thickness increased significantly in SHP2KO mice, compared to SHP2KO controls (Fig. 1a). The increase in AC thickness was also accompanied by an increase in the abundance of anabolic gene transcripts, such as Col2a1 and Acan, and SOX9 protein, and the cellularity of Sox9+ AC cells (Figs. 1b, 1c). These observations were replicated in mice and in cultured chondrocytes following treatment with the SHP2 PROTAC inhibitor, SHP2D26, which efficiently and rapidly depleted intracellular SHP2 [2]. Our mechanistic studies indicate that SHP2 negatively regulates SOX9 stability and attenuates its degradation by proteosomes, since MG132 treatment of Control Prg4+ AC cells markedly increased SOX9 in vitro (Fig. 1c). Additional evidence that SHP2 negatively regulates SOX9 stability include the significant increase in SOX9 expression in cultured cells (Fig. 1d) and SOX9+ cells in both AC and GPC in vivo (Fig. 1e) after SHP2D26 administration.

DISCUSSION Articular cartilage homeostasis is crucial for life-long joint function, but the underlying cellular and molecular mechanisms governing chondrocyte stability remain poorly understood. In this study, we have found the SHP2 supports AC development and homeostasis, in part, by influencing SOX9 stability and SOX9 responsive anabolic gene expression in the Prg4+ cartilage cells. SHP2 regulation of SOX9 stability is mainly mediated by PTM, since SOX9 abundance is comparable in Prg4+ AC cells between SHP2Knockout and SHP2KO mice. Importantly, MG132 but not CHX treatment stabilizes SOX9 in control cells, suggesting that SHP2 positively modifies lysosome-mediated SOX9 degradation. This finding is further supported by our in vivo findings.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that SHP2 is indispensable for joint development and homeostasis, and that targeting the SHP2 signaling pathway has a potential to mitigate AC degeneration by sustaining SOX9 expression and anabolic gene expression. Our findings could form the basis for novel therapeutics to a series of cartilage degenerative diseases.

REFERENCES:

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