PTPN11 Gene Is Required For Chondrogenesis and Cartilage Homeostasis

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Introduction: Cartilage plays an important role in supporting skeletal growth, reducing frictions at joints, and facilitating the normal functions of certain organs. Chondrocytes are the solely cellular component in cartilage; their development and maturation involve multifaceted and finely regulated processes. However, the molecular and cellular mechanisms underlying chondrogenesis remain incompletely understood. Shp2, encoded by the Ptpn11 gene, is one of two Src homology 2 domain-containing protein-tyrosine phosphatases, and is required for most, if not all, receptor tyrosine kinases (RTKs), cytokine, and integrin signaling pathways. Global deletion of Shp2 in mice results in early embryonic lethality, whereas postnatal Shp2 deficiency in various tissues/cells has diverse effects on their development and function. Ptpn11 loss-of-function (LOF) mutations in cartilage were recently reported to cause a human cartilage tumor syndrome metachondromatosis, suggesting a critical role for Shp2 in chondrogenesis. We hypothesize that Shp2 regulates chondrogenesis by influencing RTKs and cytokine signaling and therefore investigated the role of Shp2 in the development and homeostasis of growth plate and articular cartilage via a genetic LOF approach in vivo and in vitro.

Methods: Mice carrying Ptpn11 floxed (fl), Col2a1-Cre and Col2a1-CreERtm alleles were described previously (1,2). To generate Col2a1-expressing-cell specific Shp2 deficient mice and study the role of Shp2 in chondrogenesis and cartilage homeostasis, Ptpn11 floxed mice were bred to Col2a1-Cre and Col2a1-CreERtm alleles to generate Ptpn11fl/fl;Col2a1-Cre (Col2a1-KO), Ptpn11fl/+;Col2a1-Cre (Col2a1-Control), Ptpn11fl/fl;Col2a1-CreERtm (Col2a1ER-KO), and Ptpn11fl/+;Col2a1-CreERtm (Col2a1ER-Control) animals. To trace the fate of Col2a1+ chondroid cells in vivo in the presence or absence of Shp2, Roza26mTmG (R26mTG) reporter alleles (3) were also bred to Col2a1ER-KO and Col2a1ER-Control animals. Chondroprogenitor ATDC5 cells with stable Shp2 knockdown were established by retroviral shRNA technology. For histological analysis, skeletal tissues were fixed, decalcified, embedded, and sectioned following a standard protocol. Tissue sections were stained with H&E, alcian blue, and Safranin-O to visualize general histology and ECM content. µ-CT and X-ray radiographs were conducted to visualize joint structures. Quantitative RT-PCR was carried out with total RNA extracted from femoral heads of Col2a1ER-KO and Control mice post tamoxifen treatment or from ATDC5 cells at different chondrocytic stages. Immunoprecipitation and western blot analysis were performed following a standard protocol.

Results: Shp2 deficiency in Col2a1-expressing cells leads to dwarfism and multiple joint dysplasia: By taking a cartilage-specific gene ablation approach and biochemical studies, we found that Shp2 is essential for chondrogenesis and that its deficiency in Col2a1-expressing cells causes midgestation embryonic lethality. Postnatal Shp2 deletion in chondrocytes upon tamoxifen induction leads to dwarfism, multiple joint dysplasia, and impairment of the articular and growth plate cartilage development and homeostasis (Fig. 1A).

Shp2 regulates chondrocyte maturation: Examining the role of Shp2 in cartilage formation and chondrocyte differentiation in 6-week-old KO-Col2a1CreER mice demonstrated that Shp2 was necessary for forming well-organized growth plate cartilage. This organized columnal structure was disrupted in Shp2 deficient mice, instead featuring a broadened growth plate with an increase in proliferating and hypertrophic chondrocytes (Fig. 1B). These histological alterations were further enhanced by aging and accompanied by the decrease of ECM content as revealed by the reduction of Safranin O staining (Fig. 1C). These characteristics in growth plate cartilage were also observed in articular cartilage. Gene expression analysis demonstrates that Shp2 deletion in Col2a1-expressing cells compromises the expression of chondrocytic genes, such as Sox9, Runx2, Col2a1, and aggrecan. Shp2 negatively regulates IGF1-evoked Erk and Akt activation: Upon focusing on IGF1 signaling in ATDC5 cells, Shp2 was found to negatively regulate IGF1-evoked Erk and Akt activation by modulating the tyrosyl phosphorylation of IGF1R and IRS1, its recruitment of p85 subunit of PI3 kinase, and other signal relaying molecules.

Discussion: We reported an important role for Shp2 in embryonic and postnatal cartilage development and homeostasis. Shp2 regulates chondrogenesis possibly by influencing multiple RTK and cytokine receptor signaling pathways and the expression of Sox9 and Runx2. At the very least, Shp2 negatively regulates IGF1-evoked chondrogenesis by modulating Erk and Akt activation in vitro.

Significance: Our study has uncovered an important role for Shp2 in chondrogenesis and cartilage homeostasis. It is therefore apparent that manipulating Shp2 and Shp2-regulated signaling pathways can potentially facilitate the development of novel therapeutics to treat developmental and degenerative cartilage diseases.

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References:
Figure 1. Shp2 deletion in Col2a1-expressing cells causes dwarfism, scoliosis, and multiple joint dysplasias. A. X-ray and μ-CT radiographs showed that TM-treated 1-week-old KO"Col2a1" mice compared to Controls, had dwarfism and scoliosis (top), joint dysplasia of hip (middle) and knee (low) (n=5). Notice that KO"Col2a1" mice had a shallow acetabular socket and misshapen and improperly mineralized femoral head and greater trochanter (arrows); a broad growth plate cartilage (arrows) was noticeable in the knee joints of KO"Col2a1" mice (arrows). B. Images of Safranin O-stained knee joint sagittal sections showing the broad and disorganized growth plate cartilage in 6-week-old KO"Col2a1" mice compared to that of Controls. Images of i, ii and iii, iv are enlarged view of the boxed area (top) of the growth plate and articular cartilage, respectively. C. Images of H&E (i, ii, v, vii) and Safranin O-stained (i, ii, iii, vii) proximal tibia sections demonstrated that long-term Shp2 deletion caused distorted growth plate cartilage, reduced ECM content and cellularity in the articular cartilage of 12-week-old KO"Col2a1" mice (iii, vii).