Activation of Indian Hedgehog Promotes Chondrocyte Hypertrophy and Upregulation of MMP-13 in Osteoarthritic Cartilage

Frank Wei¹, Jingming Zhou¹, Xiaochun Wei², Wesley Wu¹, Richard Terek¹, Qian Chen¹, +Lei Wei³

¹Department of Orthopaedics; Brown Medical School/Rhode Island Hospital, CORO West, 1 Hoppin Street, 402A, Providence RI 02903
²Department of Orthopaedics; Shanxi Medical University, Taiyuan, Shanxi, 030001
³Lei_Wei@brown.edu

Introduction. Osteoarthritis (OA) is the most common degenerative joint cartilage disease but its exact pathogenesis is still largely unknown. Indian hedgehog (Ihh) is principally synthesized in the prehypertrophic chondrocytes during growth plate development. It plays a crucial role in regulating the onset of chondrocyte hypertrophy and endochondral bone formation. Recent mouse genetic studies show that Ihh promotes chondrocyte hypertrophy in the developmental growth plate and may play a role in articular chondrocytes. However, direct genetic evidence for Ihh in adult mice and OA patients has not been reported because tissue-specific deletion of Ihh (targeted by Col2al-Cre) mice died shortly after birth. In this study, we examine its role by comparing the level of Ihh in OA cartilage to normal cartilage and by quantifying collagen type X and MMP-13 after up-regulating or knocking-down Ihh gene expression in human OA chondrocytes.

Material and Methods. Knee joint cartilage and synovial fluid (SF) were obtained during patient OA knee joint replacements (N=36). Normal control samples were obtained from tumor amputations and healthy volunteers (N=5). X-ray demonstrated cartilage damage in OA patients and no joint changes was found in normal controls. Expression of Ihh on the cartilage and in SF were determined by immunohistochemistry and western blot respectively. The density of Ihh expression and the size of chondrocytes were calculated using Ivision software. mRNA levels of Ihh, type X, and MMP-13 were compared between OA cartilage and its adjacent normal cartilage. To determine whether Ihh plays a role in chondrocyte hypertrophy and matrix degradation, mRNA levels of collagen type X and MMP-13 were determined by real time PCR from human OA chondrocytes incubated with Ihh protein and Ihh SiRNA.

Results. Expression of Ihh protein was undetectable in normal articular cartilage, but was significantly increased in cartilage of OA patients (Fig. 1). The expression of Ihh was mainly located in the superficial zone of articular cartilage, which was correlated with the severity of OA cartilage damage as determined by modified Mankin score. Western blot analysis indicated that the concentration of Ihh in synovial fluid was much higher in OA patients compared to the age-matched controls (Fig. 2). High levels of mRNA of Ihh, collagen type X, and MMP-13 were found in OA cartilage compared to the adjacent normal cartilage. Furthermore, treatment of OA chondrocytes with Ihh protein resulted in an increase in collagen type X, a marker for chondrocyte hypertrophy and MMP-13, a critical enzyme for cartilage matrix degradation (Fig. 3). Conversely, knockdown of Ihh by transfecting chondrocytes with Ihh SiRNA inhibited the expression of collagen type X and MMP13 in those cells.

Discussion. Recent studies indicate that OA articular chondrocytes recapitulate the differentiation process that happens during fetal development, which does not occur to an appreciable degree in normal adult articular cartilage. In the developmental growth plate, Ihh is expressed by the prehypertrophic chondrocytes. In this study, we demonstrate that the expression of Ihh is associated with the severity of OA cartilage damage. We also found the levels of Ihh, collagen type X, and MMP-13 in OA samples to be much higher in comparison to normal controls. Our results indicate that Ihh may regulate the chondrocyte hypertrophic phenotype and cartilage degradation during OA development. Furthermore, we demonstrate that upregulated Ihh signaling is correlated with accelerated chondrocyte hypertrophy as determined by collagen type X and cartilage matrix degradation by MMP-13. Direct evidence from the normal and OA patients indicates that OA cartilage degeneration is accompanied by a response of chondrocytes to this damage which involves enhanced Ihh synthesis. This study provides strong evidence regarding the role of Ihh in promoting chondrocyte hypertrophy and the pathological progression of OA.

Fig. 1. The expression of Ihh is significantly increased in OA cartilage comparison to normal control.

Fig. 2. An increase of Ihh was found in the synovial fluid of OA patient compared to aged matched healthy controls.

Fig. 3. Knockdown of Ihh by Ihh SiRNA decreases the expression of collagen type X and MMP-13 while overexpression of Ihh significantly increases the expression of collagen type X and MMP-13.