Cholesterol Homeostasis mediates Hedgehog Signaling in Osteoarthritis

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Introduction: Current evidence suggests that mechanical, genetic, and metabolic factors likely play a role in the pathogenesis of osteoarthritis (OA). The changes that occur to chondrocytes during disease progression, such as hypertrophy, recapitulate some of the changes that occur to growth plate chondrocytes during elongation of the long bones. Hedgehog (Hh) signaling regulates chondrocyte differentiation in the growth plate and has been shown to regulate osteoarthritic changes in the articular cartilage, with higher levels of Gli-mediated transcriptional activation associated with increased severity1,2. To elucidate the mechanism through which Hh signaling exacerbates OA, we identified novel Hh target genes in human osteoarthritic cartilage.

Methods: Microarray analyses were performed to detect changes in gene expression when the Hh pathway was modulated in human OA cartilage samples. Results from the Affymetrix Human Gene 1.0 ST microarray were analyzed for differentially expressed genes and grouped into functional networks using Ingenuity® Pathway analysis. From this, several genes known to be involved in sterol homeostasis were found to be modulated by Hh signaling. To investigate the function of sterol homeostasis in cartilage, mice with chondrocyte-specific cholesterol accumulation were generated. This was achieved by excising Insig1 and Insig2, major negative regulators of cholesterol homeostasis3, under Col2a1 regulatory elements (designated InsigDKO). To assess sterol and lipid accumulation, Oil-Red-O staining and quantification was performed. To assess OA development, histology, radiography, and gene expression analyses were conducted.

Results: With aging or surgically induced joint instability, mice with chondrocyte-specific cholesterol accumulation (InsigDKO) developed more severe OA than control littermates (Fig. 1A). They expressed markers of chondrocyte hypertrophy in the articular cartilage (Fig. 1B), exhibited subchondral bone sclerosis (Fig. 1C), and had increased expression of proteases including Adamts5 and Mmp13. Statin treatment to inhibit cholesterol production rescued this phenotype and reduced the severity of OA (Fig. 1D). Genetic manipulation of Hedgehog signaling in these mice altered the accumulation of sterol. Higher levels of Gli-mediated transcription in Col2a1-Gli2 mice caused accumulation of sterol, and lower levels of Gli-mediated transcription in Gli2+/- mice reversed this, both in the presence and absence of Insig1 (Fig. 1E).

Discussion: We identified cholesterol homeostatic genes as novel Hh target genes in chondrocytes, and found that cholesterol dysregulation in the chondrocytes predisposes to OA. We show that Hh signaling regulates sterol accumulation in chondrocytes, and through the use of InsigDKO mice, we show that this relationship exists independently of Insig1. Future experiments will determine the exact mechanism through which sterol accumulation results in OA. Our data suggest that pharmacologic correction of intra-articular sterol imbalance, by either statin treatment of inhibition of Hh signaling, can be used as a treatment for osteoarthritis.

Significance: This is the first study to demonstrate that Hedgehog signaling regulates genes that govern cholesterol homeostasis, and that intracellular cholesterol accumulation contributes to osteoarthritis.
pathogenesis. Our findings have therapeutic implications since statin treatment attenuated the severity of osteoarthritis, and reduction of Hedgehog signaling reversed cholesterol accumulation.

Figure 1. (A) Representative sections of mouse knees showing Safranin-O staining at 6 months of age. Arrows point to loss of proteoglycan (red) and cartilage defects in InsigDKO. (B) Representative sections showing Col2a1 immunohistochemistry (brown/arrow) as a marker of hypertrophic chondrocytes in the articular cartilage of the femur at 6 months of age. (C) Representative micrographs of mouse knees showing a lateral view at 4 months of age. Arrows point to areas of subchondral sclerotic (whitening) in InsigDKO. (D) Representative sections showing haematoxylin and eosin staining of the knees in 4-month-old mice that were implanted with a slow release Placebo or Statin pellet following medial meniscectomy surgery. Arrows point to cartilage fibrillation in Placebo-treated mice. (E) Representative images of primary chondrocytes stained with Oil-Red-O to show sterol and lipid accumulation according to genotype. All scale bars = 100 μm.

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