expression of collagen type X, whereas Wnt5a, Wnt5b that delay hypertrophy of cartilage were down-regulated coinciding with down-regulation of Sox9, Collagen II, and Aggrecan. Finally, increased levels of β-catenin have been observed in degenerative cartilage.
Conclusions: Our data suggest that Wnt/beta-catenin signaling involved in endochondral ossification may be important for the onset and progression of OA. Also these molecular changes involved in endochondral ossification coincided with molecular changes of IL1β, IFNγ and TNFα, known to be part of mechanisms leading to excessive remodeling and degradation of cartilage matrix in OA.
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AUTOPHAGY IS A PROTECTIVE MECHANISM IN NORMAL CARTILAGE AND ITS AGING-RELATED LOSS IS LINKED WITH CELL DEATH AND OSTEOARTHRITIS

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Purpose: Autophagy is a process for turnover of intracellular organelles and molecules that protects cells during stress responses. This study evaluated the potential role of ULK1, an inducer of autophagy, Beclin1, a regulator of autophagy and LC3, which executes autophagy, in the development of osteoarthritis (OA) and in cartilage cell death.
Methods: DNA array studies were performed to analyze differences in the expression of ULK1 in normal and OA human knee cartilage. Protein expression of ULK1, Beclin1 and LC3 was analyzed in human normal and OA chondrocytes and cartilage by western blot and immunohistochemistry (IHC). Autophagy markers were also studied in mouse models of aging-associated and surgically-induced OA. The apoptosis marker poly-ADP(ribose) polymerase (Parp p85) was used to determine the relationship between cell death and autophagy.
Results: In normal human articular cartilage ULK1, Beclin1 and LC3 were constitutively expressed. ULK1 gene expression and ULK1, Beclin1 and LC3 protein expression were reduced in OA chondrocytes and cartilage but these three proteins were expressed in the cell clusters in OA cartilage. In mouse knee joints loss of glycosaminoglycans (GAGs) was observed at 9 months of age and in the surgical OA model 8 weeks after knee destabilization. Expression of ULK1, Beclin1 and LC3 decreased together with loss of GAGs, suggesting decreased autophagy correlates with extracellular matrix changes in OA. The decrease in autophagy in the same human and mouse OA cartilage was associated with an increase in Parp p85.
Conclusions: Autophagy may be a protective or homeostatic mechanism in normal cartilage. By contrast, human OA, spontaneous and surgically-induced OA in mice are associated with a reduction and loss of ULK1, Beclin1 and LC3 expression and a related increase in apoptosis. These results suggest that compromised autophagy represents a novel mechanism in the development of OA.

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HYPEROSMOTIC STIMULATION OF CHONDROCYTES LEADS TO DIFFERENTIAL POST-TRANSCRIPTIONAL REGULATION OF SOX9 AND COL2A1 mRNA

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Purpose: Post transcriptional control of SOX9 mRNA has been shown to occur following exposure of human articular chondrocytes (HAC) to stress conditions (cycloheximide, hyperosmolarity). These conditions lead to stabilisation of the mRNA and an increase in its steady state levels. In this study we have started to examine how sequences within the SOX9 3'UTR may control this process and how the chondrocyte specific collagen gene COL2A1 responds under similar conditions.
Methods: Freshly isolated HAC were plated as high density monolayer cultures and used within 48 hours. Cells were cultured for 5 hours under 380mOsm (control) or 550mOsm (hyperosmotic) conditions before RNA was isolated. To measure t1/2 of mRNA, actinomycin D chase experiments were performed under these conditions. SOX9 and COL2A1 mRNA levels were determined by qPCR. SOX9 3'UTR was amplified by RT-PCR and cloned into pGEMT-Easy before being subcloned into the pBBB reporter vector and transfected into NIH/3T3 cells.
Results: Hyperosmotic stimulation of HAC led to increased SOX9 mRNA levels which was associated with an increase in the mRNA t1/2. Conversely, COL2A1 mRNA levels were slightly decreased and this change was associated with a decrease in the mRNA t1/2. Introduction of the SOX9 3' UTR sequence into the pBBB reporter vector destabilised the encoded β-globin transcript (pBBB = 17.7 hours, pBBB_S9UTR = 2.8 hours).
Conclusions: Despite increased SOX9 levels, COL2A1 mRNA levels decrease in HAC exposed to hyperosmotic conditions. Changes in both genes appear to involve a post transcriptional mechanism but display opposite responses. Elements within the SOX9 3'UTR act to destabilise transcripts and are likely to interact with the RNA regulatory proteins. Understanding this post transcriptional control mechanism could help us to regulate cartilage gene expression during tissue regeneration and disease.

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ENDOTHELIAL NITRIC OXIDE SYNTHASE DEFICIENCY RESULTS IN REDUCED CHONDROCYTE PROLIFERATION AND ENDOCHONDRAL BONE GROWTH

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Purpose: Nitric oxide (NO) has been implicated in the local regulation of bone metabolism. However, the contribution made by specific nitric oxide synthase (Nos) enzymes to bone development is unclear. Here we describe the effects of inactivation of endothelial nitric oxide synthase (eNos) on cartilage development and early endochondral bone formation in mice.
Methods: We used eNos-deficient mice to address the role of this gene in cartilage development in vivo. Methods used in this projects include bone growth, histological staining, immunohistochemistry, BrdU incorporation assay, real-time RT-PCR and western-blotting.
Results: Mice carrying this mutation show increased lethality and marked abnormalities in bone formation. Tibiae and femurs from newborn mutant mice were significantly shorter. Histological sections demonstrated thinner cortical bone and fewer trabeculae in mutant mice, delayed primary and secondary ossification and a larger area of collagen X expression (a marker of late chondrocyte differentiation). eNos-deficient growth plates are disorganized and