Analysis of BMP-2 signaling under hypoxia showed an unexpected inhibition of Smad1/5/8 phosphorylation, whereas p38 MAPK was activated. Inhibition of p38 MAPK with SB202190 prevented Sox9 binding onto the type II collagen promoter and subsequent type II collagen production.
Conclusions: in addition to the HIF pathway, hypoxia could help to promote the chondrocyte phenotype through p38 MAPK signaling. These results suggest that hypoxia environment could be a not only an anabolic signal per se, but also a physiological signal that boosts the action of anabolic growth factors on the resident chondrocytes in human cartilage. Thus, combination of hypoxia and BMP-2 could be considered for a better control of the chondrocyte phenotype during the amplification step used in the cell therapy protocols such as Autologous Chondrocyte Implantation.

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HYPERCHOLESTEROLEMIA AND LIPID ACCUMULATION IN CHONDROCYTES

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Purpose: Patients with primary OA commonly have cardiovascular disease (CVD) and it has been reported that cardiovascular mortality is directly proportional to the extent of OA in affected individuals. Although the high incidence of concurrent OA and CVD may be merely an independent feature of advanced age and/or obesity (major risk factors for both), one can speculate that there is a direct link between the two. In an attempt to investigate the effects of hypercholesterolemia and exercise on lipid accumulation in chondrocytes, we examined articular cartilage from hypercholesterolemic pigs. Our hypotheses were that lipid accumulation in chondrocytes would be elevated in hypercholesterolemic pigs and lipid accumulation in chondrocytes would be altered by exercise.
Methods: All procedures were approved by the institution's animal care and use committee. Familial hypercholesterolemic pigs (FMH, n=6) were randomized to either a sedentary (Sed, $n = 3$) or exercise (Ex, $n = 3$) group. The Ex group was trained between 20 and 24 months of age using an established progressive treadmill-based training program. The Sed group was caged alongside the Ex animals but did not undergo training. Blood and medial femoral condyles were obtained after euthanasia at 24 months of age. Articular cartilage was obtained from the weight bearing portion of the medial femoral condyle of the left knee for quantification of glycosaminoglycan (GAG) content using DMMB assay. Medial femoral condyles of the right knees were fixed in 10% neutral buffered formalin fixative, decalcified in 10% EDTA solution, cryosectioned, and stained with oil red O for examination of lipid accumulation. The remaining right medial femoral condyles were routinely processed, sectioned and stained with H&E and Toluidine blue for histopathologic examination. Blood and medial femoral condyles obtained from size matched regular crossbred pigs served as a normocholesterolemic control (n=3). Three consecutive images of mid-central articular cartilage of each section were captured using a microscope with a 40x objective lens and a digital camera. The number of lipid particles, the total area of lipids, and the number of chondrocytes were quantified using the computer software Image-Pro Plus, ver. 7 (Media Cybermetrics, Inc. Bethesda, MD). Data from each group were combined and mean ± S.D. determined. Significance was set at $p < 0.05$.
Results: Total cholesterol and low-density lipoprotein in FMH (both Ex and Sed) were significantly higher than in controls (P<0.005). None of the plasma lipid indices were altered between Ex and Sed. The number of lipid particles per 100 chondrocytes was significantly higher in the Sed (15.9 ± 6.9) and Ex (7.9 ± 2.6) compared to controls (4.6 ± 3.1) (P<0.005). While total lipid area was largest in Sed, followed by Ex and controls, there were no statistical differences between the groups (P=0.096). GAG content [μg/dry weight (mg)] was significantly higher in the controls (216.8 ± 19.2) compared to Ex (126.4 ± 18.2) and Sed (129.5 ± 23.2). There was not a statistically significant difference between Ex and Sed for GAG concentration. Subjectively, the articular cartilage of the FMH groups had reduced toluidine blue staining compared to controls.
Conclusions: Loss of GAG, which is often the earliest histology finding associated with osteoarthritis, was evident in FMH pigs. This study also demonstrated increased chondrocyte lipid accumulation in hypercholesterolemic animals providing further evidence for a potential link between cardiovascular disease and osteoarthritis. Exercise may be associated with benefits to chondrocyte lipid metabolism at the cellular level, however, further research is necessary to delineate these findings.

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ABERRANT EXPRESSION OF AUTOPHAGY MARKERS DURING OSTEOARTHRITIS

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Objectives: Recent studies suggest that the process of autophagy, a form of programmed cell survival, is impaired during osteoarthritis (OA) and may contribute towards decreased chondroprotection in the articular cartilage associated with OA pathophysiology. To further explore the role of autophagy in OA, we determined the expression of known autophagy genes in human OA, mouse and dog model of OA.
Methods: Human normal and OA cartilage was subjected to human autophagy PCR array and Heatmap was generated. The expression of key autophagy markers was further investigated by quantitative PCR (qPCR) and immunohistochemistry and compared with mouse and dog OA model. The effect of major OA pro-inflammatory cytokine (IL-1β) on the expression of autophagy markers was also determined.
Results: The Heatmap and GEDI images obtained from human autophagy PCR array demonstrated a down-regulation of 16 and up-regulation of 17 autophagy genes in human OA cartilage versus normal cartilage, with a fold change of <-1.5 or >1.5 respectively. Data further demonstrated a significant up-regulation in the expression of autophagy master regulator mTOR, and significant reduction in the expression of key autophagy markers including ULK1 (most up stream autophagy inducer), LC3B (critical factor for autophagy vacuole formation), ATG5 (required for autophagosome formation) and BNIP3 (interactor of LC3) in human OA compared to normal cartilage. Similarly, a significant up-regulation in the expression of mTOR and downregulation of autophagy-specific genes (LC3B and ATG5) was observed in mouse and dog experimental OA. Treatment of normal human cartilage explants with IL-1β resulted in a significant reduction in the expression of LC3B, ATG5 and BNIP3.
Conclusions: This study is the first to provides a global view of dysregulation in the expression of mTOR and various autophagy-specific genes in human OA compared to experimentally-induced OA in mouse and dogs. Targeting autophagy could open up new therapeutic avenues for OA treatment or prevention.

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MITOCHONDRIAL DNA REPAIR ENZYME OGG1 IS ESSENTIAL FOR PROTECTION AGAINST THE DOWNREGULATION OF CHONDROCYTE ACTIVITY IN OA

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Purpose: During the development of osteoarthritis (OA), mechanical and chemical stresses on articular cartilage change the stable cellular activities of chondrocytes and produce excess amounts of reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines. Previous studies have provided ample confirmation of the generation of ROS and the depletion of cellular antioxidants in degenerated articular cartilage.
An oxidized form of guanine, 8-oxo-7,8-dihydroxyguanine (8-oxoguanine), is a major causative lesion for mutagenesis by ROS, because it can cause a stable base pair with adenine or cytosine during DNA replication. These mutations are thought to be involved in the pathogenesis of a variety of diseases, including degenerative diseases. In our previous study, we have found that 8-oxoguamine level was increased in the degenerated articular cartilage in OA, suggesting that the oxidative damage may accumulate in the OA cartilage. 8-oxoguanine DNA glycosylase (Ogg1) repairs 8-oxoguanine, one of the most abundant DNA adducts caused by oxygen free radicals. We postulated that depletion of cellular antioxidant, Ogg1, in degenerated articular cartilage participates in the development of cartilage degeneration. The aim of the study was examined the potential involvement of accumulation of 8-Oxoguanine and impairment of mitochondrial DNA repair enzyme Ogg1 in the pathogenesis of OA.
Methods: The expressions of 8-oxoguanine and Ogg1 were immunohistologically investigated in articular cartilage samples from patients