OGT and O-GlcNAcase expression were assessed by western blot analysis employing specific antibodies. Furthermore OGT expression was localized in the cartilage by immunohistochemistry.

Results: In human OA cartilage, the level of O-GlcNAcylated proteins was significantly increased in comparison to that of healthy cartilage. We also observed an increased expression of the different isoforms of OGT in OA cartilage and OA chondrocytes when compared to healthy controls. The level of O-GlcNAcylated proteins was also increased in OA rabbit cartilage at all the weeks studied. We also observed an increased expression of the different isoforms of OGT in the OA cartilage in comparison to control animals. The peak of expression of these proteins was observed 12 weeks after surgery. Furthermore, in OA cartilage we observed a decreased expression of O-GlcNAcase at all the times studied. By immunohistochemical studies, OGT was mainly localized inside the chondrocytes in all the samples analyzed, including both superficial and deeper zone chondrocytes.

Conclusions: These results demonstrate that OA could be associated with a dysregulation in the hexosamine biosynthesis pathway that could lead to an accumulation of O-GlcNAcylated proteins. Our data support the hypothesis that O-GlcNac formation may play an important role in the development of chronic and age-related diseases.

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INHIBITING CALCINEURIN ACTIVITY UNDER PHYSIOLOGICAL TONICITY: A WIN-WIN SITUATION FOR CELL-BASED CHONDRAL LESION REPAIR
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Purpose: Osteoarthritis (OA) is characterized by an imbalance between matrix synthesis and degradation: increased synthesis of catabolic enzymes (e.g. MMPs and ADAMTSs) by hyaline chondrocytes causes collagen (i.e. mainly collagen type II, COL2) and proteoglycan (i.e. aggrecan, AGC1) depletion in the extracellular matrix (ECM). Both molecules are major structural components of cartilage's ECM: COL2 networks provide biomechanical strength, while sulfated proteoglycans ensure the characteristic high fixed negative charge density (FCD) of this tissue. The latter binds mobile cations (e.g. Na+) and builds the tissue's hydrostatic pressure by attracting water, which is confined by an intact collagen network limiting tissue swelling. The FCD determines the physiological extracellular toxicity of healthy hyaline articular chondrocytes in vivo to between 250 and 480 mOsm, while OA is associated with a severity-depending drop in toxicity to between 280 and 350 mOsm.

We showed that calcineurin inhibitor FK506 modestly improves chondrogenic marker expression in these cells. We recently further showed that culturing osteoarthritic chondrocytes (OA-HACs) under elevated toxicity (380 mOsm) is beneficial too.

The aim of this study was to investigate whether a combined treatment with elevated toxicity and FK506 exerts synergistic effects on anabolic (COL2, AGC1, TIMPs) and catabolic (MMPs, ADAMTs) marker expression by human articular chondrocytes.

Methods: Human articular cartilage was explanted from macroscopically normal areas of the femoral condyles and tibial plateau of 4 patients undergoing total knee replacement surgery for OA. Human articular chondrocytes (HACs) were isolated under standard culture conditions (280 mOsm) and expanded to medium toxicity (380 mOsm). Primary HACs were culture expanded in monolayer (7,500 cells/cm2) at both isolation toxicities (280 or 380 mOsm). Passage 1 (P1) and P2 cells were seeded in high-density cultures (20,000 cells/cm2) and FK506 (0, 50 or 500 ng/ml) was added for 24 hours prior to six days of in vitro culture, followed by mRNA (QPCR) and protein (Western Blotting) analysis.

Results: Isolation and subsequent 24-hour passage of P1 and P2 of OA-HACs under physiological toxicity (380 mOsm) significantly increased mRNA levels of important anabolic markers such as AGC1, SOX9 and COL2 compared to standard culture. Under this condition, addition of FK506 dose-dependently increased this expression even further: SOX9 was induced 6-times, COL2 increased to 50-fold in P1 chondrocytes, COL2 protein 14-fold and AGC1 up to 2.6-times. In contrast, unwanted COL1 mRNA expression was suppressed to 1% of control. In addition, expression of anti-catabolic genes such as TIMP-1 and -2 significantly increased 4-times by 380 mOsm and FK506.

Interestingly, physiological toxicity slightly increased mRNA levels of hypertrophy markers COL10, alkaline phosphatase and MMP-13. However, in combination with FK506 all these markers were suppressed to or below control levels (280 mOsm).

Conclusions: Physiological toxicity provides a simple, yet effective, means to improve marker expression during cytokine-free isolation and in vitro expansion of human articular chondrocytes. Compared to FK506 alone, elevated toxicity significantly improved chondrogenic marker expression. Combining FK506 and elevated toxicity significantly improved chondrogenic marker expression even further, while suppressing the few toxicity-induced catabolic and hypertrophic marker genes. Our findings will lead to the development of improved cell-based repair strategies for chondral lesions and may provide novel insights into mechanisms underlying osteoarthritic progression.

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EXPRESSION OF P53R2 IN CHONDROCYTES IS REGULATED BY SHEAR STRESS
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Purpose: Chondrocyte apoptosis plays an important role in cartilage degeneration in osteoarthritis (OA), and mechanical injury to cartilage induce chondrocyte apoptosis. In response to DNA damage, p53 expression is up-regulated and regulates the p53-regulated apoptosis-inducing protein 1 (p53AIP1). We previously showed that mechanical stress induced chondrocyte apoptosis via p53 and p53AIP1 pathway. While, p53R2 expression is regulated in response to DNA damage. However, p53R2 repairs damaged DNA, and it protects from catabolism of chondrocytes. In this study, we evaluated the p53R2 expression of OA and normal chondrocytes in response to shear stress.

Methods: OA cartilage samples were obtained from total knee replacement surgery, and normal cartilage samples were from femoral neck fracture. Chondrocytes were isolated from cartilage. Two, five and ten% shear stress was introduced to chondrocytes for 12 hours by using Flexer cell system. Expression of p53R2 in chondrocytes was detected by western blotting.

Results: The expression of p53R2 in OA chondrocytes was increased by 2 and 5% shear stress but decreased by 10% shear stress in comparison with control (non stress).

Conclusions: We previously demonstrated that phosphorylation of p53 was elevated in OA chondrocytes in comparison with normal chondrocytes. In vivo, p53R2 is produced by binding p53 and R2 when p53 is activated. We demonstrated here that p53R2 expression was increased in OA chondrocytes when mild shear stress was introduced. However, p53R2 expression was decreased when excessive shear stress was introduced. Therefore, excessive shear stress may spend all the p53R2 in OA chondrocytes and allow the induction of apoptosis. We are doing further investigation to analyze p53R2 expression and function in chondrocytes.

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IL-6, A HIF-2α TARGET GENE, REGULATES OSTEOARTHRITIC CARTILAGE DESTRUCTION BY MODULATING MATRIX METALLOPROTEINASES (MMP)3 AND 13
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Purpose: Recently we have identified that hypoxia-inducible factor (HIF)-2α is a novel transcription factor of catabolic genes involved in cartilage destruction. In an attempt to understand further regulatory mechanisms of HIF-2α in cartilage, direct target gene of HIF-2α was selected and its function in cartilage was elucidated.

Methods: For finding novel target genes of HIF-2α, cDNA microarray assay was performed. As a result of microarray, interleukin (IL)-6 was selected as a novel target gene which is dramatically up-regulated cytokine by HIF-2α in articular chondrocytes. To further elucidate the role of IL-6 in cartilage, the expression of IL-6 in several arthritic cartilages from both human and mice was determined by RT-PCR and immunohistochemistry. Recombinant IL-6 protein was injected into knee joint for gain-of-function study, and neutralizing anti-IL-6 antibody was used for loss-of-function study in vivo.

Results: HIF-2α directly regulates IL-6 expression by binding to its promoter region. We determined that HIF-2α mediated IL-1β-induced IL-6 expression and recombinant IL-6 treatment in articular chondrocytes caused MMP3 and MMP13 production, which induced cartilage destruction. Moreover, we observed that IL-6 is up-regulated in articular cartilage from both...