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and thapsigargin was attenuated by AICAR in articular chondrocytes.

Conclusions: Certain inflammatory stimuli suppress AMPK activation in articular chondrocytes. Moreover, AMPKα knockdown by RNAi enhances pro-catabolic responses of articular chondrocytes. Conversely, pharmacologic AMPK activation attenuates articular chondrocyte pro-catabolic and hypertrophic differentiation responses to inflammatory cytokines and alleviates the ER stress response. Because human OA knee articular chondrocytes exhibited attenuated AMPKα activation, our results provide the first evidence that therapeutic activation of AMPK has the potential to inhibit the progression of OA.

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SELENOMETHIONINE INHIBITS IL-1β INDUCED NITRIC OXIDE SYNTHASE (INOS) AND CYCLOOXYGENASE 2 (COX2) IN PRIMARY HUMAN CHONDROCYTES

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Purpose: Inflammatory cytokine Interleukin-1 (IL-1β) stimulates reactive oxygen species (ROS), which mediates downstream cell signaling, modulating catabolic and inflammatory gene expression such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). The nutritional and antioxidant factor selenium (Se), has long been considered to play an important role in Osteoarthritis (OA). The ability of Se to neutralize reactive oxygen and nitrogen species is one potential chondroprotective mechanism against OA progression. In this study, we test if Se can neutralize inflammatory and catabolic effects of IL-1β and examine the common signaling pathways involved.

Methods: *Primary chondrocytes and treatment.* Primary human chondrocytes were isolated by enzymatic digestion as previously described from nonlesional cartilage of patients undergoing total knee replacement surgery. Chondrocytes were pretreated with and without 0.5 μM SelenoMethionine (SeMet) for 24 hrs and then followed by 50 pg/ml or 100 pg/ml 1L-1β treatment for up to 24 hrs.

RNA isolation and Real Time RT-PCR. Total RNA was extracted and reverse transcribed into cDNA for Real Time RT-PCR analysis. The ABI Prism 7000 sequence detection system and relative quantification software (Applied Biosystems, Foster City, CA) were used for real-time analyses. Analysis of relative mRNA expression levels of iNOS and COX2 were normalized to 18S gene expression. Values are the mean (SEM) percentage as a percentage of IL-1β induced iNOS and COX2 mRNA levels for three independent experiments, conducted in triplicate. The relative mRNA level in cells treated with IL-1β alone was set at 100%.

Immunoblotting and Phosphorylation Assay. Whole cell lysates from chondrocytes for each tested condition were separated by electrophoresis on reducing gradient gels, and transferred to nitrocellulose for immunoblotting. Membranes were blocked with 5% BSA in TBS/0.1% Tween 20 (TBS-T). Polyclonal primary antibodies against Phospho-p44/42 MAPK 3(Erk1/2), Phospho-p38 MAPK (Thr180/Tyr182), Phospho-SAPK/JNK (Thr183/Tyr185), Phospho-IKKα/β (Ser 176/180) Phospho- NF-kB p65 (Ser 536) were obtained from Cell Signaling Technology (Danvers, MA) and used at 1:1000 dilution. A monoclonal antibody against tubulin (Sigma) was used as a normalization control at 1:10,000 dilution. Anti-rabbit and anti-mouse IgG-HRP (Jackson ImmunoResearch, West Grove, PA) secondary antibodies were diluted 1:50,000. The results shown are representative of two independent experiments, conducted in duplicate.

Results: Pretreatment of chondrocytes with SeMet significantly inhibited the IL-1β induced iNOS gene expression (by ~30%) and COX2 gene expression (by ~40%), Figure 1. Furthermore, SeMet

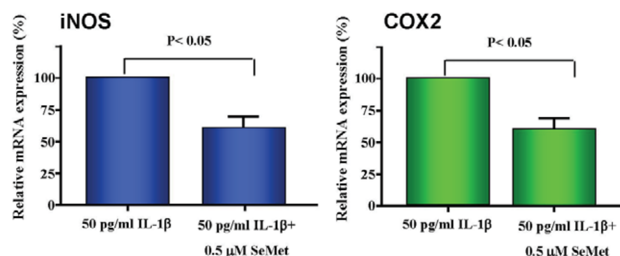

Figure 1. Pretreatment of SeMet inhibits IL-1β induced iNOS and COX2 mRNA levels.

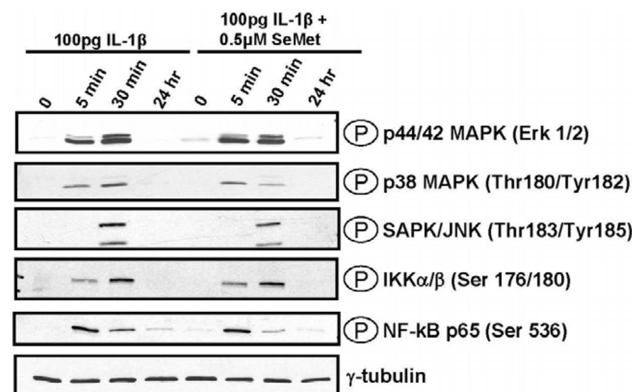

Figure 2. The effect of selenium pretreatment on the IL-1β induced signaling pathways.

selectively inhibited IL-1β activated p38 MAPK and NF-kB activity, but not JNK, ERK and IKKα/β activity (Figure 2, Western blot).

Conclusions: Taken together, our data show that Se can partially reverse IL-1β induced iNOS and COX2 gene expression, and shows evidence of altering IL-1β activated cell signaling. These results suggest that one mechanism by which Se may exert a chondroprotective effect is through regulation of cell signaling pathways induced by pro-inflammatory cytokines.

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CHONDROCYTE PHENOTYPE IS DETERMINANT FOR THE EXPRESSION OF ADIPOKINES AND THEIR RECEPTORS

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Purpose: As adiposity rather than simply excess in body mass has been shown to be detrimental to the knee joint, increasing studies have been undertaken to determine the role of adipokines in osteoarthritis (OA). However, contradictory data have been found for the expression and the effects of these adipose-derived proteins in chondrocytes. This study investigated therefore the effects of phenotypic modulation on the expression of adipokines and their receptors in human chondrocytes.

Methods: The expression of adipokines (leptin and adiponectin) and their receptors (AdipoR1, AdipoR2 and Ob-R) was examined by quantitative real-time RT-PCR in chondrocytes obtained from patients with osteoarthritis (OA) either directly after cells harvest or after culture in monolayer or in alginate bead which is known to restore and/or maintain the differentiated state of chondrocytes. The change in transcript level of collagens type 1, 2A and 2B, aggrecan and Sox9 was used to evaluate phenotypic modulation.

Results: The results indicated that leptin, adiponectin, AdipoR1 and Ob-R were expressed in freshly isolated chondrocytes while AdipoR2 was barely detected. Major changes in the gene expression pattern occurred after culture in monolayer with a shift from

the adipokines to their receptors. Interestingly, the downregulation of adipokines was associated with the reduced expression of the cartilage-specific transcription factor Sox9 and a strong increase in the transcript level of collagen type 1. Chondrocytes recovered a cartilage-like expression profile of leptin and adiponectin when cultured in alginate beads, but ceased expressing their receptors.
Conclusions: The modulation of chondrocyte phenotype induced by experimental conditions affects the expression of adipokines and their receptors. These experiment-dependent changes could result in modifications of cell response to leptin or adiponectin, and could therefore contribute to the discrepancies found in different studies. These findings suggest also that adipokines may play an essential role to prevent a phenotypic loss of chondrocyte function.

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AUTOPHAGY: A NEW TARGET IN THE HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: There is considerable evidence to suggest that programmed cell death (PCD) is not confined to apoptosis and that other mechanisms may also operate. One of these mechanisms is the so-called “autophagic PCD”. Previously, some authors observed that chondrocytes in OA cartilage demonstrated morphologic changes that are characteristic features of apoptosis; however, we have observed the increase in autophagic levels in OA cultured chondrocytes compared to normal cultured chondrocytes. On the other hand, levels of microtubule-associated protein light chain 3 (LC3), specifically LC3-II, is clearly correlated with the number of autophagosomes.
The objective is to asses the levels of autophagy in normal and OA human articular cartilage.
Methods: Normal and osteoarthritic (OA) human cartilages were obtained from patients with joint replacement (femoral and knee joint) and from autopsy cases (knee joint). To carry out studies with cartilage, some pieces were frozen and subsequently pulverized and other ones were cryopreserved until histologic studies were done. The expression of the AuTophaGy-related (*ATG*) gen LC-3 was assessed by means of western-blot and immunohistochemistry using a specific policlonal antibody (Abcam, UK). Values obtained by western-blot were normalized by means of α-tubuline expression and the changes in this expression were measured using the *ImageQuant* (*Version 5.2*) program. On the other hand, 30 μg of cartilage extract was resolved using 2-DE; wide pH range (nonlinear 3-10) was used for first dimension and small format polyacrylamide gels for the second dimension. The MS-compatible silver staining was performed. The samples were analyzed using the MALDI-TOF/TOF mass spectrometer 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA).
Results: The study of LC-3 expression by means of western-blot showed that OA human cartilage has higher expression of LC3-II compared with normal cartilage (ratio >2). Densitometric analysis was carried out by Image-Quant software. These findings were corroborated with an immunohistochemistry study. On the other hand, some autophagy-related proteins were identified by means of MALDI-TOF/TOF mass spectrometer: lysozyme and phospolipase; in both cases, the levels of these proteins were significantly higher in OA samples.
Conclusions: These results show the increase in autophagic levels in OA human cartilage compared to normal human cartilage, confirming the previous results with chondrocyte cultures. The autophagy increase could contribute to the development and progression of articular cartilage degeneration.

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HEME OXYGENASE-1 REGULATES INFLAMMATORY MEDIATORS IN CARTILAGE ADJACENT TO SUBCHONDRAL BONE FROM OSTEOARTHRITIC PATIENTS

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Purpose: Osteoarthritis (OA) produces articular degeneration manifested by changes in the cartilage and subchondral bone. Previously, we reported that heme oxygenase-1 (HO-1) is down-regulated by catabolic factors in superficial chondrocytes. The function of these chondrocytes can be modulated by the cells present in deeper zones including calcified cartilage and subchondral bone. The aim of the present work was to study the possible protective effects of HO-1 against inflammatory mediators in this area.
Methods: Osteochondral explants (including cartilage and subchondral bone) of 3.5 mm of diameter were obtained from 10 patients with diagnosis of advanced OA undergoing total knee joint replacement. Explants were maintained for 24h with DMEM/F12 and antibiotic and then stimulated with IL-1β (100 U/ml) and/or the HO-1 inducer cobalt protoporphyrin IX (CoPP) during 72h. Samples of culture medium were taken to measure prostaglandin E_2 (PGE_2) by RIA and nitrite by a fluorometric method. Tissue explants were included in formalin (10%) for histological and immunohistological determinations. Cellular viability (evaluated by the LDH method) and phenotype (collagen II expression) were maintained throughout the culture period.
Results: We have shown by immunohistological analysis of explants that HO-1 and telomerase are predominantly expressed in chondrocytes in the area next to subchondral bone. This expression was down-regulated in the presence of IL-1β. Besides, this pro-inflammatory cytokine increased nitric oxide synthase-2 (NOS-2), cyclo-oxygenase-2 (COX-2) and high mobility group box 1 (HMGB1) expression in the same cells, as well as the levels of nitrite and PGE_2 in the culture medium. Induction of HO-1 by CoPP reverted the effects of IL-1β on telomerase expression. In addition, we observed a significant decrease in NOS-2, COX-2 and HMGB1 expression by HO-1 up-regulation, accompanied by reductions in the levels of corresponding metabolites.
Conclusions: In this work, we have shown a correlation between HO-1 and telomerase expression in chondrocytes adjacent to subchondral bone, accompanied by inhibition of inflammatory mediators. These results support the view that HO-1 may be a potential target for cartilage regeneration and repair.

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REGULATION OF OSTEOARTHRITIC CHONDROCYTES BY GROWTH AND DIFFERENTIATION FACTOR 5

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Purpose: Genetic studies have identified osteoarthritis (OA) susceptibility genes that are present across different populations. Interestingly, a majority of them are involved in developmental processes and/or maintenance of cartilage and bone homeostasis. Amongst these confirmed susceptibility genes is growth and differentiation factor (GDF) -5. Early in life, GDF-5 is an important mediator of skeletal formation. However, little is known about the physiologic role of this protein in adults and despite its genetic association with OA, potential implication of GDF-5 protein in this disease remains unknown. Therefore, the main objective of this study is to assess the effect of rhGDF-5 on OA human and ca-