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 assess 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) gene 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-TTOF/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-TTOF/TOF mass spectrometer: lysozyme and phospholipase; 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 downregulated 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 E2 (PGE2) 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 PGE2 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 carti-