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 osteoarthritis (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 polyclonal 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 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 and nitrate 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-oxidgenase-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 and ca-
nine chondrocytes. By looking at cartilage proteins expression and degradative enzymes activity, we hope to identify GDF-5 action on OA cartilage homeostasis.

Methods: Human OA chondrocytes were isolated from patients undergoing knee replacement. Canine OA chondrocytes were generated from the Nuki-Pond dog model. After in vitro expansion, chondrocytes were co-incubated with increasing concentrations of rhGDF-5. Expression of selected markers of cartilage metabolism (aggrecans, type-I and -II collagen, ADAMTS-4 and -5) was analyzed at the mRNA and protein levels using qRT-PCR and western blot respectively. Aggrecans degradation products, which reflect the activity of aggrecanases, were also analyzed by western blot following GDF-5 stimulation.

Results: Similar responses were recorded in human and canine chondrocytes. Stimulation of human and canine OA chondrocytes with rhGDF-5 led to a dose-dependent increase of aggrecans and type II/type I collagen ratio, with a concomitant decrease in both ADAMTS-4 and ADAMTS-5 mRNA expression. A similar action for rhGDF-5 was observed for aggrecans and ADAMTS-4 and ADAMTS-5 at the protein level. Finally, aggrecans degradation products were also significantly decreased in OA chondrocytes following rhGDF-5 stimulation.

Conclusions: Interestingly, the addition of rhGDF-5 had a dual effect on OA chondrocytes, increasing markers of anabolic activity and decreasing catabolic enzymes expression and activity. These results suggest a key role for GDF-5 in maintaining cartilage homeostasis, an action that could be lost in OA. Because GDF-5 concentrations are constant in normal and OA cartilage, interference with GDF-5 signalling may occur in OA, an anomaly that appears to be recovered by the addition of rhGDF-5. Further in vivo studies will evaluate the potential of GDF-5 as a disease modifying drug in OA.

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INTERACTION BETWEEN 4-HYDROXYNONENAL-MODIFIED EXTRACELLULAR MATRIX AND OSTEOARTHRITIC CHONDROCYTES OR OSTEOBLASTS INDUCES CHANGES IN CELL PHENOTYPE AND FUNCTION

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Purpose: Extracellular matrix (ECM) degradation and formation are closely linked and tightly regulated in order to maintain cartilage and bone homeostasis. In cartilage and bone, accumulation of 4-hydroxynonenal (HNE), a product of lipid peroxidation (LPO), undergoes considerable alterations in ECM structure and thereby rendering it more prone to mechanical damage. This study aimed to demonstrate that interaction between HNE-modified extracellular matrix and chondrocytes or osteoblasts induced changes in cell morphology and function of these cells.

Methods: Plates culture were coated with 0.1 mg/well type II collagen (Col II) and then modified by increasing concentrations of HNE (0.1 to 2 mM). Chondrocytes or osteoblasts where then incubated for 48 hrs in appropriate culture medium. Cell viability was evaluated by MTT test. The protein expression of adhesion molecules such as integrin αvβ3 and inter-cellular adhesion molecule-1 (ICAM-1) is performed by Western blot. The level of matrix metalloproteinase-13 (MMP-13), prostaglandin 2 (PGF2) and osteocalcin (OC) was determined by ELISA using commercial kits. Alkaline phosphatase (ALPase) activity was measured as the release of p-nitrophenol hydrolysed from p-nitrophenyl phosphate.

Results: After 48 hrs of incubation, the modification of Col II with 2 mM HNE induces shape change of chondrocytes and osteoblasts from elongated phenotype to rounded phenotype with a 10% decrease in cell viability. At a functional level, the modification of Col II with 0.1-0.5 mM HNE enhanced the production of integrin αvβ3, ICAM-1, MMP-13 and PGE2 in chondrocytes, and OC release and ALPase activity in osteoblasts. In contrast, the modification of Col II with 1 and 2 mM HNE reduced their production in both cell types.

Conclusions: Our results showed that interaction between HNE-modified Col II and chondrocytes or osteoblasts induced changes in cell phenotype of chondrocytes/osteoblasts and modulate the expression of catabolic and inflammatory factors. As described previously by our laboratory, we suggest that accumulation of HNE/Col II adducts in OA cartilage and bone may play a critical role in OA pathogenesis.

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DIFFERENTIAL RESPONSE OF CARTILAGE EXPLANTS TO CLINICALLY-RELEVANT LEVELS OF IL-1 AND FIBRONECTIN FRAGMENTS

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Purpose: In vitro, it is known that high doses of recombinant interleukin-1 (IL-1) causes rapid and extensive cartilage degradation characterized by loss of proteoglycan from tissue to surrounding culture medium. However, IL-1 is only one of several potentially catabolic factors detected in synovial fluid from osteoarthritic (OA) joints, and has been shown to be present at much lower concentrations than those typically employed in vitro. Our goal was to expose cartilage explants to levels of IL-1 similar to those we measured in OA synovial fluid, assess the loss of sulfated GAG from the tissue, and determine the effect of IL-1 blockade using IL-1 receptor antagonist (IL-1ra).

Methods: Levels of IL-1 were determined in synovial fluid collected from OA patients. Bovine articular cartilage was harvested from the patellofemoral groove of 3 adult animals, and prepared as full-thickness discs 6 mm in diameter. These cartilage explants were equilibrated for 48 hours in medium (DMEM with 50 μg/mL gentamicin), then transferred to culture wells containing fresh medium supplemented with IL-1 alpha ranging from 5000 ng/mL (often used for in vitro degradation studies) to more clinically-relevant levels (200, 20, 16, 8, 4, 2, 1, 0.5, and 0 ng/mL), based on measured levels from above. To assess the contribution of other potential catabolic factors, some cultures were also supplemented with 30 kDa N-terminal fragment of fibronectin (Fn-f) at reported physiologic concentration (0.8 μM). The effect of IL-1 blockade was assessed by preincubating some cartilage samples with 1.4 mM IL-1ra for 2 hours before addition of IL-1 or Fn-f. After 4 days, culture media were collected and assayed for sulfated GAG content by DMMB, normalized to initial tissue wet weight. GAG distribution was also visualized in cartilage discs by safranin-O staining.

Results: Incubation of cartilage with IL-1 gave a dose-dependent decrease in sGAG release between 5000 and 20 pg/mL. The GAG content of the culture medium was not different from control explants (no IL-1) for the range 0.5-20 pg/mL IL-1. Similarly, there was no difference in safranin-O histology in this dose range. Addition of IL-1ra to the culture medium completely inhibited this loss. However, when Fn-f was included in the culture medium, GAG release was increased, and the additional degradation was not able to be inhibited by IL-1ra.

Conclusions: In later-stage OA patients, we and others have detected IL-1 at very low levels. Incubation of cartilage with IL-1 at these concentrations did not induce GAG release over that of untreated cartilage explants. However, at least one other catabolic factor, Fn-f, induced degradation at a more clinically-relevant concentration. It may be that in advanced stages of OA, the contribution of IL-1 to degradation is not as pronounced as that of other catabolic factors. While IL-1 may be more involved in earlier OA,