Purpose: Osteoarthritis (OA) is a chronic low-grade inflammatory disease for which the diagnosis currently relies on clinical and radio-
logically means whereas sensitive serum biomarkers specific for an early stage of the disease are lacking. The aim of the study was to identify specific and early markers of cartilage degradation in vitro and in vivo.

Methods: Full thickness equine articular cartilage explants were harvested from the weight bearing part of the distal metacarpal bone III in the metacarpal-phalangeal joint of three horses. The explants were cultured in the presence or absence of interleukin (IL-1b) in vitro and cell media was changed and sampled at day 3, 6, 9, 12, 15, 18 and 22. Proteins were isolated from the harvested media and following trypsin digestion the peptides were labeled with isobaric tandem mass tags (TMT) and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The TMT reporter ion intensities in MS/MS spectra were divided by reference reporter ion intensities, giving a relative amount for each quantified peptide. The relative amount of released components in media from IL-1 stimulated and unstimulated explants were compared over time.

Results: IL-1b stimulation resulted in an abundance of proteins related to the extra cellular matrix and the proteins were released at different time points during culturing time. Release of several cartilage matrix components e.g. aggrecan, cartilage oligomeric matrix protein (COMP), chondroadherin, thrombospondin-1 and proteoglycan 4 displayed high amounts at days 3 and 6, similar to the early stage of the disease process of OA in vivo. Collagen type II release was identified at day 9 and 12 and the release of collagen type VI increased continually from day 18-22. Several small leucine-rich proteoglycans (SLRP) such as biglycan and lumican had the highest release at day 22, together with a high amount of collagen type II in IL-1b stimulated media. The unstimulated media showed signs of new synthesis of collagen type II by the presence of high amount of procollagen type II C-propeptide and C endopeptidase enhancers, and this collagen synthesis was not at all prominent in the IL-1b stimulated media.

Conclusions: This inflammatory in vitro model display structural changes seen at distinct developmental stages of OA in vivo. Novel findings presently are the release, at different time points after IL-1b stimulation, of cartilage components in a specific time pattern in the order as follows; COMP, collagen type XII, collagen type VI, SLRPs and finally collagen type II, indicating a degradation of the collagen network in a distinct pattern, without evidence for new synthesis of collagen type II. This results in the unstimulated explant having reduced capacity with an increase of collagen synthesis. The identification of early biochemical changes in cartilage would be of great importance in both finding disease modifying pharmaceutical compounds as well as in the development of biomarkers for early OA.


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Purpose: Osteoclastogenesis is enhanced in early osteoarthritis (OA). We have previously demonstrated that cartilage degradation is reduced in vivo when osteoclastogenesis is inhibited in murine models with high chondrocyte turnover. To investigate the molecules involved in osteoclast generation and their potential role in OA, we aimed to identify molecules which could be targets for therapeutic intervention. Hence, we hypothesized that a reduction of osteoclast generation would lead to an increase in bone mass in mice. To test this hypothesis, we used a murine model of OA to investigate the role of various molecules involved in the differentiation and function of osteoclasts.

Methods: Murine osteoclasts were isolated from the femora of wild-type and Notch1 deficient mice. The osteoclasts were then cultured under standard conditions in the presence of osteoclast conditioned medium (OC-CM) for 48h. The gene and protein expressions of catatolism and anabolism were analyzed using quantitative real-time PCR and immunohistochemistry. The results were compared to those obtained from wild-type and Notch1 deficient mice using Student's t-test.

Results: The expression of the osteoclast marker RANKL was reduced in Notch1 deficient osteoclasts compared to wild-type osteoclasts. Additionally, the expression of the osteoclast marker TRAP was also reduced in Notch1 deficient osteoclasts. Moreover, the expression of the osteoclast marker OC were also reduced in Notch1 deficient osteoclasts. Furthermore, the expression of the osteoclast marker OC-CM was also reduced in Notch1 deficient osteoclasts.

Conclusions: These results suggest that Notch1 is involved in the differentiation and function of osteoclasts. This study provides a potential target for the treatment of OA and other bone diseases.
were analyzed by RT-qPCR and western blot. To investigate the regulation of canonical Wnt pathway, transactivation assay was performed in primary chondrocytes derived from Topgal mice and cultured with Oc-CM. Western blot and immunocytochemistry from osteosarcoma patients. OA chondrocytes were isolated and cultured as monolayers. Galectin expression in cultured chondrocytes was determined at the mRNA level using validated RT-qPCR assays (for LGALS1, -2, -3, -4, -7, -8, -9) in human OA and non-OA cartilage. 

Results: Oc-CM induced a marked decrease in proteoglycan release by chondrocytes. This is the result of both the reduction of the expression of anabolic genes such as collagen type II, Aggrecan and Sox-9 and the increase of the expression of catabolic genes such as metalloproteases (MMP-3 and -13) and aggrecanases (Adams-4 and-5). We then monitored the nuclear translocation of β-catenin induced by Oc-CM. We observed an abolition of the translocation of β-catenin and subsequently Topgal activity along with the reduction of Wnt target genes (Axin, Wisp1, C-Myc). In order to rescue the inhibition of canonical pathway induced by Oc-CM, a dose-effect of LiCl was performed (1 - 5 mM). Low dose of LiCl totally reversed the effect of Oc-CM while it was aggravated with the highest dose.

Conclusion: We here demonstrated that osteoclasts secrete soluble factors able to disrupt the balance of chondrocyte metabolism via the inhibition of the Wnt canonical signaling. Therefore, manipulating bone may affect chondrocyte function.

GALECtin FINGERPRINTING IN OA CARTILAGE

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Purpose: Enormous amounts of biological information are triggered by the interaction between glycans and their endogenous receptors (e.g., galectins). How these potential implications of cartilage glycobiology in the pathophysiology of osteoarthritis (OA) remain largely unknown. We recently showed that interleukin-1beta and tumor necrosis factor-alpha can alter distinct aspects of the glycan pattern of cultured human chondrocytes, such as the status and type of N glycan sialylation. Notably, glycans structures of OA chondrocytes are not only altered in vitro but also in situ with progressing cartilage degeneration. Addressing the protein part of the galectin-glycan recognition system, the present study was designed to assess the expression and localization of endogenous galectins (Gal-1, Gal-3) and cathepsin D in human OA and non-OA cartilage.

Methods: OA cartilage was received from total knee replacement surgery (56 ± 9 years) or OA donors undergoing knee replacement surgery (56 ± 9 years) were grown in monolayer cultures until confluence. For cell viability and caspase 3/7 activation assays, hAC were treated with 0.5 mM palmitate, 0.5 mM oleate or vehicle, in the presence or absence of 1 µg/ml IL-1β for 72 hours. For gene expression studies, shorter treatment time (24-hours) and lower IL-1β concentration (10 µg/ml) were used. Expression levels of proinflammatory factors, extracellular matrix proteins and extracellular proteases were measured. In addition, chondrocyte viability and matrix damage were assessed in bovine articular cartilage explants cultured with FAH or without IL-1β.

Results: Palmitate induced caspase 3/7 activation and cell death in IL-1β-stimulated normal hAC, and upregulated IL6 and Cox2 expression through toll-like receptor-4 (TLR-4) signaling. Palmitate proinflammatory and proapoptotic effects were synergistically increased upon IL-1β co-treatment. Exposure to FFA did not modify type II collagen, aggrecan, ADAMTS-4 or -5, and MMP13 gene expression after 24 hours. Ex vivo experiments in bovine cartilage explants showed that palmitate accelerated cartilage destruction driven by IL-1β, as evidenced by a significant increase in cell death and glycosaminoglycan release. Lastly, pharmacological inhibition of caspases and TLR4 signaling significantly reduced palmitate and IL-1β-induced cartilage damage.

Conclusions: Palmitate acts as a pro-inflammatory and catabolic factor that, in synergy with IL-1β, induces chondrocyte apoptosis and articular cartilage breakdown. Collectively, our data suggest that elevated levels of saturated FFA often found in obesity may contribute to OA pathogenesis.

THREE NEW FUNCTIONALLY CONSERVED CIS-REGULATORY ELEMENTS IN THE ACAN GENE

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Purpose: Aggrecan gene (AcAn) codes for the chondroitin sulfate proteoglycan, a key core protein responsible for the unique mechanical properties of cartilage. Aggrecan protein is required both for proper cartilage formation in development and maintenance of mature cartilage as illustrated by the phenotypes of chicken and mouse mutants. The transcriptional regulation of this important gene is not fully understood. This investigation was undertaken to shed light on the cis-acting sequences that define the Acan expression in skeletal elements.

Methods: The expression of AcAn in cartilage is highly conserved in evolution, and has been described in Xenopus and zebrafish as well as chicken, mouse, and other mammals.