inadequate barium perfusion. Future studies of cartilage canals using SWI are likely to improve our understanding of the pathogenesis and progression of OC and other developmental orthopedic diseases by providing a non-invasive method to evaluate the vascular supply and associated pathology of the AEC.

191 VERIFICATION OF THREE DIFFERENT MMP-13 ISOFORMS EXPRESSION IN HUMAN OSTEOARTHRITIC CARTILAGE
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Purpose: Collagenase-3 (MMP-13) is a human matrix metalloproteinase highly overexpressed in diseases where tissue repair and remodelling is needed, such as rheumatoid arthritis (RA), osteoarthritis (OA) and some cancers. However, its expression in normal tissues is limited to fetal ossification processes. MMP-13 can digest fibrillar collagen, preferentially type II collagen, but also glomerular collagen and other components of the extracellular matrix (ECM). MMP-13 structures is composed of 4 domains: a hydrophobic pre-domain necessary for the secretion, a pro-domain involved in enzyme latency, a catalytic domain, and a hemopexin-like domain This last is essential for the substrate recognition and collagenolytic activity.

Three different MMP-13 transcripts of 3.0, 2.5 and 2.2/2.0 kb have been described to be expressed in human cells. The 2.5kb transcript (isoform 205) corresponds to the MMP-13 original form, while the 3.0kb transcript (isoform 344) presents an insertion at the C-terminal produced by lack of exon 9B splicing. Finally, the smaller transcript of 2.2/2.0kb (isoform 205) presents a deletion (probably due to alternative splicing) of 88 amino acids, also affecting the C-terminal domain. Our objective is to demonstrate the presence of these three MMP13 protein isoforms in human OA samples.

Methods: A specific peptide from each isoform was synthesized and two rabbits were immunized with each peptide. The antibodies recognizing each isoform were purified from the total antiserum by sulfolink column. On the other hand MMP-13 isoforms were synthesized in vitro in a Sf9 insect cells system, with a recombinant baculovirus containing MMP-13 transcripts cloned. The insect cells were culture to produce the respective isoforms, and they were purified by molecular exclusion chromatography. The antibodies were used to test the specificity of the polyclonal antibodies obtained by Western Blot. Finally, ECM proteins were extracted from cartilage samples obtained from knee and hip arthritic patients undergoing arthroplasty, using Guanidinium chloride (4M) method. The presence of the 3 isoforms in these samples was assayed by western blot with the specific isoform polyclonal antibodies obtained.

Results: The purified polyclonal antibodies show immunoreactivity only in front of its specific isoform, showing that they are useful for the detection of each specific isoform in the samples. In all the samples tested, different intensities of the bands corresponding of each antibody were detected. No relative quantification could be performed due to the lack of a standard of each MMP-13 isoforms that allows us to compare the affinity of each polyclonal antibody to each specific isoform.

Conclusions: The MMP-13 mRNA isoforms are translated to protein in cartilage OA samples. The transcripts containing the deleted and the alternatively spliced exons differ from the original sequence in the region coding for the hemopexin-like domain. This domain is necessary to cleave native triple helical collagens and provide to the protease its substrate specificity and affinity. The expression of 205 and 344 isoform could have clinical implications in OA if they present different substrate recognition and/or speed of degradation, explaining some differences observed in the pathophysiology of the disease.

192 QUANTITATIVE ECHOGENICITY OF SMALL INTRA-ARTICULAR DEPOSITS OF CALCIUM, HYALINE CARTILAGE THICKNESS AND SONOGRAPHIC ASPECTS OF THE SYNOVITUM IN PATIENTS WITHOUT RADIOGRAPHIC CHONDROCALCINOSIS

Purpose: Ultrasonography is very helpful in the detection of intra-articular crystal deposits and also in distinguishing gout from chondrocalcinosis (CC). However, only three patients without CC on radiographies with evidence of calcium deposits within the cartilage detected by ultrasound (US) have been reported.

Methods: In a cohort of 406 patients who underwent musculoskeletal US from January 2010 to June 2011, 30 patients with chondrocalcinosis were identified by the presence of calcium deposits using a machine equipped with an 8–13 MHz linear transducer. The presence of chondrocalcinosis was sonographically recognized as hyperechoic foci within the articular cartilage or fibrocartilage. Once aggregates of calcium were identified, the size was measured unless the deposit burden was too large to quantify. The quantitative echogenicity was assessed among all patients with chondrocalcinosis. A sampling of the area of the calcium aggregates with apparent highest echogenicity was matched with the gray scale bar and measured in a scale of 0 to 10 centimeters. In addition, cartilage thickness was measured on transverse view obtained with the knee fully flexed. In the same view, a minimal-crystal distance of the deposit (distance of the superior margin of the deposit to the cartilage surface) was calculated.

Results: We identified 30 patients with US evidence of aggregates of calcium within the wrist or knee cartilage. Two patients were excluded from the study since prior radiographs of the joint were not available. Of the 28 patients included in the study, 9 (32%) showed radiographic evidence of chondrocalcinosis and 19 (68%) patients had no evidence of chondrocalcinosis on prior radiographs ($p = 0.001$). Those patients with sonographic, but not radiographic CC, predominately had small calcium deposition [$mean: 1.55mm$ (range: $0.8mm- 4.6mm$)] vs. the group with radiographic CC that showed extensive deposition (Panel A). The group with radiographic CC showed deposits in more than one compartment (82%), which was observed in only 33% in the group with no radiographic CC. The patients with CC on x-ray had deposits with higher echogenicity ($mean: 6.2$) compared with those without ($mean: 5.7$). However, it was not statistically significant ($p=0.16$). Cartilage thickness was not different between patients with or without radiographic CC [$mean: 1.96mm vs. 2.0mm$ respectively, $p=0.84$]. Patients without radiographic CC showed a small minimal-crystal distance [$mean: 0.5 mm$ (range: 0–1.3mm)] compared with the group with CC on radiographies [$mean: 0.8mm$ (range: 0.3–1.6)]. In the first group (21 knees), this distance was similar in presence or not of non-shadowing hyper-echoic dots within the synovium or effusion [$mean: 0.5mm vs. 0.4 mm$, $p=0.25$]. Effusion and synovial hypertrophy were detected in the majority of patients irrespective of the presence of small or extensive deposits of calcium. Roughly one-third of all patients showed hyper-echoic dots within the synovium. The presence of dots within the effusion was detected in 42% in the group with occult CC vs. 33% with radiographic CC (Panel B, C and D). Ultimately, a calcified meniscus was identified in 26% of patients without radiographic CC. Conversely, all patients with radiographic evidence of CC showed a meniscocalcinosis sonographically (Panel B).

Conclusions: Ultrasound was better than conventional radiography in detecting small deposits of calcium within the cartilage. Knee cartilage thickness was similar in both groups with and without radiographic CC. We speculate that small size of the calcium deposits, mainly localized in one compartment of the knee, lower echogenicity, and the low rate of
meniscocalcinosis contributed to the absence of CC on x-rays. Studies in a lapar region might clarify whether these small deposits of calcium represent a different morphopathologic pattern of CC and whether the minimal crystal distance might be considered as a predictor of synovitis.

193 ATP-STIMULATED ATP RELEASE AND METABOLIC ACID PRODUCTION: REGULATING LIFE AND DEATH DECISIONS IN ARTICULAR CHONDROCYTES
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Purpose: There is convincing evidence that extracellular ATP, signalling through P2 purinoreceptors, plays a major role in the regulation of bone remodelling particularly in mechanotransduction. P2 receptors are known to be expressed in cartilage but their role in regulating chondrocyte physiology is still largely unexplored. The aim of this research was to identify which P2 receptors are expressed in chondrocytes, and to determine the consequences of receptor activation.

Methods: Initial experiments were undertaken on the C20 chondrocyte cell line, cultured in monolayer or alginate beads. Subsequently, we investigated primary human chondrocytes isolated by enzyme digestion. Primary chondrocytes were cultured in 1, 5, or 20% oxygen. P2 receptor expression was determined by RT-PCR. ATP concentration in conditioned medium was measured using the luciferin/luciferase assay in a Berthold Tube Luminometer. Lactate was measured using a colorimetric kit supplied by Cobas.

Results: Chondrocytes expressed a range of P2 receptors including P2Y1, P2Y2, P2Y4, P2Y6 and P2X7. Addition of ATP had little effect on the production of extracellular matrix by chondrocytes. However, there were biphasic and time dependent effects on cell proliferation. Addition of ATP at concentration below 1 micromolar led to an initial increase in cell number, whereas addition of 10-100 micromolar ATP resulted in a dose-dependent decrease in cell number. One of the most striking effects of ATP treatment was a dose dependent acidification of the culture medium over the first 24 hours following initial exposure. Further investigation revealed that the acidification was the result of an increase in lactate production. Treatment with 100 micromolar ATP induced a massive release of ATP from the cells, which would have effectively depleted them of ATP. The release of ATP appeared to be a result of P2X7 activation as it could be replicated by addition of 10 micromolar Ba2+ATP, a prototypic P2X7 receptor agonist with 10 fold greater potency than ATP. The ATP-induced ATP release was observed in both C20 and primary chondrocytes, and was unaffected by oxygen tension.

Conclusions: The results of this study demonstrate that chondrocytes express a range of P2 receptors including P2X7. This latter is a pore-and channel forming receptor which can induce proliferation or apoptosis. Low concentrations of extracellular ATP appear to have a positive effect on chondrocyte cell number, indicating that there might be a trophic effect on cell growth/survival. However when extracellular ATP was elevated to concentrations which might occur following localised mechanical injury or inflammation, activation of P2X7 receptors resulted in release of more ATP. Diffusion of released ATP to adjacent chondrocytes could lead to further activation of P2X7 receptor possibly resulting in the death of cells close to lesions. Diffusion of ATP through cartilage is likely to play a significant role in regulating cell function in this aneural, avascular tissue.

194 INTRA-ARTICULAR ADMINISTRATION OF GELATIN HYDROGELS INCORPORATING RAPAMYCIN-MICELLE REDUCES DEVELOPMENT OF EXPERIMENTAL OSTEOARTHRITIS IN A MURINE MODEL
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Purpose: Autophagy is a cellular homeostasis mechanism to degrade macromolecules and organelles damaged by various stresses. The dysfunction of autophagy has been recently reported to be associated with degenerative diseases and aging. We previously reported that autophagy regulates osteoarthritic gene expression of human chondrocytes and the rapamycin, a potent activator of autophagy, plays a preventive role against an inflammatory stress. In addition, a recent study revealed that intraarticular injection of rapamycin reduces the development of experimental osteoarthritis (OA) in a murine model. However, the local effect of intra-articular administration of rapamycin on the development of OA remains unknown and we attempted considering the side effect of systemic administration of rapamycin. The aim of this study was to investigate the therapeutic effect of intra-articular administration of rapamycin using a murine OA model.

Methods: To release rapamycin in a controlled manner, gelatin hydrogels incorporating rapamycin-micelle was created. Prior to administration, the efficacy of the controlled release of rapamycin from gelatin hydrogels incorporating rapamycin-micelle was examined in vitro. The therapeutic effect of intra-articular administration of rapamycin was examined using a murine OA model in vivo. OA was mechanically induced by destabilizing the medial meniscus under a microscope using knee joints of C57BL/6J mice. Mice (n=42) were divided into 3 groups. Group 1 was used as a control group. Group 2; Treated with gelatin hydrogels incorporating 100ng of rapamycin-micelle. Group 3; treated with gelatin hydrogels incorporating 1ug of rapamycin-micelle. Gelatin hydrogels were administered intra-articularly at the time of the surgery. Mice were sacrificed 10 weeks after surgery. The efficacy of the controlled release of rapamycin in vivo was examined using an autophagic marker, microtubule-associated protein 1 light chain 3 (LC3) by immunohistochemistry. OA progression was evaluated using the Osteoarthritis Research Society International cartilage OA histopathology grading system. In addition, total number of cells was counted using sections stained with hematoxylin-eosin.

Results: The controlled release of rapamycin from the hydrogels incorporating rapamycin-micelle was confirmed by the in vitro release test. Immunohistochmical analysis showed an increased LC3 expression in the rapamycin-treated groups 10 weeks after surgery compared with control group. The histological OA score was significantly decreased in both two rapamycin-treated groups compared with control group. Additionally, cellularity was significantly higher in the rapamycin-treated groups compared with control group.

Conclusions: We observed that the intra-articular administration of gelatin hydrogels incorporating rapamycin-micelle suppressed development of OA in the surgically-induced murine OA model. Our observations suggested that intra-articular gelatin hydrogels incorporating rapamycin-micelle can be a new therapeutic approach for treating patients with OA.

195 DATA MINING OF SEQUENCES STORED IN PUBLIC DATABASES REVELS CSDA AS A POTENTIAL MEDIATOR OF CARTILAGE INTEGRITY

Purpose: As the result of various efforts of sequencing of partial cDNAs coming from a wide variety of cells, more than eight million human cDNA sequences resulting from short single-pass sequencing reads are stored in the EST division of the NCBI database. Among them, several thousands correspond to healthy (HC) or osteoarthritic (OAC) cartilage. Although the technology has become outdated, it provides a wealth of useful data largely underused. Appropriate analysis using current bioinformatic tools might help to identify genes relevant for the maintenance of homeostasis of healthy cartilage, as well as the pathophysiology of osteoarthrosis.

Methods: dbEST division of GenBank was searched for data corresponding to human normal or OA cartilage, as well as cultured chondrocytes. Only non-normalized libraries with more than 1,000 sequences were considered. The matching genes were identified using the NCBI Blast suite. All data sets were compared to detect differentially expressed genes. A minimum level of expression of 0.6% (corresponding to presence of at least 3 transcripts in only one sample) was used as cutoff to assign specific expression in one of the conditions. Further analyses using Reactome and KEGG tools were used to identify biological processes and functions. RT-PCR and western blots were used in the validation of individual candidate genes.

Results: 4,722 and 4,621 sequences derived from HC or HOA cartilage, respectively, were identified, corresponding to 2,384 and 2,009 genes. Regarding cultured chondrocytes, more than 16,000 sequences were found coming from different libraries. A first approach revealed notable differences in gene expression between intact cartilage and cultured cells, with the latter actively expressing IGFBP3 or ELN, virtually absent...