meniscocalcinosis contributed to the absence of CC on x-rays. Studies in a lager population 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.

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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 purinoceptors, 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 BzATP, 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 unaffectedby 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

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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 intraperitoneal 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 needs to be tested 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 1μg of rapamycin-micelle. Gelatin hydrogels were administered intra-articulary 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.

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

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Purpose: As the result of various efforts of sequencing of partial cDNAs coming from a wide variety of human cells and tissues, 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