meniscocalcinosi 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 micromol led to an initial increase in cell number, whereas addition of 10-100 micromol 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 micromol ATP induced a massive release of ATP from the cells, which would have increased in lactate production. Treatment with 100 micromolar ATP resulted in cell death, 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 micromol 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 100 micromolar ATP, effectively depleted them of ATP.

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, this local effect of intrarticular administration of rapamycin on the development of OA remains unknown and is considered as a side effect of systemic administration of rapamycin. The aim of this study was to investigate the therapeutic effect of intraarticular 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 intraarticular 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/6 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. Immunohistochemical 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.
FRZB IS A CRITICAL MODULATOR OF CANONICAL WNT SIGNALLING IN CARTILAGE BIOLOGY
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Purpose: Polymorphisms in Frizzled-related protein (FRZB), a WNT antagonist, have been associated with osteoarthritis (OA). However, a recent meta-analysis failed to find a consistent effect of FRZB genetic variants on OA susceptibility. Our transcriptomics analysis in Frzb/-/- mice provided evidence for a tight regulation of WNT signalling and highlighted the complex role for FRZB in joint homeostasis. We previously demonstrated that Frzb/-/- mice have increased damage when dramatically challenged by papain, collagenase or severe inflammation. As these models are acute and short-term, we aimed to further investigate the effect of Frzb loss in a true translational model of OA and to delineate effects of FRZB genetic variation on canonical WNT signalling pathway, respectively. Fluctuating levels of Frzb did not in increase in the activation of canonical WNT signalling pathway, and OA cartilage. Conclusions: Our data mining of sequences stored in public databases can be a useful tool to identify genes involved in biological process. We have been able to identify a set of genes differentially expressed in normal and OA cartilage. Confirmation of the results on clinical samples has revealed CSDA as a potential mediator of cartilage integrity.

AGRN EXPRESSION IS DOWNREGULATED IN OA AND IS REQUIRED FOR CHONDROCYTE DIFFERENTIATION
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Purpose: Background: AGRN is a large basement membrane heparan sulphate proteoglycan best known for its function at the neuromuscular junction. It is responsible for synapse formation via binding to the MuSK receptor complex, leading to Acetylcholine receptor aggregation. Mice deficient of Agrin die perinatally as a result of respiratory failure. Agrin is also expressed in other cell types including chondrocytes of the developing growth plate, and agrin-deficient embryos in which agrin expression had been rescued at the neuromuscular junction develop skeletal abnormalities 1. Therefore it is possible that Agrin may play a role in cartilage homeostasis.

Aims: The aim of this project is to determine if agrin plays a functional role in the homeostasis of the articular cartilage and in osteoarthritis.

Methods: Human adult knee articular cartilage samples were obtained from preserved or damaged cartilage from individuals undergoing joint replacement for osteoarthritis. Experimental osteoarthritis was induced in 10 week old mice by destabilization of the medial meniscus2. Sham-operated controlateral knees were used as controls. Knees were collected 8weeks after surgery, decalcified and embedded in paraffin. Bone primary articular chondrocytes (BPAC) were isolated by