INTERACTIONS BETWEEN S100A8 AND DDR2 MECHANISMS IN CARTILAGE DEGRADATION IN OSTEOARTHRITIS

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Purpose: The surface bound tyrosine kinase receptor Ddr2 has been reported to play a role in early osteoarthritis (OA) pathogenesis. Increased levels of HtrA1 and degradation of the chondrocyte pericellular matrix expose collagen II fibrils, which activate Ddr2 upon binding, and lead to increased expression and activation of MMP-13. Pathology is delayed in surgically induced OA in Ddr2 Δ/Δ mice, and Ddr2, HtrA1 and MMP-13 are all increased in a spontaneous model of OA in Col11a1 Δ/Δ mice from 8 months of age. S100A8 and S100A9 have also been implicated in the induction of chondrocyte MMP-13, and are well known to be up-regulated at both the mRNA and protein level in inflammatory mouse models of arthritis. In contrast to inflammatory models, in a surgically-induced post-traumatic OA, chondrocyte S100A8 and S100A9 mRNA are up-regulated but they are not detected in cartilage at the protein level. The aim of this study was to determine if there is a link between the Ddr2 pathway and S100A8 and/or S100A9 in the pathogenesis of OA cartilage pathology by examining S100A8 and A9 expression in the Col11a1 Δ/Δ model where Ddr2 and HtrA1 are present, and by determining the effect of S100A8 and S100A9 on Ddr2 and HtrA1 expression in mouse cartilage.

Methods: S100A8 and S100A9 expression was examined in the knee cartilage of Col11a1 Δ/Δ mice and wild-type (WT) littermates at 3 and 9 months of age using immunohistochemistry (n = 3 of both genotypes at both times; archival sections from mice with antigen-induced arthritis (AIA) as a positive control. Mouse femoral head cartilage explants were dissected from 15 WT mice between the ages of 6-8 weeks old, homogenised, and the explanted cartilage distributed evenly amongst wells, and cultured in serum-free media ± 0.1μM murine S100A8 or S100A9 for 24 hours (n=2 wells/treatment). Gene expression of Ddr2, HtrA1 was determined by quantitative RT-PCR and normalised to Gapdh expression.

Results: S100A9 protein was not detected in the cartilage of 3 or 9 month old mice of either genotype although strong positive staining was observed in bone marrow of all mice, as well as cartilage from AIA animals. In addition to marrow and AIA cartilage samples, S100A8 protein was weakly detected in some chondrocytes/pericellular matrix in non-calcified cartilage in 1 of the 3 WT and Col11a1 Δ/Δ mice at 3 months of age. In contrast S100A8 was localized to chondrocytes/pericellular matrix of non-calcified cartilage and meniscal cells in all 9 month old animals, with no difference observed between genotypes. In mouse femoral cartilage explants cultures, gene expression of Ddr2 was increased by S100A8, but decreased by S100A9, while HtrA1 was decreased by both S100A8 and S100A9.

Conclusions: Whilst S100A8 staining was observed in non-calcified cartilage of some 3 month old and all 9 month old mice, there was no differential regulation between genotypes, suggesting that whilst an increase in S100A8 in cartilage may be an age-related change, it is not associated with the accelerated cartilage degradation seen in the Col11a1 Δ/Δ model. In contrast, no S100A9 staining was observed in the cartilage of any mice from either genotype or age, and therefore may not be associated either with OA or age-related cartilage changes. Gene expression data from femoral head cartilage cultures, however, suggests that S100A8 but not S100A9 can increase Ddr2 mRNA, providing a link between these degradative cartilage pathways. However the down-regulation of HtrA1 mRNA by the S100 proteins suggests that an alternative mechanism for exposure of the Ddr2 ligand would be required. Taken together, this data suggests that whilst S100A8 is not associated with cartilage degradation in Col11a1 Δ/Δ, it may pre-dispose the cartilage to degradation via up-regulation of Ddr2.

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PPARDELTA AS A NOVEL TARGET FOR OSTEOARTHRITIS THERAPY

Introduction: Osteoarthritis (OA) is a degenerative disorder associated with the breakdown of articular cartilage. The mechanisms responsible for this condition are not well understood and therefore no treatments exist to halt or delay the progression of OA. Recent findings from our laboratory indicate that activation of the transcription factor PPARdelta induces the expression of enzymes involved in proteoglycan breakdown and can lead to cartilage degeneration in OA, prompting us to speculate whether inhibition of PPARdelta, can protect from cartilage breakdown in OA.

Purpose: To evaluate the role of PPARdelta in Osteoarthritis through all encompassing in-vitro, ex-vivo and in-vivo models of disease.

Methods: To test this, human chondrocytes and mouse femoral head cartilage explants were treated with pharmacological agonist (GW501516) and antagonists of PPARdelta (GSK3787/0660) to evaluate changes in gene expression (qPCR), and histology (Safranin-O, immunohistochemistry) consistent with OA, and to determine if recovery was possible.

Our in-vivo approach uses the Cre-Lox system to inactivate PPARdelta specifically in the cartilage of using a surgical model of OA. Mutant and control mice aged 20 weeks are being compared 8 weeks after a destabilization of medial meniscus (DMM) surgery, based on the premise that changes in biomechanical load drive cartilage degeneration. In order to assess the progression of OA between groups, histopathological scoring (OARSI) and immunohistochemistry for known markers of OA, (MMP 13, cartilage matrix breakdown products) are being analyzed. Serum analyses for extracellular matrix markers of cartilage breakdown are being conducted. To investigate changes in joint loading during OA, mutant and control mice are being compared through gait analyses using the CatWalk system that measures load on