DNA methylation in the IL-1β promoter in 3/5 patients. Further work concentrated on this gene. Bisulfite modification suggested that the CpG sites at -290bp and - 247bp were important for the epigenetic regulation of IL-1β, and the site at -290bp was selected for quantification of DNA methylation. We then determined which experimental conditions resulted in the greatest loss of DNA methylation and whether this paralleled the increase in mRNA expression. Culture with the de-methylating factor 5-aza-dC halved DNA methylation, which resulted in 4-8 fold increases in IL-1β expression compared with cultured controls. This demonstrated that DNA de-methylation *per se* increased gene expression. However, this was not the only factor involved, since far greater effects were seen with the inflammatory cytokines: IL-1β reduced methylation to ~15%, cresulting in 50-100 fold increases in gene expression. The greatest effect was seen with TNF-α/OSM, which abolished DNA methylation almost completely and caused IL-1β expression to increase 500-1000 fold. The cytokine-induced expression was translated into protein. Unlike IL-1β, TNF-α expression was not susceptible to auto-induction or epigenetic control.

Conclusions: A system has been developed in which loss of DNA methylation in combination with 100-1000 fold increases in gene expression can be induced experimentally - a crucial prerequisite for mechanistic studies. Moreover, we demonstrated for the first time that inflammatory cytokines can cause loss of DNA methylation in addition to all other known effects, and that IL-1β (but not TNF-α) induces its own expression.

146

MECHANISMS OF ACTION OF IKKα IN REGULATING THE HYPERTROPHIC TRANSITION OF PRIMARY OSTEOARTHRITIC CHONDROCYTES

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Purpose: Our previous work revealed differential roles for the NF-κB activating kinases in controlling the progression towards terminal differentiation of primary osteoarthritic chondrocytes. Here we elaborate some of the multiple steps whereby IKKα controls the terminal chondrogenic differentiation program.

Methods: Primary chondrocytes were derived from 15 Osteoarthritis (OA) patients undergoing joint arthroplasty. IKKα or IKKβ shRNAs were stably expressed by pSuper retroviral transduction of IKKα or IKKβ specific shOligos followed by selection of puromycin resistant cells and KD efficiencies were verified by immunoblotting. High density monolayer and micromass cultures under mineralizing conditions were selected to investigate the mechanisms whereby the IKKs control chondrocyte terminal differentiation. Runx2 was evaluated by both REAL TIME PCR in 1 week old micromasses and by immunohistochemistry in 3 week old micromasses and Sox9 expression in high density monolayer cultures by immunoblotting. The effects of IKKα KD on type 2 collagen accumulation at the transcriptional or post-translational levels were investigated in micromasses. ECM remodelling was directly evaluated by staining for neoepitopes (Col2 3/4 C and DIPEN and NITEGE). Moreover, IKKα expression was rescued in IKKα KD chondrocytes with either a wild type or kinase-dead IKKα(K44M) mutant to explore the connection between IKKα's effects on chondrogenesis and the NF-κB pathway.

Results: IKK KDs were at least 80% and generally greater than 90%. Both IKK KD were found associated with increased Col2 mRNA expression and also resulted in defective ECM remodelling as shown by IHC detection of neoepitopes. Silencing IKKβ markedly increased sGAG accumulation, in conjunction with increased Sox9 expression. However, IKKα ablation markedly enhanced collagen 2 deposition independently of Sox9 but instead in association with a strong remodelling block in conjunction with Runx2 suppression. In addition ablation of either IKKα or IKKβ also inhibited Collagen X deposition thus providing additional evidence, along with blocks in calcium deposition, that each IKK (albeit via different mechanisms) is of central importance for progression towards terminal differentiation.

Conclusions: We have uncovered an unexpected, pivotal role for IKKα in the terminal chondrogenesis of primary osteoarthritic human chondrocytes. IKKα controls the transition to the hypertrophic phase at multiple levels, while IKKβ appears to mimick the effects of canonical NF-κB activation. Preliminary indications of an IKKα rescue experiment suggest that its more profound effects could be independent of the NF-κB pathway. In conjunction with the healthier status of IKKα KD chondrocytes, with respect to their extensive ECM and high viability, our results support the view that IKKα could be a novel therapeutic target for Osteoarthritis with its ablation causing a profound blockage towards terminal differentiation.

Acknowledgements: Supported by the CARISBO Foundation (Contract Grant number: 2005-1059); grants from Bologna University; Ricerca Corrente Istituti Ortopedici Rizzoli. KBM greatfully acknowledges the support of the CRBA, Bologna University.

147

APOLIPOPROTEIN A-1 IS A NOVEL INDUCER OF AGGRECAN BREAKDOWN IN CULTURED ARTICULAR CARTILAGE

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Purpose: While investigating the effects of sharp injury upon inflammatory signalling pathways in articular cartilage, we found that synovial fluid strongly activated MAP kinases in cartilage explants adapted to culture. We set out to identify the activator and investigate its effects on chondrocyte function.

Methods: Cartilage from porcine metacarpophalangeal joints was cultured in serum-free medium for 24h before treatment with porcine synovial fluid. Tissue extracts were analysed for MAPK (ERK, p38 and JNK) and ATF-2 (a preferred JNK substrate) activation by phospho-Western blotting. Culture medium samples were analysed for the presence of aggrecanase-generated aggrecan fragments by using antibodies against the aggrecan ARGS neoepitope. Porcine synovial fluid was subjected to sequential chromatography (Resource Q, Resource S and Blue Sepharose) and the MAPK activator was identified by mass-spectrometry.

Results: The porcine synovial fluid activator was identified as *apolipoprotein A-1* (*APOA-1*) the major component of high density lipoprotein (*HDL*) in tissue fluids. Porcine APOA-1 purified from synovial fluid and commercially available human APOA-1 activated MAPKs and also induced aggrecan breakdown in cultured cartilage. APOA-1 induced mRNA of *ADAMTS-4* and *-5* and *MMP-13*. APOA-1 differed from typical inflammatory mediators that cause cartilage catabolism (IL-1, TNF, LPS), in that it did not induce cyclooxygenase-2 (COX-2), a typical inflammatory response gene.

Conclusions: APOA-1 in synovial fluid causes MAPK activation and induces aggrecanases in cartilage. It appears to cause a matrix-resorbing response without inducing inflammatory response genes. APOA-1 (apparent Mr ~120kD) may normally be excluded from the cartilage matrix but is perhaps able to access