The WISP-3 gene was stably expressed in an immortalised chondrocytic cell line (C-28/I2) to investigate the effects of WISP-3 on chondrocyte biology. The expression of cartilage specific genes was compared by real time PCR in clonal cells stably transfected with WISP-3 or the empty vector alone. Results in the WISP-3 stably expressing cells were verified by treating untransfected parental C-28/I2 cells with recombinant human WISP-3 protein.

The potential role of WISP-3 in cell signalling was investigated by treating C-28/I2 cells with WISP-3 alone, IGF-1 alone or IGF-1 and WISP-3 together. Erk1/2 phosphorylation was then measured by western blotting after treatment for 10 minutes or 1 hour.

Results: Real time PCR revealed WISP-3 mRNA was upregulated in osteoarthritic cartilage compared to normal post mortem cartilage. Immunohistochemical staining of osteoarthritic and post mortem cartilage sections showed WISP-3 protein was present in damaged areas. In severely damaged cartilage sections, WISP-3 often appeared as strong halos around chondrocytes.

Stable over-expression of WISP-3 in C-28/I2 cells led to a down-regulation of *Col2a1* mRNA. Preliminary results suggest this effect can also be produced by treatment of parental C-28/I2 cells with recombinant WISP-3 protein.

Treatment of C-28I/2 cells with IGF-1 induced Erk1/2 phosphorylation after 10 minutes. WISP-3 could also induce Erk1/2 phosphorylation, although when in combination with IGF-1 it did not appear to enhance or prolong Erk1/2 phosphorylation.

Conclusions: Our data suggest that WISP-3 may be a marker for damaged cartilage and may exert effects on matrix synthesis and chondrocyte signalling. Further work is required to determine the role of WISP-3 in cartilage matrix metabolism. We intend to assess the effec ts of WISP-3 on metalloproteinase expression, proliferation and IGF-1 induction of *Col2a1* in C-28/I2 cells.

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IKKα AND IKKβ ARE BOTH REQUIRED FOR THE CHEMOTACTIC ACTIVITY OF MONOCYTES TOWARDS PRIMARY OSTEOARTHRITIC CHONDROCYTES

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Purpose: IKKα and IKKβ are essential kinases for activating NF-κB transcription factors that regulate cellular differentiation and inflammation. By virtue of their small size and ECM diffusibility chemokines could invoke cross-talk between osteoarthritis (OA) chondrocytes with other joint compartments and also contribute to immune cell chemotaxis into the synovial space. Because many chemokines are known to be direct NF-κB targets, here we employed shRNA retroviruses to ablate the expression of each IKK to determine their individual contributions for monocyte chemotaxis in response to chondrocyte conditioned media.

Methods: Primary chondrocytes were derived from 6 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 or micromass cultures were established for each patient. Conditioned media was collected, with or without 72 h of prior exposure to IL-1β. Chemotaxis of human monocytes towards chondrocyte conditioned media was assessed with boyden chambers. The chemokine expression repertoires under basal and stimulated conditions in wild type and IKK KD chondrocytes were evaluated by focused Oligo GEarrays (SuperArray) and the concentrations of monocyte active chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES) were also quantified by Multiplex fluorescence-based assays.

Results: Penetrant IKKα or IKKβ KDs of 80-90% blunted the monocyte chemotactic potential of chondrocyte conditioned media. Under basal conditions CCL2/MCP-1 was the chemokine of highest concentration (nanogram levels) in wild type chondrocyte conditioned media, which presented the strongest association with monocyte chemotaxis (Spearman=0.943, $p<0.05$). Interestingly CCL2/MCP-1 production was co-dependent on IKKα and IKKβ, while stimulus induced expression of CCL3/MIP-1α, CCL4/MIP-β and CCL5/RANTES appeared to be largely dependent on IKKα and not IKKβ. Similar results were obtained for CCL5/RANTES at the RNA level after IL-1 stimulation for either 2 or 8 hours.

Conclusions: Results of targeted retroviral mediated RNA interference revealed a co-dependency on IKKα and IKKβ for monocyte chemotaxis in response to chondrocyte conditioned media, even in the absence of an extracellular pro-inflammatory stimulus (i.e., IL-1β). Interestingly at least part of the dual IKK dependency for this inflammatory-like migration response was associated with the magnitude of MCP-1 production. Moreover IKKα and IKKβ appear to be constitutively active in OA chondrocytes, because chondrocyte conditioned media induced a strong migration response by primary monocytes under basal conditions, likely reflecting the synovitis often complicating OA. Our findings reveal important roles of both NF-κB activating kinases in driving a key inflammatory process underlying this debilitating disease.

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Hsp90 AND p130^{cas}: NOVEL REGULATORY FACTORS OF MMP-13 EXPRESSION IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Purpose: MMP-13 is a key protease in osteoarthritis (OA) pathophysiology, and this protease is able to degrade a wide range of cartilage matrix molecules. Our previous studies have shown that human OA chondrocytes could be classified into two broad categories, L- (Low) and H- (High) OA chondrocytes according to their MMP-13 basal levels and IL-1β inducibility. L-OA chondrocytes had low MMP-13 basal levels and high IL-1β inducibility, whereas H-OA chondrocytes showed high basal levels and low IL-1β inducibility. Moreover, MMP-13 is regulated in H-OA chondrocytes by the proteins NMP4 and p130^{cas} acting at the AGRE site in the MMP-13 promoter. The aim of this study was to identify factors involved in MMP-13 regulation in human L-OA chondrocytes and to determine the effect of IL-1β on these factors and their roles. Furthermore, the effects of these factors were also assessed on the expression of other MMPs.

Methods: Gel shift assays were done with AGRE-oligonucleotides and human L-OA chondrocyte nuclear extracts; the

proteins in the specific DNA/protein complex were identified by mass spectrometry. siRNAs specific for $p130^{cas}$ and Hsp90 were transfected, alone or in combination, into L-OA chondrocytes, and the cells were incubated in the absence or presence of IL-1β (100 pg/ml). Total RNA was extracted and processed for real-time PCR with primers specific for the MMP-1, -2, -3, -9, -13 and -14 genes. MMP-1 and -13 protein production was determined by specific ELISAs.

Results: Mass spectrometry data revealed the presence of the Hsp90 protein in the L-OA gel shift complex. siRNA experiments on L-OA chondrocytes showed that $p130^{cas}$ and Hsp90 silencing significantly increased the expression of MMP-13 (3.5 fold; $p<0.0001$, and 1.8 fold; $p<0.05$, respectively) and, to a lesser extent, MMP-1 (2.2 fold; $p<0.0001$, and 1.6 fold; $p<0.05$), whereas NMP4 showed no effect. These results point to an in vivo inhibitory role of $p130^{cas}$ and Hsp90 on these MMPs. The expression of MMP-2, -3, -9 and -14 was not affected by either factor. Interestingly, the double silencing Hsp90/$p130^{cas}$ produced an additive effect on MMP-13 expression (4.9 fold; $p<0.0001$), while the effect on MMP-1 was minimal (2.3 fold; $p<0.005$). MMP-13 and MMP-1 protein levels are reminiscent of the expression pattern. The presence of IL-1β with the $p130^{cas}$-silenced cells, and to some extent with the Hsp90-silenced cells, further stimulated MMP-13 expression, suggesting that in vivo the presence of these factors prevents a maximal stimulation by IL-1β.

Conclusions: This study identifies the intracellular proteins $p130^{cas}$ and Hsp90 as inhibitors of MMP-13 expression in L-OA chondrocytes, and demonstrates the complexity of MMP-13 regulation. Studies are underway to determine if $p130^{cas}$ and Hsp90 act independently or in cooperation.

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RESISTIN IS ELEVATED FOLLOWING TRAUMATIC JOINT INJURY AND CAUSES MATRIX DEGRADATION AND RELEASE OF INFLAMMATORY CYTOKINES FROM ARTICULAR CARTILAGE *IN VITRO*

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Purpose: Determine if resistin is present in the joint following joint injury and in primary osteoarthritis (OA) and to elucidate the role of resistin in cartilage inflammation and degradation *in vitro.*

Methods: The level of resistin was measured in paired synovial fluid and serum samples from patients following joint injury (ACL or meniscus tear), primary OA, and non-injury/non-OA controls using adipokine multiplex kits. Localization of resistin in joint tissues was visualized by immunohistochemistry (IHC) in synovial tissue and cartilage from OA, rheumatoid arthritis (RA), and normal controls. Murine cartilage explants were treated with recombinant resistin for three days in culture. Release of cytokines was assayed in the supernatants using a cytokine multiplex kit. Inflammatory marker, PGE_2, was measured in culture supernatants by ELISA. Matrix degradation was visualized in Toluidine Blue stained cartilage sections and quantified by measuring release of sulfated GAG (sGAG) to the culture media using the dimethylmethylene blue (DMMB) dye assay.

Results: Resistin was detected in synovial fluid and serum samples immediately after joint meniscus and ligament injury and in the early stage of OA. The level of resistin in both synovial fluid and serum declined with increasing time after injury from 3000 pg/ml to 900 pg/ml in synovial fluid and 8550 pg/ml to 5200 pg/ml in serum. Resistin protein was detected by IHC in macrophages and mast cells in synovial tissue from both OA and RA patients. Cartilage was negative for resistin staining in 3/3 OA and 2/3 RA samples. Cartilage explants treated *in vitro* with resistin at 10 μg/ml released increased levels of MCP-1, KC (functional homolog of human IL-8), IL-6, and PGE_2 compared to untreated control cultures (Fig. 1). Resistin stimulated degradation and release of sGAG from the cartilage extracellular matrix.

Conclusions: Resistin is present in the joint following injury and in primary OA and causes the release of inflammatory markers and cartilage matrix degradation in vitro. Inhibition of resistin in a diseased joint has the potential to prevent joint inflammation and cartilage degradation.

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MITOGEN-ACTIVATED PROTEIN KINASE-ACTIVATED PROTEIN KINASE 2 MODULATES THE CYTOKINE-INDUCED PRODUCTION OF MATRIX METALLOPROTEASES AND THE PUTATIVE PAIN MEDIATOR PGE2 IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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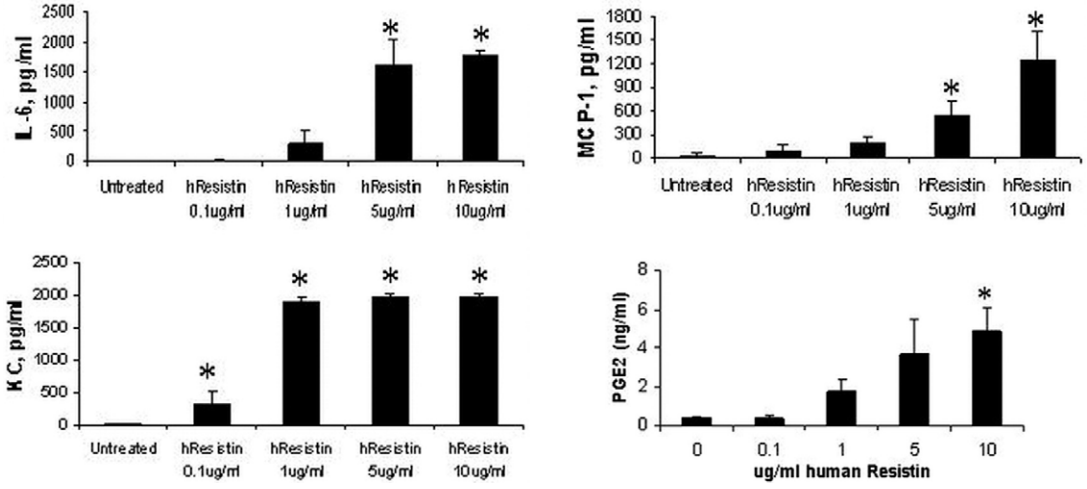
Purpose: Mitogen-activated protein kinase-activated protein



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