advanced OA cartilage correlated significantly with BMI values of the patients (r=0.610, p<0.05). SF had higher leptin concentrations than the paired serum sample, while a significant correlation was observed between serum and SF leptin levels (r=0.672, p<0.01). In normal chondrocyte cultures (48 hrs), leptin enhanced cell proliferation but reduced it in osteoarthritic chondrocytes, while in long term cultures leptin had a similar detrimental effect on chondrocytes. Furthermore, leptin induced the production of IL-1β in long term cultures.

Conclusions: For the first time to our knowledge, using quantitative real-time PCR, we observed leptin and Ob-Rb mRNA expression in chondrocytes of osteoarthritic cartilage. The observed intrajoint difference in leptin and Ob-Rb mRNA expression between advanced and minimally affected knee and hip osteoarthritic cartilage, along with the higher observed leptin concentrations in SF than in paired serum samples indicate a local role of leptin in joint tissues. The increased leptin’s mRNA expression in obese compared to normal weight patients suggested that mechanical overload may alter chondrocytes’ phenotype. Also the decrease in chondrocyte’s proliferation after induction with leptin points toward a long term detrimental effect on cartilage, while the observed IL-1β production suggests an inflammatory role of leptin, which may account for cartilage degradation. Taken together our findings support the hypothesis that leptin is involved in OA development and that may be the missing link between biomechanical and metabolic factors involved in OA. However, further studies are needed to confirm the involvement of leptin in OA and to investigate the link between obesity, inflammation, leptin and osteoarthritis.

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ACETYLATION REGULATES EXPRESSION OF THE MMP28 GENE

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Purpose: The matrix metalloproteinase (MMP) family consists of 23 structurally related proteases that are able to degrade a wide range of substrates, including many components of the extracellular matrix (ECM). MMP-28 (epilysin), is a recently cloned member of the MMP family. We have recently described MMP28 expression in cartilage with significant increase in osteoarthriti-
sis. The principle level of control of MMP expression is gene transcription. The promoter region of both human and mouse MMP28 has been cloned and characterised. Conserved motifs were identified that could control the expression of MMP28 including a GT-box situated 300 bp upstream from the transla-
tion start site that binds both Sp1 and Sp3 proteins and is essential for MMP28 basal expression in keratinocytes. We showed recently that histone deacetylase inhibitors (HDACi) are chondroprotective. Whilst HDACi downregulate expression of a number of MMPs, a small subset are induced and this includes MMP28.

Methods: Promoter-reporter constructs were made in pGL3 and transiently transfected into HeLa or SW1353 cells using FuGene6. DNA-protein interaction was assessed by gel mobility shift assay. Acetylation of Sp1 and Sp3 was probed using immunoprecipitation with a specific antibody and Western blotting with an anti-(pan acetyl lysine) antibody.

Results: Transient transfection assays with deletion constructs of the MMP28 gene promoter, showed that HDACi, including trichostatin A (TSA), valproic acid, sodium butyrate, upregulate transcription from the promoter via the promoter proximal GT described above. This was confirmed using point mutants in this site. Gel mobility shift and supershift assays using Sp1/2/3 antibodies showed that Sp1 and Sp3 bind to the MMP28 promoter via the GT-box. Using transient transfection assays with the wild type and mutant promoters we showed that Sp1, but not Sp3, can induce the MMP28 promoter via the GT-box. HDACi induce transcription from the promoter via Sp1 action at this site. Sp1 is known to interact with the histone acetyltransferase (HAT) p300 and histone deacetylase 1 (HDAC1). Forced overexpression of either p300 or PCAF, but not CBP (all of which are known HATs), induces the MMP28 promoter, as does siRNA knockdown of HDAC1. Sp1 and 3 can both be acetylated and phosphorylated which is reported to alter their DNA-binding properties. In immunoprecipitation experiments, the acetylation of Sp1 and Sp3 was increased by TSA treatment. Furthermore, the ser-

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OVER-EXPRESSION OF RHOB IS ASSOCIATED WITH OSTEARTHRITIS AND DRIVES APOPTOSIS IN A CHONDROSARCOMA LINE

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Purpose: We recently identified RHOB as a gene over-expressed in OA patients. The analysis of RHOB expression revealed allelic imbalances indicating cis-regulatory mechanisms and indeed, a single nucleotide polymorphism (SNP) at -165 in the RHOB promoter turned out to be associated with OA. We here investigate in detail the regulation of RHOB expres-
sion and the functional consequences of its over-expression in a chondrosarcoma line.

Methods: Apoptosis following over-expression of RHOB in the human chondrosarcoma line SW1353 was assayed via quantifi-
cation of cells in the sub-G1 phase of the cell cycle and con-

Conclusions: Our results support the concept of OA being the consequence of active disease processes. We show that...