Conclusions: We found that N-domain and full-length TIMP-3 are similarly effective in most in vitro and cell-based assays, although full-length was more potent against MMP-13. The N-domain was more effective in blocking IL-1-induced GAG release from bovine nasal cartilage explants. Both forms, but most dramatically the full-length protein, inhibited cartilage degradation, osteophyte growth and bone lesions in a rat meniscal tear model of osteoarthritis. The greater efficacy seen with the full-length form could be due to its greater potency against MMP-13.

A15
ELEVATED EXTRACELLULAR MATRIX PRODUCTION AND GAG DEGRADATION UPON BMP-2 STIMULATION POINT TOWARDS A ROLE FOR BMP-2 IN CARTILAGE REMODELING

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Purpose: BMP-2 has often been proposed as a good tool for cartilage repair and as a strong stimulant of chondrogenesis. Cells modified to overexpress BMP-2 and scaffolds coated with BMP-2 have been placed into a cartilage defects for reparative purposes. However, it is unknown what BMP-2 does on intact cartilage that is present in the treated joints. To study the effect of BMP-2 on intact cartilage in vivo, we overexpressed BMP-2 in normal murine knee joints and evaluated the effects on proteoglycan synthesis and degradation.

Methods: C57Bl/6 mice were injected intra-articularly with an adenovirus overexpressing BMP-2. Mice were injected i.p. with 35SO2- 24 hours before sacrifice. After 3, 7 and 21 days knee joints were isolated for histology to perform Safranin O/Fast Green staining and autoradiography to assess anabolic effects of BMP-2. Immunohistochemical staining of the aggregan neo-epitopes VDIPEN and NITEGE was carried out to investigate GAG degradation. Histological quantification of staining in patellar and tibial cartilage was performed with a computerized imaging system. In addition, patellar and tibial cartilage were isolated for measurement of proteoglycan (PG) synthesis by means of 35SO2- incorporation or RNA isolation for quantitative PCR.

Results: BMP-2 overexpression resulted in altered chondrocyte appearance, which appeared to be larger than chondrocytes in control cartilage. Furthermore, BMP-2 stimulated PG-synthesis in patellar cartilage significantly on all days (up to two fold) and in the tibia on day 21. Stimulation was less prominent in tibial cartilage than in patellar cartilage. On mRNA level collagen type II expression had increased on all days in the patella with the highest expression on day 7 (17-fold) Aggrecan expression showed the same pattern with a a 13-fold increase on day 7. On the tibia collagen type II expression had increased 12-fold on day 7 and 14-fold on day 21 and aggrecan expression was elevated 15-fold on day 7 and 12-fold on day 21. In addition to stimulation of extracellular matrix production, BMP-2 overexpression also resulted in MMP- as well as ADAMTS-mediated cartilage degradation. VDIPEN staining (indicating MMP activity) was elevated upon BMP2 stimulation on day 3 on tibial cartilage and on day 3 and 7 in patellar cartilage, but no longer by day 21. NITEGE staining (indicating aggregcanase activity) was not found on day 3, but this is likely due to elevated MMP-activity cleaving off the NITEGE epitope. On day 7 NITEGE staining had increased 2-fold in the lateral tibial condyle and 3.5 fold in patellar cartilage, which was still increased 2-fold on day 21. On RNA levels we found elevated MMP3 expression on both tibia and patella, and elevated ADAMTS4 expression in the patella. BMP-2 overexpression did not result in detectable cartilage damage in Safranin O/Fast Green stained sections.

Fig. 1

Conclusions: Adenoviral overexpression of BMP-2 shows that BMP-2 is able to elevate proteoglycan synthesis in cartilage and stimulates collagen type II and aggrecan mRNA expression. At the same time increased catabolic activity was observed indicated by elevation of VDIPEN and NITEGE neo-epitopes. These data show that BMP2 not only boosts matrix synthesis in normal cartilage but that BMP-2 increases matrix turnover. Increased matrix turnover might be functional to replace matrix molecules in the repair of a damaged cartilage matrix.

A16
DIFFERENTIAL ROLES OF IKKα AND IKKβ IN CHONDROGENESIS AND OA INFLAMMATION REVEALED BY RETROVIRAL MEDIATED RNA INTERFERENCE

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Purpose: IKKα and IKKβ are essential kinases for activating NF-κB transcription factors that regulate cellular differentiation and inflammation. Each IKK was targeted for knockdown (KD)
by transducing human chondrocytes with shRNA expressing retroviruses to determine their roles in chondrocyte micromass differentiation and in fostering monocyte chemotaxis.

**Methods:** Primary chondrocytes were derived from 15 Osteoarthritis (OA) patients undergoing joint arthroplasty. Stable expression of IKKα or IKKβ shRNAs was achieved by transduction with a pSUPER retroviral vector harbouring an IKKαalpha or IKKbeta specific shOligo. Populations of shRNA expressing cells were selected for puromycin resistance. KD efficiencies were determined by Western blot compared to cells transduced by a firefly luciferase shRNA retrovector. Multiple physiological parameters in high density monolayer or micromass cultures under basal conditions or IL-1 stimulation were studied including: (1) cellular morphology & ECM via Transmission Electron Microscopy (TEM), (2) GAG accumulation by the DMMB method, (3) MMP-13 release by ELISA, (4) attraction of human monocytes by chondrocyte conditioned media in boyden chambers. Off target effects were ruled out by using up to 3 shOligos targeted to different regions of the IKKα or IKKβ mRNAs.

**Results:** IKK KDs were at least 80% and generally greater than 90%. GAG accumulation in micromasses increased after IKKα and IKKβ KD. TEM analysis also revealed an ECM increase as well as enhanced cellular viability. Noteworthy, the IKKα KDs displayed organized and abundant collagen fibrils. MMP-13 release in response to IL-1 was dependent on IKKβ but either unaffected or slightly increased without IKKα. KDs of either IKKα or IKKβ blunted the monocyte chemotactic potential of chondrocyte conditioned media.

**Conclusions:** Retroviral transduction efficiently delivers specific shRNAs into primary human chondrocytes. Our data reveal that IKKα and IKKβ have differential roles in chondrogenesis (ECM remodeling and terminal differentiation) in the micromass culture model. IKKα appears to selectively control collagen fibers, while IKKβ mainly affects accumulation of the GAG component. MMP-13 release upon IL-1 stimulation was dependent on IKKβ in keeping with its regulation by the canonical NF-κB activation. Moreover, we observed that chondrocyte conditioned media strongly attracts primary monocytes, likely reflecting the synovitis often complicating OA. Monocyte chemotactic potential was dependent on IKKα and IKKβ, thus confirming the inflammatory characteristics of OA chondrocytes and revealing that both IKKs are involved in this key inflammatory process underlying osteoarthritis.

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A17

**LOCATION SPECIFIC RADIOGRAPHIC JOINT SPACE WIDTH FOR OSTEOARTHRITIS PROGRESSION: VALIDATION OF A SOFTWARE METHOD**

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**Purpose:** To establish the performance of location specific computer measures of joint space width. We hypothesize that location specific computer measures of radiographic joint space width (mJSW) will outperform measurements of minimum joint space width (JSW) for the assessment of medial compartment knee osteoarthritis (OA). The study also determined the most disease-sensitive location for measuring JSW.

**Methods:** Serial bilateral PA conventional radiographs were obtained 36 months apart in persons with knee OA participating in the Health ABC study, a community based, multi-center cohort study of 3,075 White and Black men and women aged 70-79 at enrollment. We randomly selected a sample of 40 participants with radiographs and excluded those with lateral compartment joint space narrowing and knees with Kellgren and Lawrence scores of zero at baseline, leaving 51 knees from 29 subjects for analysis. The radiographs were digitized using a Vidar film digitizer with a pixel spacing of 0.085 mm and transferred to a personal computer for analysis.

To enable consistent measures of JSW, a coordinate system was defined based on anatomical landmarks (See Figure 1) The x axis was set tangent to both femoral condyles. The y-axis was tangent to the medial epicondyle and the line x = 1 was defined as the tangent to the lateral epicondyle of the knee.

**Results:** Table 1 provides the results. The accuracy for mJSW at eight fixed locations was facilitated by the use of automated software that delineated the femoral and tibial margins of the joint. (See Figure 2.) mJSW was defined as the minimum distance between the delineated femur and tibia margins. Measures of JSW were defined as the distance from the tibial margin to the femur margin at fixed locations on the coordinate system shown in Figure 1.

**Figure 1. Definition of coordinate system**

**Figure 2. Locations of JSW measurements**

A human reader operated custom software to verify and correct the software-drawn margins where necessary. Paired images were displayed with the reader blinded to the time point. As a metric to quantify performance we used the accuracy of each method to correctly order the time point of the films. This evaluation was based on the reasonable assumption that structural changes in OA do not improve over time.

**Results:** Table 1 provides the results. The accuracy for mJSW was 75%. Accuracy ranged from 67% to 82% for JSW at fixed locations, outperforming mJSW in 6 of 8 positions. In this study we found that the optimal location for measurement was at the location x = 0.225.