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cartilage changes. Calcitonin is well-known for its inhibitory actions on osteoclasts and bone resorption. We investigated the effect of endogenous increased levels of salmon calcitonin (sCT) on the articular cartilage and subchondral bone, after destabilization of the medial meniscus (DMM) in normal and sCT over-expressing mice.
Methods: Mice over-expressing sCT and wild-type littermates were used for the experiments. Genotyping was performed by measuring the circulating serum levels of sCT by ELISA. Northern Blot expression analysis was carried out to investigate the liver-specific expression. Non-decalcified plastic embedded vertebrae from mice of 6 and 12 month of age were used for histomophometric investigations. Serum samples were collected for osteocalcin measurements and urine for Dpd/creatinine measurements. For evaluation of cartilage and subchondral bone changes, forty-four 10 week old mice were divided into four groups and subjected to destabilization of the medial meniscus (DMM) or sham operation. After 7 weeks the study was terminated and the knee joints isolated for histological analysis by Safranin'O staining and biochemical markers were measured in fasting blood samples. The proteoglycan volume of the tibia and femur was quantitative assessed from histology sections by using the VisioPharm image technology. Individual pixels were labeled for multiple variables including bone, proteoglycans and bone marrow to create a working algorithm. Still images were taken of the knee joint histology sections and the images were processed and classified by masking the area of interest. The algorithm was then applied to the processed area for calculating proteoglycan loss.
Results: Transgenic mice had >800 pg/mL serum sCT and wild-type mice had no detectable levels. Von Kossa staining revealed an increase in trabecular bone volume (BV/TV) of 100% after 6 month and 150% after 12 month in sCT mice when compared to controls ($P<0.05$). Trabecular number and thickness increased significantly in transgenic mice of both 6 and 12 months of age ($P<0.05$), and trabecular separation decreased ($P<0.05$). Osteoblasts number, bone formation rate and osteocalcin measurements was not affected in transgenic mice. Wild-type DMM operated mice had major damage to the articular cartilage, compared to DMM operated transgenic mice. In wild-type animals a 5-fold increase in the quantitative erosion index was observed after DMM, and the semi-quantitative OARSI score showed more than 400% ($P<0.001$) increase, compared to sham-operated wild-type. DMM operated transgenic mice were protected against cartilage erosion and showed a 65% and 64% ($P<0.001$) reduction, respectively, for the two histopathological evaluation methods, compared to DMM wild-type littermates. Preliminary quantitative analysis of proteoglycan loss revealed a trend towards sCT protection against cartilage erosion, with DMM operated WT mice showing a 40% increase in proteoglycan loose compared to WT sham, while DMM operated sCT mice showed a 20% reduction compared to sCT sham. DMM operated WT mice showed a 30% reduction in proteoglycans compared to sCT DMM.
Conclusions: sCT over-expressing mice had higher bone volume compared to wild-type, and were protected against cartilage erosion compared to wild-type litter mate controls. This data suggest that increased levels of sCT may hamper the pathogenesis of OA however more studies are necessary to confirm these preliminary results.

017

EFFECTS OF DYNAMIC LOADING ON INJURED ARTICULAR CARTILAGE IN VITRO AND IN VIVO

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Purpose: Recent clinical studies in patients showed that ACL deficiency not only shifts tibiofemoral contact location but significantly increases the magnitude of cartilage contact deformation: from 10-20% compressive deformation in contralateral normal knees to 20-30% in ACL-deficient knees of the same patients. In addition, previous in vitro models have shown that mechanically injured cartilage cultured in the presence of inflammatory cytokines, known to be upregulated in human synovial fluid after joint injury, potentiates matrix catabolism. The purpose of this study was to examine whether increased loading after injury further exacerbates cartilage degradation using both in vitro and in vivo models.
Methods: *In vitro studies:* Bovine cartilage explants (3mm diam x 1mm thick disks) were subjected to a single radially-unconfined compression to 50% final strain at a velocity of 1 mm/second (100%/second strain rate). Explants were then incubated with TNF-α (10 ng/mL) and IL-6/sIL-6R (20/100 ng/mL) over 8 days. Intermittent dynamic compression was applied from days 2-8 (0.5 Hz haversine waveform, 1 hr on, 5 hr off, 4 times per day), with dynamic compression amplitude varying from 0-30%, n=6 disks per condition. *In vivo studies:* IACUC approval was given by Rush University. Ten week old C57Bl/6 male mice were subjected to a surgical Destabilization-Induced Joint Injury (DMM) model developed by Glasson et al., alone or in combination with TM running (14 days 15° incline, 32 cm/sec, 20 min per day, for 2 weeks starting on day 4 post-surgery). For DMM surgery, the anterior medial meniscotibial ligament was completely severed. Control mice were subjected to only cage activity. At 3 weeks post surgery, mice were euthanized and joints were stained with India Ink for gross observations or protein was extracted for Luminex (23-plex mouse cytokine kit), n=3 per condition. Statistics: Data were analyzed by ANOVA with Tukey post-hoc test, $p<0.05$. Luminex data were normalized to the total protein extracted per joint.
Results: *In vitro studies:* Dynamic compression applied to bovine cartilage initially subjected to injury and cytokine treatment caused additional loss of matrix GAG that increased with dynamic compression amplitude in a dose-dependent manner (ANOVA, $p<0.05$). *In vivo studies:* C57Bl/6 mice developed marked cartilage roughening and lesions 18 days post DMM surgery on the medial aspects of the operated joint, and fibrotic remodeling along the tibial and femoral joint margins was readily detected. The DMM model alone resulted in significantly elevated levels of IL-1β, G-CSF, KC (mouse IL-8-related), and MCP-1 compared to controls. With DMM+TM, the extent of erosion near the patellar groove was much more severe on both the lateral and medial sides and was accompanied by an additional fibrous ingrowth from the margins. Along with the increased joint damage, DMM+TM resulted in significantly increased protein levels of G-CSF, IL-1β and KC (murine related IL-8) over controls; IL-9 and IFN-γ were significantly decreased compared to controls. RANTES was increased over DMM alone.
Conclusions: In this study, we showed that dynamic compression further increases GAG loss from injured cartilage explants in a dose-dependent manner. Additionally, a murine mimic of knee joint injury followed by dynamic loading revealed significantly different levels of specific inflammatory cytokines that have been previously found in human synovial fluid post-injury. These results demonstrate the potential usefulness of these in vitro and in vivo models for studying the mechanisms underlying cartilage degradation following joint injury and for testing the effects of therapeutics to ameliorate this degradative response.

018

HIF2A/NF-κB SIGNAL IN CHONDROCYTES CONTROLS EXTENSIVE STEPS OF OSTEOARTHRITIS DEVELOPMENT IN MICE AND HUMANS

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Purpose: Endochondral ossification is a crucial process not only for skeletal growth but also for osteoarthritis (OA) development. This process starts from chondrocyte hypertrophy characterized by type X collagen (COL10A1) expression, which is followed by cartilage degradation, vascularization, and osteogenesis. This study sought to identify the molecular mechanism that extensively controls these sequential steps in mice and humans.
Methods: We performed screenings of transcription factors using chondrogenic ATDC5, OUMS27 and SW1353 cells transfected with luciferase-reporter constructs containing the target promoters. For functional studies, we used stable lines of ATDC5 cells with retroviral overexpression of the genes, the dominant negative mutants, the siRNA, and the mutants at the oxygen-dependent hydroxylation residues. Promoter analyses were performed by luciferase assay, electrophoretic mobility shift assay, and chromatin immunoprecipitation assay using the chondrogenic cells above. An experimental OA model was created surgically by inducing instability in the knee joints of 8-week-old mice, and OA severity was quantified by the OARSI histopathology grade. Human joint cartilage was obtained as surgical specimens of total knee arthroplasty. Using a population-based cohort of the ROAD study, we searched for common SNPs with minor allele frequency >0.1 in the 1.5 kb 5'UTR of the human gene, and genotyped the SNP by RFLP.
Results: A *COL10A1* promoter assay identified hypoxia-inducible factor 2α (HIF2A) as the most potent transactivator among transcription factors expressed in chondrocytes. The HIF2A expression increased with chondrocyte differentiation in cultured ATDC5 cells and in the mouse limb cartilage,

as well as with OA progression in the knee joint cartilages of the mouse experimental model and human surgical specimens. In all systems, the expression was associated with factors related to endochondral ossification such as COL10A1, MMP3, 9, 13, VEGF, Indian hedgehog, PTH/PTHrP type I receptor, and Runx2. HIF2A enhanced promoter activities of these factors through specific binding to the respective hypoxia-responsive elements. The *Hif2a* heterozygous deficient (*Hif2a*+/-) mice exhibited slight growth retardation and notable resistance to OA development with decreased expressions of all factors above. The loss- and gain-of-function analyses in the cultures of ATDC5 cells and primary *Hif2a*+/- chondrocytes revealed that HIF2A was crucial for endochondral ossification, independently of the oxygen-dependent hydroxylation. Our further study on the upstream signal using the *HIF2A* promoter assay identified RELA, an NF-κB family member, as the most potent transactivator, and determined an NF-κB motif as the core responsive region by mutagenesis analysis. TNF-α and IL-1β, putative ligands for the NF-κB signal, increased the HIF2A expression in chondrogenic cells. In the mouse joint cartilage, the RELA expression was induced alongside the HIF2A expression during OA development. Finally, we have identified one common SNP (rs17039192; +18C/T) in 5'UTR of the human *HIF2A* gene in the ROAD population-based cohort, and the case-control association study using individuals over 50 years of age with (K/L grade $\geq$ 3; n=397) and without (K/L$\leq$1; n=437) knee OA showed a significant association of this SNP with knee OA (*P*=0.013, odds ratio=1.44). The *HIF2A* promoter containing the susceptibility allele (18C) showed higher transactivity than that containing 18T in chondrogenic cells with and without the RELA co-transfection; however, the difference was abrogated by the mutagenesis in the responsive NF-κB motif above, indicating the mediation of HIF2A transactivation by the NF-κB signal in the regulation of OA by the SNP.

Conclusions: The HIF2A/NF-κB signal controls extensive steps of endochondral ossification in OA development of mice and humans, so that this signal may represent a therapeutic target for OA.

019

A POSITIVE ROLE OF DBC1, A SIRT1 REPRESSOR, IN THE ARTHRITIC RESPONSE IN HUMAN CHONDROCYTES

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Purpose: Osteoarthritis (OA) is a multi-factorial disease featuring an imbalance between cartilage anabolism and catabolism. Chronic inflammatory stress contributes to enhanced matrix degradation and chondrocyte apoptosis in OA cartilage. We have shown that SirT1, a protein deacetylase known to prolong lifespan, is able to enhance both chondrocyte viability and cartilage specific gene expression. Here we evaluate the ability of DBC1 (Deleted in Breast Cancer 1), a repressor of SirT1, to regulate expression of the cartilage-specific genes collagen type 2 and aggrecan.

Method: Overexpression of DBC1 and Sirt1 in human osteoarthritic chondrocytes were performed by Amaxa electroporation. Antibodies against Sirt1 (Millipore), aggrecan, collagen 2, (Santa Cruz) and DBC1 (Abcam) were used in immunoblotting, immunohistochemistry and immunoprecipitation. PCR utilized aggrecan, collagen type 2, GAPDH primers and Taqman PCR mix (Applied Biosystem)

Results: Overexpression of SirT1 in human chondrocytes led to enhanced expression of aggrecan and collagen type 2. Further, SirT1 repress expression of many matrix metalloproteinases (MMPs). In contrast, tissue sections from OA patients revealed reduced protein levels of SirT1 and aggrecan and collagen type 2, with elevated levels of MMPs. We find that DBC1 is upregulated in OA cartilage compared to Normal cartilage. Moreover, when expressed in human chondrocytes, DBC1 represses these aggrecan and collagen type 2. Further, DBC1 is upregulated by TNFα in chondrocytes where cartilage genes are repressed and MMPs are elevated.

Conclusion: Our results indicate that the longevity protein SirT1 is a positive regulator of cartilage matrix in chondrocytes. Further, DBC1 displays characteristics of a pro-arthritic protein, due to its ability to block SirT1 enzymatic activity. That DBC1 is upregulated in OA and is induced by TNFα. Thus SirT1 has features of an anti-osteoarthritic enzyme, consistent with its ability to reduce the severity of age-associated diseases.

020

THE EXPRESSION OF THE ANTI-APOPTOTIC TRANSCRIPTION FACTOR NF-kappaB-P65 IS MARKEDLY DIMINISHED IN CHONDROCYTES OF MURINE OSTEOARTHRITIC CARTILAGE AND IN A SUBSET OF HUMAN OSTEOARTHRITIC CARTILAGE SAMPLES

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Purpose: Chondrocytes play a central role in cartilage pathology as seen in rheumatoid arthritis (RA) and osteoarthritis (OA) patients by a deranged synthesis of extracellular matrix (ECM) components and the enhanced release of ECM destructive metalloproteinases (MMPs). Nuclear factor-kappaB (NF-kappaB) is an important transcription factor in the regulation of MMPs, but is also regarded as a survival factor in cells. We studied the regulation of NF-kappaB-P65 in chondrocytes in rheumatoid arthritis, osteoarthritis, mouse models of arthritis and osteoarthritis and the functional consequences of decreased level of NFkappaB-P65.

Methods: We measured the level of NF-kappaB-P65 in freshly isolated chondrocytes of arthritic cartilage obtained from joint replacement surgery, cartilage of a spontaneous osteoarthritis mouse model (STR/ORT), cartilage of a streptococcal cell wall- and antigen-induced arthritis and cartilage from young (14 weeks) and old (>12 months) mice by Western blotting, immunohistochemistry or RT-qPCR. To study the functional consequences of decreased level of NF-kappaB-P65 in chondrocytes the murine H4 chondrocyte-cell line was stably transduced with a lentivirus expressing a short-hairpin RNA against NF-kappaB-P65 to reduce the NF-kappaB-P65 protein levels by a RNA interference approach. We selected several celllines that expressed different amounts of NF-kappaB-P65 protein. To study the biological consequences, conditioned medium of OA synovium was added to the murine chondrocyte cell line with the lowest NF-kappaB-P65 level.

Results: In all chondrocytes of RA patients high NF-kappaB-P65 levels were detected, by immunohistochemistry and Western blot, whereas in chondrocytes of a subset of OA cartilage samples levels were unexpected low (6 out of 12). In mouse models the level of NF-kappaB-P65 showed the same regulation. NF-kappaB-P65 levels in cartilage from murine arthritis models was increased up to 250% at day 2 after induction of streptococcal cell wall- or antigen-induced arthritis and at day 7 returned to the basal level of naive knee joints, whereas in STR/ORT mice levels were diminished more than 75% when joints became affected, as determined by immunohistochemistry. Levels of NF-kappaB-P65 in young and old mice were equal, but the older groups showed more variation, detected by immunohistochemistry. *In vitro*, we selected chondrocyte cell-lines with different levels of NF-kappaB-P65. By adding TNFalpha, cell death was only induced in the cells with low levels of NF-kappaB-P65, as detected by 7-AAD staining. A clear negative correlation between TNFalpha induced cell death and the levels of NF-kappaB-P65 in chondrocyte cell-lines was found. Adding conditioned medium of synovial explants from different OA patients to the murine chondrocyte cell-line with the lowest NF-kappaB-P65 level, resulted in more than 60% chondrocyte death in 3 of the 5 conditioned medium samples tested which could be prevented by preincubation of these media with soluble-TNFR1 (Enbrel). TNFalpha was detected using a Luminex assay in the same samples that caused cell death.

Conclusions: This study clearly demonstrated that lower levels of NF-kappaB-P65 makes chondrocytes more vulnerable for TNFalpha, a cytokine which can be produced during OA, and that this anti-apoptotic transcription factor is downregulated in chondrocytes in murine OA and in 50% of OA patients.

021

RESPONSIVENESS AND RELIABILITY OF MRI IN OSTEOARTHRITIS: ANALYTIC LITERATURE REVIEW

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Purpose: To summarize literature on the responsiveness and reliability of MRI-based measures of osteoarthritis (OA) structural change.

Methods: An online literature search was conducted of the OVID, EMBASE, CINAHL, PsychInfo and Cochrane databases of articles published up to the