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