

by reporter assay, nuclear accumulation of β -catenin (Western blotting), or expression of Axin-2 mRNA. Gene expression analysis of chondrocyte markers including Col2A1, Col1A1, Col10A1, Aggrecan, Sox9, MMP3, MMP13, ADAMTS5 was performed by quantitative real time PCR.

Results: Wnt3a induced the activation of both the canonical and the CaMKII-dependent pathways. More importantly, using a systematic strategy of blockade of the different pathways, we have separated the effects that are directly dependent on the activation of the canonical pathway, including proliferation, from those that are non-canonical such as disruption of the chondrocyte phenotype, as evaluated by downregulation of differentiation markers, and cell survival. Most importantly we have built up a model in which, through reciprocal inhibition of these two pathways, either excessive Wnt3a stimulation or blockade equally leads to cartilage breakdown.

Conclusions: – Wnt3a can activate either the canonical or the CaMKII-dependent Wnt signaling pathway in adult human articular chondrocytes
– The β -catenin and the CaMKII-dependent Wnt pathways are reciprocally inhibitory
– The Wnt3a-dependent canonical and non canonical pathway activation has distinct outcomes

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ALARMIN S100A8 AND S100A9 SKEW CHONDROCYTES TOWARDS A CARTILAGE BREAKDOWN PHENOTYPE IN HUMAN OSTEOARTHRITIS

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Purpose: Alarmins S100A8 and S100A9 are members of the S100 family of Ca²⁺-binding proteins that are associated with inflammation and bone erosion during human rheumatoid arthritis (RA). Recently, we found that S100A8 and S100A9 are also associated with cartilage degradation in murine collagenase-induced osteoarthritis (OA). We also showed that S100A8 and S100A9 stimulate expression and activity of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines in murine chondrocytes. We now investigated whether S100A8, S100A9 and/or S100A8/S100A9 complex could also activate human chondrocytes from OA patients and skew them towards a cartilage breakdown phenotype.

Methods: Cartilage was collected from human OA patients undergoing joint replacement. Immunostaining was performed on paraffin sections of OA cartilage using antibodies against S100A8 and S100A9 and against VDIPEN and NITEGE, which are MMP- and A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-induced cartilage breakdown neopeptides. Chondrocytes were isolated from OA cartilage and stimulated with recombinant S100A8, S100A9, S100A8/A9 heterodimer and/or interleukin 1 beta (IL-1beta). mRNA levels of MMPs, cytokines and cartilage matrix molecules were determined with RT-qPCR and protein levels of MMP and cytokine using Luminex.

Results: Immunostaining on OA cartilage showed that both S100A8 and S100A9 protein were expressed in and around chondrocytes. Furthermore, immunostaining of breakdown neopeptides VDIPEN and NITEGE was found in the same areas as the S100 proteins.

Stimulation of OA chondrocytes with the monomers S100A8 and S100A9 for 24 hours strongly stimulated cartilage degrading molecules like MMPs and cytokines. mRNA expression of MMP1, -3, -9 and -13 was upregulated 4-, 4-, 3- and 3-fold respectively. Protein levels of MMP1, -3 and -13 were upregulated 234, 27 and 136% respectively. Moreover, particularly interleukin 6 (IL-6) levels were strongly upregulated on mRNA level (11-fold) and protein levels reached 5 ng/ml. The catabolic effect of S100A8 and S100A9 was almost comparable to IL-1beta effects, but there was no additive effect of S100 with IL-1beta, suggesting independent mechanisms. Apart from stimulating cartilage degradation, S100A8 and S100A9 monomers also inhibited new formation of cartilage matrix molecules. mRNA levels of aggrecan and collagen type II were significantly decreased 2- to 3-fold, suggesting that these proteins inhibit repair.

In contrast to the monomers, the heterodimer S100A8/A9 neither had effect on cartilage degradation nor on cartilage matrix production.

Conclusions: S100A8 and S100A9 expression is found in areas with cartilage breakdown in OA cartilage. Moreover these proteins inhibit new formation of matrix molecules and stimulate production of cartilage degrading molecules thereby skewing chondrocytes towards a cartilage breakdown phenotype.

S100A8 and/or S100A9 may serve as therapeutic targets for the treatment of cartilage damage during OA.

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EFFECTS OF TGF-BETA ON OA CHONDROCYTE MITOCHONDRIA

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Purpose: TGF- beta is a powerful anabolic factor for chondrocytes and plays an important role by enhancing cartilage repair. Oxidative stress and mitochondrial damage have been found to promote cell death, functional failure and degeneration. There is a growing body of evidence that mitochondrial dysfunction is present during the progression of osteoarthritis. The purpose of the present study was to evaluate whether TGF-beta enhances cartilage repair through the prevention of mitochondrial DNA damage and dysfunction in human OA chondrocytes subjected to experimentally-induced oxidative stress.

Methods: Primary OA chondrocytes cultures, generated from cartilage from patients undergoing total knee replacement, were exposed for 30 min to reactive oxygen species (ROS) generators xanthine oxidase/hypoxanthine or peroxyxynitrite. Some cells were pre-incubated with 5 ng/ml of TGF-beta for 24 h prior to treatment. In some experiments cells were incubated for 48 h with IL-1 beta or TNF-alpha alone or in combination with TGF-beta. Following exposure, cells were immediately lysed for DNA isolation, protein isolation, ATP analysis, or replenished with normal growth media and left for recovery and then analyzed. Mitochondrial dysfunction was assessed in terms of mitochondrial DNA damage (Quantitative Southern blot analysis), ATP content (Bioluminescence kit), mitochondrial proteins levels (Western blot). Cell viability and apoptosis were evaluated by flow cytometry. Additionally, Western blot was applied to perform caspase 9 cleavage assay.

Results: When OA chondrocytes were exposed to ROS generators, mitochondrial dysfunction and apoptosis were accumulated as a consequence. Pretreatment with TGF- beta preserved mitochondrial DNA integrity, ATP levels, and restored drop in mitochondrial protein levels. Moreover, TGF-beta enhanced chondrocytes viability and diminished the appearance of apoptosis. The similar results were obtained for IL-1 beta and TNF-alpha. Interestingly, TGF-beta did not have effect on nitric oxide levels enhanced following pro-inflammatory cytokine exposure of chondrocytes.

Conclusions: The present results demonstrate for the first time the mitoprotective properties of TGF-beta and offer additional explanation of the mechanisms through which TGF-beta enhances cartilage repair. More studies required evaluating more precisely the observed TGF- beta effects.

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P38 AND MEK/ERK MAP KINASES REGULATE COMMON AND UNIQUE GENE SETS IN CHONDROCYTES

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Purpose: The cartilage growth plate regulates growth of endochondral bones, and ectopic initiation of growth plate events (e.g. chondrocyte hypertrophy) in articular chondrocytes contributes to the development of osteoarthritis. MAPK signaling pathways have been implicated in different aspects of growth plate development. In this study we focus on two MAPK pathways: p38 and MEK/ERK. We analyzed the effects of these two pathways on mouse growth plate chondrocyte gene expression and bone growth in culture. Some of the specific roles of the p38 and MEK/ERK pathways have been already investigated, but no study so far reported on the effects of their simultaneous inhibition. We hypothesized that these two signaling pathways have antagonizing roles in different aspects of the growth plate development and the simultaneous inhibition will result in a reduction of their specific effects.

Methods: RNA was isolated from mouse growth plate chondrocytes after 24 hours of culture in the presence of UO126 (MEK/ERK inhibitor), SB202190 (p38 inhibitor) or DMSO (control) and hybridized to Affymetrix MOE 430 2.0 gene chips (n = 3). The data were normalized and analyzed using GeneSpring GX 7.3.1 and Ingenuity Pathway Analysis (IPA). The expression of selected genes was confirmed by real-time PCR. In addition, mouse embryonic tibiae were cultured for 6 days in the presence of DMSO, SB202190 (SB), UO126 (U) or SB202190+ UO126 (SU). Bone growth was