then treated with mixed secondary antibodies, Alexa Fluor488 donkey anti-rabbit IgG and Alexa Fluor594 donkey anti-goat IgG for 30 min.
Results: There were no significant differences in the level of HTRA1 mRNA between control and experimental chondrocytes treated with 42oC, TNFα, or the high hydrostatic pressure. In contrast, the level of HTRA1 mRNA increased by ~5-fold in human chondrocytes treated with TGF-β1. The level of HtrA1 mRNA was also increased in mouse chondrocytes treated with TGF-β1. Expression of p-Smad1 was hardly detected in Col11a1+/- and DMM mice and corresponding control mice. However, the expression of Tgf-β1 and p-Smad2 was increased. The increased expression of p-Smad2 and HtrA1 was co-localized in Col11a1+/- and DMM mice. Interestingly, expressions of p-Smad2 and HtrA1 were hardly detected in Col11a1+/- mice treated with the neutralizing TGF-β1 antibody.
Conclusions: These results indicate that TGF-β1 may induce HTRA1 in chondrocytes. Therefore, current OA therapy using TGF-β1 may be contraindicated as it may do more harm than good in the protection of articular cartilage against OA in matured joints.

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IKKα MODULATES OXIDATIVE STRESS-INDUCED DNA DAMAGE AND REPAIR IN PRIMARY HUMAN OA CHONDROCYTES

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Purpose: Functional derangement of osteoarthritic chondrocytes make them more susceptible to stressful conditions such as oxidative stimuli within aging tissue, which can further provoke extrinsic senescence by DNA damage responses (DDR). Our previous observation that IKKα knockdown increased the replicative potential of primary human OA chondrocyte monolayer cultures and the survival of the same cells undergoing hypertrophic-like differentiation, prompted us to investigate whether IKKα knockdown could modulate the stress-induced senescence of OA chondrocytes as assessed by their DDR.
Methods: We assessed the effects of IKKα loss on ROS-induced DDR by exposing control and IKKα KD chondrocytes (derived from 10 patients) in low density in monolayer cultures to 1 hr of 100 μM hydrogen peroxide and then scored them for their relative degrees of microsatellite instability (MSI) and double strand breaks (DSB).
Mismatch repair (MMR), the main post-replicative correction pathway has a fundamental role in maintaining genomic stability and its efficiency can be monitored by assessing MSI. Part of the cells, left untreated or exposed to 100 μM H_2O_2, were collected after 6 hour recovery to evaluate MMR enzymes (i.e., MLH1, MSH2, MSH6, MSH3, PMS1 and PMS2) mRNA expression by quantitative RT-PCR using GAPDH as a reference control. Other cells were further cultured for 72 hours and analyzed for MSI at five different genomic DNA loci (CD4,VWA, FES, TPOX, and P53).
DDR was evaluated in cells immediately after H_2O_2 exposure by flow cytometric analysis for γH2AX foci in chromatin. γH2AX foci serve as a marker of double strand breaks representing sites of H2AX phosphorylation, which can subsequently lead to the recruitment of DNA repair factors.
Results: Real time PCR data show increased expression of MSH3 and PMS1 mRNAs in IKKα KD chondrocytes after H_2O_2 exposure. MSI was detected in control cells but not in their IKKα KD counterparts. Under basal conditions, preliminary flow cytometry results indicate the comparable presence of γH2AX DNA foci in IKKα KD and controls in 6 out of 7 patient chondrocytes. However, after H_2O_2 exposure, preliminary results indicate a higher percentage of γH2AX positive cells and more foci per cell in the absence of IKKα.
Conclusions: Thus far our preliminary data indicate that IKKα controls the intensity of primary human OA chondrocyte DDR induced by oxidative stress. Thus IKKα loss appears to increase cell susceptibility to ROS-mediated DDR as indicated by their augmented DSB induction, perhaps due in part to the faster doubling time of IKKα deficient cells. However, MSI and MMR gene expression data suggest that IKKα deficient primary OA chondrocytes also possess a higher capacity to repair DNA damage. More experiments are underway to confirm and extend our results with cells at comparable passage numbers to determine if H_2O_2-mediated stress induces these effects or exacerbates a pre-existing physiological state reflecting the faster division rate of IKKα compromised cells.

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AN INVESTIGATION OF THE EFFECT OF EXOGENOUS GROWTH FACTOR GDF5 ON PRIMARY OA CHONDROCYTES - IS THERE A PREDICTABLE RESPONSE?

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Purpose: A genetic deficit mediated by SNP rs143383 and involving reduced expression of the growth differentiation factor 5 gene GDF5 is strongly associated with large-joint OA. We speculated that this deficit could be attenuated by the application of exogenous GDF5 protein and as a first step we have assessed what effect such application has on primary OA chondrocyte gene expression.
Methods: Chondrocytes were isolated from the cartilage of OA patients who had undergone elective surgery of the hip or knee and cultured in the presence of 100ng/ml of wild-type recombinant human GDF5 protein for 6, 12, 24 and 48 hours. We also studied variants of GDF5 that have a higher affinity for the BMP Ia receptor, which is highly expressed by chondrocytes. As a positive control, chondrocytes were treated with TGFβ, which is known to elicit a predictable anti-catabolic response. The expression of genes coding for catabolic, anti-catabolic, and structural proteins of cartilage were measured by quantitative PCR (qPCR).
Results: The expression of the relevant GDF5 receptor genes BMPRII, BMPRIA and BMPRIB was confirmed by qPCR. The capacity of GDF5 to initiate cell signaling in chondrocytes was demonstrated by the phosphorylation of intracellular SMADs, and the ability of the signal to then translocate to the nucleus was demonstrated by the activation of a luciferase reporter construct harbouring SMAD response elements. Chondrocytes cultured with TGFβ demonstrated a consistent down-regulation of the catabolic metalloproteinase genes MMP1 and MMP13, of the cartilage differentiation transcription factor gene SOX9, and of the aggrecan gene ACAN. They also showed a consistent up-regulation of TIMP1, which codes for an inhibitor of MMPs, and of the cartilage type II collagen gene COL2A1. In contrast, chondrocytes cultured with wild-type GDF5, or its variants, did not show any consistent response, with variation observed relative to the length of time of culture, and with an inconsistent response between individuals irrespective of the donor's sex or of the original site of the chondrocytes (hip or knee).
Conclusions: OA chondrocytes do not respond in a predictable manner to culture with exogenous GDF5. This may be a cause or a consequence of the OA disease process and will need to be surmounted if treatment with exogenous GDF5 is going to be advanced as a potential means of alleviating the genetic deficit mediated by OA susceptibility at the gene GDF5.

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THE ROLE OF NOREPINEPHRINE IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Norepinephrine belongs to the catecholamine family of tyrosine-derived neurotransmitters of the sympathetic nervous system. Tyrosine-hydroxylase positive sympathetic nerve fibers have been identified in bone marrow, in the periosteum and in bone-adherent ligaments indicating that growth and metabolic activity of bone and joint tissues is regulated by sympathetic neurotransmitter. It is known that norepinephrine can regulate cell proliferation or apoptosis in several cell types, such as osteoblasts. It is further described that norepinephrine modulates inflammation during rheumatoid arthritis and gut inflammation. Here, we aim to