Aggrecanase activity was assessed by measuring in the glycosaminoglycan (GAG) release and ARGSVIL neoeptide formation. Explants were further examined by histology after safranin O proteoglycans (PG) staining/light green counterstaining, and by immunohistochemistry for aggrecan NITEGJE neoeptide. PG and neoeptide content was then quantified applying a scoring system to individual images.

In addition, cytokine-induced matrix degradation was assessed in a new rat chondrosarcoma cell model (‘RCS assay’) in which the amount of sulphated proteoglycan was quantified using Alcian blue staining and image processing.

Results: TNFα+OSM or IL1β+OSM strongly induced ADAMTS4 and ADAMTS5 in human cartilage explants. OSM is strictly needed for induction of aggrecanases but not for MMP13. A specific ADAMTS5 inhibitor (cpd A, IC50 80% inhibition of GAG release, >80% inhibition of neoeptide release at 3μM). In contrast, a selective ADAMTS4 inhibitor (cpd B, IC50 <100 nM) did not inhibit cartilage degradation by more than 50%. Cpd C, a non-selective 3-OH-3-methylpypecolic hydroxamate type metalloproteinase inhibitor completely inhibited GAG and Neoeptide release.

Histological evaluation confirmed that cpd A prevented PG loss and inhibited neoeptide formation in cartilage matrix but not in the pericellular matrix (PM), while cpd B only inhibited neoepti- tope formation in PM. Cpd C prevented PG loss and inhibited neoeptide formation in both cartilage matrix and PM.

In addition, the compound ranking obtained in the explant model was reproduced with the simple ‘RCS assay’.

Conclusions: This study represents the first pharmacological evidence for the importance of ADAMTS5 over ADAMTS4 in human cartilage degradation. Strong induction of ADAMTS4 by cytokines and significant prevention of cartilage degradation by its pharmacological inhibition using a selective inhibitor are demonstrated. This observation underscores the important role of ADAMTS5 in the degradation of human cartilage. Furthermore, these ADAMTS5 effects were recapitulated in a new RCS-based assay, which provides a good substitute for the complex cartilage explant model.

Finally, histological analysis delivered the unexpected observation that matrix aggrecan is subject to different proteolytic events depending on its localization, providing mechanistic information on the cartilage degradation process.

THE MELANOCORTIN SYSTEM IN HUMAN CHONDROCYTES

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Purpose: Melanocortins (MCs) are structurally related peptides that elicit their actions via binding to melanocortin receptors (MCRs). Originally defined as neuropeptides these hormones exhibit an astonishing spectrum of effects on many peripheral cell types and tissues. However, our present knowledge on the function of the MC system in cartilage is scarce. We could show that MC-1R is expressed in human articular cartilage and chondrocytes. α-Melanocyte-stimulating hormone (α-MSH), a prototype of MCs, is capable of regulating a number of important genes involved in extracellular matrix (ECM) composition and inflammation in chondrocytes. The major goal of this study is to define the role of MCs and the MC-1R in the context of osteoarthritis (OA).

Methods: Cartilage slices were aseptically dissected from femoral condyles of OA patients who underwent total knee arthroplasty. Articular chondrocytes isolated from OA-cartilage were proliferated in monolayer until passage one and either cultured in 3D-micromass pellets for 7 days or kept in monolayer. After stimulation for 48h with 10-6 M α-MSH only (micromass pellet culture) or in presence of 1 ng/ml IL-1α (monolayer culture); the cDNA copy number of Sox9, collagen, MMPs; TGF-β1, TNF-α and interleukins was assessed with quantitative RT-PCR. POMC, converting enzymes and MC-receptor gene expression was determined by end point RT-PCR and, MC-R1 protein expression in situ on cryosections of OA-cartilage by immunohistochemistry. Signal transduction of α-MSH was evaluated by cAMP ELISA and Ca2+-assays using Fura2-AM. Protein concentration of MMP-2, MMP-7, MMP-9 and MMP-13 was induced, however, MMP-13 protein concentrations in culture supernatant revealed profound interpatient fluctuations. MMP-2 protein concentration was consistent within the sample collective and remained unaltered in the presence of α-MSH. IL-1α and TNF-α gene expression was reduced, IL-6 remained mostly unchanged and Sox9 and TGF-β1 were induced upon stimulation with α-MSH.

IL-6 and TNF-α protein concentrations were upregulated. α-MSH attenuates IL-1β-induced expression of IL-4 and IL-6, MMP-2, -9 and -13 in articular OA-chondrocytes.

Conclusions: Human articular chondrocytes are target cells for α-MSH. The effects of α-MSH on cytokine- and MMP expression suggest a chondroprotective role of this neuropeptide in inflammatory and degenerative processes in cartilage. It is conceivable that inflammatory reactions can be mitigated by induction of endogenous melanocortins or administration of α-MSH to the affected joints. The induction pattern of regulatory and structural ECM components as collagens, Sox9, anabolic and catabolic cytokines points towards a function of α-MSH as a trophic factor in skeletal development during endochondral ossification or as anti-inflammatory agent in degenerative joint pathologies.

THE FUNCTION OF p53 ON HUMAN CHONDROCYTE APOPTOSIS IN RESPONSE TO SHEAR STRESS

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Purpose: Chondrocyte apoptosis plays an important role in cartilage degeneration in osteoarthritis (OA), and mechanical injury to cartilage induces chondrocyte apoptosis. p53 has been identified as a 53 kDa cellular protein and ‘ultimate tumor suppressor gene’. p53 is recognized as a pivotal regulatory protein that responds to a variety of signals and recruits an array of biochemical activities to trigger diverse biological responses, most notably cell cycle arrest and apoptosis. In response to DNA damage, p53 expression is upregulated, increases transcriptional activity, and initiates apoptosis signals. p53-regulated apoptosis-inducing protein 1 (p53AIP1) is one of the p53-regulated genes, and is activated in response to severe DNA damage with the phosphorylation of p53 at Ser-46. In this study, we speculated that p53 and p53AIP1 might play an important role in DNA damage-induced apoptosis of articular carti-
lager, and analyzed the function of p53 and p53AIP1 in chondrocyte apoptosis by shear stress.

Methods: Shear stress was introduced to NHAC-kn cells (human normal chondrocytes) with Flexcell system. DNA damage was detected and quantified by comet assay, DNA fragmentation, and phosphorylation of ataxia-telangiectasia mutated kinase (ATM). To explore the p53 function, NHAC-kn cells were pretreated with pifithrin-α, which is specific inhibitor of p53, or p53 siRNAs before induction of shear stress. Chondrocyte apoptosis was detected by expression of cleaved caspase-9 with Western blotting and TUNEL staining. Expressions of p53 and p53AIP1, and phosphorylation of p53 at Ser-15 and -46 were analyzed by Western blotting.

Results: DNA damage was actually induced by shear stress, with the results of comet assay, DNA fragmentation, and phosphorylation of ATM. After loading shear stress, TUNEL positive cells and expression levels of p53, p53AIP1 and cleaved caspase-9 were increased in a force and time dependent manner. Phosphorylation of p53 at Ser-15 and Ser-46 were analyzed by Western blotting. Moreover, p53AIP1 act as an important molecule for chondrocyte apoptosis via p53, mainly phosphorylation at Ser-46, and the mitochondrial pathway. In conclusion, we demonstrated that p53 and p53AIP1 act as an important molecule for chondrocyte apoptosis induced by elongation shear stress, and down regulation of exogenous or endogenous p53 expression inhibited chondrocyte apoptosis.

Conclusions: We demonstrated that expression of p53 and p53AIP1 increased with the activation of apoptotic processes in human normal chondrocytes following shear stress. However, pifithrin-α reduced chondrocytes apoptosis after loading shear stress. Furthermore, down regulation of endogenous p53 by transfection of p53 siRNA inhibited chondrocytes apoptosis after loading shear stress. Our results suggest that shear stress induces apoptosis via p53, mainly phosphorylation at Ser-46, and the mitochondrial pathway. In conclusion, we demonstrated that p53 and p53AIP1 act as a focal point for functional cross-talk between early (Sox-9) and late (β-Catenin) positive effectors of chondrocyte differentiation, thereby pushing chondrocytes towards a more hypertrophic-like state.

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CROSSTALK BETWEEN RESIDENT CELLS IN THE JOINT: IMPACT ON CHONDROCYTE METABOLISM

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Purpose: Osteoarthritis (OA) is a multi-factorial disorder that results in a net loss of cartilage from synovial joints, often leading to pain. Cartilage lesions are a product of blunted extracellular matrix (ECM) synthesis by chondrocytes, and enhanced enzyme-mediated ECM degradation. Although often characterized by the single pathological feature of cartilage degeneration, OA is increasingly recognized as a disease of the whole joint in which interplay between the different cells within the joint are important. Here we explore the impact of various joint resident cells on ECM metabolism of chondrocytes in an in vitro co-culture system.

Methods: Primary human chondrocytes were isolated by enzymatic digestion of adult articular cartilage and encapsulated in 3D alginate beads. Primary human osteoblasts, purchased from ProcSet, were isolated by enzymatic digestion of bone from patients undergoing knee endoprosthesis due to arthrosis of the knee joint. Human macrophages were differentiated from CD14+ peripheral blood monocytes (AllCells) by treatment with macrophage colony stimulating factor.