of the UGDH gene and may offer new approaches to investigate its potential alteration in joint diseases, including osteoarthritis and rheumatoid arthritis.

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ARTICULAR CARTILAGE CATABOLISM IN THE PATHOGENESIS OF KASHIN-BECK DISEASE

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Purpose: Kashin-Beck Disease (KBD) is an endemic, chronic, degenerative osteoarthropathy which affects 2.5 million of 30 million people living in the KBD region of China. The symptoms include joint pain, stiffness of the joints after getting up in the morning, disturbances of flexion and extension in the elbows, enlarged phalangeal joints and limited motion in the middle-sized and large joints of the body. It manifests as cartilage degeneration and necrosis, with pathological changes occurring in growth plate and articular cartilage. At present the etiology and pathogenesis of KBD is unclear. One of the most popular hypotheses is that KBD is caused by mycotoxins. The other etiologies include selenium deficiency in soil and water in KBD area, nutrition deficiency and virus infection etc. However, all of the hypotheses lack adequate experimental evidence to support any particular conclusion. This study investigated the extracellular matrix metabolism in cartilage and serum levels of IL-1 beta, TNF-alpha, MMP-1 and CD44 in serum of KBD patients.
Methods: Immunohistochemical analyses of normal and KBD patient cartilage (16 adults and 4 children and normal age-matched patient tissue 4 adults and 3 children) was performed using a monoclonal antibody recognising CD44, the IGD aggrecase-generated neoepitope (monoclonal antibody BC-13 recognising ... EGE373) and monoclonal antibody 3-B-3(-) which recognizes thee nonreducing terminal of CS chains in osteoarthritic cartilage. In addition, the serum levels of soluble CD44 (sCD44), IL-1 beta, TNF-alpha and MMP-1 were determined in KBD patients (20 adults and 18 children) and normal patients (20 adults and 18 children) using a competitive ELISA.
Results: Toluidine Blue and Saffron O staining indicated there was a proteoglycan loss in both of KBD children and adult cartilage. Strong immunohistochemical staining for CD44 occurred in 14 of 16 adult KBD patients with moderate staining present in the remaining two patients. Similarly, strong CD44 immunostaining occurred in 3 of the 4 KBD children with moderate staining in the fourth KBD child. In contrast, weak CD44 immunostaining was only found in two of the four normal adult cartilage and none of the three normal child samples. Immunohistochemical staining with MAb BC-13 and 3-B-3(-) showed an intensive staining in both of KBD children and adult cartilage when compared with the normal cartilage samples. Furthermore, statistically significant elevated levels of sCD44, IL-1 beta and TNF-alpha were found in the sera of both adult and child KBD patients when compared to serum levels of normal adult and child controls. However, there was no difference in MMP-1 level between KBD and normal children.
Conclusions: Our results demonstrate that altered IL-1 beta, TNF-alpha, aggrecan and CD44 metabolism occurs in the pathogenesis of KBD and there is an increased aggrecanase-generated proteoglycan loss from KBD adult and children cartilage. These primary metabolic changes are a contributing factor causing pathology in joint formation and instability which in turn leads to the onset of secondary osteoarthropathy in the major load-bearing joints of KBD patients.

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PROTECTIVE ABILITIES OF INTERLEUKIN-10 IN BLOOD-INDUCED CARTILAGE DAMAGE

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Purpose: Joint bleeds occur frequently in patients suffering from haemophilia or can occur as a consequence of a trauma. It has been demonstrated that even a limited number of bleeds in time leads to severe joint damage. With respect to the mechanisms of blood induced joint damage, we have demonstrated that monocytes/macrophages within the mononuclear cell population together with the red blood cells as present in blood are responsible for the irreversible inhibition of cartilage matrix synthesis. Small amounts of IL-1 that are produced by activated monocytes/macrophages increase the production of hydrogen peroxide (H_2O_2) by chondrocytes. The hydrogen peroxide reacts with haemoglobin-derived iron from damaged and phagocytosed red blood cells, which results in the formation of hydroxyl radicals in the vicinity of chondrocytes. This leads to chondrocyte apoptosis and with that the irreversible inhibition of cartilage matrix synthesis.
In search of possible interventions to prevent or limit the cartilage damaging effects of joint bleeds, we tested interleukin-10 (IL-10) as an inflammation-controlling cytokine on blood-induced cartilage damage.
Methods: Healthy human articular cartilage tissue explants were cultured in the presence or absence of 50% v/v blood for 4 days, followed by a recovery period of 12 days. IL-10 was added in 0.1, 1 or 10 ng/ml. The effect on cartilage matrix proteoglycan synthesis, -release, and -content were determined.
Results: IL-10 was able to prevent the decrease in proteoglycan synthesis and the increase in proteoglycan release of cartilage exposed to blood dose dependently. As a consequence, the decrease in proteoglycan content after blood exposure could be prevented dose dependently. With 10 ng/ml IL-10, which is still a low dose for local therapeutic treatment, at least 50% reduction in adverse effects was observed.
Conclusions: The present results show that interleukin-10 prevents the direct harmful effects of blood on articular cartilage. This effect of IL-10, in addition to its anti-inflammatory properties, may add to prevention of irreversible degenerative joint damage as a consequence in time of joint haemorrhages. Although treatment studies in addition to prevention studies have to be performed, IL-10 might be of use for local treatment of joint.

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VENOUS ENDOTHELIAL GROWTH FACTOR PRODUCTION BY CHONDROCYTES CULTURED IN MONOLAYER AND IN MICROMASS

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Purpose: To verify the involvement of proinflammatory cytokines IL-17, IL-1β and TNF-α in cartilage vascularization on the production of vascular endothelial growth factor (VEGF) by chondrocytes isolated from patients with osteoarthritis (OA), rheumatoid arthritis (RA) and patients with femoral neck fracture (FP), and to evaluate the production of VEGF by chondrocytes freshly isolated from patients with OA, in comparison with chondrocytes redifferentiating in micromass.
Methods: Chondrocytes isolated from patients with OA were maintained in monolayer culture for several passages. Chondro-

cyte dedifferentiation was monitored by the synthesis of cathepsin B by these cells. Chondrocyte redifferentiation was studied by micromass three-dimensional (3D) culture of freshly isolated cells. Chondrocytes freshly isolated, at each monolayer subculture (subculture 1 to 3) and in 3D cultures were stimulated with IL-17, IL-1β or TNF-α. Supernatants were collected, immunoassayed for the production of VEGF and cathepsin B and assayed as the source of VEGF on the VEGF sensible ECV304 cell line. The cells were used to quantify intracellular cathepsin B enzymatic activity.

Results: Freshly isolated chondrocytes maintained in 3D culture secreted amount of VEGF lower than in monolayer culture and comparable with the amount of VEGF secreted by cells in subculture 1. In 3D cultured chondrocytes the cathepsin B synthesis shown a differentiation degree such as that of freshly isolated chondrocytes. In differentiated conditions of freshly isolated chondrocytes in monolayer, IL-1β and TNF-α, but not IL-17, can inhibit the spontaneous secretion of VEGF by human OA, RA and FP chondrocytes, and IL-17 can restore the decrease in VEGF secretion caused by TNF-α. IL-17, together with IL-1β and TNF-α, can enhance VEGF secretion to various extents by dedifferentiated OA chondrocytes. This change in effect with respect to primary culture was observable for all cytokines at the beginning of dedifferentiation, when the production of VEGF by chondrocytes had dramatically fallen and the cathepsin B synthesis had increased. The amount of VEGF induced by cytokines on dedifferentiated chondrocytes never reached the amount of VEGF produced by differentiated chondrocytes. In contrast with freshly isolated chondrocytes cultured in monolayer, TNF-α was able to enhance VEGF secretion by chondrocytes in 3D culture. VEGF produced by chondrocytes stimulated the ECV304 cell line proliferation.

Conclusions: These results indicate that freshly isolated chondrocytes maintained in 3D culture secrete VEGF but show some characteristics of chondrocytes from monolayer subculture. Dedifferentiated OA chondrocytes secrete VEGF after stimulation with proinflammatory cytokines and this event may be responsible for neovascularization found in OA cartilage.

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FUNCTIONAL ANALYSES OF MMP-28 AND ADAMTS-16

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Purpose: MMP-28 (epilysin) and ADAMTS-16 are recently discovered members of the matrix metalloproteinase and 'a disintegrin and metalloproteinase domain with thrombospondin motifs' families respectively. We have previously reported expression of *MMP28* and *ADAMTS16* in normal human articular cartilage and significant induction of expression in osteoarthritis. The physiological function and substrates of MMP-28 or ADAMTS-16 are currently unknown.

Methods: Expression constructs for wild-type proteinases and inactive mutants were cloned into pcDNA4-FLAG. Protein expression after transfection into a variety of cell types was assessed by western blotting. Stable cell lines were generated using SW1353 chondrosarcoma cells and C28/I2 immortalized chondrocytes were generated by transfection with linearised vector and zeocin selection; clonal lines were then generated. Processing events were probed using both furin and MMP inhibitors. Functional analyses included cell proliferation, adhesion and migration.

Results: HeLa cells transiently transfected with full length MMP28 cDNA showed strong expression of MMP-28. Cell lysates contained pro-MMP-28, conditioned medium contained pro-MMP-28 and a cleaved C-terminal domain, and ECM fractions contained some pro-MMP-28 and also the active form. Using a furin inhibitor, we found that the activation of MMP-28 was furin dependent. Addition of an MMP inhibitor showed the MMP-dependence of cleavage of the C-terminal domain, but comparison with the EA mutant MMP-28 suggested that this was not due to autoproteolysis. Stable expression of MMP-28 in chondrocyte cell lines increased the capacity to adhere to a number of extracellular matrix components. Similar experiments are ongoing with ADAMTS-16 expressing cell lines.

Conclusions: In initial studies to characterise the biochemistry and function of MMP-28 and ADAMTS-16, we observed that the activation of pro-MMP-28 is furin dependent. Interestingly, the active enzyme preferentially associates with the ECM. This may suggest a matrix substrate for MMP-28. We have successfully generated SW1353 and/or C28/I2 cell lines stably transfected with MMP28 (full length, EA mutant and pro-cat) and ADAMTS-16 (full-length and EQ mutant). A more complete analysis including adhesion, migration and proliferation assays, as well as in candidate protein and proteomic screens of potential substrates is currently underway.

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β1-INTEGRIN REGULATION OF MECHANOTRANSDUCTION IN OSTEOARTHRITIC HUMAN ARTICULAR CHONDROCYTES

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Purpose: Normal and osteoarthritic (OA) human articular chondrocytes (HAC) respond to 0.33 Hz cyclical mechanical stimulation (MS) via a $\alpha_5\beta_1$ integrin mediated mechanotransduction pathway although downstream signalling pathways are different. In both normal and OA cells, the pathway involves tyrosine kinase activity and protein kinase C (PKC). However, while normal HAC respond to 0.33 Hz mechanical stimulation with increases in aggrecan gene expression and cell membrane hyperpolarisation, in OA HAC aggrecan mRNA levels are unchanged and a cell membrane depolarisation response is observed.

Experiments were performed utilising a panel of function-modifying antibodies to investigate whether modulation of β_1 integrin function influenced OA chondrocyte mechanotransduction.

Methods: OA HAC were incubated with the function modifying anti-β_1 integrin receptor antibodies JB1a, P4C10 and TS2/16 for 30 min prior to 0.33 Hz MS. Membrane potentials were assessed before and after addition of the relevant antibody and following MS. RNA was extracted 24 hours post-MS and relative levels of aggrecan mRNA were assessed by semi-quantitative RT-PCR. PKC translocation and FAK phosphorylation in response to MS were assessed by western blotting.

Results: Following MS, OA cells exhibit significant cell membrane depolarisation. Treatment with JB1a and P4C10 for 30 minutes did not alter cell membrane potential, but treatment with TS2/16 caused significant cell membrane depolarisation. Both JB1a and P4C10 inhibited MS induced cell membrane depolarisation and MS had no effect on TS2/16 induced cell membrane deplolarisation.

Treatment with anti β_1 integrin antibodies for 30 minutes did not alter aggrecan mRNA levels, however, following 24 hours antibody incubation significant decreases in aggrecan mRNA levels were seen with P4C10 but not JB1a or TS2/16. Following MS, there was no alteration in aggrecan mRNA levels in untreated OA cells or cells treated with TS2/16 and MS had no effects on