their isolation. The absence of cross-reaction with the IIA isoform was established by ELISA and WB. In addition, the Saos-2 cell line was chosen to test a possible labelling of other fibrillar procollagens, mainly type I, V and XI. In fact, this cell line is described to synthesize the (α1)I, (α2)I, (α1)V, (α2)V, (α1)XI and (α2)XI, but no (α3)XI chains. No signal was detected on WB of cellular extracts or conditioned media with anti-pNIIB52, whereas antibodies to the collagen I, V and XI triple-helical parts revealed indeed the presence of proforms of these collagens.

Conclusions: Anti-pNIIB52 antibodies allow a very sensitive and specific detection of the procollagen IIB, the protein synthesized by mature chondrocytes. No cross-reactions with the IIA or with the major fibrillar procollagens synthesized by cells with an osteoblastic phenotype were observed. These antibodies offer a new tool in basic as well as in translational research concerning cartilage differentiation, homeostasis and repair.

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INFLUENCE OF OXYGEN TENSION ON THE ANTI-INFLAMMATORY AND CHONDROPROTECTIVE EFFECTS OF HEME OXYGENASE-1 IN HEALTHY AND OSTEOARTHRITIC HUMAN CHONDROCYTES

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Purpose: Articular chondrocytes are adapted to live in conditions of low O_2. Such hypoxic conditions in the avascular cartilage play an important role in extracellular matrix synthesis and survival of chondrocytes. In previous work we have shown anti-inflammatory and chondroprotective effects of heme oxygenase-1 (HO-1) on osteoarthritic (OA) condrocytes in primary cultures in 20% O_2. However, the influence of O_2 tension on HO-1 function in healthy and OA chondrocytes remains unknown.

Methods: Human chondrocytes were obtained from healthy donors and patients with diagnosis of advanced OA undergoing total knee joint replacement. The chondrocytes were isolated by digestion with collagenase and used in primary culture. Healthy and OA chondrocyes were cultured in 20% or 1% O_2 tension, and in 5% CO_2. Cells were stimulated with IL-1β (10 ng/ml) for 48h. HO-1 was induced by incubation with 10 μM cobalt protoporphyrin IX (CoPP). Protein expression was assessed by Western blot, ELISA and immunocytochemistry. Nitrite production and matrix metalloproteinase (MMP) activity were evaluated by fluorometric methods. HO-1 gene silencing was achieved by using a gene-specific siRNA.

Results: HO-1 protein was expressed in both healthy and OA chondrocytes in hypoxia (1% O_2) and normoxia (20% O_2). IL-1β down-regulated HO-1 expression in all conditions whereas CoPP treatment counteracted this effect. CoPP treatment was able to reduce the levels of TNFα and MMP activity after IL-1β stimulation. CoPP also decreased the production of nitrite induced by IL-1β in healthy and OA cells at both O_2 concentrations. This effect was accompanied by a reduction in iNOS expression at 24h. In hypoxic conditions HIF-2α and SOX9 expression was decreased by IL-1β in both healthy and OA chondrocytes. However, HO-1 induction was able to reverse this effect and prevented the decrease in type collagen II. In addition, IL-1β induced HIF-1α expression irrespective of O_2 tension whereas HO-1 induction by CoPP down-regulated HIF-1α expression in OA chondrocytes only.

Conclusions: HO-1 induction in primary chondrocytes cultured in hypoxic conditions resulted in stronger anti-inflammatory and chondroprotective effects compared with normoxia. These results suggest that HO-1 could be a physiologically important chondroprotective factor.

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HUMAN OSTEOARTHRITIS SYNOVIUM CONTAINS AN ALTERNATIVELY SPLICED TRANSCRIPT OF ADAMTS4

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Purpose: The characterization of an alternatively spliced transcript of the ADAMTS4 aggrecanase.

Methods: In human OA synovial cell cultures, RT-PCR was performed using oligonucleotide primers designed to amplify across the exon 8/9 region of human ADAMTS4. The PCR products were purified using a QIAquick purification kit (Qiagen) and sequenced using in house facilities. A pCEP4 (Invitrogen) mammalian expression vector containing ADAMTS4 plus a FLAG epitope was mutated using the QuikChange II site directed mutagenesis kit (Stratagene) to contain the ADAMTS4 splice variant plus a FLAG epitope. The recombinant proteins were purified from HEK293 transfected cells using Anti-FLAG M2 affinity gel (Sigma). Polyclonal antibodies were raised against synthetic peptides representing sequences within the C-terminal region of the splice variant of ADAMTS4 and the raised antibodies were characterized using the recombinant splice variant of ADAMTS4. The antibodies were used in immunohistochemical analysis of human osteoarthritic synovium. The proteolysis of aggrecan and other proteoglycans by the recombinant spice variant of ADAMTS4 was investigated.

Results: The degradation of aggrecan is mainly mediated by the aggrecanases, of which ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2) are the best known. We here characterize an alternative splice variant of ADAMTS4.

RT-PCR performed as described above resulted in the amplification of normal ADAMTS4, and also a smaller product missing 161 base pairs from the 5' end of exon 9, the result of alternative splicing in which exon 8 joins to a cryptic 3' splice site within exon 9. The protein produced by this alternative splicing would lack the spacer domain and have a C-terminus lacking any homologies with the normal ADAMTS4 spacer domain. The alternatively spliced transcript of ADAMTS4 was found in cultured OA synovial cells and in freshly digested OA synovium, but not in human brain, cervix or lung, or in normal bovine synovium. The protein synthesized from this alternatively spliced transcript of ADAMTS4 would lose functions dependent on its spacer domain, like substrate and matrix binding, and inhibition through fibronectin. Removal of the spacer domain from ADAMTS4 has been reported to increase its ability to cleave aggrecan at the Glu373-Ala374 bond, and it may well be that the alternatively spliced transcript produces a protein that is secreted in a more active form.

HEK293 cells transfected with a pCEP4 vector containing the cDNA sequence of the splice variant of ADAMTS4 produced the corresponding protein in both the pro and active form. This protein could be found in the media, but mostly associated with the cells, as confirmed using antibodies specific for the splice variant that were produced using synthetic peptides. Immunohistochemical analysis of osteoarthritic synovium using these antibodies showed staining of cells within the synovium. Proteins purified by immunoprecipitation by Anti-FLAG M2 affinity gel from transfected and untransfected HEK293 cells were analysed using the ANASpec SensoLyte 520 Aggrecanase I assay kit. The splice variant had aggrecanase activity comparable to a commercially available ADAMTS4. The splice variant cleaved aggrecan at the G1u373-A1a374 site, as assessed by the neoepitope monoclonal antibody BC3, with activity comparable to ADAMTS4.

Conclusions: ADAMTS4 is regulated at multiple levels through control of gene expression, mRNA splicing and protein processing, as well as the expression of naturally occurring inhibitors. We here describe the characteristics of the first known splice variant of ADAMTS4. This alternative splice transcript of ADAMTS4 is expressed as a protein in vivo and can be found in the synovium. It can be speculated that the changes in the C-terminal domain of the protein resulting from this alternatively spliced transcript would have changes in its substrate specificity. The protein produced by the alternative spliced transcript of ADAMTS4 has aggrecanase activity, and the release of low levels of this fully active variant of ADAMTS4 might be a factor in the slow process of superficial zone aggrecan loss in osteoarthritis.

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ANGIOPOIETIN-LIKE 4 PROMOTES TERMINAL CHONDROGENIC DIFFERENTIATION

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Purpose: Mesenchymal stem cells (MSCs) are an attractive cell source for cartilage tissue engineering given their ability to differentiate into