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Oral Abstract Presentations

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AN ALTERNATIVELY SPLICED TRANSCRIPT OF ADAMTS4 IN HUMAN OSTEOARTHRITIS SYNOVIUM

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Purpose: To describe and characterize a newly discovered alternatively spliced transcript of the ADAMTS4 aggrecanase.
Methods: Human OA synovium was digested and synovial cell cultures established. RT-PCR analysis was performed using oligonucleotide primers designed to amplify across the exon 8/9 region of human ADAMTS4. RT-PCR was performed and PCR products 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 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).
Results: Destruction of articular cartilage is a key feature of osteoarthritis. Aggrecan degradation is followed by irreversible collagen degradation. The degradation of aggrecan is mainly mediated by the aggrecanases, multidomain metalloproteinases belonging to the ADAMTS family, in which ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2) are the most studied. Although alternative splicing has previously been described in other ADAMTS members, we here describe and characterise the first known alternative splice variant of ADAMTS4.
RT-PCR on human OA synovial cells using the above primers resulted in the amplification of two products: normal ADAMTS4 and 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 would lack the spacer domain and have a different C-terminus lacking homologies with the normal human ADAMTS4 C-terminal spacer domain.
The alternatively spliced transcript of ADAMTS4 has been 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 predicted 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 increases its ability to cleave aggrecan, and it may well be that this alternatively spliced transcript produces a protein that is secreted in an active form, capable of cleaving the Glu373-Ala374 bond in the interglobular domain of aggrecan.
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. 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 which showed that the splice variant had aggrecanase activity comparable to a commercially available ADAMTS4. The purified splice variant was also found to cleave the Glu373-Ala374 aggrecanase site of aggrecan, as assessed by the neoepitope monoclonal antibody BC3, and again this activity was 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. Although alternative splicing has been described in other ADAMTS family members, we here present the first known splice variant of ADAMTS4, occurring in human OA synovium, but not in other human tissues tested, or in normal bovine synovium.
It is likely 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 resulting protein has aggrecanase activity, and it is possible that 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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MATRIPTASE IS A NOVEL INITIATOR OF CARTILAGE MATRIX RESORPTION IN OSTEOARTHRITIS

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Purpose: Although matrix metalloproteinases (MMPs) are most strongly associated with the proteolytic destruction of articular cartilage in osteoarthritis (OA), increasing data implicate serine proteinases in such pathological tissue turnover. We have consistently shown that serine proteinases interact with the MMPs in the cascades leading to cartilage collagen resorption. Since collagenolysis is initiated around chondrocytes, membranous serine proteinases may well contribute to disease. We therefore screened OA cartilage for serine proteinases including the transmembrane serine proteinases (TTSPs) as candidate enzymes with a role in tissue destruction.
Methods: Serine proteinase gene expression in femoral head cartilage from either hip OA or fracture to the neck of femur (NOF) patients was assessed using a custom Taqman low density array. The effect of matriptase on collagen breakdown was determined in cartilage degradation models, whilst the effect on MMP expression was analyzed by real-time PCR. Activation of proMMPs was determined using SDS-PAGE and N-terminal sequencing. Activation of proteinase-activated receptor-2 (PAR-2) was performed in a synovial perfusion assay in mice.
Results: Matriptase gene expression is significantly elevated in cartilage from OA patients compared to NOF and matriptase is immunolocalized to OA chondrocytes. We show matriptase can activate proMMP-1 and -3 to its fully active form. Matriptase alone when added to OA cartilage in explant culture causes significant collagenolysis which is metalloproteinase-dependent. Matriptase also induces MMP-1, -3 and -13 gene expression in OA cartilage. Synovial perfusion data confirmed matriptase to activate

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