Review

The regulation of the ADAMTS4 and ADAMTS5 aggrecanases in osteoarthritis: a review

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ABSTRACT

Destruction of articular cartilage is a key feature of a number of arthritides, osteoarthritis prominent among them. Aggrecan degradation, caused by increased activity of proteolytic enzymes that degrade macromolecules in the cartilage extracellular matrix, is followed by irreversible collagen degradation. The degradation of aggrecan is mediated by various matrix proteinases, mainly the aggrecanases, multidomain metalloproteinases belonging to the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. There has been much interest in the possible role of these aggrecanases, mainly ADAMTS4 and ADAMTS5, as therapeutic targets in osteoarthritis. There is still debate which of them is the major aggrecanase in osteoarthritis, however, as well as major issues concerning how they are regulated, with possible discrepancies between murine models and results obtained using human osteoarthritis tissue. This review discusses some recent data regarding the regulation of ADAMTS4 and ADAMTS5 gene expression in osteoarthritis, with emphasis on the role of proinflammatory cytokines in driving these enzymes, and of the transcription factor NFκB in mediating their expression.

Introduction

Cartilage consists of a relatively small amount of chondrocytes, embedded in abundant extracellular matrix (ECM) that contains numerous macromolecules, major constituents being collagen fibrils and the large aggregating proteoglycan aggrecan. Aggrecan fills the interstices of the collagen meshwork by forming large aggregated complexes interacting with hyaluronan and link proteins. The high negative charge density of the glycosaminoglycan chains on aggrecan monomers, with the associated water molecules, is essential for the ability of articular cartilage to withstand compressive deformation during joint articulation (1). The chondrocytes synthetize and catabolize ECM macromolecules, which in turn serve to maintain the homeostasis of the cellular environment and the cartilage structure. In diseases like rheumatoid arthritis (RA) and osteoarthritis (OA), degradation of ECM macromolecules exceeds their synthesis, resulting in a net decrease in the amount of cartilage matrix, eventually leading to total or partial erosion of the cartilage. The depletion of aggrecan from articular cartilage, as evidenced by the release of aggrecan catabolites into the synovial fluid, is an essential early pathophysiological event in OA (1-2). The aspect most studied has been the proteolysis of the interglobular domain of aggrecan with release of the glycosaminoglycan (GAG)-attachment regions, since it appears to be the most destructive to tissue function.

It has been debated whether the proteolysis of the interglobular domain of aggrecan is mediated by matrix metalloproteases (MMPs) or by aggrecanases. The aggrecanases are members of the family of disintegrin and metalloproteases with thrombospondin motifs (ADAMTS) that were first characterised for their ability to cleave the Glu^{373}-Ala^{374} bond in the interglobular domain of aggrecan (1). These enzymes are regulated at multiple levels through control of gene expression, mRNA splicing and protein processing, as well as regulation of the expression of various naturally occurring inhibitors (3). Several ADAMTS aggrecanases have been identified, among them aggrecanase-1 (ADAMTS4) and aggrecanase-2 (ADAMTS5). Recent studies suggest that one or more aggrecanases

are responsible for cleavage of the interglobular domain with destructive loss of GAG (4-5). ADAMTS4 and ADAMTS5 are multi-domain metalloproteases secreted from the cell into the extracellular space as furin active proteases. They both consist of a catalytic metalloprotease domain and a series of other ancillary domains that play a role in regulating their activity and substrate specificity (Fig. 1). For example, ADAMTS4 activity for the interglobular domain cleavage site is increased when the C-terminal spacer domain is removed, and additional C-terminal truncation also leads to a preferred substrate specificity for small leucine-rich proteoglycans and other proteins (6). Another recent study suggests that blocking aggrecanase cleavage in the interglobular domain of aggrecan diminished aggrecan loss and cartilage erosion in murine models of surgically induced osteoarthritis and inflammatory arthritis, and appeared to stimulate repair following acute inflammation (7).

It has long been debated which of the ADAMTSs is the main aggrecanase in OA. Due to observations of ADAMTS4 mRNA being inducible through interleukin (IL)-1 in chondrocytes, this enzyme has attracted a good deal of attention (8-10). But in models of murine OA induced by antigen or surgical joint destabilisation, ADAMTS4-null mice did not show any protective effect on cartilage aggrecan loss compared with wild-type mice, whereas there was a marked protective effect in ADAMTS5-null mice (11-13). These studies suggest that in this model of murine degenerative arthritis, ADAMTS5 plays a key role in aggrecan degradation. The significance of this important finding for idiopathic human OA is not yet known.

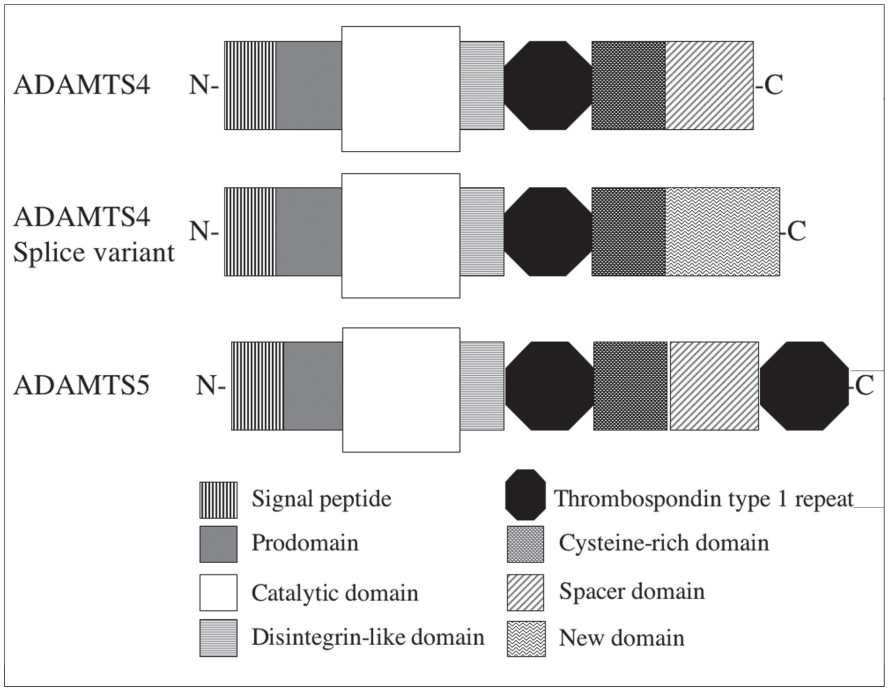

Fig. 1. The structure of ADAMTS4, its only known splice variant, and ADAMTS5.

The role of proinflammatory cytokines in the regulation of ADAMTS4 and ADAMTS5

In models of cultured bovine and porcine chondrocytes or cartilage explants, ADAMTS4 is induced following stimulation with IL-1, tumour necrosis factor (TNF)α, oncostatin M or transforming growth factor β, but ADAMTS5 is not (8-10,14). A recent study (15) indicated that although ADAMTS4 gene expression could be upregulated through treatment with either IL-1β, TNF-α or oncostatin M, there was little effect on ADAMTS5 in either human chondrocytes or cultured human cartilage explants. In contrast, there was an additive effect of combination treatment with oncostatin M and either IL-1β or TNF-α in these systems, leading to marked induction of ADAMTS4 gene expression and also some induction of ADAMTS5 (15). In OA synovium or cartilage, aggrecanase activity and expression of ADAMTS4 and ADAMTS5 is present constitutively, without any requirement for any catabolic stimulation (15-16).

To investigate the role of TNF-α, and IL-1 in driving ADAMTS4 and ADAMTS5 expression in the human OA synovium, we used a model of cultures of synovial cells from digested OA synovium (17). These cells have the advantage of spontaneously producing a variety of both pro- and anti-inflammatory cytokines, including TNF-α, IL-1 and IL-10, as well as the major MMPs and TIMPs. By means of specific neutralization of macrophage-produced TNF-α and IL-1, it was possible to assess the contribution of these two proinflammatory cytokines on ADAMTS4 and ADAMTS5 gene expression.

We used a model to effectively and specifically neutralise the endogenous production of these cytokines from the OA synovial macrophages (18). Cultures were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), incubated with a neutralizing anti-IL-1β antibody, or incubated with a combination of Enbrel and anti-IL-1β. There was no effect of either Enbrel or the neutralizing anti-IL-1β antibody on ADAMTS5 expression, nor was it at all affected by a combination of these treatments (Fig. 2). Thus ADAMTS5 appears to be constitutive in OA synovial cells. In contrast, ADAMTS4 was significantly ($p < 0.05$) inhibited by Enbrel, and more potently ($p < 0.01$) inhibited by a combination of Enbrel and the neutralizing anti-IL-1β antibody (Fig. 2). This would indicate that in the human OA synovium, the upregulation of ADAMTS4 is dependent on TNF-α and IL-1 produced by the synovial macrophages, whereas the level of ADAMTS5 is not changed by these cytokines (18).

In contrast to this wealth of data from human, porcine and bovine models indicating that ADAMTS4 mRNA responds to IL-1, there are two recent papers indicating that this is not the

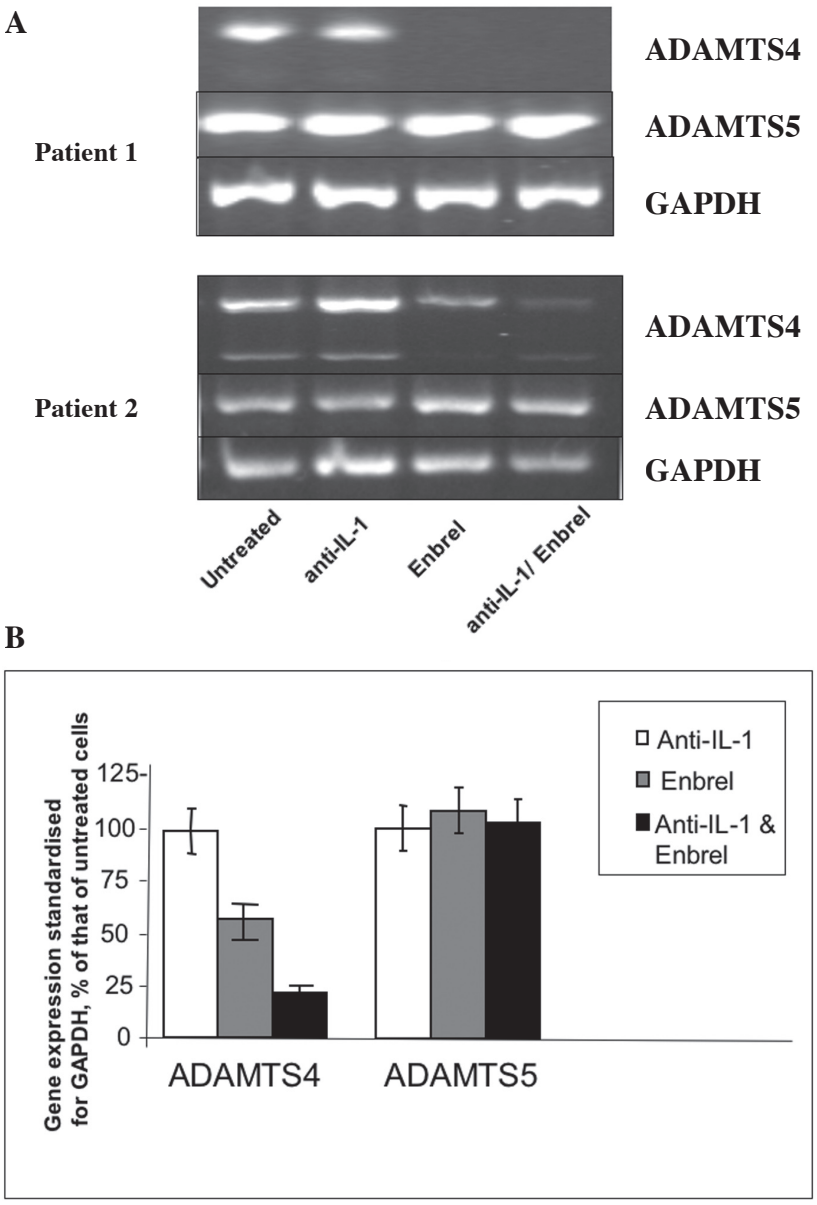

Fig. 2. Effect of neutralization of TNF-α and/or IL-1 on the expression of ADAMTS4 and ADAMTS5 in the OA synovium.
The cells were either left untreated, incubated with a neutralizing anti-IL-1β antibody, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), or incubated with a combination of etanercept and anti-IL-1β. After incubation for 48 h the cells were washed with PBS and the RNA extracted using Tri-reagent for RT-PCR analysis using oligonucleotide primers specific for ADAMTS4 and ADAMTS5 in two patients, with analyis of GAPDH used for comparison of gene expression **(A)**. In **(B)**, the PCR data is expressed as percentage of the gene expression in untreated cells, as standardised for GAPDH, with the SEM given (n = 4-5).

case in mouse cells. In murine femoral head explant cultures, ADAMTS4 mRNA levels were unaffected by IL-1 (19). Monocytes from wild-type mice, but not monocytes from IL-1 deficient mice, upregulated ADAMTS5 mRNA in chondrocytes without affecting ADAMTS4, again suggesting that murine ADAMTS4 is unresponsive to IL-1 (20).

The role of NFκB in the regulation of ADAMTS4 and ADAMTS5

It has been shown that both in RA and in OA, the transcription factor NFκB plays an important part in regulating various proinflammatory and destructive mediators, including several matrix metalloproteases, *i.e.* MMP-1, 3, 9 and 13 (17, 21). Recently, the role of this transcription factor in regulating ADAMTS4 and ADAMTS5 gene expression has also become clearer. ADAMTS4, but not ADAMTS5, has several NFκB binding sites on its 5' flanking region that are conserved between species (22). In bovine chondrocytes, ADAMTS4, but not ADAMTS5, could be upregulated by IL-1 stimulation (Fig. 3A). Using a model of transfecting bovine chondrocytes with the 5' flanking region of the ADAMTS4 or ADAMTS5 gene luciferase receptor vector, it was observed that the IL-1-induced upregulation of the ADAMTS4 gene involved its 5' flanking region, whereas the 5' flanking region of the ADAMTS5 gene played no part (Fig. 3B). Mutation of any one of the three identified NFκB binding sites resulted in the loss of the IL-1 response to the ADAMTS4 gene luciferase reporter vector (Fig. 3C), indicating that the IL-1-stimulated increase in ADAMTS4 gene transcription depends on two or more NFκB binding sites located in the 5' flanking region of this gene (22).

In bovine nucleus pulposus tissue, TNF-α treatment induced upregulation of aggrecanase activity, ADAMTS4 in particular, in a NFκB dependent manner, although the specificity of the small molecule NFκB inhibitor used in this study remains unproven (23). In human OA synovial fibroblasts, treatment with IL-1 or TNF-α, but not treatment with phorbol ester, resulted in upregulation of ADAMTS4, whereas ADAMTS5 was unaffected (Fig. 4A). In this model, it was possible to use adenoviral gene transfer of the endogenous inhibitor IκBα to specifically inhibit NFκB without affecting other signalling pathways or causing apoptosis (24). Whereas ADAMTS5 gene expression was not changed by gene transfer of IκBα, the ADAMTS4 induction by IL-1 or TNF-α was potently inhibited by NFκB downregulation (Fig. 4).

These three papers (22-24) strongly suggest that the upregulation of ADAMTS4 induced by IL-1 or TNF-α is NFκB dependent. There is no evidence that NFκB plays any part in regulating ADAMTS5 expression, however.

An ADAMTS4 splice variant in human OA synovium

Post-transcriptional regulation through alternative splicing has been recognised

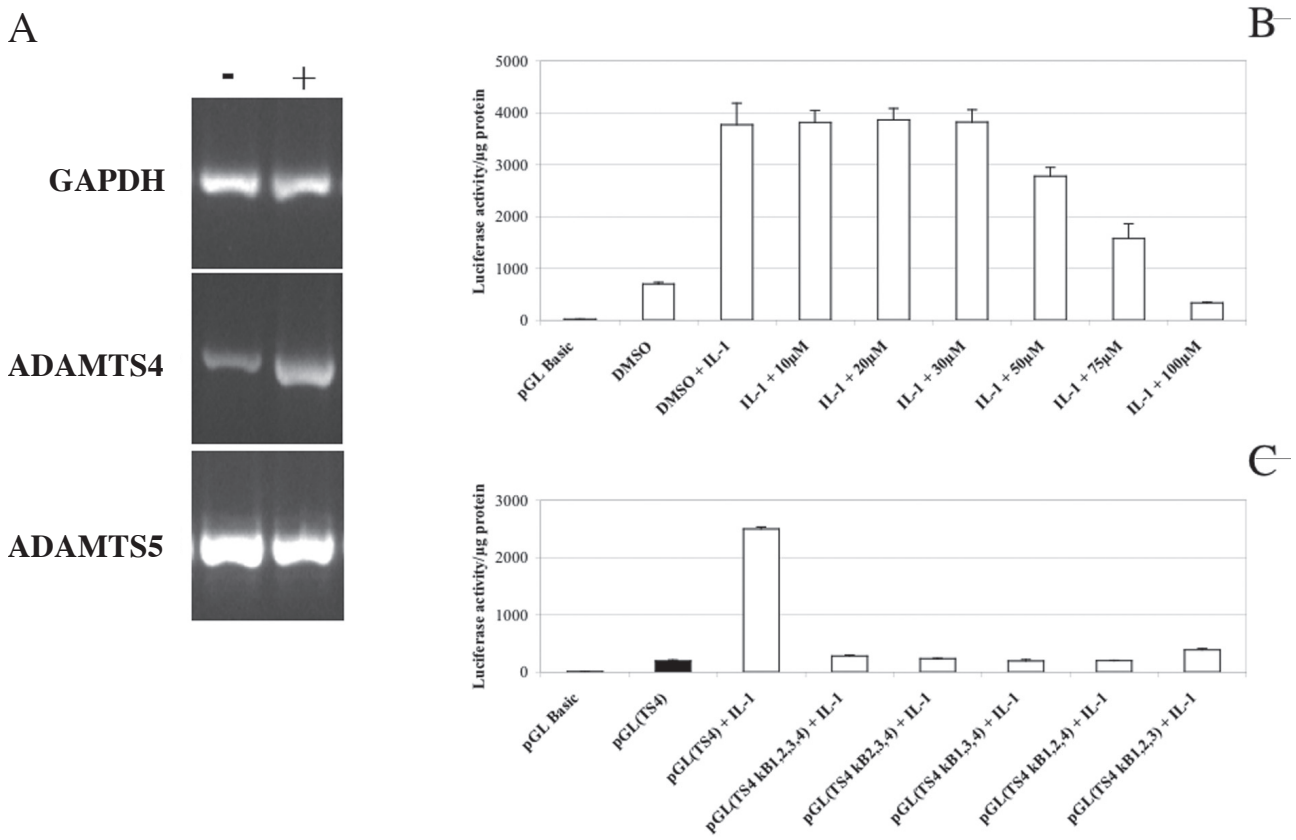

Fig. 3. IL-1 upregulates ADAMTS4, but not ADAMTS5, in an NFκB dependent manner.
Bovine chondrocytes were either left untreated or stimulated with IL-1α (10 ng/ml) before RNA was extracted and RT-PCR analysis carried out using oligonucleotide primers specific for bovine GAPDH, ADAMTS4 and ADAMTS5 **(A)**. In **(B)**, a luciferase assay was performed using pGL3 basic plasmids containing the 5' flanking region of the ADAMTS4 gene (pGL(TS4) transfected into bovine chondrocytes treated with the solvent DMSO, DMSO and IL-1α (10 ng/ml), or IL-1α and various concentrations of the NFκB inhibitor weldolactone. In **(C)**, a luciferase assay was performed using either the wild-type 5' flanking region of the ADAMTS4 gene (pGL (TS4)), a construct in which this region had all four kB sites inactivated by mutation (pGL(TS4kB 1, 2, 3, 4) or a series of constructs in which each member had a different kB site unmutated.

for several of the ADAMTS proteins, including ADAMTS-6, -7 and -9 (3, 25). We recently described the first known splice variant of ADAMTS4 (Fig. 1) in human OA synovium, using an oligonucleotide primer pair designed to amplify across the exon 8/9 junction of human ADAMTS4 (26). This alternatively spliced transcript of ADAMTS4 is missing 161 base pairs from the 5' end of 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 (Fig. 1).

This protein would lose functions dependent on its spacer domain, like substrate and matrix binding and inhibition through fibronectin (27). It is known that removal of the spacer domain from ADAMTS4 increases its ability to cleave aggrecan, and it may well be that this alternative splice variant produces an ADAMTS4 protein that is secreted in an active form that can cleave the Glu373-Ala374 bond in the interglobular domain of aggrecan (6, 28). This splice variant has hitherto only been detected in human OA synovium, and not in other human tissues like brain, cervix or lung; nor has it been detected in normal bovine synovium (26). It may be speculated that the release of low levels of this fully active variant of ADAMTS4 may be a factor in the slow progress of superficial zone aggrecan loss in OA.

Another recent study has demonstrated the presence of several C-terminally truncated isoforms of ADAMTS4 and ADAMTS5 in porcine articular cartilage explants and porcine chondrocyte-agarose cultures exposed to IL-1 (29). In particular, IL-1 treatment induced production of a low molecular weight (37 kD) isoform of ADAMTS4, which was capable of degrading exogenous aggrecan at the interglobular domain site, and required *de novo* protein synthesis for its generation. In porcine chondrocyte-agarose cultures, this 37kD ADAMTS4 appears to be the major protease responsible for IL-1-induced interglobular domain aggrenanase activity. Due to its low molecular weight, it is obviously not the same protein as the high-molecular weight ADAMTS4 splice variant described above, but it might represent another yet uncathergorised splice variant or protein cleavage product.

Which is the main aggrecanase in osteoarthritis?

There is good evidence that in OA synovium and cartilage, ADAMTS4 is the aggrecanase induced by proinflamma-

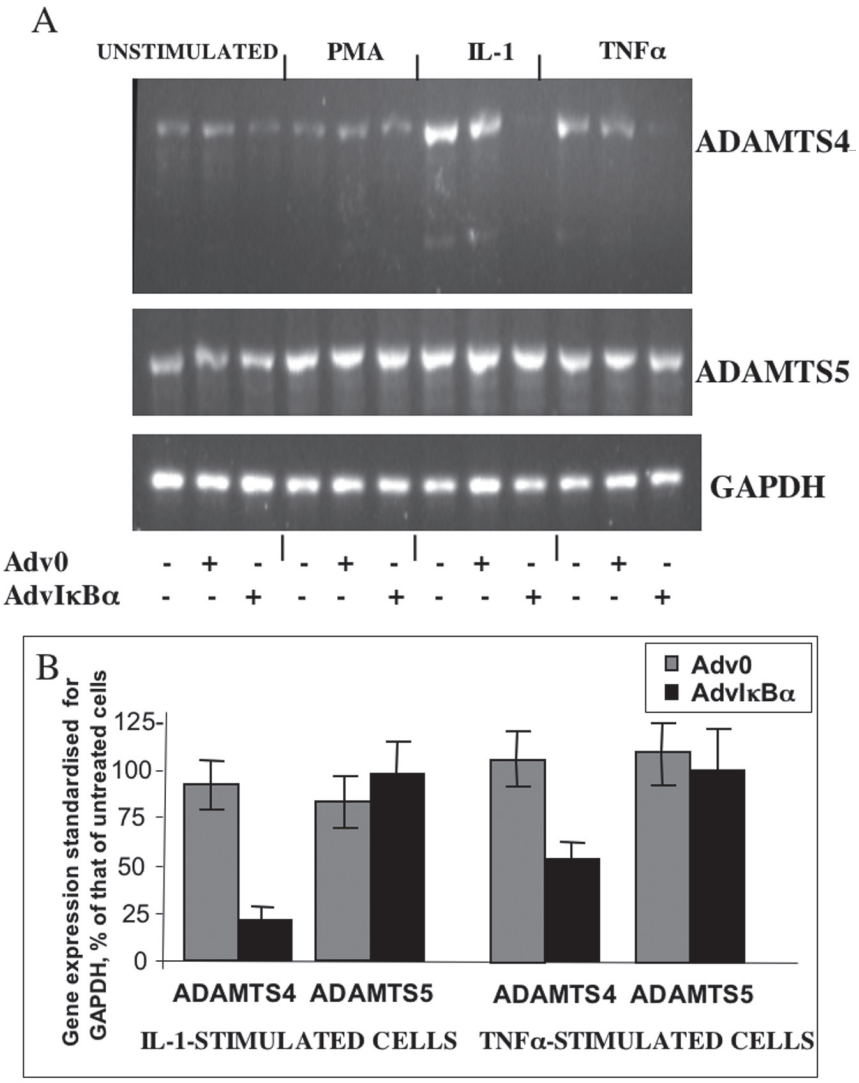

Fig. 4. The role of NFκB in the regulation of ADAMTS4 and ADAMTS5 expression in the OA synovium.
OA synovial fibroblasts were either left uninfected or infected with 30:1 of either AdvIκBα or Adv0. After 24 hrs, cells were stimulated with either PMA, IL-1β or TNF-α. RT-PCR analysis was carried out using oligonucleotide primers specific for ADAMTS4 and ADAMTS5, with analysis of GAPDH gene expression used for comparison **(A)**. In **(B)**, the PCR data is expressed as percentage of the gene expression in untreated cells, as standardised for GAPDH, with the SEM given (n = 3-5).

tory cytokines, whereas ADAMTS5 appears to be constitutive (9-10, 18, 24). If it is accepted that OA is a cytokine driven disease, as indicated by some recent papers suggesting that macrophage-produced IL-1 and TNF plays a role in driving destructive responses in OA, this finding would render it likely that ADAMTS4 is the aggrecanase responsible for aggrecanolysis in OA (18, 30-33).

In marked contrast to this *in vitro* data derived from studying human tissue, recent studies using transgenic mice suggest that in murine models of degenerative joint disease, ADAMTS5 is the pathologically induced aggrecanase. Mice lacking ADAMTS4 develop normally and develop surgically induced OA in a similar manner to wild-type mice, but deletion of ADAMTS5 protects mice from developing OA (11-13). These results suggest that at least in murine models of OA, ADAMTS5 is the major aggrecanase. The only caveat to this conclusion is the apparent discrepancy between human and murine cells with regard to the regulation of ADAMTS4 (19-20). If the human, but not murine, ADAMTS4 gene responds to IL-1 stimulation, this brings into question the use of a murine model for the study of human aggrecanolysis.

Studies on the effect of ADAMTS5 deficiency on aggrecanolysis in mouse cartilage have provided contradictory results: in one study, spontaneous aggrecan degradation was also ablated in murine epiphyseal chondrocyte cultures from ADAMTS5-null mice as compared to cultures from wild-type mice (34), in another, ADAMTS5 deficiency did not block aggrecanolysis at preferred cleavage points (19).

Another recent study used a small interfering RNA approach to assess the effect of the inhibition of ADAMTS4 and ADAMTS5 in human chondrocytes and cartilage explants. Suppression of either ADAMTS4 or ADAMTS5 led to siginificant inhibition of the degradation of aggrecan induced by a combination of TNF-α and oncostatin M (15). Studies of the mRNA levels of ADAMTS4 and ADAMTS5 in normal and osteoarthritic human cartilage have as yet failed to provide consistent results. In some studies, ADAMTS5 expression is higher than that of ADAMTS4, in others the opposite is true (9, 15, 35-36). In a study of the effect of salt concentration on aggrecanolysis, recombinant ADAMTS4 and ADAMTS5 had similar general proteolytic effects, but the aggrecanase effect of ADAMTS5 was markedly higher (37). Importantly, a reduction of aggrecan breakdown was observed after the suppression of either ADAMTS4 or ADAMTS5 through a small interfering RNA approach in unstimulated human OA cartilage. This would indicate that, in contrast to the situation in genetically modified mice, both ADAMTS4 and ADAMTS5 contribute to the structural damage in human OA (15).

Aggrecanases as potential therapeutic targets in OA

The recent data presented in this review suggests that although both ADAMTS4 and ADAMTS5 cleave aggrecan, they are two very different enzymes with regard to their regulation. At least in human cells, ADAMTS4 responds to IL-1 and TNF-α, but ADAMTS5 does not (9-10, 18, 24). Another difference is that whereas the upregulation of

ADAMTS4 depends on the transcription factor NFκB, ADAMTS5 is NFκB independent and lacks kB elements on its promoter (22-24). With this in mind, it is interesting to note that treatment of bovine cartilage explants with a small molecule IκB kinase inhibitor led to prevention of IL-1-induced aggrecan depletion, suggesting that this process occurred in a NFκB dependent manner (38). This differential regulation of ADAMTS4 and ADAMTS5 has implications for the potential development of disease-modifying osteoarthritis drugs (39). A therapeutic strategy that would inhibit the cytokine-driven inflammatory response would be likely to downregulate ADAMTS4, as would an inhibitor of NFκB. However, neither strategy would be likely to influence ADAMTS5.

The design of small molecule aggrecanase inhibitors is an area of considerable interest for the pharmaceutical industry (40-44). For such approaches to meet with success, there is a need to appreciate that ADAMTS4 and ADAMTS5 are differentially regulated. The primary aggrecanase (ADAMTS4 or ADAMTS5) involved in human OA also needs to be conclusively identified.

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