Identifying the human aggrecanase

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**Summary**

It is clear that A Disintegrin And Metalloproteinase with Thrombospandin motif (ADAMTS)-5 is the major aggrecanase in mouse cartilage, however it is not at all clear which enzyme is the major aggrecanase in human cartilage. Identifying the human aggrecanase is difficult because multiple, independent, molecular processes determine the final level of enzyme activity. As investigators, we have good methods for measuring changes in the expression of ADAMTS mRNA, and good methods for detecting aggrecanase activity, but no methods that distinguish the source of the activity. In between gene expression and enzyme action there are many processes that can potentially enhance or inhibit the final level of activity. In this editorial we discuss how each of these processes affects ADAMTS activity and argue that measuring any one process in isolation has little value in predicting overall ADAMTS activity.

**Introduction**

It is now over a decade since A Disintegrin And Metalloproteinase with Thrombospondin motif (ADAMTS)-4, and ADAMTS-5 were identified as aggrecanases, based on their ability to cleave at the Glu373
374Ala bond in the aggrecan interglobular domain (IGD). Since then ADAMTS-1, -8, -9, -15, -16 and -18 family members have also been identified as aggrecan-degrading enzymes in vitro, but none have seriously challenged ADAMTS-4 and ADAMTS-5 as the candidate human aggrecanases because these have substantially greater aggrecan-degrading activity in vitro. ADAMTS-4 and -5 are expressed in human cartilage and in many cases their expression levels are increased in joint disease, and at sites of localised aggrecan loss. The results of a recent genome-wide association study revealed eight polymorphisms in the ADAMTS-5 gene, two of which caused amino acid changes. However neither polymorphism was associated with difference in susceptibility to osteoarthritis (OA) in a large cohort of patients.

It is not clear whether ADAMTS-4 or ADAMTS-5 is the aggrecanase in human cartilage. Indeed there is evidence suggesting that both aggrecanases have key roles in degrading aggrecan in vivo. Identifying the human aggrecanase is a major challenge because the final level of enzyme activity is determined by multiple, independent and interacting molecular processes, including promoter activity, epigenetic modifications, regulation by non-coding RNA (ncRNA) including microRNA (miRNA), and alternative splicing. At the protein level, ADAMTS activity is determined by the summed effect of prodomain processing, C-terminal processing, the availability and binding of endogenous enhancers or inhibitors, and the extent of glycosylation on both the aggrecan substrate and the enzymes themselves (Fig. 1). We herein discuss how each of these processes affects ADAMTS activity and argue that measuring any one parameter in isolation is an inadequate surrogate for determining the identity of the human aggrecanase.

**Regulating ADAMTS-4 and -5 mRNA expression**

Apart from activity studies that tell us a great deal about aggrecanases collectively, the most abundant studies on human ADAMTS-4 and -5 are those that have analysed mRNA expression either in vitro with and without catabolic stimuli, or immediately ex vivo, from normal or OA cartilage. Most but not all the in vitro studies in human cartilage or chondrocytes found that ADAMTS-4 mRNA was induced by catabolic cytokines such as interleukin-1β (IL-1β) or Tumor necrosis factor α (TNFα) and that ADAMTS-5 mRNA was not regulated by cytokines but expressed constitutively. These findings differ from most animal studies which show that ADAMTS-5 mRNA expression is upregulated by catabolic cytokines (reviewed in Ref. 11).

The absence of ADAMTS-4 and -5 in early OA gene profiling studies suggests that on a global scale, these enzymes are poorly expressed. Other expression studies focussing specifically on...
cartilage proteinases show that ADAMTS-5 is more highly expressed than ADAMTS-4 in both normal and OA cartilage. Some studies report increased expression of both ADAMTS-4 and ADAMTS-5 in knee cartilage from patients with late, but not early stage OA. Other studies report that only ADAMTS-4 mRNA is increased in knee cartilage or hips measured at the time of joint replacement, while others report decreases in both ADAMTS-4 and protein expression in human synovium. ADAMTS-5 plays an anabolic role in matrix metabolism, but TGF-β strongly induces aggrecanase activity in the meniscus, after Arg696. There is no information on the abundance of this transcript in synovium, its expression in cartilage and other joint tissues, or its expression in other patient groups. It will be fascinating to determine the efficacy of the ADAMTS-4 splice variant as an aggrecan-degrading enzyme. The splice variant is likely to have altered enzyme activity, given the importance of the C-terminal processing of either NFATp or Runx2 in cells expressing 4.5 kb of the ADAMTS-4 promoter resulted in transcriptional activation of the ADAMTS-4 promoter. Similarly, analysis of 2.6 kb of the human ADAMTS-5 promoter revealed four putative binding sites for the Runx family of transcription factors. Over-expression of either NFATp or Runx2 in cells expressing 4.5 kb of the ADAMTS-4 promoter resulted in transcriptional activation of the ADAMTS-4 promoter. By a variety of modulators typically found in arthritic joints. However we emphasise that to date, there are no corresponding studies linking increased mRNA expression with increased ADAMTS-4 or ADAMTS-5 activity.

In addition to modulators that are known, or thought, to act via intracellular signalling pathways, at least two transcription factors can potentially modify aggrecanase gene expression by direct binding to the ADAMTS-4 or -5 gene promoters. The human ADAMTS-4 promoter has two putative binding sites for the transcription factor nuclear factor of activated T cells-α (NFATp) and eight putative binding sites for the Runx family of transcription factors. Over-expression of either NFATp or Runx2 in cells expressing 4.5 kb of the ADAMTS-4 promoter resulted in transcriptional activation of the ADAMTS-4 promoter. Similarly, analysis of 2.6 kb of the human ADAMTS-5 promoter revealed four putative binding sites for the Runx family of transcription factors and Runx2 transactivated the ADAMTS-5 promoter in vitro. The in vivo influence of Runx2 and NFATp on aggrecanase activity via the ADAMTS-4 or ADAMTS-5 promoter is unknown.

ADAMTS-4 and -5 regulation by alternative splicing, epigenetics and ncRNA

There are other pre-translational mechanisms for modulating aggrecanase activity independently of mRNA expression; these include alternative splicing of the ADAMTS transcripts, regulation by ncRNAs including miRNA and gene silencing (or unsilencing) by epigenetic modifications to DNA and histones.

An alternatively spliced transcript of ADAMTS-4 has been identified in synovium from OA patients. The splice variation occurs between exons 8 and 9 to create a longer protein which diverges from the wildtype ADAMTS-4 near the start of the spacer region, after Arg696. There is no information on the abundance of this transcript in synovium, its expression in cartilage and other joint tissues, or its expression in other patient groups. It will be fascinating to determine the efficacy of the ADAMTS-4 splice variant as an aggrecan-degrading enzyme. The splice variant is likely to have altered enzyme activity, given the importance of the spacer region in conferring maximum aggrecanolytic activity on ADAMTS-4. There is no evidence for alternatively spliced transcripts of ADAMTS-5.

The genomes of eukaryotes studied to date are almost entirely transcribed, leading to vast numbers of ncRNAs with unknown functions. These ncRNAs and miRNAs represent a new frontier in the molecular biology of human disease. miRNAs primarly affect gene expression by inhibiting protein translation, without affecting transcription. Much less is known about ncRNAs in terms of actions in vivo but they are thought to have important roles in epigenetic, transcriptional and post-transcriptional regulation of gene expression. It is clear that miRNAs provide important regulatory checks in the immune system and inflammatory reactions, and a 16-miRNA gene signature that is differentially expressed in OA has recently been described. Two independent studies have now reported the
down-regulation of miR140 in OA cartilage compared with healthy controls. miR140 --/-- mice have significantly increased levels of ADAMTS-5 mRNA and increased aggrecan loss in vitro, compared with wildtype mice. miR140 is also predicted to target ADAMTS-4 and matrix metalloproteinase-13 (MMP-13). There is no data comparing the effects of miR140 on ADAMTS-4 vs ADAMTS-5-mediated aggrecanase activity in any species. Mimic miRNAs and anti-miRNAs are of interest for the treatment of cancers however the study of ncRNAs in cartilage, and their influence in OA and inflammatory arthritis, is very much in its infancy.

Epigenetic modifications include changes in the methylation status of CpG sites in gene promoters, and the acetylation and deacetylation of histone proteins. Epigenetic changes determine whether genes are silenced or transcribed and they lead to heritable and permanent alterations in gene expression. In arthritic diseases, epigenetic changes can potentially escalate disease progression through, for example, synovial hyperplasia in RA, or cell proliferation within chondrocyte clusters that are typically seen in human OA. Recent studies have shown that aberrant expression of ADAMTS-4 is associated with loss of DNA methylation at specific CpG sites in the ADAMTS-4 promoter. Furthermore, a related study comparing young (23 years of age) and old (80--90 years of age) OA cartilage, with non-arthritis age-matched comparators, suggests that ageing alone does not account for the epigenetic unsilencing in OA. This is based on the finding that the association between ADAMTS-4 expression and demethylation of the ADAMTS-4 promoter was a consistent feature of both the young and old OA cartilage. There are examples of abnormal ADAMTS-5 promoter methylation in some cancers. In the future it will be important to characterise the epigenetic changes in ADAMTS-4 and -5 and correlate them with changes in aggrecan-degrading activity.

**ADAMTS-4 and -5 activation by N-terminal processing**

Two early studies pointed to the possibility that aggrecanase activity might be controlled at a post-translational level. Flannery and colleagues showed that retinoic acid-induced aggrecanase activity was increased in bovine chondrocytes with no concomitant increase in mRNA levels for any ADAMTS homologues. In a separate study, Pratta and colleagues showed that IL-1-induced ADAMTS-4 activity was increased in bovine chondrocytes in the absence of any detectable increase in ADAMTS-4 protein production; these investigators concluded that enzyme activation was responsible for the increased activity of constitutively expressed ADAMTS-4.

ADAMTS-4 and -5 are synthesised as latent proenzymes and retained in their inactive state by interactions between their pro- and catalytic domains. The mechanism by which the prodomains maintain ADAMTS latency is unknown but it is unlikely to be via the well known cysteine switch mechanism found in the matrix metalloproteinase family. Instead, the ADAMTS-4 and -5 prodomains might directly inhibit enzyme activation via a non-cysteine switch mechanism, similar to that seen for ADAM17.

Furin, or furin-like proprotein convertases (PC), has major roles in activating ADAMTS enzymes. Furin, PC7 and PC5A activate recombinant ADAMTS-4 or -5 and Malfait et al. have used silencing RNA (siRNA) to show that PACE4 activates aggrecanases in OA and cytokine-stimulated human cartilage in culture. Cleavage occurs at the multibasic furin/PC recognition sites located at RRKR in human ADAMTS-4 and RRRRR in human ADAMTS-5. Importantly, N-terminal processing and activation can be mediated by more than one PC at more than one cellular location, since PCs are located in the trans Golgi network, in endosomes, at the cell surface and in the extracellular matrix. Extracellular and/or cell surface activation has been reported for ADAMTS-5 and both intracellular and cell surface activation has been reported for ADAMTS-4.

Variables in the ADAMTS activation process (which PC, which cellular site) could significantly influence the efficiency of enzyme activation and alter enzyme activity, independent of mRNA levels. Other variables include the fact that PCs themselves are subject to regulated expression and selective glycosylation of PCs can affect their activity.

**Regulation of ADAMTS-4 and -5 activities by C-terminal processing**

Processing to remove C-terminal ancillary domains of ADAMTS-4 and -5 is likely to be a common mechanism for regulating aggrecanase activity. Truncated forms of both enzymes are consistently found in tissue extracts and experimental systems following local proteolysis or autolysis. For example, the major form of ADAMTS-5 in normal human synovium is the 70 kDa mature form, whereas synovium from RA patients is predominantly the 53 kDa form lacking the last thrombospondin module and the spacer domain. The minimum domain composition for a molecule containing aggrecan-degrading activity, in both ADAMTS-4 and -5, is the catalytic domain, the disintegrin domain and the first thrombospondin module (Fig. 2); recombinant truncates lacking the first thrombospondin module are inactive against aggrecan.

Mature ADAMTS-4 and -5 containing a full suite of C-terminal ancillary domains are the most active species in vitro and, in the absence of heparin, engineered deletions of C-terminal domains diminish catalytic activity against both the IGD and chondroitin sulphate (CS)-rich regions of aggrecan. In these studies with recombinant enzymes expressed in HEK293-EBNA cells, deletion of the spacer domain of ADAMTS-4 or the cysteine-rich domain of ADAMTS-5 ablates aggrecan-degrading activity by more than 95%, while having only minor effects on cleavage of non-aggrecan substrates such as fibromodulin. It is possible that C-terminal processing of ADAMTS-4 might have different effects in other cell types, since Gao et al. have used a human chondrosarcoma cell line stably transfected with ADAMTS-4 and MT4-MMP, to show that the most active form of ADAMTS-4 is generated by MT4-MMP-mediated removal of the spacer domain. The authors propose that sites at Lys and Thr in the spacer domain are susceptible to MT4-MMP cleavage.

There is also evidence that C-terminal processing influences tissue localisation of aggrecanases. ADAMTS-5 in normal human cartilage localises around cells in the superficial zone and around clonal clusters in OA cartilage. The pericellular localisation is thought to occur via HA binding to the disintegrin domain and to a heparin-binding motif in the spacer domain. Other studies in HTB-94 chondrosarcoma cells show that the cysteine-rich domain of ADAMTS-5 is essential for binding and localisation in the extracellular matrix. In ADAMTS-4, it is the spacer domain that mediates matrix binding. Thus, C-terminal processing can influence aggrecanase activity directly via its effects on enzyme localisation.
activity, and indirectly by disrupting ADAMTS localisation in extracellular matrices.

Proteinases that cleave ADAMTS C-terminal domains include MT4-MMP\(^{54}\), PACE4\(^{52}\) and ADAMTS-4 and -5 themselves\(^{56}\). In addition, two putative PC cleavage sites are located at RRTR\(^{565}\) and RRKR\(^{650}\) in ADAMTS-4\(^{46}\). The presence of two established and two putative C-terminal cleavage sites, combined with apparent susceptibility of ADAMTS enzymes to at least three different families of proteinases, suggests that ADAMTS-4 and -5 C-terminal processing is a key mechanism for regulating aggrecanase activity. In addition to proteolysis, alternative splicing of ADAMTS-4 provides another means of disrupting C-terminal ancillary domains to modulate enzyme activity\(^{27}\).

### Regulation of ADAMTS-4 and -5 activities by glycosylation: enzyme and substrate

Almost all secreted proteins undergo some form of post-translational modification and the most common type of modification is glycosylation. In many cases glycosylation confers essential functional properties on secreted proteins. All ADAMTS proteinases are predicted to be glycoproteins carrying at least one mannosyl modification on tryptophan residues within prescribed motifs in the first thrombospondin motif\(^{62}\). This prediction has been confirmed experimentally for ADAMTS-5 in studies which identified two C-mannose substitutions as well as a glucose-fucose disaccharide on the thrombospondin domain of recombinant enzyme\(^{62}\). Two sites for C-mannosylation are also predicted for the thrombospondin domain of ADAMTS-4. N-linked glycosylation in the ADAMTS-5 spacer domain was predicted\(^{47}\) and has been confirmed in a separate study by a change in electrophoretic mobility of recombinant ADAMTS-5 after digestion with PNGase\(^ F^{55}\). There is no evidence that ADAMTS-4 carries N-linked glycosylation\(^{63}\) and there is no evidence that underglycosylation affects ADAMTS-4 or -5 activity. Mannosylation in the thrombospondin domain regulates secretion of some members of the ADAMTS family\(^{62}\). It is possible that secretion of ADAMTS-4 and -5 is also influenced by mannosylation. ADAMTS enzymes degrade aggrecan extracellularly so controlling their secretion will affect aggrecanase activity directly.

Glycosylation of the aggrecan substrate is another variable that markedly affects aggrecanase activity. The signature activity of the aggrecanases is cleavage within the IGD. The IGD contains keratan sulphate (KS) but no CS substitutions, so it is not surprising that removal of KS alone blocks aggrecanase cleavage in the IGD\(^{54}\)\(^\text{-}^{56}\). The KS content of human aggrecan increases from a minimal percentage at birth, to over a quarter of the glycosaminoglycan content at maturity, and KS microstructure also changes dramatically during this time\(^{67}\). In addition, the microstructure of KS in the IGD is distinctly different to the microstructure of KS elsewhere on aggrecan\(^{68}\), suggesting that KS microstructure might contain functional information. Furthermore, whereas ADAMTS-5 cleavage in the IGD is highly efficient in both mature and juvenile cartilage, ADAMTS-4 cleavage in the IGD is inefficient in juvenile cartilage\(^{54}\)\(^\text{-}^{59}\). Collectively these studies suggest an intriguing link between increased susceptibility to aggrecanalysis with ageing, and increased KS substitution with ageing. Further work is required to investigate this potential link and to determine whether ADAMTS-4 and -5 have age-specific functions in cartilage and OA.

### Inhibitors and enhancers of ADAMTS-4 and -5 activity

#### Inhibitors of ADAMTS-4 and -5 activity

Once aggrecanase enzymes have been synthesised, secreted from the cell, activated by prodomain removal and modified by C-terminal processing, their activity can be further modified by endogenous inhibitors. The Tissue Inhibitor of Metalloproteinases (TIMP) family of inhibitors are expressed in joint tissues, and are regulated in arthritis. Of the four family members, TIMP-3 is the most potent inhibitor of both ADAMTS-4\(^{70}\)\(^\text{-}^{71}\) and ADAMTS-5\(^{70}\)\(^\text{-}^{70}\) and there is negligible inhibition of aggrecanases by TIMPs-1, -2 or -4\(^{70}\). In the TIMP-3 null mouse there is increased aggrecan loss and increased NITEGE\(^{72}\) neoepitope, concomitant with mild cartilage degradation\(^{72}\), suggesting that TIMP-3 is indeed an endogenous aggrecanase inhibitor. TIMP-3 binds tightly to negatively charged glycosaminoglycans\(^{72}\) and TIMP-3 potency against ADAMTS-4 is enhanced by ADAMTS-4 binding to aggrecan\(^{55}\); it appears that TIMP-3 has a greater affinity for ADAMTS-4 complexed with aggrecan, than it does for ADAMTS-4 alone\(^{54}\). A recent, exciting finding is that TIMP-3 potency against ADAMTS-4 and -5 is greatly enhanced in the presence of calcium pentosan polysulphate (CaPPS)\(^{72}\). CaPPS itself inhibits aggrecanase activity via interactions with the spacer domain of ADAMTS-4 and the cysteine-rich domain of ADAMTS-5\(^{75}\). The levels of TIMP-3 in cartilage are very low due to rapid internalisation by the cell via Lipoprotein receptor-related proteins (LRP)-mediated endocytosis\(^{75}\).

Other proteinase inhibitors in cartilage include \(\text{\footnotesize{\text{G}}}_2\)-macroglobulin, which is a membrane anchored glycoprotein, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), \(\text{\footnotesize{\text{G}}}_2\)-macroglobulin inhibits ADAMTS-4 and -5 in vitro by trapping the enzymes in a 1:1 complex\(^{76}\) however, there is no evidence for such a complex in synovial fluid, nor evidence that \(\text{\footnotesize{\text{G}}}_2\)-macroglobulin is cleaved by either enzyme\(^{76}\). Similarly, there is no evidence that RECK inhibits ADAMTS-4 or -5. Thus, Timp-3 appears to be the only endogenous inhibitor of aggrecanase activity in cartilage. Interestingly, ADAMTS-4 activity is blocked when the C-terminal domain of fibronectin binds the ADAMTS-4 spacer domain\(^{64}\). Enzyme inhibition following interactions between fibronectin and ADAMTS-4 could be relieved by C-terminal processing of ADAMTS-4 to displace fibronectin or fibronectin fragments. The concentrations of fibronectin and fibronectin fragments are increased in OA and rheumatoid synovial fluids.

#### Enhancers of ADAMTS-4 and -5 activity

There is potential for increased aggrecanase activity following interactions between ADAMTS enzymes and other cell surface or extracellular matrix molecules. For example, the interaction of ADAMTS-177 and ADAMTS-5\(^{78}\) with the matrix molecule fibrin-1 increases catalytic activities toward aggrecan or versican substrates, respectively. Fibrin-1 itself is not a substrate for these enzymes. The C-terminal thrombospondin domain of ADAMTS-1 interacts with fibrin-1, however, it is not clear which domain(s) of ADAMTS-5 might interact with fibrinulin. The epidermal growth factor (EGF)-like repeats in domain II of fibrin-1 interact specifically with the G3 domain of aggrecan\(^{79}\), and since aggrecan is typically with the G3 domain of aggregan\(^{56}\), and since aggrecan is a major ADAMTS-5 substrate, the interaction between fibrin-1 and ADAMTS-5 might provide a mechanism for localising ADAMTS-5 in close proximity with its substrate.

Recent \textit{in vitro} studies have shown that ADAMTS-5 interacts directly with syndecan-4, and mice deficient in syndecan-4 are protected against aggrecan loss and cartilage damage in a running wheel model of joint damage\(^{80}\). These results suggest that syndecan-4 might enhance ADAMTS-5 activity, however the mechanism is unknown. Perhaps syndecan-4 is required to tether ADAMTS-5 to the cell surface\(^{80}\), remain-anchored of the model in which syndecan-1 is thought to tether ADAMTS-4 to the cell surface\(^{80}\). High molecular weight HA is also thought to have a role in sequestering ADAMTS-5 in the pericellular matrix of OA cartilage\(^{16}\). No complexes between HA and ADAMTS-4, or HA and ADAMTS-9, have been detected in
OA cartilage. It is worth considering the possibility that HA, syndecan-4 and syndecan-1 enhance aggrecanase activity, not via their interactions with ADAMTS-4 or -5, but by virtue of anchoring these enzymes at their sites of action and limiting their ability to diffuse away. ADAMTS-4 and -5 are certainly diffusible enzymes; ADAMTS-4 and -5 synthesised by mouse synovial fibroblasts in co-cultures can penetrate and degrade aggrecan within femoral head cartilage explants. Together these findings suggest firstly, that there are mechanisms for helping to colocalise aggrecanase enzymes with aggrecan substrate and secondly, that ADAMTS activity detected in one joint tissue might be the product of ADAMTS mRNA produced in a different joint tissue.

**Perspectives and challenges for the future**

Aggrecanase research to date has been hampered by two major, technical limitations. The first is the inability to measure the products of aggrecanase activity in patient serum or urine. In this issue of *Osteoarthritis and Cartilage*, Thirunavukkarasu and colleagues describe successful reverse engineering of the original aggrecanase neoepitope antibody, BC31. The authors have generated antibody BC3-C2 with a 4-log improvement in affinity for the 394 TYV slewed peptide, BC3 and the specificity of this new antibody will enable the measurement of aggrecanase fragments in human serum and urine by ELISA assay and facilitate clinical testing of aggrecanase inhibitors. However BC3-C2 will not solve the thorny issue of identifying the human aggrecanase. The second technical limitation is that we have no methods at all for distinguishing the products of ADAMTS-4 activity, from the products of ADAMTS-5 activity; clearly, because the products are identical. At present this limitation seems insurmountable; we are stymied on this front! And to complicate matters further, there is a third aggrecan-degrading enzyme that cleaves at the well-recognised aggrecanase cleavage sites in the CS-rich region. This enzyme cleaves at the Glu1279Gly and Glu1467 Gly of aggrecanase sites in the CS-rich region of mouse aggrecan and its activity is detected in the ADAMTS-4/-5 double-deficient mouse.13 While this third aggrecanase is unlikely to be the aggrecanase in humans, it is nevertheless important that the enzyme be identified and characterised. If it has a role in OA pathology, then it will be necessary to inhibit this enzyme along with ADAMTS-4 and/or ADAMTS-5. Conversely, if it has a role in normal skeletal development and endochondral ossification, it will be important to ensure that aggrecanase inhibitors currently in development do not inhibit this enzyme.

Despite intensive research over the last decade we are still unable to positively identify the human aggrecanase and indeed the possibility remains that both ADAMTS-4 and ADAMTS-5 play equally important roles in aggrecanolyis. In this editorial we have highlighted the difficulties in finding definitive answers to this quandary; the regulation of ADAMTS-4 and -5 enzymes is complex and there are significant pre- and post-transcriptional events that contribute to the final level of enzyme activity. These are likely to be dynamic events that are themselves under tight control. The simplest and most abundant studies have examined regulation of ADAMTS-4 and -5 mRNA expression, comparing OA cartilage with normal cartilage, or the regulatory effects of cytokines, growth factors and other exogenous modulators, in vitro. However, we caution that no reliable conclusions about the source of the aggrecanase activity can be made from mRNA studies, either in vitro or in vivo; the number and range of processes (Fig. 1) that determine the final level of ADAMTS-4 or ADAMTS-5 activity is significant, and likely to overwhelm the effects of increased or decreased mRNA expression, making absolute conclusions about the contributing aggrecanase doubtful. Although ADAMTS-4 mRNA is reproducibly upregulated by cytokines in a number of experimental systems, it is important to counter that in the same or similar systems, ADAMTS-5 is often expressed at much higher levels than ADAMTS-4, and that ADAMTS-5 has a 30-fold greater intrinsic activity than ADAMTS-4. Accordingly, the dilemma of identifying the human aggrecanase remains.

**Author contributions**

AJF role: conception, drafting, critical revision and final approval of the manuscript.

RFM role: drafting, critical revision and final approval of the manuscript.

**Conflict of interest**

The authors have no conflicts of interest to declare.

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