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ADAMTS-5: THE STORY SO FAR

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Abstract

The recent discovery of ADAMTS-5 as the major aggrecanase in mouse cartilage came as a surprise. A great deal of research had focused on ADAMTS-4 and much less was known about the regulation, expression and activity of ADAMTS-5. Two years on, it is still not clear whether ADAMTS-4 or ADAMTS-5 is the major aggrecanase in human cartilage. On the one hand there are *in vitro* studies using siRNA, neutralising antibodies and immunoprecipitation with anti-ADAMTS antibodies that suggest a significant role for ADAMTS-4 in aggrecanolysis. On the other hand, ADAMTS-5 (but not ADAMTS-4)-deficient mice are protected from cartilage erosion in models of experimental arthritis, and recombinant human ADAMTS-5 is substantially more active than ADAMTS-4. The activity of both enzymes is modulated by C-terminal processing, which occurs naturally *in vivo*. The most interesting finding to emerge from our comparison of ADAMTS-5 and ADAMTS-4 is that in terms of gene regulation, these two enzymes are the antitheses of each other. In most cases, ADAMTS-5 is constitutively expressed in human chondrocytes and synovial fibroblasts, whereas ADAMTS-4 expression is induced by proinflammatory cytokines. This paper reviews the data on ADAMTS-5 so far. It represents a snapshot in time of a field that is fast-moving and very exciting.

Key Words: Aggrecanase, ADAMTS-5, ADAMTS-4, aggrecanolysis, metalloproteinase, cartilage, arthritis.

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List of abbreviations

ADAM	A disintegrin and metalloproteinase
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
CS	Chondroitin sulphate
CS-2	Second chondroitin sulphate domain
G1	First (N-terminal) globular domain of aggrecan
G2	Second (N-terminal) globular domain of aggrecan
IGD	Interglobular domain of aggrecan
KS	Keratan sulphate
MMP	Matrix metalloproteinase
TIMP	Tissue inhibitor of matrix metalloproteinase
TS	Thrombospondin
α2M	α2-Macroglobulin

Introduction

ADAMTS-5 is an important aggrecanase that cleaves at key sites in the aggrecan core protein, in healthy and diseased cartilage. By the mid 1980s it was apparent that during aggrecanolysis, the aggrecan core protein was cleaved close to the G1 globular domain by an unknown "aggrecanase", to release the majority of the aggrecan molecule from the cartilage matrix (Campbell *et al.*, 1986; Ratcliffe *et al.*, 1986). These early results were from explant culture experiments and came at the same time that the aggrecan G1 and G2 globular domains were identified by rotary shadowing electron microscopy (Paulsson *et al.*, 1987) and peptide sequencing (Perkins *et al.*, 1989). In 1991 a novel glutamyl endopeptidase activity was identified; it cleaved the aggrecan core protein at several sites, but most notably at the $E^{373}\downarrow^{374}A$ bond in the aggrecan interglobular domain (Ilic *et al.*, 1992; Loulakis *et al.*, 1992; Sandy *et al.*, 1991). This was an exciting discovery because aggrecan fragments resulting from cleavage at the $E^{373}\downarrow^{374}A$ site were simultaneously identified in synovial fluids from osteoarthritic, joint injury and inflammatory joint disease patients (Lohmander *et al.*, 1993; Sandy *et al.*, 1992). The findings prompted vigorous research efforts to identify the aggrecanase, and in 1999, after a few false starts that revealed aggrecanase activity in MMP-8 (Arner *et al.*, 1997; Fosang *et al.*, 1994) and MMP-14 (Büttner *et al.*, 1998), an enzyme cloned from bovine articular cartilage was revealed as aggrecanase-1 (ADAMTS-4) (Tortorella *et al.*, 1999). This finding was closely followed by the identification of aggrecanase-2 (ADAMTS-5) (Abbaszade *et al.*, 1999). Because ADAMTS-4 is upregulated by inflammatory cytokines in joint tissues (Bau *et al.*, 2002; Curtis *et al.*,

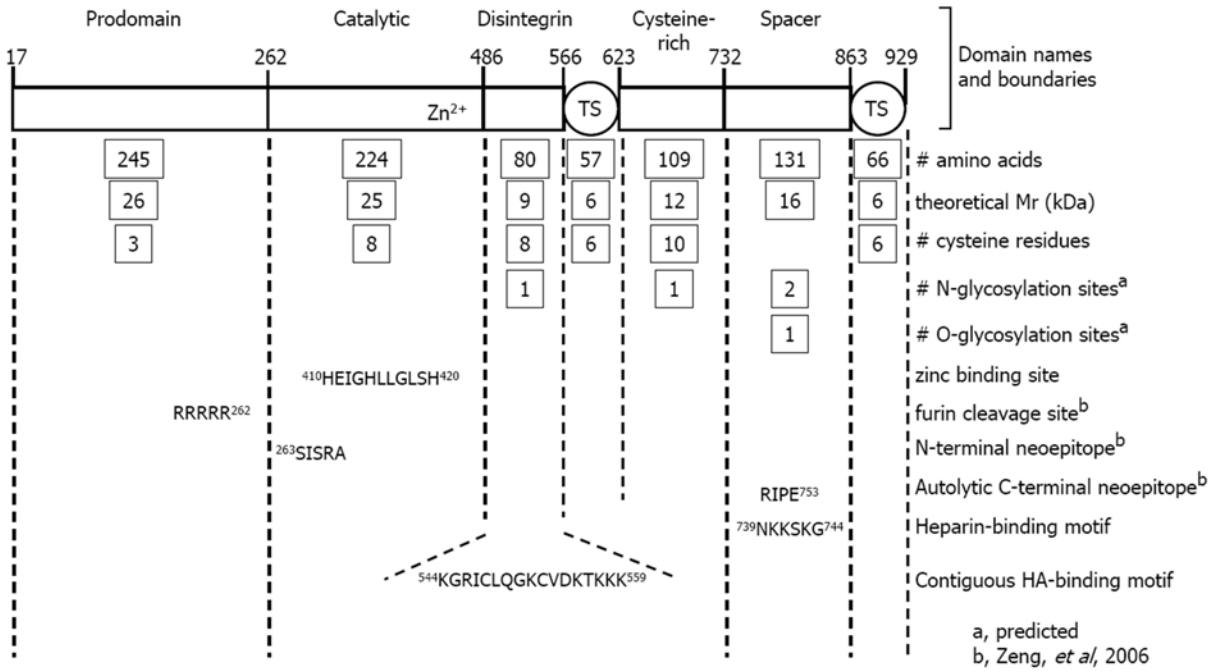

Figure 1. The domain structure and features of ADAMTS-5. The schematic (adapted from Gendron *et al*. (2007)) shows the composition of the structural motifs in human ADAMTS-5. The theoretical Mr and the number of amino acids, cysteine residues and glycosylation sites are shown for each domain. Sites for furin cleavage, heparin binding and HA binding are shown. Human and mouse ADAMTS-5 are 89% identical at the amino acid level. They are located on chromosomes 21 and 16 respectively (Hurskainen *et al*., 1999).

2000; Koshy *et al*., 2002; Little *et al*., 2002; Moulharat *et al*., 2004; Tortorella *et al*., 2001; Yamanishi *et al*., 2002), much work on the characterisation, activation and regulation of ADAMTS-4 followed. It was therefore a surprise when, in 2005, ADAMTS-5 was revealed as the principal aggrecanase in mouse cartilage (Glasson *et al*., 2005; Stanton *et al*., 2005); whether it is the principal aggrecanase in human cartilage remains unclear. This paper reviews the literature on ADAMTS-5, focussing on its role in joint tissues. Other excellent reviews on ADAMTS proteins can be found elsewhere (Flannery, 2006; Jones and Riley, 2005; Kaushal and Shah, 2000; Nagase and Kashiwagi, 2003; Porter *et al*., 2005).

Structural Domains of ADAMTS-5

The ADAMTS proteins are a family of zinc-dependent enzymes within the metzincin family of metalloproteinases (Bode *et al*., 1995; Gomis-Ruth, 2003; Hooper, 1994; Tang, 2001). ADAMTS proteins comprise an N-terminal prodomain, a catalytic domain, a disintegrin domain, one or more thrombospondin (TS) motifs, a cysteine-rich domain and a spacer domain of variable length (Jones and Riley, 2005; Porter *et al*., 2005). ADAMTS-5 (Fig. 1) is one of the shorter members of the family, having only two TS motifs, and ADAMTS-4 is the shortest member with one TS motif.

Although some ADAMTS enzymes including ADAMTS-7, -9 and -13, are active with their prodomains intact (Koo *et al*., 2007; Majerus *et al*., 2003; Somerville *et al*., 2004), full length ADAMTS-5 is thought to be an inactive zymogen that is activated by removal of its prodomain by furin or furin-like enzymes. The prodomain of human and mouse ADAMTS-5 contains three potential furin recognition sites (Hurskainen *et al*., 1999) within a multibasic sequence ^{258}RRRRR^{262} (Fig. 1) and although the predicted activating cleavage at R^{262}↓^{263}S is detected in an *in vitro* expression system (Zeng *et al*., 2006), activation by cleavage at this site has not been confirmed *in vivo*. Whether proprotein convertases other than furin can activate ADAMTS-5 and whether removal of the prodomain is required for its secretion has yet to be resolved (Wang *et al*., 2004a).

The ADAMTS-5 active site (**H**EIG**H**LLGLS**H)** conforms to the consensus sequence, **H**EBx**H**xBGBx**H**, where **H** (histidine) represents strictly conserved zinc ligands and B represents bulky apolar residues. The conserved methionine residue located 19 amino acids C-terminal to the third histidine, forms the 'met-turn' characteristic of the metzincin family (Gomis-Ruth, 2003). The crystal structure of the ADAMTS-5 catalytic domain has been determined to 0.14nm resolution in the presence of a pan metalloprotease inhibitor (Shieh *et al*., 2007). The structure reveals that in addition to the zinc binding site, ADAMTS-5 contains two calcium binding sites critical for maintaining the structural integrity of the protein. One site is occupied by a single calcium ion and the other contains two calcium ions in a calcium cluster, not previously reported for other metalloenzymes (Shieh *et al*., 2007). The overall fold of the catalytic domain resembles other metalloproteinases, but the shape of the substrate-binding site is unique. This unique binding site suggests that ADAMTS-5 recognises different substrate motifs than MMP, ADAM and other ADAMTS enzymes.

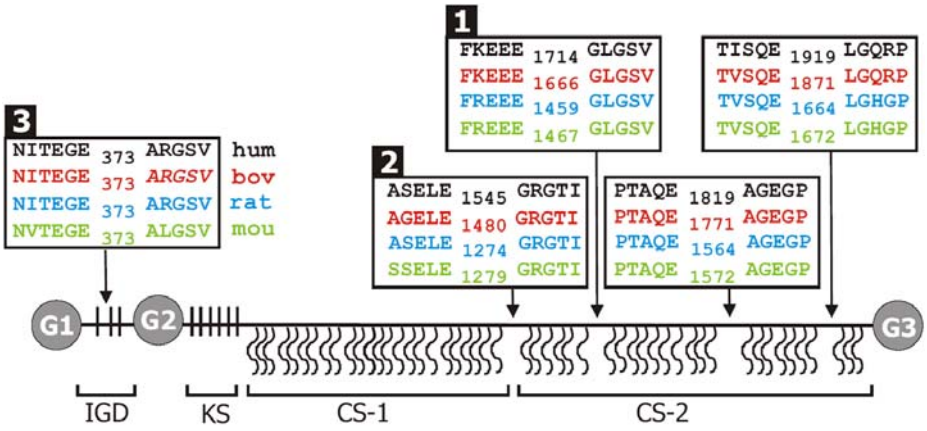

Figure2. Aggrecanase cleavage sites in the aggrecan core protein. The schematic shows the aggrecan core protein with globular G1, G2 and G3 domains. The core protein is substituted with CS (wavy lines) and KS (straight lines) chains. The KS-attachment region between the G2 and CS-1 domains of human and bovine aggrecan is absent in rat and mouse aggrecan. Some KS is present in the mouse IGD (AJ Fosang, unpublished data). Aligned aggrecanase cleavage sites are shown for human (bold), bovine (bold, italics), rat and mouse (italics). The numbered flags above the boxed sequences denote the preferred order of cleavage (Sandy *et al*., 2000; Sandy and Verscharen, 2001; Tortorella *et al*., 2002; Tortorella *et al*., 2000).

The unique binding site also increases the likely success of developing inhibitors that are specific for ADAMTS-5 (Bursavich *et al*., 2007b; Gilbert *et al*., 2007).

The catalytic domain is followed by the disintegrin domain (Fig. 1). The disintegrin domain lacks the classical integrin binding sequence (RGD) (Ruoslahti and Pierschbacher, 1986) and does not interact with integrins. The first TS module located C-terminal to the disintegrin domain is highly conserved within the ADAMTS family, including the CSR(T/S)C pentapeptide and conservation of selected glycine, tryptophan and proline residues (Hurskainen *et al*., 1999). The cysteine rich-region contains ten cysteine residues that are also highly conserved in ADAMTS enzymes. The ADAMTS-5 spacer region has no distinguishing structural features. It has no cysteine residues, which possibly makes it more flexible than other regions of the molecule. The second TS module located at the C-terminus is less conserved among the ADAMTS enzymes than the first TS module, even though the spacing and number of cysteine residues is strictly conserved (Hurskainen *et al*., 1999). ADAMTS-5 is predicted to contain four *N*-glycosylation sites (Abbaszade *et al*., 1999); one located in the disintegrin domain, one in the cysteine-rich region and two in the spacer domain, and they are conserved in mouse and human. One *O*-linked glycosylation site is also predicted in the spacer domain (Gendron *et al*., 2007) (Fig. 1).

Aggrecanase Activity and the Aggrecan Substrate

Aggrecan is the major proteoglycan in cartilage and it gives this tissue its unique capacity to bear load and resist compression. In arthritic cartilage, aggrecan and type II collagen are degraded, then lost, causing the tissue to become thin and mechanically weak. Because aggrecan loss from cartilage is an early (Pond and Nuki, 1973) and reversible (Thomas, 1956) event, *in vitro* and *in vivo*, and because aggrecan may have a protective role in preventing collagen degradation (Pratta *et al*., 2003b), a great deal of research has focused on aggrecanolysis at the molecular level.

Aggrecan has two globular domains, G1 and G2 at the N-terminus, and a third globular domain, G3 at the C-terminus (Fig. 2). An extended sequence between the G2 and G3 domains is heavily decorated with chondroitin sulphate (CS) and keratan sulphate (KS) chains, organised into distinct chondroitin sulphate-1 (CS-1) and chondroitin sulphate-2 (CS-2) domains and a KS-rich region. An interglobular domain (IGD) of approximately 150 amino acids separates G1 from G2 and, *in vitro*, can be cleaved by almost any proteinase. However, *in vivo*, the majority of fragments are products of aggrecanase or MMP activity; aggrecanase fragments predominate, but MMPs are also involved (Chambers *et al*., 2001; Fosang *et al*., 1996; Lark *et al*., 1997; Struglics *et al*., 2006a; Struglics *et al*., 2006b; van Meurs *et al*., 1999a), particularly in late stage disease (van Meurs *et al*., 1999b; van Meurs *et al*., 1998; van Meurs *et al*., 1999c). The cysteine proteinase, m-calpain, also produces specific aggrecan fragments in human cartilage *in vivo* after digestion at VPGVA^{709}↓^{710}AVPVE in the KS-rich region (Maehara *et al*., 2007).

Proteolysis in the IGD releases the entire CS-rich region essential for the biomechanical properties of aggrecan, and is therefore the most detrimental for cartilage function. Cleavage at the E^{373}↓^{374}A bond in the IGD is the signature activity of the aggrecanases and is widely reported in humans and in animals as an activity marker. However, cleavage at E^{373}↓^{374}A is not the preferred action of these enzymes. *In vitro,* recombinant ADAMTS-4 and -5 preferentially cleave aggrecan in the CS-2 domain (Tortorella *et al*., 2002; Tortorella *et al*., 2000b). The two most preferred cleavage sites in bovine aggrecan are at KEEE^{1667}↓^{1668}GLGS, followed by GELE^{1480}↓^{1481}GRGT. Thereafter, cleavage occurs at NITEGE^{373}↓^{374}ARGS in the

IGD and at TAQE^{1771}↓^{1772}AGEG and VSQE^{1871}↓^{1872}LGQR in the CS-2 region. A similar hierarchy of cleavage preferences is shown by native aggrecanases in cell culture (Sandy *et al.*, 2000; Sandy and Verscharen, 2001). These cleavage sites are highly conserved in humans, mice and rats (Fig. 2). Although it has not been proved experimentally, the NVTEEEARG sequence in the IGD of chicken aggrecan might represent an aggrecanase cleavage site (Hering *et al.*, 1997; Li *et al.*, 1993). Four TSQEARG motifs in the chicken CS-2 domain might also represent aggrecanase sites; there is one corresponding in position and sequence to the human PTAQE^{1819}↓^{1820}AGE site, and three additional TSQEARG motifs more C-terminal, with no similarity to sites in other species.

The evidence that aggrecanases show differential activities with preferences for separate regions of the core protein is not new. The earliest indication that this might be the case came from studies showing that glucosamine inhibits aggrecanase cleavage in the CS-rich region more efficiently than it inhibits cleavage in the IGD (Sandy *et al.*, 1998), and more recently from studies showing that there are differential aggrecanase activities with preferences for separate regions of the core protein (East *et al.*, 2007b; Gao *et al.*, 2002; Gendron *et al.*, 2007; Kashiwagi *et al.*, 2004). Ideally, a measure of each different activity is needed to obtain an accurate readout of total aggrecanase activity. For example, total aggrecanase activity can be measured *in vitro* by loss of aggrecan using a dye-binding assay, by Western blotting with antibody 2B6 that detects all CS-containing fragments, or by ^{35}S-radiolabelling that detects all newly-synthesised CS and KS-containing fragments. Cleavage in the IGD, and in the CS-2 domain, can be detected with highly specific neoepitope antibodies. In mouse cartilage there is a positive correlation between total aggrecanase activity and IGD-cleavage with respect to ADAMTS-5 (East *et al.*, 2007b). However, for recombinant full length ADAMTS-4 there is a negative correlation between total aggrecanase activity and IGD-cleavage, and a positive correlation between total aggrecanase activity and CS-2-cleavage (Kashiwagi *et al.*, 2004) (Table 1).

Neoepitope Antibodies Identify ADAMTS Activity

Most aggrecanolysis studies have relied on neoepitope antibodies to detect specific aggrecan fragments as evidence of enzyme activity. Neoepitope antibodies detect epitopes at the newly-created N- or C-terminus of aggrecan fragments, but fail to recognise the same sequence of amino acids present in intact or undigested aggrecan. Aggrecan neoepitope antibodies recognising the products of aggrecanase (Billington *et al.*, 1998; East *et al.*, 2007b; Hughes *et al.*, 1995; Lark *et al.*, 1995a; Mercuri e*t al.*, 1999; Sandy *et al.*, 2000; Sztrolovics *et al.*, 1997b), MMP (Büttner *et al.*, 1998; Fosang *et al.*, 1995; Hughes *et al.*, 1995; Lark *et al.*, 1995b; Lee *et al.*, 1998; Mercuri *et al.*, 1999; Sztrolovics *et al.*, 1997a) and calpain (Oshita *et al.*, 2004) cleavage have been described. Neoepitope antibodies are specific for the products of a single proteolytic event, but do not distinguish between enzymes with identical activities. The aggrecan neoepitope antibodies do not, therefore, distinguish between the activities of ADAMTS-4, ADAMTS-5 or other potential aggrecanases including ADAMTS-1, -8, -9, -15 (Collins-Racie *et al.*, 2004; Demircan *et al.*, 2005; Kuno *et al.*, 2000; Somerville *et al.*, 2003; Zeng *et al.*, 2006) or an uncharacterised membrane aggrecanase (Billington *et al.*, 1998; Hui *et al.*, 2005). Nevertheless, neoepitope antibodies have been instrumental in:

- studies seeking to distinguish MMP-driven aggrecan loss from aggrecanase-driven aggrecan loss (Chambers *et al.*, 2001; Fosang *et al.*, 1996; Lark *et al.*, 1997; Struglics *et al.*, 2006a; Struglics *et al.*, 2006b; van Meurs *et al.*, 1999a)

- the discovery of ADAMTS-4 and -5 (Abbaszade *et al.*, 1999; Tortorella *et al.*, 1999) which relied upon the anti-^{374}ARGS antibody BC-3 (Hughes *et al.*, 1995) to track enzyme activity during purification

- the discovery of a third cartilage aggrecanase, other than ADAMTS-4 and -5 (Rogerson *et al.*, 2008), based on the upregulation of FREEE^{1467} neoepitope by retinoic acid in ADAMTS-4/-5-double deficient mice.

One aggrecan fragment can be the product of two distinct enzyme activities. For example, human OA cartilage and synovial fluids contain fragments with MMP-derived ^{342}FFGVG N-termini and aggrecanase-derived KEEE^{1714} or SELE^{1545} C-termini (Struglics *et al.*, 2006b). Similarly, large fragments (~250kDa) with KEEE^{1666}/FREEE^{1467} C-termini but lacking aggrecanase-derived N-termini have been detected in bovine and mouse cartilage explants respectively (East *et al.*, 2007b; Tortorella *et al.*, 2001) suggesting that these fragments are also the products of an aggrecanase at one end, and a non-aggrecanase, possibly an MMP[1], at the other. Identifying the enzyme families responsible for creating the fragments described above is not straightforward because there are exceptions to the rules for aggrecan neoepitope specificity. At high concentrations *in vitro*, MMP-8 (Fosang *et al.*, 1994) and MMP-14 (Büttner *et al.*, 1998) cleave at the E^{373}↓^{374}A aggrecanase site, and ADAMTS-1 (Rodriguez-Manzaneque *et al.*, 2002), ADAMTS-4 (Westling *et al.*, 2002) and cathepsin B (Mort *et al.*, 1998) can cleave at the N^{341}↓^{342}F MMP site.

Aggrecanolysis in cartilage explant cultures is highly dynamic and there is a hierarchy of aggrecanase cleavage preferences in the CS-2 domain. In the mouse, cleavage at FREEE^{1467}↓ ^{1468}GLGS precedes cleavage at SELE^{1279}↓^{1280}GRGT, and FREEE^{1467} fragments are readily converted to SELE^{1279} fragments. In explant cultures the level of FREEE^{1467} in mouse cartilage is often inversely proportional to the level of SELE^{1279} in the medium, since FREEE^{1467} is a transient intermediate (Rogerson *et al.*, 2008). FREEE^{1468} epitope, on its own, is therefore a poor indicator of aggrecanolysis. An absence of FREEE^{1467}

[1] We have found that the ^{342}FFGVG neoepitope is labile in mouse explant cultures [East *et al.,* 2007] consistent with a number of studies reporting immunodetection of DIPEN^{341} (or DIPES^{341} in the bovine) neoepitope without detection of the corresponding ^{342}FFGVG neoepitope

Table 1. Modulation of aggrecanase activity by C-terminal ancillary domain deletions.

	ADAMTS-5	ADAMTS-4
Full length	Maximum IGD-activity[C]* Maximum CS-2-activity[C]*	Minimal IGD-activity [C,E &] Maximum IGD-activity[H] Maximum CS-2-activity[C,E&]
Δ C-terminal TS	Reduced IGD-activity[C]* Maximum CS-2-activity[C]*	N/A
Δ spacer domain	Reduced IGD-activity[C] * Reduced CS-2-activity[C]*	Maximum IGD-activity[C,E,G,H] Maximum CS-2-activity[C,E]
Δ cysteine-rich domain	Reduced IGD-activity[C]* Reduced CS-2-activity[C]* IGD and CS-2-activity present[D]	Reduced IGD-activity[C,E&] Reduced CS-activity[C,E&]
Δ TS	No IGD or CS-2-activity[C] Minimal CS-2-activity[D]	No IGD or CS-2-activity[E,F]
Δ disintegrin	No IGD or CS-2-activity[C]	

*relative to full length ADAMTS-5

&relative to maximum ADAMTS-4 activity in the truncate lacking the C-terminal spacer domain

C (Gendron *et al.*, 2007)(CS-2-activity, cleavage at GELE^{1480}↓^{1481}GRGT)

D (Zeng *et al.*, 2006)(CS-2-activity, cleavage at TAQE^{1771}↓^{1772}AGEG)

E (Kashiwagi *et al.*, 2004)(CS-2-activity, cleavage at GELE^{1480}↓^{1481}GRGT)

F (Tortorella *et al.*, 2000a)(CS-2-activity, cleavage at GELE^{1480}↓^{1481}GRGT and KEEE^{1666}↓^{1667}GLGS)

G (Gao *et al.*, 2002)

H (Hashimoto *et al.*, 2004)

epitope could either be due to an absence of aggrecanolysis (the fragment is not formed), or abundant aggrecanolysis which converts the entire fragment to SELE^{1279} (see Fig 2). Thus, more than one neoepitope is needed to characterise the full extent of aggrecanolysis. Similarly, for *in vitro* studies, analysis of both cartilage and medium compartments is needed to give a complete picture of aggrecanase activity.

Neoepitope antibodies recognising the N-terminus of ADAMTS-4 or ADAMTS-5 following cleavage by furin-like enzymes have also been made (Mort *et al.*, 2003; Powell *et al.*, 2007). Unlike the aggrecan neoepitopes that are markers of enzyme activity and do not distinguish between ADAMTS-4 and ADAMTS-5, the ADAMTS neoepitopes detect the enzymes directly, and because the sequences are distinctly different, antibodies raised against the N-termini of the active enzymes readily distinguish between them. Anti-^{213}FASLS antibodies recognising active ADAMTS-4 have been used to immunolocalise ADAMTS-4 protein in the tibial growth plate of young rats (Mort *et al.*, 2003). Anti-^{263}SISRA antibodies on the other hand failed to detect active ADAMTS-5 in the same rat growth plates, suggesting either that ADAMTS-5 has a minimal role in resorbing growth plate aggrecan, or that the levels of ADAMTS-5 expressed in cartilage are exceptionally small, as proposed by others (Gendron *et al.*, 2007; Glasson *et al.*, 2005; Hurskainen *et al.*, 1999).

Functional Roles for the C-terminal Ancillary Domains of ADAMTS-5

Ancillary domains that modulate the affinity of proteinases for their substrates are called exosites (Overall, 2002). The disintegrin, TS, cysteine-rich and spacer domains of ADAMTS-5 are ancillary domains with potential exosite functions. Exosites can broaden substrate specificity by providing additional substrate binding sites in a region remote from the active site, and can increase substrate-binding affinity, impacting on Km. There is a distinction between (a) a true exosite that modulates enzyme activity as a consequence of ligand binding, and (b) ligand binding that has no influence on enzyme activity, and hence no

exosite function. For example, the spacer and C-terminal TS module of ADAMTS-5 bind heparin, but the binding does not confer increased or decreased aggrecanase activity (Zeng et al., 2006); these heparin-binding sites are therefore not true exosites. Several ADAMTS enzymes have evolved C-terminal exosites that bind glycosaminoglycans (Flannery *et al*., 2002; Kuno and Matsushima, 1998; Tortorella *et al*., 2000a) and docking of ADAMTS enzymes onto glycosaminoglycans might help orientate the active site with the scissile bond in the substrate. Exosites are not restricted to ancillary domains; they can also be found in catalytic domains but in these situations they do not form part of the active site.

ADAMTS-5 in normal human articular cartilage localises around cells in the superficial zone, and around clonal cell clusters in OA cartilage (Plaas *et al*., 2007). The pericellular localisation is thought to occur *via* binding to hyaluronan (HA) and interactions between ADAMTS-5 and HA complexes in cartilage extracts can be disrupted by treatment with *Streptomyces* hyaluronidase (Plaas *et al*., 2007). The interaction between ADAMTS-5 and HA might be mediated through the disintegrin domain which contains a pair of contiguous HA binding motifs (BX7B) (Plaas *et al*., 2007; Yang *et al*., 1994) (Fig. 1). A single heparin-binding motif in the spacer domain might contribute further to the pericellular localisation of ADAMTS-5. Full length recombinant ADAMTS-5 expressed in the HTB-94 human chondrosarcoma cell line also localises at the cell surface and in the extracellular matrix. However, in this case, the cysteine-rich domain appears to be essential for the binding and localisation because sequential deletion of the C-terminal TS and spacer domains had no effect on ADAMTS-5 localisation in the matrix, whereas further deletion of the cysteine-rich domain released ADAMTS-5 to the medium (Gendron *et al*., 2007). Notably, the spacer domain is responsible for ADAMTS-4 binding to extracellular matrix (Flannery *et al*., 2002; Gao *et al*., 2002; Hashimoto *et al*., 2004; Kashiwagi *et al*., 2004), possibly through spacer-domain interactions with fibronectin (Hashimoto *et al*., 2004). It is not known whether there are similar interactions between fibronectin and the spacer domain of ADAMTS-5. Both ADAMTS-4 and ADAMTS-5 have weak activity against fibronectin (Gendron *et al*., 2007; Hashimoto *et al*., 2004).

It is interesting that the spacer domain has distinctly different roles in regulating the catalytic activities of ADAMTS-5 and ADAMTS-4. Deletion of the spacer domain increases (Gao *et al*., 2002; Kashiwagi *et al*., 2004) or maintains (Hashimoto *et al*., 2004) the IGD-activity of ADAMTS-4, but decreases the IGD-activity of ADAMTS-5 by four-fold (Gendron *et al*., 2007) (Table 1). In addition, deletion of the spacer domain has no effect on the CS-2 activity of ADAMTS-4 (Gendron *et al*., 2007; Kashiwagi *et al*., 2004), but reduces the CS-2 cleaving activity of ADAMTS-5 by 100-fold (Gendron *et al*., 2007). Thus, the aggrecan-degrading activity of recombinant ADAMTS-5 appears to be more sensitive to C-terminal truncations than ADAMTS-4 (Table 1); not only is the CS-2 activity of ADAMTS-5 reduced 100 fold by deletion of the spacer domain, it is reduced a further 20 fold following deletion of the cysteine-rich region (Gendron *et al*., 2007). ADAMTS-4 activity in the CS-2 domain, in contrast, is unaffected by deletion of the spacer domain and only minimally reduced by deletion of the cysteine-rich domain (Gendron *et al*., 2007; Kashiwagi *et al*., 2004). Autolytic processing to remove most of the ADAMTS-5 spacer domain occurs at $RIPE^{753}\downarrow^{754}GATH$ *in vitro*, creating a C-terminal $RIPE^{753}$ neoepitope (Fig. 1) (Zeng *et al*., 2006). The minimum domain composition for a molecule containing aggrecan-degrading activity, in both ADAMTS-4 and ADAMTS-5, is the catalytic domain, the disintegrin domain and first TS module. Recombinant truncates lacking the TS module adjacent to the disintegrin domain are inactive against aggrecan (Gendron *et al*., 2007; Kashiwagi *et al*., 2004; Tortorella *et al*., 2002; Zeng *et al*., 2006). Alternative splicing of ADAMTS-4 provides another potential means of modulating enzyme activity in different tissues (Wainwright *et al*., 2006). There is no report that ADAMTS-5 is alternatively spliced.

C-terminal processing to remove exosites that positively or negatively regulate activity might be a common mechanism for the post-translational regulation of this family of proteinases. ADAMTS-1 and ADAMTS-4 activities are increased by post-translational processing (Gao *et al*., 2002; Kashiwagi *et al*., 2004; Kuno *et al*., 2000; Pratta *et al*., 2003a) whereas ADAMTS-5 activity is decreased by post-translational processing, *in vivo* (Malfait *et al*., 2002; Plaas *et al*., 2007; Vankemmelbeke *et al*., 2001; Yamanishi *et al*., 2002) and *in vitro* (Zeng *et al*., 2006) (Table 1). These observations highlight the point that exosites might broaden substrate specificity, but equally, they might restrict it. MT4-MMP is involved in the further activation and processing of ADAMTS-4 (Gao *et al*., 2004), but there is no evidence that MT4-MMP has a role in processing ADAMTS-5. Aggrecanolysis in cultured chondrocytes isolated from wildtype and MT4-MMP null mice is indistinguishable, suggesting that in the mouse, MT4-MMP does not modulate ADAMTS-5 activity (Stewart *et al*., 2006).

ADAMTS-5-Deficient Mice are Phenotypically Normal

ADAMTS-5 is expressed in many tissues (Abbaszade *et al*., 1999; Glasson *et al*., 2005; Stanton *et al*., 2005), yet mice deficient in ADAMTS-5 catalytic activity have no apparent phenotype (Glasson *et al*., 2005; Stanton *et al*., 2005). It is perhaps surprising that the ADAMTS-5-deficient mice do not have skeletal or growth plate abnormalities. In skeletogenesis, mesenchymal stem cells increase their expression of ADAMTS-5 at late stage chondrogenesis (Djouad *et al*., 2007), and effectors of growth plate organisation and endochondral ossification, such as the Wnt/β-catenin signalling pathway (Tamamura *et al*., 2005) and the thyroid hormone tri-iodothyronine T3 (Makihira *et al*., 2003), also increase the expression of ADAMTS-5 suggesting it has a role in skeletogenesis. However, there is no growth plate phenotype at any age in ADAMTS-5-deficient mice. This finding could be interpreted in several ways. Aggrecan resorption in the growth plate could be driven by proteinases other than

ADAMTS-5; for example, ADAMTS-4 could be involved given the presence of anti-^{213}FASLS neoepitopes in rat tibial growth plates (Mort *et al*., 2003). An aggrecanase other than ADAMTS-4 or -5 could be involved, and indeed cartilage explants from mice deficient in both ADAMTS-4 and ADAMTS-5 activity generate aggrecanase neoepitopes (Rogerson *et al*., 2008) suggesting that an aggrecanase, yet to be identified (Billington *et al.*, 1998; Demircan *et al*., 2005; East *et al*., 2007b), could compensate for the loss of ADAMTS-4 and -5 activity. Alternatively, glycosidases such as hyaluronidase could have more prominent roles in resorbing the aggrecan aggregate in growth cartilage (Durigova *et al*., 2007; Sztrolovics *et al*., 2002). The latter hypothesis is supported by data showing a lack of skeletal abnormality in ADAMTS-4-deficient mice (Glasson *et al*., 2004; Stanton *et al*., 2005), ADAMTS-5-deficient mice (Glasson *et al*., 2005; Stanton *et al*., 2005), ADAMTS-4/-5 double-deficient mice (Ilic *et al*., 2007; Majumdar *et al.*, 2007; Rogerson *et al*., 2008), and aggrecan knockin mice resistant to aggrecanase cleavage in the IGD (Little *et al*., 2007). Aggrecan knockin mice resistant to MMP cleavage in the IGD are also skeletally normal (Little *et al*., 2005).

One unresolved issue with the ADAMTS-5-deficient mice (Glasson *et al*., 2005; Stanton *et al*., 2005) is that although the in-frame deletion generates shortened transcripts that are not removed by nonsense-mediated decay, to date it has been impossible to confirm that mutant ADAMTS-5, lacking 56 amino acids, is translated and survives in tissues. Attempts at Western blots of mouse cartilage extracts with home-made and commercial antibodies from a number of independent laboratories have been futile. This could be due to the low level of ADAMTS-5 antigen present in cartilage (Gendron *et al*., 2007), and indeed in most tissues after the pre-implantation stage in embryos (Hurskainen *et al*., 1999). On the other hand, Wyeth researchers report that, after cloning and attempts to express the identical human version of the exon 3-deleted mouse construct in CHO cells, no mutant ADAMTS-5 protein was detected in media or cell extracts, even though recombinant wildtype ADAMTS-5 protein was secreted by CHO cells (Glasson *et al*., 2005). This is an intriguing result. Ongoing collaborative studies in our laboratory comparing ADAMTS-5-deficient mice lacking the catalytic domain, with ADAMTS-5 null mice lacking the entire gene, might provide some answers. These studies might also reveal exosite functions in ADAMTS-5 ancillary domains.

Regulation of ADAMTS-5 Expression

Because of its predicted involvement in human arthritis, many studies have examined ADAMTS-5 regulation by catabolic agents such as IL-1α and β, oncostatin M (OSM), TNFα and retinoic acid (Table 2). ADAMTS-5 is expressed in both a constitutive and an inducible manner, depending on the species, cell type or experimental conditions. ADAMTS-5 is constitutively expressed in human chondrocytes and synovial fibroblasts (Table 2). In contrast, whereas its expression appears to be constitutive in mouse and bovine synovial fibroblasts, ADAMTS-5 expression in mouse and bovine chondrocytes is consistently induced by inflammatory cytokines and retinoic acid. Discrepancies in the literature might reflect differences in cell *versus* explant culture, primary cell *versus* passaged or transformed cell, variations in culture conditions including the presence or absence of serum, cell culture in two *versus* three dimensions, or the health status of human tissue at the time of harvest. In many cases, ADAMTS-5 regulation is distinctly different from ADAMTS-4 (Table 2). This could have important implications, and complications, for therapeutic strategies, should it be necessary to inhibit both ADAMTS-4 and ADAMTS-5 to manage human arthritis. For example, ADAMTS-4 expression by human synoviocytes is significantly inhibited by the TNFα blocker etanercept and an anti-IL-1β neutralising antibody, whereas the expression of ADAMTS-5 in the same cells was not affected by neutralisation of IL-1β and/or TNFα (Bondeson *et al*., 2006).

Molecules other than cytokines can also modulate the expression of ADAMTS-5 *in vitro*, though in general the effects are modest. Adenovirus infection of mouse synovium with connective tissue growth factor induces ADAMTS-5 expression and synovial fibrosis (Blaney Davidson *et al*., 2006). Galectin-3, a soluble lectin increased in human OA cartilage (Guevremont *et al.*, 2004), weakly stimulates ADAMTS-5 expression in human OA chondrocytes (Janelle-Montcalm *et al*., 2007). Conversely, nutraceuticals such as glucosamine and chondroitin sulphate at biologically relevant concentrations decrease IL-1-induced ADAMTS-5 expression in bovine cartilage explants (Chan *et al*., 2006). In addition to the anti-inflammatory properties of n-3 fatty acids, incorporation of this dietary compound into chondrocyte membranes *in vitro* decreases expression of IL-1-induced ADAMTS-5 (Curtis *et al*., 2000). Catechin gallate esters found in green tea, potently inhibit ADAMTS-5 activity with an IC_{50} of 100-150 nM (Vankemmelbeke *et al*., 2003). Pentosan polysulphate, used for the management of advancing arthritis in veterinary settings, also inhibits IL-1 and retinoic acid-induced aggrecan loss *in vitro* (Munteanu *et al*., 2000; Munteanu *et al*., 2002), although ADAMTS-5 was not measured in these experiments.

ADAMTS-5 gene expression can be regulated by epigenetic modifications including histone acetylation and deacetylation and changes in the methylation status of CpG sites in the gene promoter. Histone acetylation and deacetylation is generally associated with transcriptional activation and repression, respectively, although the inverse relationships have also been reported (Bernstein *et al*., 2000; Nair *et al*., 2001). In SW1353 chondrosarcoma cells and primary human chondrocytes, histone deacetylase inhibitors potently inhibit IL-1α/OSM-induced cartilage degradation and suppress the IL-1α/OSM-induced upregulation of ADAMTS-5 (Young *et al*., 2005). Methylation of CpG islands is associated with gene silencing. Although there are no reported changes in methylation of the ADAMTS-5 gene promoter in cartilage, ADAMTS-4 expression in OA cartilage is regulated

Table 2. Regulation of ADAMTS-5 mRNA expression by pro-catabolic agents.

Species	Cell type	constitutive expression	mRNA expression increased	mRNA expression unchanged by	ADAMTS-4 expression different from ADAMTS-5?	Reference
Human	OA synovial fibroblast[f]	Yes		IL-1, TNF	Yes. Increased by IL-1 and TNFα.	(Bondeson *et al.*, 2007)
Human	OA synovial fibroblasts[h]	Yes		anti-IL-1β, Enbrel	Yes. Decreased by Enbrel ± anti-IL-1β	(Bondeson *et al.*, 2006)
Human	synovial fibroblasts[c]	Yes		IL-1β, TNFα, TGFβ	Yes. Increased 13-fold by TGFβ	(Yamanishi *et al.*, 2002)
Human	synovial fibroblasts[l]	Yes				(Blaney Davidson *et al.*, 2006)
Bovine	synovium[d]	Yes		IL-1α, retinoic acid	No	(Vankemmelbeke *et al.*, 2001)
Mouse	synovial fibroblasts[c]	Yes		IL-1α		(East *et al.*, 2007a)
Human	chondrocytes[d]	Yes		IL-1β, TNFα, TGFβ	Yes. Increased by IL-1β, TNFα, TGFβ	(Moulharat *et al.*, 2004)
Human	chondrocytes[d,i]	Yes		IL-1β	Yes. Increased by IL-1β	(Bau *et al.*, 2002)
Bovine	chondrocytes[g]	Yes		IL-1α ±IL-1ra, TNFα ± anti-TNF	Yes. Increased by IL-1α and TNFα	(Tortorella *et al.*, 2001)
Bovine	chondrocytes[d]	Yes		IL-1α	Yes. Increased by IL-1α	(Curtis *et al.*, 2000)
Human	chondrocytes[l]; normal and OA	Yes			Yes. Detected in OA cartilage but not normal.	(Malfait *et al.*, 2002)
Human	chondrocytes[l]; normal and OA	Yes				(Bau *et al.*, 2002; Blaney Davidson *et al.*, 2006; Kevorkian *et al.*, 2004)
Human	chondrosarcoma (SW-1353)			IL-1β	No	(Pattoli *et al.*, 2005)
Human	chondrocytes[d]		8-fold by IL-1α and OSM		Yes. 35-fold greater induction	(Young *et al.*)
Human	immortalised chondrocytes[k]		8-fold by IL-1α		Yes. Increased by IL-1α and OSM but not by IL-1α alone	(Koshy *et al.*, 2002)
Bovine	chondrocytes[b]		4-fold by IL-1α; 14-fold by TNFα		Yes. Increased 25-fold by IL-1α; 13-fold by TNFα	(LaVallie *et al.*, 2006)
Bovine	chondrocytes[e]		10-fold by IL-1β		Yes. Increased 54-fold by IL-1β	(Cortial *et al.*, 2006)
Bovine	chondrocytes[b]		10-fold by IL-1α; 8-fold by TNFα		Similar	(Arai *et al.*, 2004)
Bovine	chondrocytes[g]		Increased by IL-1α		No	(Little *et al.*, 2002)
Bovine	chondrocytes[g]		10-fold increase by IL-1α		Yes. Increased 3-fold by IL-1α	(Patwari *et al.*, 2005)
Bovine	chondrocytes[g]		Increased by IL-1β			(Chan *et al.*, 2006)
Mouse	chondrocytes[g]		17-fold by IL-1α; 8 fold by retinoate		Yes. No increase by IL-1α or retinoate	(East *et al.*, 2007b; Stanton *et al.*, 2005)

[a]agent over-expressed *in vivo*
[b]pellet culture
[c]cells used at passage 3-9
[d]primary cell cultures
[e]primary cells seeded onto collagen sponges
[f]cells used at passage 4-6 ± adenoviral infection
[g]cartilage explant culture
[h]macrophage and T-cell-depleted primary cell cultures
[i]alginate culture
[k]immortalised cells in monolayer
[l]Uncultured, *ex vivo* analysis

epigenetically, with the percentage of non-methylated sites in the ADAMTS-4 promoter increased to nearly 50% in OA cartilage, from 0% in control cartilage (Roach *et al.*, 2005). In the cancer field, a high frequency (57%) of aberrant ADAMTS-5 gene methylation is associated with T-cell acute lymphoblastic leukemia (Roman-Gomez *et al.*, 2005).

A 2.6 kb promoter region of the human ADAMTS-5 gene has response elements for interactions with Runx2; ADAMTS-5 promoter activity is upregulated in response to Runx2 over-expression in primary bovine chondrocytes and human chondrosarcoma cells (Thirunavukkarasu *et al.*, 2007). Runx2 might be an important driver of cartilage degradation in human OA since it also stimulates expression of ADAMTS-4 (Thirunavukkarasu *et al.*, 2006) and MMP-13 (Jimenez *et al.*, 1999; Wang *et al.*, 2004b). ADAMTS-5 expression is thought to be NF8κB-independent in synovial fibroblasts (Bondeson *et al.*, 2007), but NFκB-dependent in chondrocytes (LaVallie *et al.*, 2006).

Glycosaminoglycans Modulate Aggrecanase Activity

Deglycosylated aggrecan is a poor substrate for aggrecanases (Gendron *et al.*, 2007; Kashiwagi *et al.*, 2004; Pratta *et al.*, 2000; Tortorella *et al.*, 2000a) suggesting that substrate glycosylation is important for enzyme activity and that CS and KS chains might contribute to exosite activity. The CS-2 activity of ADAMTS-5 at the GELE^{1480}↓^{1481}GRGT site in bovine aggrecan was markedly reduced following aggrecan deglycosylation (Gendron *et al.*, 2007). There are no data reporting on the effects of glycosylation on IGD cleavage by ADAMTS-5, specifically. A number of studies have shown that exogenous glycosaminoglycans (Munteanu *et al.*, 2002; Sugimoto *et al.*, 1999; Vankemmelbeke *et al.*, 2001) and sulphated sugar polymers (Munteanu *et al.*, 2000) inhibit cleavage at the E^{373}↓^{374}A site in the IGD. Several KS chains are substituted in the IGD between the MMP N^{341}↓^{342}F and aggrecanase E^{373}↓^{374}A cleavage sites, at residues T^{352}, T^{357}, T^{370} and N^{368} (Barry *et al.*, 1992; Barry *et al.*, 1995). KS in the IGD might therefore be an important modulator of ADAMTS-5 IGD-activity. Studies comparing the presence or absence of endogenous KS chains have suggested that aggrecanase cleavage in the IGD might be increased in the presence of KS and reduced when KS is enzymatically removed (Pratta *et al.*, 2000; Tortorella *et al.*, 2000a). We have shown that N-linked KS in the IGD potentiates ADAMTS cleavage at the E^{373}↓^{374}A site (Poon *et al.*, 2005) and that the microstructure of KS chains in the IGD is completely different to the microstructure of KS in the KS-rich region (Fosang *et al.*, 2004). These findings suggest that not only might there be competition between endogenous and exogenous glycosaminoglycans for binding to ADAMTS enzymes, but also that KS might have a direct effect on aggrecanolysis. Further studies are required to elucidate the role of KS and CS on ADAMTS-5 activity.

Inhibition of ADAMTS-5 activity

TIMP-3 is a potent inhibitor of ADAMTS-4 and ADAMTS-5 (Hashimoto *et al.*, 2001; Kashiwagi *et al.*, 2001) with Ki values in the subnanomolar range. TIMP-1, -2 and -4 do not inhibit ADAMTS-4 or ADAMTS-5 (Arner *et al.*, 1999; Kashiwagi *et al.*, 2001) whereas TIMP-3 inhibits both IL-1α and retinoic acid-stimulated aggrecanase activity in a dose-dependent manner (Gendron *et al.*, 2003). *In vivo*, there is increased aggrecan loss and increased NITEGE^{373} neoepitope, concomitant with mild cartilage degradation in the TIMP-3 null mouse (Sahebjam *et al.*, 2007), suggesting that TIMP-3 is indeed an endogenous aggrecanase inhibitor. TIMP-3 binds tightly to negatively charged glycosaminoglycans (Yu *et al.*, 2000) and TIMP-3 potency against ADAMTS-4 is enhanced by ADAMTS-4 binding to aggrecan (Wayne *et al.*, 2007); it appears that TIMP-3 has a greater affinity for ADAMTS-4 complexed with aggrecan, than it does for ADAMTS-4 alone (Wayne *et al.*, 2007). To date there are no studies comparing the effect of aggrecan or CS on the affinity of TIMP-3 for ADAMTS-5. α2-macroglobulin (α2M) present in the circulation and synovial fluid is another endogenous inhibitor of ADAMTS-4 and ADAMTS-5. *In vitro*, ADAMTS-4 and -5 cleave α2M in the bait region at M^{690}↓^{691}G, causing the enzymes to become trapped and consequently inactivated by the inhibitor (Tortorella *et al.*, 2004). However it is not clear whether α2M inhibits ADAMTS activity *in vivo*.

Small molecule inhibitors designed to block MMP active sites were developed more than 30 years ago and several entered clinical trials for evaluation as cancer and/or arthritis therapies. When none were successful, due mainly to their unacceptable side effects, industry enthusiasm (and budgets) for pursuing them consequently waned. When aggrecanases were finally identified (Abbaszade *et al.*, 1999; Tortorella *et al.*, 1999), they sparked a new wave of interest in metalloproteinases as targets for arthritis therapies. Designing ADAMTS inhibitors that do not inhibit MMPs was a priority; several broad spectrum MMP inhibitors also inhibit aggrecanases, reflecting conservation of the active site consensus sequence within the metzincin family of enzymes. Synthetic inhibitors with high specificity for ADAMTS enzymes are in development (Bursavich *et al.*, 2007a, 2007b; Cherney *et al.*, 2003; Gilbert *et al.*, 2007; Yao *et al.*, 2002; Yao *et al.*, 2001) and the aggrecanase inhibitor AGG-523 is in phase I clinical trials as an osteoarthritis drug. To maximize the usefulness of such drugs, further research is needed to resolve when ADAMTS enzymes are active, which cells they come from, and whether they have different roles, or different prominence, in inflammatory joint disease, compared with mechanically-induced joint injury. Studies in ADAMTS-deficient mice designed to disrupt only the ADAMTS catalytic site without affecting the biological functions invested in the C-terminal domains, might be useful for predicting potential effects of active-site inhibitors.

Perspectives

There is no easy way to second guess which enzyme is the critical aggrecanase in humans. *In vitro* studies using siRNA directed towards ADAMTS-4 and ADAMTS-5 in human cartilage (Song *et al.*, 2007), neutralising antibodies to ADAMTS-4 in pig cartilage (Powell *et al.*, 2007) and immunoprecipitation with anti-ADAMTS-4 and anti-ADAMTS-5 antibodies in bovine cartilage (Tortorella *et al.*, 2001) have demonstrated a significant role for ADAMTS-4 in aggrecanolysis. On the other hand, mice deficient in ADAMTS-5 (but not ADAMTS-4) are protected from early aggrecan loss and cartilage erosion in inflammatory and non-inflammatory models of arthritis (Glasson *et al.*, 2005; Stanton *et al.*, 2005), and recombinant human ADAMTS-5 is substantially more active than ADAMTS-4 (Gendron *et al.*, 2007).

The picture becomes even less clear when enzyme expression is analysed in human cartilage, because often, the results are counter-intuitive (Bau *et al.*, 2002; Kevorkian *et al.*, 2004). For example, compared with normal articular cartilage, mRNA expression of the proteinases MMP-3, ADAMTS-4, and ADAMTS-5 is

decreased in OA cartilage whereas mRNA expression of the inhibitor TIMP-3 is increased. The regulation of proteinase gene expression can differ between knee, hip and ankle joints in humans (Chubinskaya *et al*., 1996; Kang *et al*., 1998; Kevorkian *et al*., 2004) and clearly it differs between species. Whereas ADAMTS-5 is significantly decreased in human OA cartilage, it is increased in a rat model of OA compared with the sham and contralateral control (Appleton *et al*., 2007). ADAMTS-5 is more highly expressed than ADAMTS-4 in both normal and OA cartilage at the mRNA level (Bau *et al*., 2002; Kevorkian *et al*., 2004), but if ADAMTS-4 activity is predominantly modulated by post-translation processing, or alternative splicing, is this a meaningful comparison to make?

The most interesting finding to emerge from our review of ADAMTS-5 and its comparison with ADAMTS-4 is that for modulation of enzyme activity and gene regulation, ADAMTS-4 and ADAMTS-5 are the antitheses of each other (Tables 1 and 2). ADAMTS-5 activity is reduced by C-terminal processing whereas ADAMTS-4 activity is maintained or increased. In most cases, human ADAMTS-5 is constitutively expressed in chondrocytes, whereas ADAMTS-4 expression is induced by proinflammatory cytokines. Similarly, synovial fibroblasts from all species tested constitutively express ADAMTS-5 but ADAMTS-4 is induced. There is still much to be learned about the enigmatic ADAMTS-5.

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Discussion with Reviewer

Editor: Are there any "notes in proof" that you would like to add?

Authors: The authors wish to acknowledge two key papers published since the time of submission and peer review: (1) Fushimi *et al*. (2008), and (2) Mosyak *et al*. (2008). The paper by Fushimi *et al*. (2008) reports that the ADAMTS-5 catalytic domain has higher intrinsic catalytic ability than that of ADAMTS-4, and that non-catalytic domains of ADAMTS-5 are more effective modifiers of activity than those of ADAMTS-4. It also reports that the low levels of aggrecanase activity previously reported for full length ADAMTS-4 are due to inhibition by heparin *in vitro*. These results suggest that *in vivo*, ADAMTS activity might be sensitive to, and modified by, the biochemical composition of its environment. According to the paper by Mosyak *et al*. (2008), the crystal structure suggests that mature aggrecanase exists as an ensemble of at least two isomers, only one of which is proteolytically active.

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