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Review

Proteoglycan degradation by the ADAMTS family of proteinases

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ABSTRACT

Proteoglycans are key components of extracellular matrices, providing structural support as well as influencing cellular behaviour in physiological and pathological processes. The diversity of proteoglycan function reported in the literature is equally matched by diversity in proteoglycan structure. Members of the ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) family of enzymes degrade proteoglycans and thereby have the potential to alter tissue architecture and regulate cellular function. In this review, we focus on ADAMTS enzymes that degrade the lectican and small leucine-rich repeat families of proteoglycans. We discuss the known ADAMTS cleavage sites and the consequences of cleavage at these sites. We illustrate our discussion with examples from the literature in which ADAMTS proteolysis of proteoglycans makes profound changes to tissue function.

1. Introduction

Cells in all tissues are surrounded by extracellular matrix (ECM). As well as a scaffold providing structural support, the ECM has an active role in regulating the activity and behaviour of cells, including cell shape, survival, differentiation, movement, proliferation and in some cases, cell death. These cellular activities and behaviours are mediated by specialised ECM proteins and glycoproteins, for example, proteoglycans with their highly charged glycosaminoglycan side chains, as well as fibril- and network-forming molecules such as collagen, elastin, laminin and fibronectin. Many physiological processes such as morphogenesis, ovulation, parturition and wound healing require closely regulated degradation of the ECM, whereas in other cases matrix degradation leads to pathology. Proteoglycans are key components of extracellular matrices and have been recognised as such for many decades. In contrast, the family of ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) proteinases was only recently discovered [1]. Since then several ADAMTS enzymes, including ADAMTS-5 and ADAMTS-13, have made a big splash in the literature with the discovery that their proteolytic actions have key roles in arthritis and blood-clotting diseases, respectively. Here we will review the proteolysis of proteoglycans by ADAMTS enzymes, focussing particularly on the consequences of these proteolytic events.

Proteoglycans are present in the ECM of almost all eukaryotes and have a diverse range of functions that influence both the physical and biomolecular properties of tissues. Many proteoglycans have modular domain structures with interaction sites that allow them to form molecular bridges between cells and matrix. Other proteoglycans influence extracellular architecture by regulating collagen fibrillogenesis. Cell surface and extracellular proteoglycans that bind growth factors can act as co-receptors for signalling, or depots for sequestration. Accordingly, proteolysis of proteoglycans by ADAMTS enzymes can have detrimental effects, creating matrices with disrupted cell-matrix communication, matrices with impaired fibrillar networks, or matrices with altered biomechanical properties. In other situations, proteolysis of proteoglycans by ADAMTS enzymes is critical for normal physiological processes. Given the large number and diversity of proteoglycans, this review is necessarily restricted to only two subfamilies of proteoglycans: the lecticans, including aggrecan, versican, brevican and neurocan; and the small leucine-rich repeat proteoglycans, or SLRPs (reviewed in [2–7]).

2. The ADAMTS family of proteinases

The ADAMTS family belongs to the metzincin superfamily of metalloendopeptidases and now numbers 19 members, including enzymes with broad catalytic activities against a range of substrates. The

Abbreviations: ADAMTS, A Disintegrin And Metalloproteinase with ThromboSpondin motifs; CS, chondroitin sulphate; DS, dermatan sulphate; ECM, extracellular matrix; G1, G2, G3, first, second and third globular domains of lecticans; HA, hyaluronan; IGD, interglobular domain; KS, keratan sulphate; HS, heparan sulphate; PTR, proteoglycan tandem repeat; MV, mother vessels; SMC, smooth muscle cell; TS, thrombospondin

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ADAMTS enzymes are secreted proteinases, and accordingly, the known ADAMTS substrates are extracellular also. It is likely that other substrates are yet to be discovered.

2.1. Domain structure and conserved motifs

All ADAMTS enzymes share a common domain organisation comprising, from the N-terminus, a signal peptide, a pro-domain, a catalytic domain, a disintegrin-like domain, a central thrombospondin (TS) repeat, a cysteine-rich and a spacer domain. With the exception of ADAMTS-4, all other ADAMTS enzymes have further TS repeats and several family members have additional, unique modules at the C-terminus (Fig. 1). There are several, excellent reviews on ADAMTS domain organisation and function [8–12].

The pro-domain is generally considered to be essential for proper folding of the metzincins. It is also credited with maintaining enzyme latency, by virtue of its proximity to the catalytic pocket, where it impedes substrate access and hydrolysis. Excision of the ADAMTS propeptide is typically a prerequisite for ADAMTS activity; however there is no evidence for the cysteine switch mechanism that has been so well described for MMP activation. Rather, every ADAMTS enzyme has at least one consensus motif recognised by proprotein convertases (such as furin or furin-like enzymes) and it is likely that convertase processing is common to all ADAMTS enzymes.

The catalytic domain of the ADAMTS family members has the highest degree of sequence homology. ADAMTS enzymes have in common with other clan members the active-site consensus sequence **H**EBx**H**xBGBx**H**, in which the three histidines coordinate a zinc ion essential for hydrolysis and where B represents bulky apolar residues. A short distance C-terminal to the third histidine is a highly conserved methionine that constitutes the 'Met-turn', a tight turn common to the catalytic domain of all metzincins (reviewed in [13]).

The ancillary domains C-terminal to the catalytic domain show less sequence homology. The disintegrin-like domain was named for its sequence similarity to snake venom disintegrins, but there is no evidence to date that it binds integrins. Crystal structures of ADAMTS-1, -4 and -5 show that the disintegrin-like domain lies adjacent, and in close proximity to the active site, implying that it functions to regulate activity, perhaps by providing an auxiliary substrate-binding surface [14,15]. The remaining ancillary domains have roles in substrate recognition and matrix localisation. C-terminal to the disintegrin domain is a central TS repeat that is highly conserved and shows sequence homology to thrombospondins 1 and 2. Conserved amino acid residues include the CSR(T/S)C pentapeptide and several glycine, tryptophan and proline residues [16]. Further towards the C-terminus is a cysteine-rich domain containing 10 well conserved cysteine residues.

Following the cysteine-rich domain is a cysteine-free spacer domain of variable length. The spacer is the least homologous of the ADAMTS domains. With the exception of ADAMTS-4, between one and fourteen further TS repeats follow immediately C-terminal to the spacer domain, often in combination with other modules including a mucin module in ADAMTS-7 and -12, a GON-1 module in ADAMTS-9 and -20, two CUB-like domains in ADAMTS-13 or protease and lacunin (PLAC) domains in more than half the family (reviewed in [8–12]). The sequence of the C-terminal TS modules is more variable than the central thrombospondin module, but their function appears also to be matrix binding.

Fig. 1 shows the domain structure and phylogenetic analysis of the ADAMTS family members, based on full length protein sequence analysis [8,12]. The proteoglycan-degrading enzymes, ADAMTS-1, -4, -5, -8 and

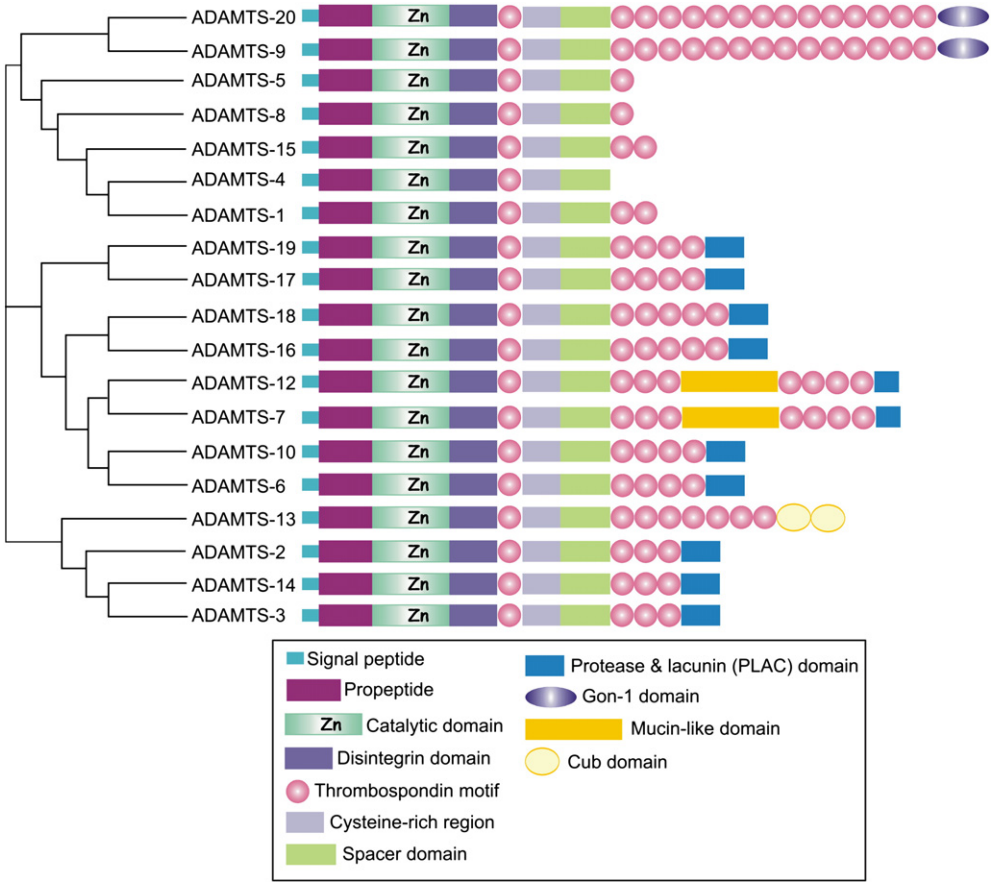

Fig. 1. The domain structure and phylogenetic analysis of the ADAMTS family. Adapted from [8,11]. Note that domains are not to scale.

-15 cluster together on one branch, forming a subgroup. Closely related are ADAMTS-9 and -20, having an additional GON-1 domain at their C-terminus.

2.2. Structural features determining catalytic activity, inhibition, substrate recognition and tissue localisation

2.2.1. The propeptide

Proprotein convertases (PCs) cleave ADAMTS enzymes at the consensus motif R/KX*n*R/K↓, where $n = 0$, 2, 4 or 6 and X is any amino acid. Of the nine mammalian PCs, seven recognise this motif (PC1/3, PC2, furin, PC4, PC5/6A/B, PACE4 and PC7) and their activities partition to different cellular compartments, (reviewed in [17]). The PCs furin and PC7 are membrane-bound PCs that are active in the trans-Golgi network and shuttle to the cell surface and back via endosomes. Furin has also been shown to operate extracellularly, in a shed, soluble form. Other PCs are found in dense core granules and/or constitutively secreted, allowing them to operate extracellularly [18]. The advantage of a system that targets zymogen processing to multiple locations is that specific ADAMTS enzymes can be maximally active at a precise location, perhaps co-localised with a preferred substrate.

Furin is the archetypal and best studied of the PCs, and many of the studies examining ADAMTS processing have been done in cell lines, using furin inhibitors, transfection of furin constructs or furin siRNA. ADAMTS-1 and -4 can be activated by furin *in vitro*, in the trans-Golgi network [19,20], whereas ADAMTS-5 activation by furin is thought to be an extracellular event [21]. PACE4 and PC5/6 can activate recombinant ADAMTS-4 in a cell-free assay [22] and co-transfection studies show that PC7 can process ADAMTS-5 (16). Malfait and co-authors have shown that the majority of PC activity extracted from human osteoarthritic cartilage is PACE4 and that PACE4 can activate both ADAMTS-4 and -5 in cartilage, *in situ* [23]. The authors ruled out a role for furin in this system.

Convention would dictate that the pro-domain of ADAMTS enzymes must be removed to allow substrate access to the catalytic pocket, prior to hydrolysis. Certainly, removal of the pro-domain by a PC is vital for ADAMTS-4 [22] and -5 [21] activity against aggrecan, or aggrecan and versican respectively. Surprisingly, some ADAMTS enzymes are active with an intact pro-domain. For example, ADAMTS-13, which has a relatively short pro-domain, cleaves von Willebrand factor with its pro-domain intact [24] and ADAMTS-7 can cleave alpha-2-macroglobulin [25], also with an intact prodomain. ADAMTS-9 further tests convention because propeptide excision acts to reduce is its catalytic activity [26]. Although the variable nature of ADAMTS activation presents a challenge to investigators, it may be a boon for ADAMTS inhibitor design, if individual convertases can be targeted for inhibition, and the cellular location at which activation takes place can be exploited.

2.2.2. The active site

Crystal structures have been solved for truncated ADAMTS-1, -4 and -5 enzymes bound to an inhibitor [14,15,27]. Several features of the catalytic domain distinguish ADAMTS enzymes from other metzincins. The ADAMTS enzymes have a unique calcium binding cluster in the catalytic domain that is essential for structural integrity [27]. Also unique to ADAMTS-4 and -5 is the potential for the active site to assume two different conformations, an active 'open' form and an inactive 'closed' form [15]. The authors propose that these forms might exist in equilibrium; this dynamic state sets the ADAMTS enzymes apart from other metzincins. One interesting feature of the ADAMTS catalytic domain is its close association with the disintegrin domain which forms part of the substrate binding pocket in the active site cleft [28]. Most promising for ADAMTS enzymes with roles in pathology, is that the ADAMTS active site pocket is sufficiently different from its counterpart in the MMP and ADAM families to facilitate design of ADAMTS-specific inhibitors. Although inhibitors selective for ADAMTS subgroups should be achievable by exploiting differences in key residues in the active site [12], the design of active site inhibitors that can distinguish between ADAMTS-4 and ADAMTS-5 could be problematic since their active sites are well conserved and their structural topography is virtually identical [15].

2.2.3. Ancillary domains

The many and varied modules of the ADAMTS ancillary domains regulate ADAMTS activity, inhibition, tissue localisation and substrate specificity. Focussing first on activity, studies with domain deletion mutants have shown that removal of C-terminal ancillary domains of ADAMTS-1, -4, -5, -8, and -9 greatly reduces their activity against natural substrates [29–34]. For ADAMTS-4 and -5, isolated catalytic domains are inactive against native aggrecan [29]. The minimum domain composition permitting aggrecanolysis is the catalytic domain, the disintegrin domain and the first TS1 repeat [29,31,35]. The TS repeats are also involved in binding to the naturally-occurring ADAMTS inhibitor, TIMP-3, which inhibits ADAMTS-1 [36] and is a potent inhibitor of ADAMTS-4 and -5 [37–39].

The cysteine-rich and spacer domains of ADAMTS-1, -4, and -5 determine binding to sulphated glycosaminoglycans and tissue localisation [29–31,35,40–42]. The spacer domain of ADAMTS-4 interacts with cell surface and pericellular proteoglycans [42], localising the activity of full length ADAMTS-4 close to the chondrocytes. Similarly, ADAMTS-5 activity is located at the cell surface and the ECM via interactions between the cysteine-rich domain and sulphated glycosaminoglycans [29] and/or hyaluronan (HA) [43]. Post-translational processing of the ADAMTS C-terminus is common amongst the ADAMTS enzymes [31,34,42,44–46] and can be autolytic [35,40,42].

3. Extracellular proteoglycans: diverse structure and function

Although proteoglycans can be classified into loose sub-groupings based on regions of primary sequence homology, collectively, proteoglycan core proteins are diverse and unrelated in their structure and function. Instead, the single unifying feature of proteoglycans is the presence of one or more glycosaminoglycan chains, covalently attached to the core protein. By this criterion, molecules from many and varied phylogenetic groupings qualify as proteoglycans. For example, type IX collagen with a 70% incidence of a single CS glycosaminoglycan substitution in the α2(IX) chain [47] can be considered a part-time proteoglycan, and ADAMTS-7 with a CS chain substituted in its mucin domain [25] is also a proteoglycan.

Glycosaminoglycans are linear carbohydrate chains comprising repeating disaccharide units carrying negatively-charged sulphate and carboxylate groups (Fig. 2). The chains may be chondroitin sulphate (CS), dermatan sulphate (DS), keratan sulphate (KS), heparan sulphate (HS) or heparin, with multiple disaccharide combinations possible for each chain type. Some proteoglycans have two or even three different chain types attached to a single core protein. For example, aggrecan is substituted with CS and KS chains (Fig. 3), the cell surface HA receptor CD44 can carry CS and/or HS chains [48] and perlecan can carry CS, HS and KS chains [49]. Many other proteoglycans can have different glycosaminoglycan chain types attached, depending on the tissue type, stage of development, maturation or disease state [48,50,51]. Superimposed on these variations in chain type, is the more subtle variation in the pattern of sulphation within a single species of glycosaminoglycan chain on a common core protein. For example, the ratio of chondroitin-4-sulphate to chondroitin-6-sulphate on aggrecan in human knee cartilage changes markedly with age, topographical position, zone of cartilage and tissue composition [52]. In humans, the amount of KS on the lectican, aggrecan (see below) increases from a minimal percentage at birth to over a quarter of the glycosaminoglycan content at maturity [53] and compared with aggrecan from immature cartilage, aggrecan from adult cartilage is also substantially more sulphated and more highly modified by fucosylation and sialylation. Clearly, there is infinite scope for heterogeneity in glycosaminoglycan and proteoglycan

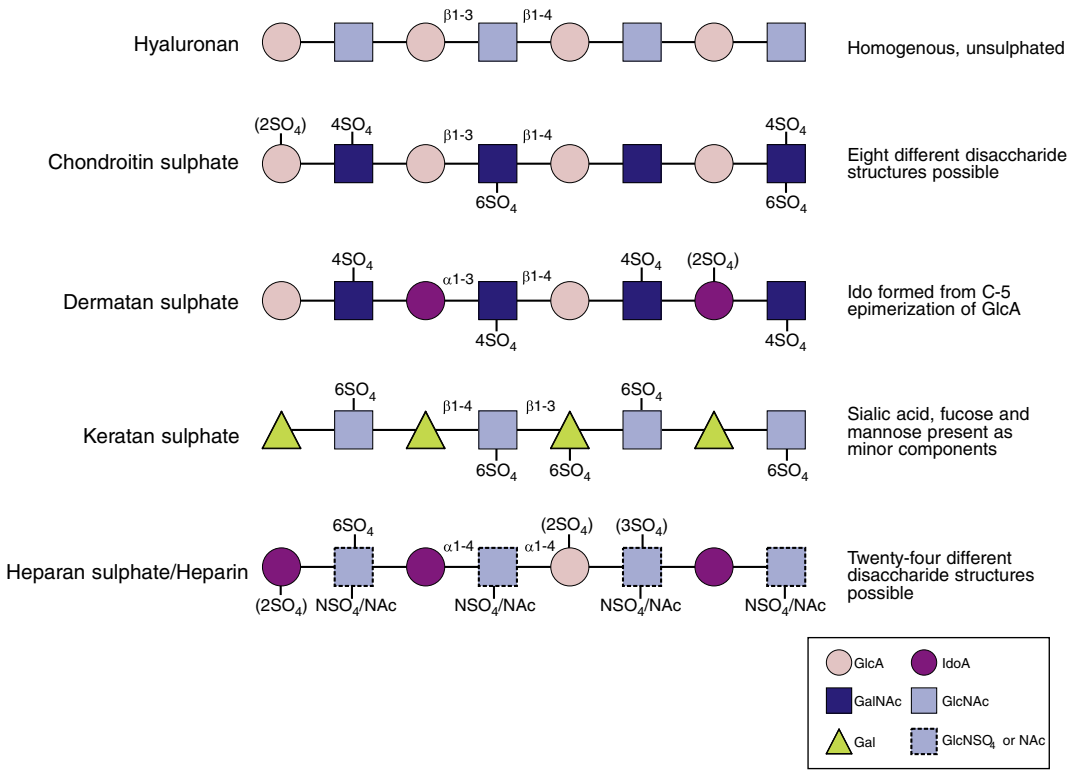

Fig. 2. The repeating disaccharide structure of glycosaminoglycans. Hyaluronan is synthesised without a covalent link to any protein. DS is formed from CS by intracellular epimerisation of glucuronate (GlcA) to iduronate (IdoA). 2-sulphated hexuronate residues are more common in DS than CS. Heparin is a more extensively epimerized and sulphated form of HS. HS frequently contains some chain segments with little or no epimerization or sulphation. Heparin is synthesised only on a mast cell granule proteoglycan, whereas HS is found on many cell surface intracellular and extracellular proteoglycans. Reproduced with permission from [185].

structure. The analytical tools for detecting the subtleties of such heterogeneity are developing rapidly, however understanding the functional consequences of a seemingly boundless range of heterogeneity within proteoglycans is an ongoing challenge. Excellent reviews of proteoglycan structure and function can be found elsewhere [4,5,7,54,55].

Finally, it is perhaps not surprising to find examples of ADAMTS activity being directly influenced by the presence or absence of glycosaminoglycans. For example, compared with fully glycosylated aggrecan, deglycosylated aggrecan is a poor substrate for ADAMTS-4 and -5 [29,31,56,57]. The sites of interaction between aggrecan's glycosaminoglycans, and ADAMTS-4 and -5, have been mapped to the spacer domain of ADAMTS-4 [31] and the cysteine-rich domain of ADAMTS-5 [29]. Other studies have suggested that KS might have a role in modulating ADAMTS activity since cleavage in the aggrecan interglobular domain (IGD) is increased in the presence of endogenous KS and reduced when KS is removed by treatment with keratanases [56–58]. KS attachment sites in the aggrecan IGD map to amino acids T^{352}, T^{357}, N^{368} and T^{370} [59,60], and are strictly conserved in humans, cattle, horses, rodents, dogs, sheep, pigs and tree shrews. Furthermore, KS in the IGD of pig aggrecan has a uniquely undersulphated microstructure compared with KS elsewhere on aggrecan [61], suggesting that this KS might also have a specific function.

3.1. The lectican family of proteoglycans

The lectican [62] (or hyalectan [2]) subfamily of CS proteoglycans includes aggrecan, versican, neurocan and brevican (Fig. 3). These proteoglycans are related by the presence of homologous N-terminal G1 and C-terminal G3 globular domains, with a centrally-located glycosaminoglycan attachment region. The G1 domain is highly homologous between the lecticans. It contains an immunoglobulin fold that mediates lectican binding to the family of small globular link proteins [63], and two homologous proteoglycan tandem repeat (PTR) motifs responsible for the high affinity binding to (HA) [64], itself an unsulphated glycosaminoglycan. The HA-binding properties of lecticans are a defining feature of this family.

The name “lectican” reflects the structural homology between the modular components of the G3 domain, and the selectin family of carbohydrate binding molecules, with each domain comprising a C type lectin, EGF-like and complement regulatory (CRP)-like domains. However, despite the sequence homology, distinct carbohydrate ligands for lectican G3 domains have not been reported. Instead the G3 domain binds tenascins [65,66], fibrillins [67] and fibulins [68,69]. Structural diversity amongst lecticans arises through alternative splicing [70–72] and versican is the best example of an alternative spliced lectican (see Section 5.3). In addition, alternative splicing of G3 domains can create functional diversity amongst the lecticans; further diversity occurs when the G3 domain is lost following ADAMTS cleavage.

Lectican CS chain synthesis is initiated by addition of a xylose residue at Ser-Gly or Gly-Ser dipeptides in the glycosaminoglycan attachment region (Fig. 3). The number of CS chains ranges from 3 chains in brevican, to 7 chains in neurocan, ~30 chains in the unspliced variant of versican and as many as 100 chains in full length aggrecan. The glycosaminoglycan chains contribute significantly to the functional properties of the lecticans. For example, aggrecan and versican carry a large number of CS chains and form large multi-molecular aggregates by binding to HA. The compact distribution of sulphate groups on CS chains in lectican aggregates creates a high fixed-charge density that attracts counter ions, and consequently water, creating large hydrodynamic domains. As a consequence there is a high osmotic swelling pressure in cartilage that confers load-bearing properties, and viscoelastic properties in blood vessels that are needed to accommodate high shear stresses and cyclical forces. Most KS in aggrecan is located on repeating hexapeptide motifs in the KS-rich region [73,74]. While these motifs number 23 and 13 in bovine aggrecan and human respectively, there are only 4 repeats in rodents [75]. This has made KS difficult

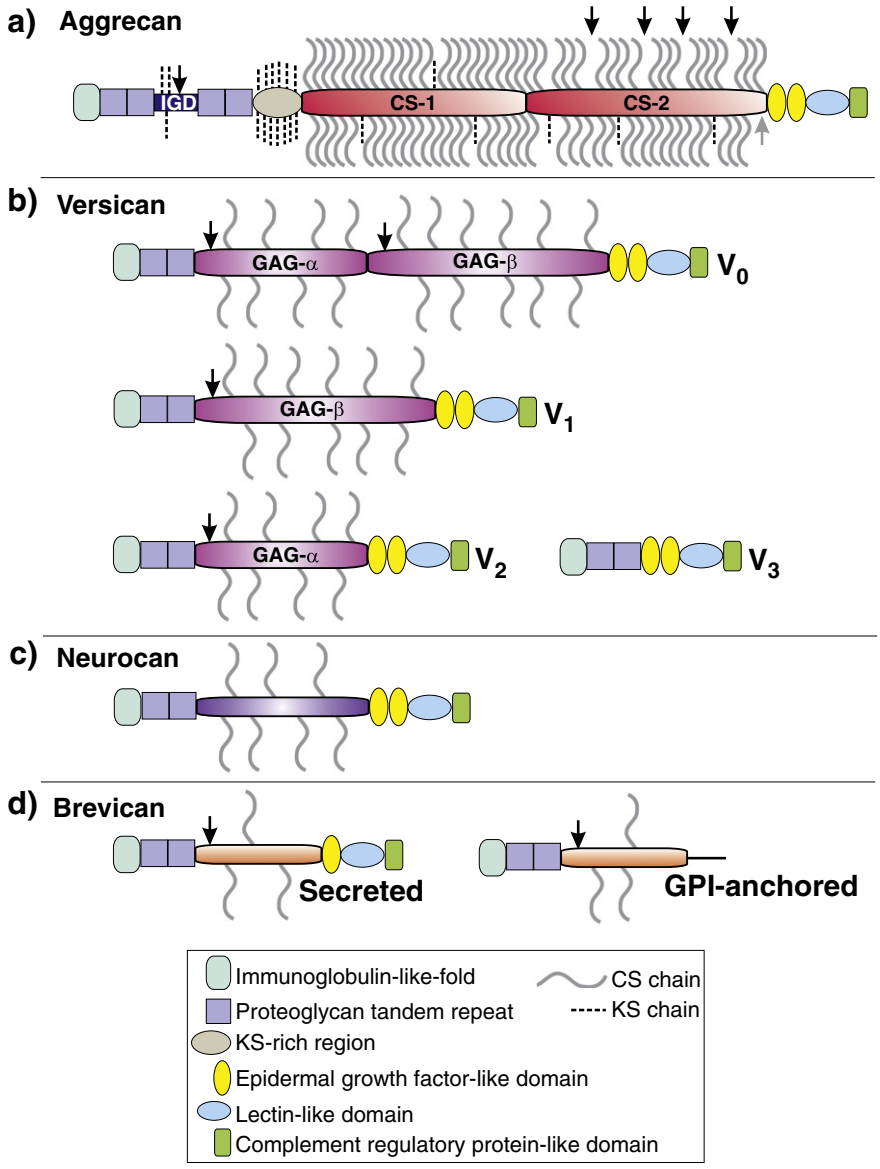

Fig. 3. The domain structure of the lectin family of proteoglycans. The modular domain structure of the lecticans (a) aggrecan, (b) versican, (c) neurocan and (d) brevican are shown. Black arrows mark the ADAMTS cleavage sites that are conserved between mammalian species. The grey arrow marks an ADAMTS-5 cleavage site in bovine aggrecan that is poorly conserved.

to detect in the mouse and has led to the dogma that mouse aggrecan does not carry KS [76,77].

3.2. The SLRP family of proteoglycans

The characteristic features of the family of small leucine-rich proteoglycans (SLRPs) include a central core protein comprising tandem, leucine-rich repeat sequences, flanked by N- and C-terminal cysteine-containing domains, and core substitution with either CS/DS or KS glycosaminoglycans. The consensus sequence for the leucine-rich repeats is LxxLxLxxNxLSxL, where L is leucine, isoleucine or valine, and S is serine or threonine [78]. A less stringent application of the consensus sequence rule allows for additional repeats. The CS/DS-containing SLRPs include the well-characterised molecules decorin and biglycan, and also epiphycan. Other SLRPs carrying *N*-linked KS or polylactosamine (unsulphated KS) chains include fibromodulin, lumican, keratocan, PRELP, osteoadherin, chondroadherin and osteoglycin (reviewed in [2–7]) (Fig. 4).

There is enormous variability within the glycosaminoglycan substitutions on SLRPs, many of which account for the functional heterogeneity within this family. For example, the core proteins of decorin and biglycan are structurally similar with 10–11 leucine-rich repeats, and depending on the tissue source and age of the sample, either one [79] or two [80] CS or DS chains of varying length, with variable sulphation and uronic acid epimerisation [5,81]. Epiphycan has fewer repeats, with an extended N-terminal sequence carrying CS/DS chains and sulphated tyrosine residues. Fibromodulin and lumican carry *N*-linked KS chains within the leucine-rich repeat regions, and also have N-terminal extensions with sulphated tyrosine residues. Many of these SLRPs can be found as non-glycanated forms that accumulate with age [82–85]. Interestingly, lumican and keratocan carry KS chains when they are expressed in the cornea, where they are thought to have critical roles in maintaining corneal transparency by precisely modulating collagen fibrillogenesis. However in other tissues, lumican and keratocan carry polylactosamine or poorly sulphated KS chains [86].

Decorin and biglycan have well-recognised roles as modulators of collagen fibrillogenesis ([87] and reviewed in [3,7]), but in addition, all members of the SLRP family are recognised as modulators of extracellular signalling (reviewed in [2,6,7]). Their portfolio of activities includes modulating cell proliferation, fibrosis, inflammation and

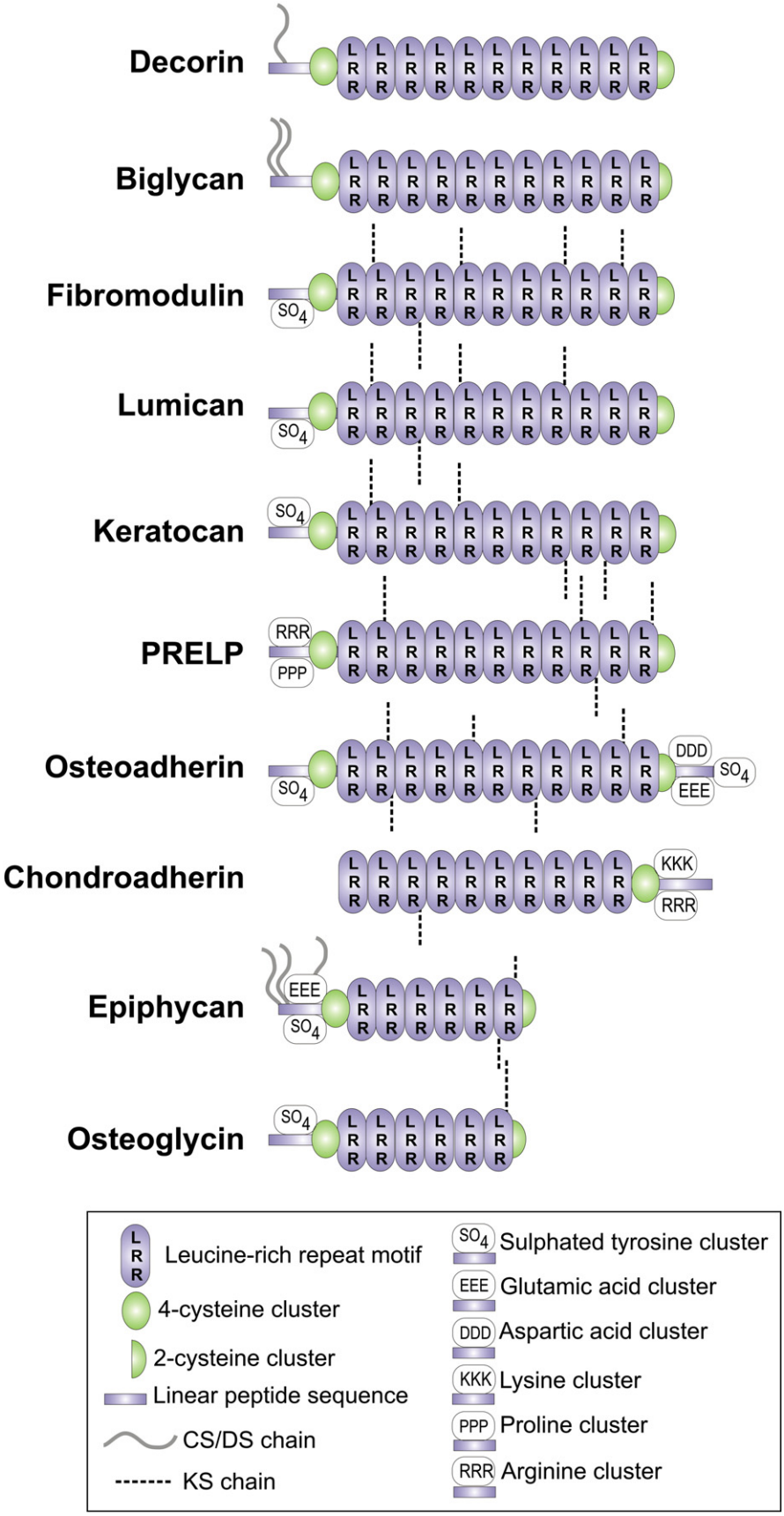

Fig. 4. The domain structure of the SLRP family of proteoglycans. Adapted from [3].

immunity, cytokine bioactivity, and receptor-binding associated with signal transduction (reviewed in [2,88,89]). These divergent functional properties reflect the presence of multiple interacting domains including the horseshoe shaped leucine-rich core domain, the highly charged N-termini of either tyrosine sulphates, glycosaminoglycans or clusters of charged amino acids, and the centrally-located KS chains. Many of the SLRP signalling activities were identified directly or indirectly from the phenotypes in SLRP-deficient mice (reviewed in [88]). There are also sites that bind integrins, and heparin binding domains (reviewed in [4]).

4. Tools for investigating proteoglycan degradation by ADAMTS enzymes

A number of valuable resources have been developed and used for the study of proteoglycan degradation by ADAMTS (and other) enzymes, including neoepitope antibodies and "cleavage-resistant" proteoglycan substrates. Neoepitope antibodies detect sequences at the newly-created N- or C-terminus of proteoglycan fragments, but fail to recognise the same sequence of amino acids present in the undigested parent molecule. Neoepitope antibodies are specific for the products of a single proteolytic event, and are therefore unambiguous markers of enzyme activity. However, they do not distinguish between enzymes with identical activities. For example, aggrecan neoepitope antibodies do not distinguish between the activities of ADAMTS-4, ADAMTS-5 and other potential aggrecanases, but they readily distinguish ADAMTS-cleaved fragments from fragments cleaved by matrix metalloproteinases [90]. The neoepitope antibody specific for the ^{374}ARGS N-terminal sequence on an aggrecan fragment [91] was instrumental in tracking the enzyme activity that led to the discovery of ADAMTS-4 and -5 as the aggrecan-degrading enzymes [4,92,93]. Subsequently, neoepitope antibodies facilitated the discovery of a third cartilage aggrecanase in *Adamts-4/-5*-double deficient mice [94]. Using a similar approach, the development of neoepitope antibodies to brevican [95] and versican [96] have helped identify ADAMTS enzymes as the proteinases responsible for cleaving these proteoglycans *in vivo*.

ADAMTS-resistant substrates have also been developed as tools to distinguish the activities of ADAMTS and MMP proteinases *in* vitro [97] and to confirm a biological role for ADAMTS activity in cartilage *in vivo* [98]. In these studies amino acids flanking the ADAMTS cleavage site in aggrecan were changed from TEGE$^{373}\downarrow^{374}$ARGS to TEGE$^{373}\downarrow^{374}$***NVYS***. The same strategy, changing amino acids flanking the ADAMTS cleavage site in brevican from TESE$^{395}\downarrow^{396}$SRGA to TESE$^{395}\downarrow^{396}$***NVYA*** has been used to successfully block ADAMTS cleavage of brevican [99] and reveal that brevican processing by ADAMTS enzymes is essential for cell motility [100] and glioma cell invasion [99].

Neoepitope antibodies have also been raised against the N-terminal sequence of PC-activated ADAMTS-4 or ADAMTS-5 enzymes [101,102]. Unlike the aggrecan neoepitopes that do not distinguish between the products of ADAMTS-4 and -5 activity, the ADAMTS neoepitope antibodies detect the enzymes directly, and readily distinguish between them. For example, anti-^{213}FASLS antibodies recognising active ADAMTS-4 have been used to immunolocalise ADAMTS-4 protein in the tibial growth plate of young rats [101].

5. ADAMTS cleavage of proteoglycans in development, disease and tissue remodelling

There are several fields of research for which a significant body of literature has developed describing ADAMTS involvement in proteoglycan degradation. We discuss these developments in the sections below, focusing on the proteoglycan cleavage sites and the biological consequences of the cleavage.

5.1. ADAMTS cleavage of proteoglycans in cartilage and tendon

The functional properties of cartilage and tendon are dependent on a number of proteoglycans. Aggrecan, versican and most members of the SLRP family are present in one or both of cartilage and tendons. ADAMTS-4 and -5 have key roles in proteoglycan processing in cartilage, and studies *in vitro* indicate that they have a similarly important role in degrading tendon proteoglycans.

Aggrecan is the archetypal lectican, named for its ability to form multimolecular aggregates. It is heavily substituted with CS chains that attract water, enabling cartilage and tendon to resist compressive and shear forces. In tendon aggrecan has the additional role of reducing the friction between adjacent collagen fibres. Aggrecan is highly sensitive to proteolysis between the G1 and G2 globular domains, in a region comprising a linear sequence of ~150 amino acids called the IGD. Proteolysis in the IGD releases the entire CS-rich region essential for the biomechanical properties of aggrecan, and is therefore the most detrimental for function. ADAMTS enzymes with aggrecanase activity cleave at the TEGE$^{373}\downarrow^{374}$ARGS bond in the IGD (Fig. 3); this signature activity of the aggrecanases is widely reported in humans and in animals, *in vitro* and *in vivo*, in cartilage and tendon. However, cleavage at TEGE$^{373}\downarrow^{374}$ARGS is the less-preferred action of these enzymes, at least in cartilage. Studies with bovine cartilage aggrecan have shown that recombinant ADAMTS-4 and -5 preferentially cleave aggrecan in the CS-2 domain [103,104]. The two most preferred cleavage sites in bovine cartilage aggrecan are at KEEE$^{1667}\downarrow^{1668}$GLGS, followed by GELE$^{1480}\downarrow^{1481}$GRGT. Thereafter, cleavage occurs at TEGE$^{373}\downarrow^{374}$ARGS in the IGD and at TAQE$^{1771}\downarrow^{1772}$AGEG and VSQE$^{1871}\downarrow^{1872}$LGQR in the CS-2 domain (Fig. 3). In cytokine-stimulated mouse cartilage, cleavage at FREEE$^{1467}\downarrow^{1468}$GLGS precedes cleavage at SELE$^{1279}\downarrow^{1280}$GRGT and FREEE^{1467} fragments are subsequently converted to SELE^{1279} fragments by ADAMTS enzymes in cartilage explant cultures [105]. Whether a similar sequence of ADAMTS cleavage events occurs in tendon has yet to be determined. Finally, an additional cleavage by ADAMTS-5 occurs in bovine aggrecan in the CS-2 domain just prior to G3, at RPAE$^{2047}\downarrow^{2048}$ARLE [106]. Unlike the other well-characterised ADAMTS cleavage sites in aggrecan, the sequences surrounding the E$^{2047}\downarrow^{2048}$A site are poorly conserved between species.

Recent studies comparing experimental arthritis in wildtype and *Adamts*-deficient mice have revealed that ADAMTS-5 is the principal aggrecan-degrading enzyme in mouse cartilage [107,108]. These studies showed that ablation of ADAMTS-5 activity blocked cleavage in the aggrecan IGD and substantially reduced the loss of aggrecan from femorotibial cartilage. ADAMTS-4 was eliminated as the primary aggrecanase in the mouse, however it is unclear whether ADAMTS-4 or -5 is the main aggrecanase in human arthritis [109,110]. Targeted knockdown of ADAMTS-4 and ADAMTS-5 in human cartilage explants has suggested that both enzymes might have roles in human arthritic disease [111].

ADAMTS cleavage in the aggrecan CS-rich region (Fig. 3) is thought to be less detrimental for the weight-bearing properties of cartilage, since most aggrecan in adult [112] and embryonic [113] cartilage lacks the G3 domain and varying portions of the CS-rich region. These finding suggest that there is early and ongoing processing of aggrecan from the C-terminus. In mice, the FREEE^{1467} neoepitope is detected in articular and growth plate cartilage from an early age (Rogerson et al., unpublished), further suggesting that ADAMTS enzymes have a role in C-terminal processing of aggrecan. The complete absence in any animal species [94,105,114–118] of a large aggrecan fragment comprising both an intact G3 domain and an ADAMTS-derived ^{374}ARGS N-terminus, is further evidence that C-terminal processing precedes proteolysis in the IGD. However, it is not clear which ADAMTS enzyme might be responsible for C-terminal processing. If C-terminal processing is indeed required for aggrecan turnover under baseline conditions, and also as a prerequisite for accelerated turnover, and if C-terminal processing is mediated by

ADAMTS-4 and/or ADAMTS-5, then it is surprising that mice deficient in ADAMTS-4 and/or -5 activity have no defects in endochondral bone formation [94,107,108,119,120]. It is possible that the activity of a third *in vivo* aggrecanase, detected in *Adamts-4/-5* double-deficient mice [110] could be the homeostatic aggrecanase.

Aggrecanase activity is more apparent in tendon than cartilage, since 65% of total aggrecan isolated from bovine tensional tendon lacks the G1 domain [121]; moreover, aggrecanase-generated aggrecan fragments are found in cultured tendon explants [121,122]. It is unclear which aggrecanases are active in tendon, however it is worth noting that aggrecan fragments with the ^{374}ARGS N-terminus are found in *un*stimulated explants of compressed and tensional tendon, and the levels of these fragments do not appear to be regulated by catabolic or anabolic agents, suggesting constitutive ADAMTS activity [122,123]. Although this is similar to human and mouse synovial fibroblasts that constitutively express ADAMTS-4 and -5 [124,125], it is in marked contrast to the situation in cartilage explant cultures, where the aggrecanases are mostly regulated by catabolic cytokines (reviewed in [126]). This raises the point that regulation of ADAMTS expression can be both inducible and constitutive, depending on the species, cell type or experimental conditions (reviewed in [126]). Overall, aggrecanolysis in cartilage [127] and tendon [128] is proposed to involve aggrecanase-mediated degradation of proteoglycans, as wells as depolymerisation of HA, and MMP-mediated destruction of the collagen II network.

The SLRPs biglycan, decorin, fibromodulin and keratocan are also substrates for ADAMTS enzymes *in vitro* [29–31,129–131]. In solution phase, bovine fibromodulin is cleaved at a single site ($Y^{44}\downarrow^{45}A$) by recombinant ADAMTS-4 and ADAMTS-5, generating a fragment approximately 5–10 kDa smaller than the full-length protein [29,31,132]. This same site is cleaved by MMP-13 [132]. When human articular cartilage was digested, ADAMTS-4 but not ADAMTS-5 cleaved fibromodulin, and the latter was a more efficient aggrecanase [130]. Biglycan and decorin are both cleaved by recombinant ADAMTS enzymes *in vitro* [29,31,129]. There is a single cleavage site in the fifth leucine-rich repeat of biglycan at the $N^{149}\downarrow^{150}C$ bond, which is recognised by ADAMTS-4 (but is distinct from the MMP-13 cleavage site) [133]. Biglycan fragments from cleavage at this site are present in cartilage from osteo- and rheumatoid arthritis patients [129]. There is some disparity in the literature about whether ADAMTS-4 or ADAMTS-5 is more active against biglycan [29,129]; Melching et al. reported more complete proteolysis of biglycan by ADAMTS-4, whereas Gendron et al. suggested that ADAMTS-5 has superior activity. Similarly, Melching and co-authors reported that decorin is not cleaved by either enzyme, whereas Gendron et al. found activity (albeit weak) with ADAMTS-4 and to a lesser extent ADAMTS-5. Keratocan was discovered in tendon relatively recently [128] and ADAMTS-4 and -5 digest this proteoglycan in bovine tendon [123]. There is the suggestion that ADAMTS-4 may be more active against keratocan in compressed regions of tendon, compared with ADAMTS-5 which is more active in tensional tendon. Whether these preferences are associated with differences in keratocan glycosylation [128], or whether other substrates such as aggrecan compete for the enzymes in the two areas of tendon is unclear.

Despite the apparent activity of ADAMTS-4 and -5 enzymes against biglycan, fibromodulin, and decorin, the acute increase in their expression and activity in cartilage following stimulation with catabolic cytokines [130,134] did not increase their cleavage of SLRPs. Rather, active degradation of SLRPs correlated with MMP activity in cartilage cultures [2,130]. Other studies characterising degradation products in cultured explants of bovine deep flexor tendon found that despite the abundant release of ADAMTS-derived aggrecan fragments, the versican fragments released from these same cultures were not produced by ADAMTS activity [121]. It is unclear why ADAMTS activity does not result in proteolysis of SLRPs or versican in these two experimental scenarios when they do so in solution phase. It is also not clear whether ADAMTS activity against SLRPS in cartilage, or ADAMTS activity against SLRPs and lecticans in tendon, is indeed pathological. Since SLRPs protect collagen fibrils from collagenase cleavage [135], it is possible that ADAMTS degradation of SLRPs could adversely affect the stability of the collagenous network and fibril cross-linking, with a concomitant negative impact on material properties. On the other hand, since SLRP fragments are readily generated in explant cultures in the absence of catabolic stimuli, an alternative possibility is that ADAMTS enzymes might be required for the normal function of SLRPs in cartilage and tendon.

5.2. ADAMTS cleavage of brevican in glioma

Brevican (also termed BEHAB) is a lectican specific to the central nervous system. Brevican has several secreted isoforms, including an isoform that is decorated with 1–3 CS chains and an isoform that is non-glycosylated. A third isoform that is membrane-bound via a GPI anchor results from alternative splicing and lacks the typical lectican C-terminus (reviewed in [136]).

Brevican mRNA expression in the brain is spatially and temporally controlled and peak expression is co-incident with periods of glial cell motility, such as in the developing brain during gliogenesis, and following experimentally-induced brain injury [137]. A dramatic increase in the expression of brevican mRNA is also a hallmark of human primary brain tumours [138]. These tumours are highly invasive, are difficult to surgically resect and resistant to conventional therapies. Significantly, brevican expression is very low in tumours of non-glial origin and in normal human brain [138].

Brevican is processed by ADAMTS enzymes at a single site at the $E^{395}\downarrow^{396}S$ bond, within the central domain of the core protein [95,139]. This site is distinct from the main MMP cleavage site that is 35 amino acids upstream [140]. ADAMTS cleavage of brevican generates two fragments of 50 kDa and 90 kDa which have long been associated with brevican's pathological properties [136]. ADAMTS-4 and -5, but not ADAMTS-1, generate these fragments in culture [95,141]. Of the three, only ADAMTS-5 mRNA is overexpressed in human glioma tissues in comparison with normal brain [141]. However, higher levels of expression alone do not necessarily indict ADAMTS-5 as the principle brevicanase in glioma because other pre- and post-translational mechanisms modulate ADAMTS activity (reviewed in (64)). What is clear is that cleavage at the $E^{395}\downarrow^{396}S$ site is a necessary step in glioma cell invasion, as seen in a study that mutated the sequence $E^{395}\downarrow^{396}$SRGA to $E^{395}\downarrow^{396}$***NVY***A, rendering it resistant to ADAMTS cleavage [99]. When CNS-1 cells stably expressing the mutant Brevican^{NVY} were implanted in rat brain, the tumours formed were no different in size to control tumours, nor was there any difference in survival endpoint from control animals. In contrast, animals implanted with CNS-1 cells overexpressing normal brevican formed tumours that were larger, more diffuse and more infiltrative than those measured in Brevican^{NVY} or control animals and the survival endpoint was significantly earlier [99].

Brevican has traditionally been described as a chemorepulsant, acting to limit cell motility and plasticity in the adult central nervous system [137], so it is somewhat counterintuitive to find that brevican promotes glioma cell motility. The reason appears to lie in the expression of altered isoforms of brevican. There are over-sialylated and non-glycosylated isoforms of brevican that are tumour-specific; the non-glycosylated form is the major isoform in high-grade human gliomas [142]. Furthermore, conditions that favour ADAMTS-cleavage also promote glioma cell movement, activation of the EGF receptor, expression of cell-adhesion molecules and secretion and accumulation of fibronectin on the cell surface [100]. Thus, reagents that target the tumour-specific isoforms of brevican, or inhibit ADAMTS-cleavage of brevican have potential as complementary therapies for primary brain tumours. Inhibiting ADAMTS cleavage of brevican might slow

the migration of glioma cells from the original tumour site(s) and increase the efficacy of surgical, radiation or chemotherapies.

5.3. ADAMTS cleavage of versican in vascular disease, angiogenesis, ovulation and tissue morphogenesis

Versican is the principal lectican in many tissues where it provides a loose and highly hydrated matrix. It has a typical lectican composition (Fig. 3) and considerable heterogeneity is provided by alternative splicing of the GAGα and GAGß domains. Further heterogeneity is conferred by the size and composition of the attached CS chains. Versican has roles in cell adhesion, proliferation, migration and extracellular matrix assembly [143].

Soluble versican V1 is degraded by ADAMTS-1, -4, -5, -9 and -20 at the $E^{441}\downarrow^{442}A$ bond [21,34,96,144,145] in the GAGβ domain. Cleavage at this site creates the G1-DPEAAE^{441} fragment and an anti-DPEAAE neoepitope antibody has been instrumental in mapping versican degradation by ADAMTS enzymes in a number of tissues, including normal and atherosclerotic human aorta [96]. The equivalent ADAMTS cleavage site in the GAGα domain of V0 is $E^{1428}\downarrow^{1429}A$ and the fragment G1-DPEAAE^{1428} has also been found in low abundance in extracts of human aorta [96]. ADAMTS-4 cleaves the GAGα domain at $E^{405}\downarrow^{406}Q$ in the versican V2 variant and cleavage of human brain V2 at this site generates glial HA binding protein [146]. These ADAMTS cleavage sites in the GAGα and GAGβ domains are distinct from the MMP and plasmin cleavage sites [147].

5.3.1. Vascular disease

All isoforms of versican are found in blood vessel walls [96,148,149]. Versican is prominent in the intima and adventitia where it forms stable complexes with hyaluronan and link protein, to provide a hydrated viscoelastic matrix. Immunohistochemical studies have shown that versican accumulates in the intima in vascular lesions including atherosclerotic plaques, restenotic lesions, lesions associated with graft repair and aneurysmal lesions [150]. It is present in early atherosclerotic intimal thickenings where it contributes to the expansion of the intima by attracting water. Versican is also present in advanced atherosclerotic lesions, at the edges of the necrotic core, where it retains lipids by binding low density lipoprotein. Multiple low density lipoprotein particles can bind to a single CS chain on versican (or other CS-bearing proteoglycans), thus the elongated CS chains seen in vascular injury contributes to lipid accumulation (reviewed in [151,152]). Versican CS chains also bind cell adhesion molecules and chemokines [153,154] and versican-HA complexes present at the plaque:thrombus interface in atherosclerotic plaques suggest a role for versican in thrombosis [155]. These complexes are found in the transitional ECM formed following injury to blood vessels, and are thought to contribute to intimal hyperplasia by permitting smooth muscle cell (SMC) migration and proliferation [156]. Over time, the intimal hyperplasia associated with balloon or stent-mediated injuries regress. Importantly, this is associated with the loss of versican [157–159].

What roles then, for ADAMTS cleavage of versican in vascular pathologies? There is mounting speculation that versican fragmentation could promote intimal regression [147,152]. Therapies aimed at preventing restenosis following vascular reconstruction have traditionally focused on inhibiting intimal hyperplasia, but therapies aimed at promoting intimal atrophy may also have merit, particularly once restenosis is evident [147]. A promising study by Kenagy et al., has shown that high blood flow/high shear conditions through aortic iliac grafts in baboons is associated with neointimal atrophy [160], SMC death and versican degradation [161]. In this model system, ADAMTS-4 mRNA levels were elevated in the neointima of the grafts, whereas the mRNA levels for ADAMTS-1, -5, -8, -9, -15 and -20 were unchanged [161]. Furthermore, ADAMTS-4 generated the DPEAEE neoepitope in this system, whereas ADAMTS-1, -5, -8, -9, -15 and -20 did not [161]. Moreover, the addition of Fas ligand to aortic SMC cultures increased ADAMTS-4 mRNA and cell-death by approximately 5-fold, suggesting a causal relationship between ADAMTS-4 cleavage of versican, cell death and tissue atrophy in aortic-iliac grafts under these conditions [161].

Jonsson-Rylander et al. [162] have shown that ADAMTS-1 protein is upregulated in intimal tissue when plaque is present. ADAMTS-1 is expressed by migrating and proliferating primary aortic SMC *in vitro*, and contributes to intimal thickening in a carotid artery flow cessation model [162]. Others have shown that ADAMTS-4 and -8 are present in advanced atherosclerotic plaques and that ADAMTS-4 mRNA is induced during lesion development in a mouse model of atherosclerosis [163]. Given the many, and diverse roles of versican in vascular disease, and the presence of the ADAMTS enzymes, the likelihood that ADAMTS cleavage of versican contributes to vascular pathology is high. It will be interesting to watch this area of research unfold.

5.3.2. Angiogenesis

Pathological angiogenesis is a fundamental process in tumour malignancy and chronic inflammatory diseases. The initial step involves breakdown of the venular basement membrane; subsequent remodelling permits vascular expansion, and the formation of highly abnormal and hyperpermeable “mother” vessels (MVs). Vascular endothelial growth factor/vascular permeability factor (VEGF-A/VPF) is a major contributor to this process. Pathological angiogenesis can be modelled *in vivo* by injecting an adenoviral vector expressing VEGF^{164} into nude mice. Using this model, Fu et al. [164] have demonstrated recently that versican in the basement membrane is degraded early in the course of pathological angiogenesis, as evidenced by the loss of versican staining and the appearance of the DPEAAE epitope, localised to MV endothelial cells. ADAMTS-1 protein increased *in vivo* concomitant with MV formation. As the MV formed daughter vessels, strong staining for versican reappeared. The data suggest a role for ADAMTS-mediated remodelling of versican during pathological angiogenesis.

5.3.3. Ovulation

ADAMTS activity against proteoglycans often leads to pathology, as discussed above. However, there are other situations in which ADAMTS cleavage of proteoglycans is part of normal physiology, and in these situations, ablation of ADAMTS activity can have detrimental effects *in vivo*. One example is the release of mature oocytes from the ovarian follicle during ovulation. Proteoglycans are important components of the ovarian follicle and follicular fluid [165]. In particular, versican is selectively localised to the expanding follicle [166] where it is thought to promote the influx of fluids and to interact with hyaluronan to help stabilise and support this rapidly expanding matrix. ADAMTS-1 also accumulates in the expanding follicle matrix where its expression is increased 10-fold [167].

Proteinases, including several ADAMTS enzymes, are expressed in the mammalian ovary (reviewed in [168]), but to date only ADAMTS-1 is known to have an essential role in normal ovulation, based on the phenotype of the *Adamts-1* null mouse [169,170]; *Adamts-1* null females are sub-fertile. They have a normal eostrous cycle but impaired oocyte release, with a significant proportion of oocytes retained within the follicle after ovulation. Thus, the *Adamts-1*null female mouse phenocopies the ovulatory failure seen in the progesterone receptor null mouse [171]. This is not surprising, given that one feature of the progesterone receptor null mouse is its inability to induce ADAMTS-1 [172]. Importantly, a positive correlation between *Adamts-1* gene expression and ADAMTS-1 activity in the ovarian follicle is confirmed by the presence of the versican DPEAAE neoepitope [167]. Russell et al. have therefore concluded that one function of ADAMTS-1 during ovulation is to cleave versican, and that failure to do so in either the *Adamts-1* null mouse, or the progesterone receptor null mouse, causes impaired

ovulation leading to sub-fertility, or infertility, respectively. Other features of the *Adamts-1* null mouse include follicular dysmorphogenesis, delayed development of the ovarian lymphatic system and impaired organisation of the medullary vascular network of the ovary [173,174].

5.3.4. Tissue morphogenesis

Versican is widely expressed in embryonic tissues where it is often co-expressed with ADAMTS enzymes. ADAMTS cleavage of versican is thought to be essential in numerous developmental processes, many of which have been elucidated in *Adamts*-deficient mice by association, or with the anti-DPEAAE neoepitope antibody. For example, bone is synthesised during skeletal development and fracture healing, initially as woven bone which is comparatively weak and less ordered than the more mature lamellar bone by which it is eventually replaced. During the transition from woven bone to lamellar bone in development of the rat mandible and hind limbs, there is a concomitant transition in the composition of the bone matrix from one rich in versican and ADAMTS-1, -4, -5, to a mature bone matrix that has a decreased content of versican and ADAMTS-proteinases [175]. The authors of this study propose that versicanolysis by ADAMTS proteinases is associated with bone maturation.

Syndactyly, or webbing of toes and fingers, occurs normally in foetal development but is usually lost by birth due to apoptosis and proteolysis of the interdigital web. Recent studies in mice with compound mutations in the genes for *Adamts-5, Adamts-9* and *Adamts-20* suggest that the catalytic activity of two or more ADAMTS enzymes against versican at the $E^{441}\downarrow^{442}A$ site is essential to allow regression of the interdigital web in mouse paws, possibly by reducing the threshold for BMP-induced apoptosis needed to ensure web regression [176].

Cleft palate describes the failure of the lateral palatine processes to fuse, and therefore close. As a consequence, the nasal cavity and the mouth are directly connected rather than separated by the roof of the mouth. Versican is abundant in palatal shelf mesenchyme and in mice, combined loss of ADAMTS-9 and -20 activity in the vascular endothelium and the palate mesenchyme, respectively, causes cleft palate [177]. The defects in versican processing were detected by a loss of DPEAAE immunoreactivity [177].

ADAMTS-20 is also involved in the development of skin pigmentation. *Adamts-20* mutant mice have a distinctive coat with a white belt in the lumbar region. Versican and DPEAAE epitopes are enriched in skin, and one of the (several) likely mechanisms by which ADAMTS-20 contributes to pigmentation is by remodelling versican in the ECM surrounding melanoblasts in the embryo [145]. Silver et al. showed that DPEAAE immunoreactivity, but not total versican immunoreactivity, is reduced in the skin of *Adamts-20* mutant mice compared with wildtypes. ADAMTS-5 also has a role in proteoglycan remodelling in adult skin [178]. In a model of excisional wound healing, versican and aggrecan accumulate in the skin of *Adamts-5*$^{-/-}$ mice during the period of wound contraction, compared with wildtype mice. In this model, the over-abundance of versican and its catabolites resolves as the wound repairs suggesting that versican is degraded by a proteinase other that ADAMTS-5. However, pericellular aggrecan is retained in the *Adamts-5*$^{-/-}$ mouse dermis. The authors propose that aggrecan retention in the *Adamts-5*$^{-/-}$ mice delays wound healing by preventing fibroblastic cells from maturing into contractile fibroblasts [178].

During development of synovial joints, a proportion of cells within the developing limb form an interzone of non-chondrogenic cells that separates the future cartilage elements. As cavitation begins to form the synovial cavity, the interzone cells show strong expression of versican and secrete high levels of HA. Recently investigators studying limb development in mice detected increased cleavage of versican by ADAMTS enzymes in the interzone, via immunodetection of the DPEAAE neoepitope [179]. This immunoreactivity overlapped with ADAMTS-1, but not ADAMTS-4 immunoreactivity, implicating the former as a key proteinase in joint cavitation. However, there must be redundancy in this process since joint dysmorphology is not a feature of the *Adamts-1* null mouse.

During foetal cardiac valve development, the provisional proteoglycan-rich ECM is replaced by a stratified ECM that includes collagen and elastin. Anomalies of the cardiac valve in early development are marked by persistence of the proteoglycan-rich matrix, suggesting a failure to resorb the provisional matrix. *Adamts-5*$^{-/-}$ mice develop enlarged cardiac valves by late foetal stages and a recent report showed that these enlarged valves are associated with reduced versican cleavage in the sub-endocardial matrix, seen by decreasing staining for the DPEAAE epitope [180]. The valve phenotype can be significantly rescued by decreasing the levels of versican in the matrix via versican heterozygosity for the *Vcan* gene in the *Adamts-5*$^{-/-}$ mouse [180]. These findings suggest that a lack of versican cleavage during development of the foetal valve may have a role in future cardiac valve disease.

A parallel study showed that *Adamts-5*$^{-/-}$ mice also have a dermal fibroblast phenotype, marked by reduced versican proteolysis in the pericellular matrix [181]. This is associated with an altered cell shape and enhanced contractility in collagen gels, typical of myofibroblasts. The fibroblast-myofibroblast transition was reversed by heterozygous expression of the inactive *Vcan* gene in the *Adamts-5*$^{-/-}$ mouse, suggesting that versican turnover by ADAMTS-5 is needed to maintain dermal fibroblast phenotype.

One mandatory consequence of versicanolysis at the $E^{441}\downarrow^{442}A$ site, be it a pathological or physiological event, is the formation of a G1-DPEAAE fragment whose size is dependent on the isoform of the parent versican molecule. There is continuing intrigue and speculation amongst the research community that this fragment, and other catalytic fragments of proteoglycan substrates, might have unique biological properties that are not invested in the parent molecules. Indeed, recombinant single domains of versican appear to have bioactivity in cell culture systems [182–184].

6. Perspectives

Proteoglycans in extracellular matrices are degraded by ADAMTS enzymes in many and varied biological situations. Given the extent of this proteolysis *in vivo*, the prospect that the products of proteoglycan proteolysis are signalling molecules that allow cells to monitor the health and integrity of their extracellular environment is an exciting, albeit not entirely new, consideration. Bioactive fragments of degraded matrix can interact with cells or other matrix components to signal in ways that are distinctly different to their parent precursors. We envisage that fragment signalling could modulate normal tissue homeostasis or arise in response to tissue pathology. These catalytic events can be mapped with neoepitope antibodies and used as potential biomarkers of destructive or regenerative proteolysis *in vivo*. Proteoglycan fragments resulting from ADAMTS cleavage could also be exploited for drug development.

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