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Review

ADAMTS: a novel family of extracellular matrix proteases

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Abstract

ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) is a novel family of extracellular proteases found in both mammals and invertebrates. Members of the family may be distinguished from the ADAM (a disintegrin and metalloprotease) family members based on the multiple copies of thrombospondin 1-like repeats they carry. With at least nine members in mammals alone, the ADAMTS family members are predicted by their structural domains to be extracellular matrix (ECM) proteins with a wide range of activities and functions distinct from members of the ADAM family that are largely anchored on the cell surface. ADAMTS2 is a procollagen N-proteinase, and the mutations of its gene are responsible for Human Ehlers-Danlos syndrome type VII C and bovine dermatosparaxis. ADAMTS4 and ADAMTS5 are aggrecanases implicated in the degradation of cartilage aggrecan in arthritic diseases. Other members of the ADAMTS family have also been implicated in roles during embryonic development and angiogenesis. Current and future studies on this emerging group of ECM proteases may provide important insights into developmental or pathological processes involving ECM remodeling.

Keywords: ADAMTS; Metalloprotease; Extracellular matrix; Aggrecanase

Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs; BMP-1, bone morphogenetic protein 1; ECM, extracellular matrix; EDS, Ehlers-Danlos syndrome; MMP, matrix metalloproteinase; TACE, TNFα converting enzyme.

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1. Introduction

The interaction with the extracellular matrix (ECM) provides cells with, in addition to mechanical support, a wealth of information necessary for the regulation of cell fate and morphology. Thus, cell-ECM interactions are important in mediating diverse physiological events such as lineage decisions during embryogenesis, differentiation, cell migration, wound repair and programmed cell death. A wide spectrum of ECM remodeling and turnover occurs during growth and development, as well as in pathological states such as cancer. Cell surface and ECM proteases play pivotal roles in these processes [1,2].

A large number of molecules with protease activities are involved in proteolytic processes in the ECM. These can be divided into several protein families based on their distinct domain structures. The first group consists of serine proteases such as thrombin, tissue plasminogen activator, urokinase and plasmin [3,4]. The second group, the matrix metalloproteinases (MMPs) is a large family (about 23 members) of highly conserved Zn-dependent endopeptidases [5,6]. The first two groups act generally as broad-spectrum proteases for major ECM degradation events and are principal participants in cancer metastasis [3,4,7,8]. The third group, the bone morphogenetic protein 1/tolloid family of metalloproteinases are linked to cellular differentiation and pattern formation through a proposed role in activating latent growth factors of the TGF-β superfamily [9,10]. Finally, the ADAMs (for a disintegrin and metalloprotease) or the MDC (for metalloprotease/disintegrin/cysteine-rich) proteins are a family of transmembrane glycoproteins with diverse roles in cell-cell adhesion and proteolysis [11–15].

The ADAM family of proteins, now numbering almost 30, has been identified in organisms ranging from *Schizosaccharomyces pombe* to humans. They all have a characteristic conserved domain structure. An N-terminal signal sequence is followed by a prodomain, a metalloprotease domain, a disintegrin domain and a cysteine-rich region usually containing an EGF repeat. Most members also have a transmembrane domain followed by a cytoplasmic tail at the C-terminus. Although the metalloprotease domains are relatively well conserved, only 15 of those identified contain the catalytic site consensus of the zinc-binding peptidase (HEXXH), and are thus, predicted to be catalytically active. The rest are probably lacking in metalloprotease activity.

The ADAMs have been implicated in diverse processes in cellular adhesion and proteolytic processing of important cell surface molecules. The heterodimeric sperm's protein — fertilin α and β (ADAMs 1 and 2) are essential for sperm-egg fusion during fertilization [16]. Meltrin α (ADAM 12) has a role in myoblast fusion during muscle development [17]. The TNFα-converting enzyme (TACE, ADAM 17) is involved in proteolytic release of the extracellular domains of cell surface transmembrane growth factor molecules such as TNFα [18,19]. As indicated by the phenotype of developmental defects resulting in embryonic lethality in TACE knockout mice, TACE is also responsible for the ectodomain shedding of TGFα [20]. The *Drosophila* metalloprotease disintegrin

KUZ (kuzbanian, SUP-17 of *Caenorhabditis elegans*, ADAM 10 of mammals) is important for neural development through its processing of Notch [21–23] and the Notch ligand Delta [24]. The physiological function for most of the other ADAM family members, however, has yet to be elucidated.

The past 2–3 years have witnessed the identification of a new family of ADAM-related proteins, collectively known as ADAMTS [25–28]. They have the characteristic ADAM-like protease domain, a disintegrin-like and a cysteine-rich domain. However, they differ from conventional ADAMs with a distinct feature — a thrombospondin type 1 (TSP-1)-repeat [29] found between the disintegrin-like and the cysteine-rich domain. This is also followed by a varying number of TSP-1-like repeats at the C-terminus. Importantly, unlike the ADAMs, these proteins lack transmembrane domains and are, therefore, secreted into the ECM. The reader is referred to other excellent articles [11–15] for a review on ADAMs. This review will discuss the new family of ADAMTS proteins, focusing on some of its members whose physiological functions are now beginning to be established

2. ADAMTS — Genes, expression and domain structure

The currently known members of the ADAMTS family are listed in Table 1. The nomenclature 'a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif' (ADAMTS) and the consecutive numbering of the mammalian ADAMTS 1–8 follow that of the human genome organization (HUGO) gene nomenclature committee (http://www.gene.ucl.ac.uk/users/hester/adamts.html). Aliases, inevitable when the same gene was discovered or cloned by more than one group, and other trivial names, are also shown. One *C. elegans* ADAMTS (Gon-1) has been reported [30] and there are two other open reading frames in the *C. elegans* genome that code for ADAMTS-like proteins. Likewise, there are three open reading frames in the *Drosophila* genome that looks like ADAMTS, and they are listed with their GeneBank accession numbers. Also shown in Table 1, are the respective chromosomal localization of the mammalian ADAMTSs. Unlike several of the MMP genes that are clustered in the mammalian genome, the ADAMTS genes are rather well distributed amongst the human chromosomes.

Most of the mammalian ADAMTS genes are expressed at low levels in adult tissues but meaningful comparison of transcript levels in different tissues had been made by Northern analysis and RT-PCR [25,27,31,32]. ADAMTS1, ADAMTS4 and ADAMTS7 are the relatively more abundant and ubiquitous ones. ADAMTS2, 3 and 8 are generally lower in abundance and are detected at low levels in particular tissues. ADAMTS5 and ADAMTS6 are exclusively expressed in placenta [27]. The expression profiles of the ADAMTS are not particularly helpful in pointing to their possible function. A low transcript abundance means that they are less likely to be represented as expressed sequence tags to facilitate their identification through database searches, and there may be more members of the family encoded by the mammalian genome that awaits discovery.

The deduced primary structure of representatives of some mammalian ADAMTS and the *C. elegans* Gon-1 are compared with that of a typical MMP and a typical ADAM in Fig. 1. The primary sequence of an ADAMTS is organized into modular structures. Like the ADAMs, there is a typical signal sequence at the N-terminus, followed by a putative prodomain of varying length. A furin cleavage site concensus (RX(K/R)R) [33] marks the end of the prodomain of all the mammalian ADAMTS proteins. The ADAM protease domain is well conserved amongst all members of the family, with a typical reprolysin-type Zn-binding signature and a critically conserved methionine which forms the 'met-turn' — a structural feature common to all metzincins [34]. The catalytic site consensus HEXXH is present in all ADAMTSs, and all the proteins are presumably catalytically active. The catalytic domain is followed by a region with 35–45% similarity to snake venom disintegrins [35]. The corresponding region in some ADAM molecules such as the

fertilins are important for their function in promoting cell adhesion and fusion. There is no evidence yet that this domain in ADAMTSs interacts with any integrin.

The disintegrin-like domain is followed by a feature that distinguishes ADAMTSs from ADAMs, a TSP-1 motif that is similar to that in thrombospondin 1 [29] and which is rather similar amongst all members of the ADAMTS family. This TSP-1 motif is followed by a cysteine-rich domain containing 10 conserved cysteine residues. A region of relatively low homology amongst the ADAMTSs and without any distinct feature, known as the spacer domain, follows the cysteine rich domain. The molecule ends with repeats of TSP-1-like motifs that are much less conserved in sequence relative to the first TSP-1 motif.

Overall, the mammalian ADAMTSs show between 20 and 40% similarity to each other. A nearest neighbor dendrogram illustrating the phylogenetic relationship between all the members of the ADAMTS family prepared by the DNASTAR MegAlign program is shown in Fig. 2. There are four recognizable subfamilies. The first

Table 1
The ADAMTS family members[a]

ADAMTS	Other names	Chromosomal location [27,60]	Remarks on function
Mammalian			
ADAMTS1	METH-1 [38]; KIAA1346	21q2I-q22	Inflammatory response [25]; Angiogenesis [38]; organ morphogenesis [39]
ADAMTS2	Procollagen N-proteinase [31]	5q23-q24	Procollagen processing [45]
ADAMTS3	KIAA0366	4q21	
ADAMTS4	Aggrecanase-1 [50] KIAA0688	1q21-q23	Aggrecan cleavage [50]; Brevican cleavage [54]
ADAMTS5	Aggrecanase-2 /ADAMTS11 [51];		
Implantin [27]	21q21-q22	Aggrecan cleavage [51]	
ADAMTS6		5	
ADAMTS7		15	
ADAMTS8	METH-2 [38]	11q25	Angiogenesis [38]
KIAA1312		3	
C. elegans			
Gon-1			Gonadal development [30]
CE02110			
CE07514			
Drosophila			
AAF46065	CG4096		
AAF46905	CG3622		
AAF55199	CG6107		

[a] All known members of the ADAMTS family, including unpublished sequences in the database are listed with their aliases, human chromosome location and remarks on possible or revealed functions. The numbering of the mammalian ADAMTS is according to that of the HUGO nomenclature committee, and a list of the known mammalian ADAMTS can be found at http://www.gene.ucl.ac.uk/users/hester/adamts.html. The *C. elegans* predicted polypeptide sequences encoding putative ADAMTS proteins are found by searching the Sanger Center's Wormpep database using the BLAST search server at http://www.sanger.ac.uk The numbers shown are Wormpep accession numbers. The putative *Drosophila* ADAMTS proteins are found by searching the *Drosophila* genome with the NCBI servers at http://www.ncbi.nlm.nih.gov The numbers shown are GeneBank Accession numbers and the *Drosophila* genome accession number. The human chrososomal assignment of mammalian ADAMTS1-8 was as reported by Apte's laboratory [27,61]. The sequence and chromosomal mapping information on KIAA1312 can be found in the Human Unidentified Gene-Encoded (HUGE) database of the Kazusa DNA Research Institute at http://zearth.kazusa.or.jp.

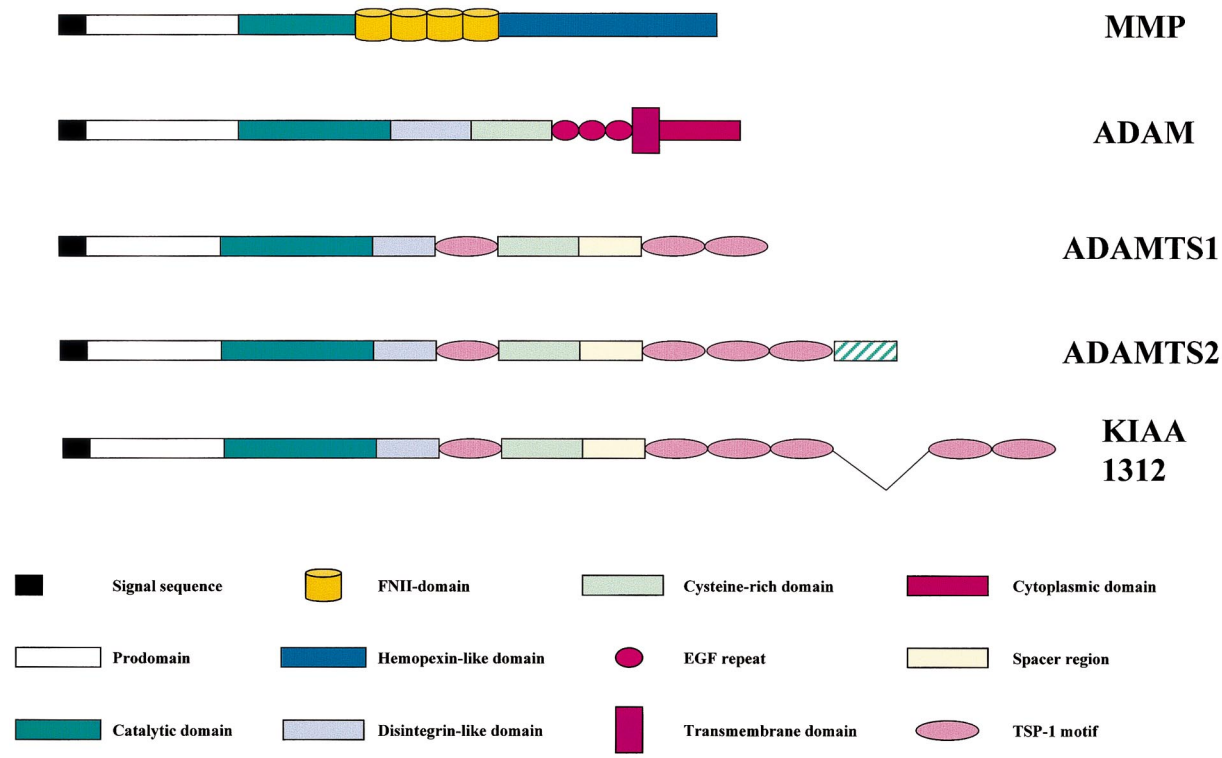

Fig. 1. Schematic representation of the domain structure of the ADAMTS family in mammals. Depicted are general structural features of a MMP, a typical ADAM and ADAMTS1, ADAMTS2 and KIAA1312. The generalized structure shown for MMP is that of MMP-3 and MMP-9 and not all the domains depicted are present in all other MMPs. Most ADAMs have a transmembrane domain that anchor them to the cell surface, a feature that is absent in the ADAMTS family. Instead, the ADAMTS have a TSP-1 motif and several other TSP-1 like motifs at the C-terminus. The box at the C-terminus of ADAMTS2 indicates an extension beyond the last TSP-1-like motif that is found in ADAMTS2 and ADAMTS3. KIAA1312 is unique in that it has 11 TSP-1-like motif at the C-terminus.

include ADAMTS1, 4, 5 and 8, the second brings together Gon-1, the *Drosophila* AAF55199 and a partial sequence in the database known as KIAA1312 (discussed later), the third subfamily include ADAMTS6, 7 and the *Drosophila* AAF46085 and the fourth lumps together ADAMTS2 and ADAMTS3. Two sequences from *C. elegans* and one from the *Drosophila* appear to be remote from each other and from others, above. They have a less homologous first TSP-1 motif and also appear to lack certain conserved cysteine residues.

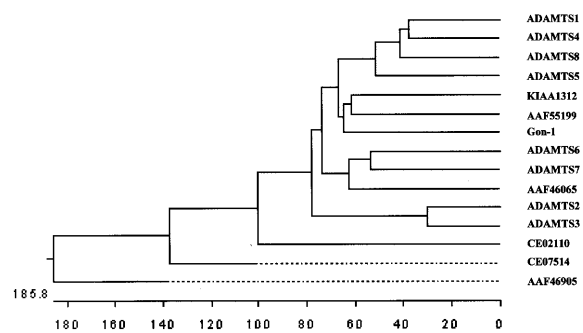

Fig. 2. Phylogenetic relationship amongst members of the ADAMTS family. A nearest neighbor dendrogram depicting the phylogenetic relationship between all the known members of the ADAMTS family (generated by the DNASTAR MegAlign program).

3. ADAMTS1 — role in inflammation, angiogenesis and development

ADAMTS1 is the prototype of the ADAMTS family of proteins, and the term ADAMTS was first coined by Kuno et al. upon the identification of mouse ADAMTS1 [25]. It is a gene that is selectively expressed in a mouse colon carcinoma cell line (colon 26) that was used as a model for cancer cachexia. The authors showed that the mouse ADAMTS1 transcripts could be induced by stimulating colon 26 cells with an inflammatory cytokine, interleukin-1, suggesting that the gene could be induced during an inflammatory response. Examination of adult tissues revealed only low transcript levels in heart and kidney. Systemic inflammation induced by intravenous administration of lipopolysaccharide resulted in enhanced expression of ADAMTS1 mRNA in these two tissues, but not other organs. This reinforces the notion that ADAMTS1 is an inflammation-associated protein.

Upon cloning of the gene, the authors immediately demonstrated that the first TSP-1 repeat of ADAMTS1 could interact with heparin, much like the TSP-1 repeats in thrombospondin 1 and 2 [25]. They followed this up with the demonstration that ADAMTS1 transiently expressed in COS-7 cells are not secreted into the culture medium, but was found associated with the ECM [36]. The protein is secreted in two forms — a larger precursor form and a smaller mature form, as expected when the proprotein undergoes proteolytic processing at its furin cleavage site. Deletion analysis revealed, interestingly, that the prodomain in conjunction with the proteinase domain has ECM binding capacity, as the precursor form of a mutant with only these domains intact can still be found in the ECM. The spacer region and the TSP-1-like motifs at the C-terminal are apparently important for the tight association of the mature protein with the ECM. The mature form of a mutant with only the first TSP-1 motif intact is found largely in the medium. Further, analysis of the carboxyl-terminal regions revealed that the spacer region alone associates with the ECM. The spacer region, thus named for its lack of recognizable homology to other proteins, is, therefore, an ECM interaction domain.

As mentioned above, all the ADAMTS described to date have a Zn-binding peptidase consensus HEXXH, and are presumably catalytically active. The metalloproteinase activity of ADAMTS1 was inferred from its ability to form a complex with α_2-macroglobulin [37]. A point mutation within the Zn-binding motif abolished complex formation. Furthermore, formation of the mature form of ADAMTS1 is impaired in the furin-deficient LoVo cell line, and can be restored by the coexpression of furin cDNA. As discussed later, physiological substrates have been identified for ADAMTS2, 4 and 5. The above data, however, remained primarily important in establishing the paradigm that ADAMTS are secreted active metalloproteinase that binds to the ECM.

The human ADAMTS1 (termed METH-1) and a close homologue ADAMTS8 (METH-2) were identified and cloned by Vazquez et al., and these authors had analyzed the proteins from a different perspective [38]. As thrombospondin-1 is an inhibitor of angiogenesis, they had looked for proteins containing TSP-1 repeats with antiangiogenic ability. Accordingly, recombinant ADAMTS1 and ADAMTS8 made in mammalian cells suppressed FGF2-induced vascularization in a cornea pocket assay and inhibited VEGF-induced angiogenesis in a chorioallantoic membrane assay. Both proteins also inhibit endothelial cell proliferation, but not the growth of fibroblast or smooth muscle cells. Although the angiogenic inhibitory capacity is rather remarkable (greater than that mediated by thrombospondin-1 or endostatin on a molar basis), it is unclear at present how that might be achieved. A possibility discussed by the authors involves the disintegrin-like domains, which may functionally inhibit neovascularization should they interact and inhibit the right type of integrins.

More insight into the physiological function of ADAMTS1 came from the recent targeted disruption of the gene in mouse, the first *ADAMTS* gene subjected to such analysis in vertebrates [39]. *ADAMTS1*$^{-/-}$ mice exhibit significant growth retardation and adipose tissue malformation. The major pathological features include significant changes in kidney structure. A particularly distinctive feature is an obstructive fibrosis extending

from the ureteropelvic junction into the ureter. Electron microscopy showed the accumulation of collagen fibers in these obstructions, suggesting that processing of collagen and related ECM substances may be impaired. This particular phenotype is consistent with ADAMTS1 transcript enrichment in the kidney and supports the notion that its protease activity is important for ECM remodeling during organ development. Female fertility is impaired in *ADAMTS1*$^{-/-}$ mice, and this is accompanied by obvious abnormalities of the uterus and ovaries. *ADAMTS1* was recently shown to be a progesterone-regulated gene in the ovulation process [40], and it is, therefore, not surprising that it has a function in shaping the female genital organs.

Another phenotype of the knockout mouse is more difficult to reconcile with the earlier findings. The adrenal gland of the *ADAMTS1*$^{-/-}$ mouse is abnormal — the reticular network of capillaries of the adrenal medulla is disrupted with cavities and few capillaries containing blood cells were observed. The function of the adrenal medulla did not appear to be severely impaired by its malformation as the levels of catecholamine metabolites in the urine of *ADAMTS1*$^{-/-}$ mice is comparable to that of wild type mice. However, this finding is not at the first glance consistent with the observed anti-angiogenic effect of ADAMTS1. In any case, the data from the knockout of *ADAMTS1* demonstrates that the gene product has multiple and non-redundant functions in organogenesis. The structural abnormalities in the kidney bears particular resemblance to that found in congenital ureteropelvic junction obstruction and primary obstructive megaureter in humans, and may be useful as a disease model.

4. ADAMTS2 — a procollagen N-proteinase

Collagen, a major constituent of the ECM, is synthesized as precursors in the form of procollagen triple helices flanked at the N-terminus and C-terminus by propeptides. The removal of these propeptides by specific proteinases is necessary for the subsequent assembly of collagen fibrils in the ECM. In 1996, two groups reported the identification of the procollagen C-proteinase as the earlier described bone morphogenetic protein-1 (BMP-1) [41,42]. A procollagen N-proteinase specific for type I and type II procollagens that was purified to homogeneity the year before, by Colige et al. [43], turned out to be an ADAMTS.

The procollagen N-proteinase activity has long been known and detected [44] but biochemical purification of the activity came very much later. Colige et al. purified a 107 kDa protein from fetal calf skin and showed that this polypeptide has the full type I procollagen N-proteinase activity. With peptide sequences from this protein, the authors cloned the bovine procollagen N-proteinase [45], or ADAMTS2. ADAMTS2 differs from most other ADAMTS in having a unique C-terminal extension after its last TSP-1-like repeat, a feature that it shares with a homologous (52% identity) human sequence in the database, KIAA0366 (ADAMTS3). It is not clear at present, which ECM components the C-terminal region of ADAMTS2 interact with, in vivo. Interestingly, although one would expect it to bind type I and type II collagens, the bovine skin ADAMTS2 was found instead to bind, rather specifically, to type XIV collagen during its purification. It is also unknown at present, if the very homologous ADAMTS3 also has procollagenase activity. In addition, a tripeptide sequence, RGD, which is a potential integrin binding site, is present on both the predicted mature protease of bovine and human ADAMTS2. The corresponding sequence in ADAMTS3 is RGE. Whether, these are authentic integrin binding sites, remains to be investigated.

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of congenital connective tissue disorders characterized by altered biomechanical properties of skins, joints, blood vessels and ligaments, mainly resulting from mutations in genes encoding collagen isoforms or genes involved in collagen processing. EDS type VIIC and dermatosparasis, the related disease in cows and sheep, are characterized by severe skin fragility resulting from a defect in the N-terminal processing of type I procollagen. These unprocessed precursors assemble into abnormal collagen fibrils that do not provide the normal tensile strength to

skin tissues. Colige et al. cloned the human ADAMTS2 gene and identified the corresponding mutations in several EDS type VIIC patients and a dermatosparactic calf [46]. So far, ADAMTS2 is the only member of the ADAMTS family whose physiological substrate and corresponding genetic disorders have been clearly characterized.

5. ADAMTS4 and ADAMTS5 aggrecanases

The articular cartilage contains large amounts of the proteoglycan aggrecan, which contributes significantly to the mechanical properties of the articular cartilage in withstanding compressive deformation during joint articulation. The loss of aggrecan fragments from the articular cartilage and their release into the synovial fluid by proteolytic cleavages is a central pathophysiological event in osteoarthritis and rheumatoid arthritis. Two major cleavage sites have been identified in the proteolytically sensitive interglobular domains at the N-terminal region of the aggrecan core protein. The first is between Asn^{341} and Phe^{342}, and several matrix metalloproteinases (MMPs) have been shown to cleave at this site [47]. The MMPs are, however, not responsible for another site of cleavage between Glu^{373} and Ala^{374}. A putative proteolytic activity, termed aggrecanase, has been postulated to be responsible for the cleavage at this site, and this activity is also relevant to cartilage degeneration in inflammatory joint diseases [47–49].

Biochemical purification of two aggrecanases and subsequent molecular cloning revealed that they are members of the ADAMTS family. By following the aggrecanase activity with a neoepitope antibody, aggrecanase-1 (ADAMTS4) and 2 (ADAMTS5, also termed ADAMTS11 by the authors), was purified from IL-1-stimulated bovine nasal cartilage conditioned media using two different purification schemes [50,51]. Gene cloning and expression of recombinant forms of the aggrecanases in *Drosophila* S2 cells confirmed their substrate and cleavage specificity. A more recent follow up of the work on aggrecanase-1 revealed four additional cleavage sites within the C-terminal region of the aggrecan core protein [52]. In another paper, the authors showed that the TSP-1 motif of aggrecanase-1 is critical for substrate recognition and cleavage [53]. The Tsp-1 motif binds to the glycosaminoglycans of aggrecan and the enzyme was not effective in cleaving GAG-free aggrecan. Furthermore, aggrecanase-1 with the TSP-1 motif deleted is not functional and peptides corresponding to different regions of the motif blocked cleavage by preventing substrate binding.

The identification of the aggrecanases and the availability of recombinant forms of the enzymes will undoubtedly facilitate the development of therapeutics for inhibiting aggrecan degradation in inflammatory joint diseases. Aggrecan, however, may not be the only physiological substrate of the aggrecanases. Brevican is a brain specific ECM protein, whose expression is dramatically increased in the human and rat gliomas. A notable recent report showed that the synthesis and cleavage of brevican may play a role in the invasiveness of intracranial grafts of the highly invasive CNS-1 glioma cell line, and that ADAMTS4 is responsible for the brevican cleavage activity [54]. Another relevant point to note concerns the functional sites of the aggrecanases in an organism. Aggrecanase-1/ADAMTS4 transcript levels are by far more abundant and ubiquitous in adult tissues. Its expression is particularly prominent in lung, heart and brain. *ADAMTS5* transcripts are expressed to a significant level only in placenta. In fact, another group had given ADAMTS5 a trivial name, implantin, based on its specific expression at the peri-implantation period of the mouse embryo [27].

5.1. Gon-1 — a role for ADAMTS in development

The phenotype of *ADAMTS1*$^{-/-}$ mouse in a way confirmed the suspicion that some members of the ADAMTS family, as ECM localized proteinases, may have rather specific and nonredundant roles in shaping organ structures during development. A direct demonstration of this role for an ADAMTS came from studies in *C. elegans* gonadal development. During development, the *C. elegans* gonad acquires a U-shape by the di-

rected migration of a specialized 'leader cell' located at the tip of the growing gonadal arm. In *gon-1* mutants, the adult gonad is severely disorganized, with essentially no arm extension and no recognizable somatic structure [55]. The *gon-1* gene product thus, appears to have two independent functions. It is required for leader cell migration and for morphogenesis of gonadal somatic structures.

Cloning of the *gon-1* gene by mapping and mutant rescue revealed that the gene product is a member of the ADAMTS family [30]. It bears significant homology to the mammalian ADAMTSs, but has an extraordinarily long C-terminal tail consisting of 17 TSP-1 like motifs. Its expression in the leader cells comes on during the period of most active gonadal-arm elongation, and is in agreement with its proposed function in directing the expansion of the gonad by remodeling of the basement membrane. Interestingly, a predicted polypeptide encoded by a partial human sequence in the database, KIAA1312, bears striking similarity to Gon-1 in also having an extraordinarily large number of TSP-1-like motifs at the C-terminal tail (11 of them in this case). KIAA1312 may well be a mammalian homologue of Gon-1.

6. Future perspectives

It is now clear that we are looking at a new family of ECM proteases with distinct structure and function from earlier described families. Investigations into their physiological functions, as well as their participation in pathological conditions are just beginning. The expression of most members of the family are probably developmentally regulated and their low abundance in adult tissues suggests that there are new mammalian ADAMTS that will come to light only when the human genome is completely sequenced. Meanwhile, there are many fundamental questions to be addressed and many fruitful lines of investigation await our exploration.

There is little doubt that most, if not all ADAMTS, will be functional proteinases, and as such, their physiological and pathophysiological roles would have much to do with their catalytic activity. It is, therefore, of great interest to identify the respective ECM substrates for the ADAMTSs, which may be specific or context dependent on the physiological environment. ADAMTS1, for example, has recently been shown to be able to cleave aggrecan, at least in vitro [56]. The substrate specificity of the protease may be determined by the protease domains themselves, but it will also be influenced to a larger extent by the C-terminal TSP-1 motifs and spacer domains that will serve as the substrate recognition and binding sites. In other words, the ECM molecule that can interact with the TSP-1/spacer motif of a particular ADAMTS would likely to be a substrate. In addition, all the ADAMTS have the furin cleavage site concensus, and prodomain cleavage by furin-like proteases could be a major way of producing active ADAMTS. However, other cell surface or ECM proteases may also serve to cleave, and, therefore, regulate, the activity of locally secreted ADAMTS. It would be of interest to see, if any of the MMPs or ADAMs have such roles, and if ADAMTS activities are regulated by cascades of proteases action in response to physiological cues.

Data from ADAMTS1 and Gon-1 suggest that ADAMTS may have specific and nonredundant roles in tissue morphogenesis during development. The severity of these phenotypes contrasts those of the soluble MMP knockouts, which are rather mild [56]. In view of the importance of MMPs in roles established by many other studies, the surprisingly mild phenotype of MMPs may be in part attributed to functional redundancy amongst its members. An alternative consideration emphasizes the primary roles of MMPs in mature tissues rather than a requisite function in morphogenesis during development. The developmentally regulated expression of some members of the ADAMTS family make them excellent candidates for fulfilling the latter role. Analysis of all the ADAMTS genes by transgenesis, targeted disruption and allelic knockin is, therefore, essential. A thorough and detail examination of the temporal and spatial expression patterns of *ADAMTS* transcripts would also be very helpful in delineating their roles in development.

Finally, the upregulation of ADAMTS1 and ADAMTS4 in a colon carcinoma and a glioma cell line, respectively, suggests that dysregulated expression of members of the ADAMTS family may be involved in tumorigenesis. In view of their enzymatic nature, and drawing analogies from the MMPs [7,8], they would serve tumor cells not just in terms of invasion and metastasis, but also in functions such as growth, angiogenesis and migration. It may, therefore, not be too far fetched to undertake a systematic examination of various tumor lines and samples for ADAMTS activity (especially the highly metastatic and invasive ones). It would also be of interest to see if some of the future *ADAMTS* gene knockouts become less susceptible to tumorigenesis, like some of the MMP knockouts.

In marked contrast to the mild phenotype of the soluble MMPs mentioned above, more global and severe connective tissue defects were recently observed in the membrane type MT1-MMP$^{-/-}$ mice [57]. In fact, it was also recently shown that MT1-MMP, but not the soluble MMPs, is capable of mediating invasion of a collagen matrix in an in vitro model system [58]. The *mig-17* mutant of *C. elegans* exhibits morphologically abnormal gonadal arms as a result of incorrect migration of the 'leader' cells, unlike *gon-1* mutants, where the migration is completely inhibited. The recent cloning of the *mig-17* gene revealed that its product is an ADAM [59]. It bears significant homology to ADAMTS1 but does not have TSP-1 motifs. Clearly, any given developmental or pathological process that require spatial movement of cells would involve coordinated action of several different ECM proteases. It will be both interesting and challenging to fit the ADAMTSs amongst the serine proteases, MMPs and ADAMs to form a comprehensive picture of ECM remodeling during cell migration and invasion [60].

7. Note added in proof

The structure shown for KIAA1312 in Fig. 1 is based on a predicted structure of a full length gene product, however currently KIAA1312 encodes a partial sequence. Recently a paper has been published describing a new ADAMTS family member, ADAMTS9 (Clark et al., ADAMTS9: a novel member of the ADAM-TS/metallospondin gene family. Genomics 67 (2000) 343–350) ADAMTS9 appears to be an alternatively spliced isoform of KIAA1312, with a complete N-terminus but ending after three TS repeats at the C-terminus. ADAMTS9 transcripts are expressed in all fetal tissues as well as some adult tissues, and the gene is localized to 3p14.2-14.3, an area known to be lost in hereditary renal tumors.

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