

## The roles of ADAMTS metalloproteinases in tumorigenesis and metastasis

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## 1. ABSTRACT

The human ADAMTS (a disintegrin and metalloproteinase with thrombospondin-like motifs) family of 19 secreted, multidomain proteolytic enzymes is involved in a wide range of biological processes including ECM assembly and degradation, hemostasis, organogenesis and the regulation of angiogenesis. Defects in certain family members give rise to inherited human genetic diseases, while aberrant expression of other ADAMTSs has been linked to the pathogenesis of arthritis and cancer. Several ADAMTSs act as tumor or metastasis suppressors whose functions are lost either by mutation or epigenetic silencing during tumor progression. This review looks in depth at the involvement of ADAMTSs as positive and negative mediators in cancer growth and spread.

## 2. INTRODUCTION

The defining characteristic of malignant tumors is their ability to invade surrounding tissues, leading to metastatic spread, the main cause of death in cancer patients. The notion that secreted proteases facilitate tumor growth and metastasis by providing a path-clearing function to degrade basement membranes and stromal extracellular matrix (ECM) dates back many years. This led to major investment by the pharmaceutical industry in the 80's and 90's in targeting enzymes such as the matrix metalloproteinases (MMPs) that had been shown to be key mediators of cancer cell invasion (1). However, broad-spectrum metalloproteinase inhibitors (MPIs) failed in cancer clinical trials (2). Although a major disappointment, this setback has energized investigations into the functions

of proteases in tumor biology and what has become clear over the last decade is that the simple concept of proteases as ECM degrading machines is no longer sufficient. These enzymes establish and regulate the extracellular microenvironment by precise cleavage of molecules such as growth, adhesion and differentiation factors and their signalling receptors, influencing their bioavailability and generating bioactive fragments that determine cell phenotype and fate (3). Moreover, it is now appreciated that proteases of multiple catalytic classes, most of which were unknown at the time of the MPI cancer trials, can have powerful actions as suppressors of malignancy (4). Among such enzymes are members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin-like motifs) family.

In the sections that follow, we will provide a general introduction to the ADAMTS metalloproteinases and then review recent developments in our understanding of their roles in tumor pathobiology. Several members of this gene family appear to act as tumor or metastasis suppressor functions that are switched off during tumor progression, and a recurrent theme will be their anti-angiogenic actions. However it is also clear that certain members, in particular ADAMTS1, may accelerate malignancy in appropriate contexts, which reflects a need to understand in greater detail the actions of this interesting gene family. There have been several excellent recent reviews of this fascinating class of enzymes which will provide the reader with further insights into their basic biological functions and involvement in cancer and other diseases (5-10).

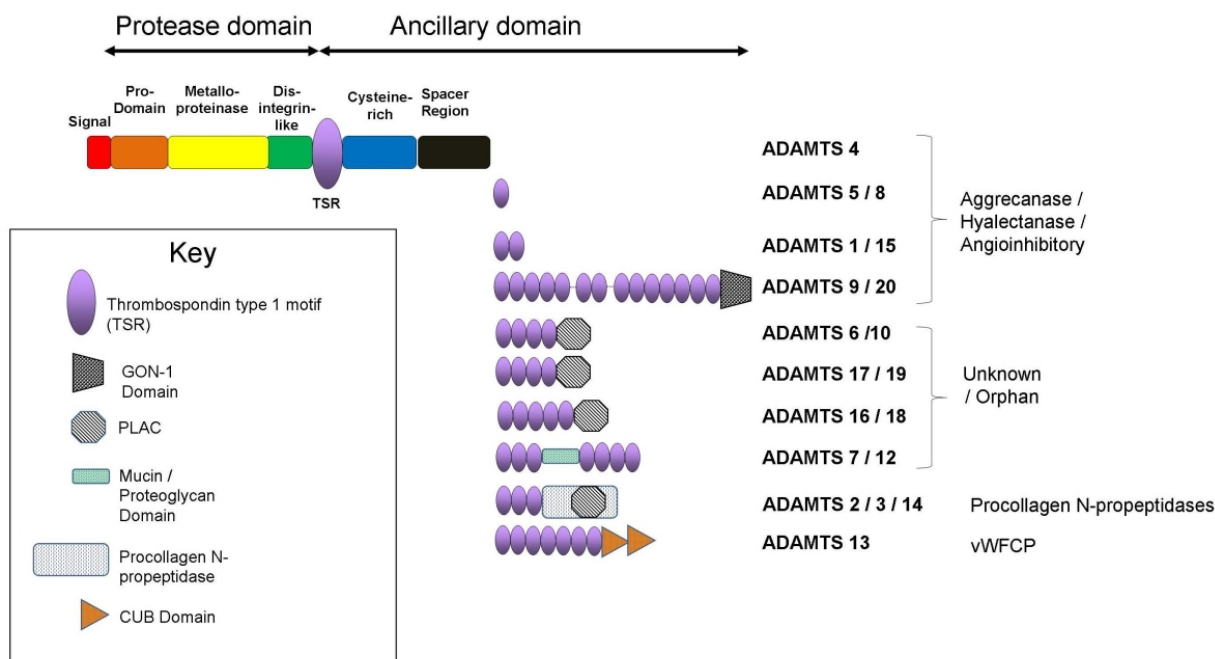
### 3. THE ADAMTS FAMILY

The ADAMTSs are extracellular, secreted enzymes that have diverse functions in animal development and disease. There are 19 members of the human ADAMTS family, of which the founding member (ADAMTS1), was identified in 1997 as a gene associated with inflammation and cancer-induced cachexia (11). The ADAMTSs are structurally related to the ADAM (a disintegrin and metalloproteinase) enzymes and more distantly to the MMPs; all of these metalloproteinases belong to the “metzincin” superfamily (5). A sub-group (with ADAMTS4 and -5 foremost) cleave ECM proteoglycans such as aggrecan and versican at specific sites, and have thus been termed “aggrecanases” or “hyalectanases”. Aggrecanase activity is pivotal in the cartilage matrix breakdown associated with arthritis, in which ADAMTS5 has emerged as the key player. Several of the hyalectanase/aggrecanase group (ADAMTS1, -4, -5, -8, -9, 15 and -20) possess anti-angiogenic properties, which involve multiple mechanisms that will be discussed in detail in a later section. Another group of ADAMTSs (ADAMTS2, -3 and -14) are pro-collagen N-propeptidases that are essential for the maturation of triple helical collagen fibrils (5). In humans, *ADAMTS2* mutations give rise to Ehlers-Danlos syndrome type VIIc, a connective tissue disorder characterized by severe skin fragility. Another rare connective tissue disorder, autosomal recessive Weill-Marchesani syndrome, results from

mutations in *ADAMTS10* that interfere with the normal function of the matrix glycoprotein fibrillin-1 and the bioavailability of TGFβ (7). *ADAMTS13* is required for hemostasis as it encodes the von Willebrand Factor (vWF)-cleaving proteinase, which processes the large multimeric vWF precursor in the circulation to an optimal size to allow proper coagulation. Mutations in *ADAMTS13* lead to formation of platelet-rich thrombi which occlude blood flow to vital organs, resulting in the inherited disease thrombotic thrombocytopenic purpura (TTP). Essential roles for other ADAMTSs in development are emerging from knockout mouse studies. Examples include the recent demonstration of a role for *ADAMTS5*, -9 and -20 in interdigital tissue regression during limb development (12), *ADAMTS1* in cardiac morphogenesis (13) and *ADAMTS9* and 20 in melanoblast development in the skin (14).

The ADAMTSs have a compound domain organization (Figure 1), the simplest form of which is characterized by ADAMTS4, which has a signal peptide followed by a pro-region of variable length (which in general preserves enzyme latency but has other functions for certain ADAMTSs as discussed further below), a metalloproteinase domain, a disintegrin-like domain, a central Thrombospondin type 1 Sequence Repeat (TSR) motif and a cysteine-rich domain followed by a spacer region. Other family members build on this basic structure with a variety of further C-terminal domains, including one or more additional TSRs. The entire C-terminal region downstream from and including the central TS repeat is termed the ancillary domain, which is where the greatest differences between ADAMTS family members occur. Entirely separate from the ADAMTS family, another group of seven ADAMTS-like genes constitutes a distinct family encoding proteins that resemble ADAMTS ancillary domains, but which lack the metalloproteinase or disintegrin-like domains (7). These ADAMTS-like proteins may function to modulate the activities of the ADAMTSs, and indeed they are also proving to have relevance to cancer (15), however in this review we will focus on the proteolytically competent ADAMTS enzymes.

Lying C-terminal to the variable number of TSR arrays, four additional modules are found in the ancillary domains of particular ADAMTSs. These include a Gon-1 module in ADAMTS9 and -20, and a PLAC (protease and lacunin domain) in several family members. In the pro-collagen N-propeptidase group (ADAMTS2, -3 and -14) the PLAC domain is embedded within a C-terminal extension. Finally, in the von Willebrand Factor-cleaving proteinase (ADAMTS13) there are two C-terminal CUB domains. The 19 human ADAMTSs can be grouped into 8 clades based on their similar structural organization and known functions, and strikingly, most are displayed in pairs in (Figure 1) which reflects their origins via a series of gene duplication events during evolution. They can be traced back to 6 ADAMTS genes in the chordate sea squirt *Ciona intestinalis* which underwent gene duplication early during vertebrate evolution, along with retrotransposition events that expanded the “hyalectanase/aggrecanase/angiogenesis” group comprising ADAMTS1, -4, -5, -8, -9, -15 and -20 (16, 17).



**Figure 1.** The domain organization of the ADAMTS family. The figure shows a schematic at the top left of the basic organization of structural modules, with separation into the proteinase and ancillary domains. The modules are identified in the key on the left of the figure. The modules include: TSR, Thrombospondin type 1 Sequence Repeat; Gon-1, a module with homology to sequences in the *gon-1* gene of *C.elegans*; PLAC, a protease and lacunin domain; CUB, complement C1r/C1s, Uegf (epidermal growth factor-related sea urchin protein) and BMP-1 (bone morphogenetic protein-1).

The basic ADAMTS structure can be considered as a protease domain (containing the metalloproteinase and the disintegrin-like modules) and an ancillary domain, the latter determining substrate specificity and enzyme localization via ECM association, the former defining substrate cleavage specificity (7). The crystal structure of ADAMTS1 reveals that the so-called “disintegrin-like” module is a misnomer and that this is actually a cysteine-rich module that is part of the protease domain (18). Certainly, there is no association of any ADAMTS protein with integrins, as has been documented for the ADAM proteins (19). Various parts of the ancillary domain are involved in binding of mature ADAMTS proteins to the ECM. The TSR modules and the spacer region of ADAMTS1 were shown to function in glycosaminoglycan (GAG) binding (20), and the Cys-rich domain/spacer region of ADAMTS4 has three GAG-binding sequences (21). A critical point of control that can determine both the localization and substrate preferences of the ADAMTSs is their proteolytic processing within the C-terminal ancillary domain. These internal cleavages may profoundly alter the catalytic functionality of the enzymes, as shown for example for ADAMTS4 (22), as well as generating fragments with novel bioactivities.

The pro-domain is generally considered to maintain enzyme latency in metalloproteinases, however for particular ADAMTSs interesting additional, and in certain cases divergent, roles have emerged, including involvement in ECM association and secretion. Proprotein convertases such as furin are involved in ADAMTS pro-

peptide removal and for ADAMTS1 and -4 this follows the conventional pathway of cleavage in the *trans*-Golgi to generate active enzyme (23, 24). However, demonstrating a paradoxical regulatory mechanism, pro-ADAMTS-9 processing by furin occurs obligatorily on the cell surface and this reduces the activity of the enzyme to cleave versican. The pro-domain of ADAMTS-9 thus acts as a chaperone to ensure secretion of the enzyme rather than latency (25). Likewise pro-ADAMTS13 does not require pro-domain removal for catalytic activity (26), however it is not clear at present how far this phenomenon extends to other ADAMTSs.

As with their ADAM relatives, ADAMTSs show restricted susceptibility to inhibition by the four members of the tissue inhibitor of metalloproteinases (TIMP) family. Where a series of TIMPs has been tested, TIMP-3 emerges as the most effective inhibitor, as is the case for several ADAMs (19). The aggrecanase activity of ADAMTS1 is inhibited by both TIMP-2 and -3, but not TIMPs-1 and -4 (27). Inhibition of the aggrecanase activity of ADAMTS4 by TIMP-3 is enhanced in the presence of aggrecan, which involves interaction of the GAG chains of aggrecan with the TSR and spacer region of ADAMTS4, generating a complex with improved affinity for TIMP-3 compared to that of the metalloproteinase alone (28). This is interesting as it exemplifies a key point that will surface again later in this review, that the cell and matrix environments in which the ADAMTS proteins operate will exert powerful effects upon their activities. Another interesting difference between the ADAMTSs and the MMPs relates to their

mode of inhibition by TIMPs. For inhibition of MMPs, the N-terminal Cys residue of the TIMP molecule is essential as it coordinates with the Zn atom at the active site (29). If TIMP-3 is extended at the N-terminus by an Ala residue, this completely abrogates MMP inhibitory activity, but potent inhibition of ADAMTS4 and -5 (and ADAM17) is retained (30). This may be significant in the design of next generation MPIs with improved selectivity for ADAMTSs.

### 4. THE ADAMTSs IN CANCER BIOLOGY

Building on this general background, in the sections that follow, we will expand on the roles of individual ADAMTSs in cancer. Expression profiling has yielded some valuable initial insights into the possible roles of the ADAMTSs in tumor progression. For example, our laboratory has profiled the expression of the full ADAMTS family in human mammary tumors and non-neoplastic breast tissue by real-time PCR (31). Ten of the 19 family members showed markedly altered expression between normal and cancer tissues, the majority (*ADAMTS1*, -3, -5, -8, -9, 10 and -18) being down-regulated in tumors. In contrast *ADAMTS4*, -6 and -14 were up-regulated. Many of the ADAMTSs were shown to be expressed by myoepithelial cells in normal tissues, which suggest that the ADAMTSs may be important in maintenance of the correct tissue architecture in the mammary gland. Expression of *ADAMTS1* has also been seen to be decreased in non small cell lung cancer (NSCLC) compared to corresponding non-diseased lung tissues (32). In contrast in head and neck squamous cell carcinomas a recent study revealed elevated expression of *ADAMTS1*, -8 and -15 in tumor tissues and tumor-derived cell lines compared to normal tissues/cell lines (33). In another study Demircan and colleagues analysed expression of *ADAMTS1*, 4, 5, 8, 9 and 15 in primary head and neck carcinomas with and without metastases and saw decreased expression compared to normal tissues, but expression of all of the genes except *ADAMTS4* was increased in metastatic foci compared to primary tumors, suggesting possible involvement in metastasis (34). *ADAMTS4* and -5 were seen to be upregulated in glioblastoma (35). Expression studies therefore need to be followed up by correlation with clinical characteristics and patient data on large patient cohorts for any of these relationships to be strengthened. However, as discussed below, for several *ADAMTS* genes there is now growing functional evidence for promoting or inhibitory roles in tumor growth or metastasis.

#### 4.1. ADAMTS1

*ADAMTS1* was identified in 1999 during a screen for new inhibitors of angiogenesis and was first named METH-1 due to the presence metalloproteinase and thrombospondin domains in its structure (36). Disruption of *Adamts1* in mice results in extensive perinatal lethality, with surviving animals showing decreased growth and abnormalities in uterine, adrenal and adipose tissues and infertility in female mice, indicating its importance in organogenesis and ovulation (37, 38).

Of all of the family, *ADAMTS1* has the most extensive literature linking it to cancer, though both promoting and antagonistic effects on tumorigenesis and

metastasis have been observed in different studies. However, it is possible that these opposing views can be reconciled. An important study by Liu and colleagues showed that over-expression of full-length *ADAMTS1* in TA3 mammary carcinoma and Lewis Lung Carcinoma cells enhanced their metastatic ability (39). Metalloprotease activity was essential for metastasis promotion. However, these authors found evidence for autolytic cleavage of *ADAMTS1* within its spacer region, and that over-expression of portions of *ADAMTS1* corresponding to the N- and C-terminal fragments inhibited pulmonary metastasis. A similar metastasis suppressing effect was previously seen for the C-terminal half of *ADAMTS1* when expressed in CHO cells (40). These studies show that proteolytic processing of *ADAMTS1* within its ancillary domain has a profound effect upon its biological function. Of note also, *ADAMTS1* has been shown to be epigenetically silenced in 85% of colon cancer cell lines, consistent with a role as a tumor suppressor (41).

#### 4.1.1. ADAMTS1 and inhibition of angiogenesis

*ADAMTS1* and *ADAMTS8* (termed METH-2 (36)) have both been shown to have powerful anti-angiogenic actions *in vivo* in the chick chorioallantoic membrane (CAM) and the corneal pocket assays. *ADAMTS1* significantly blocked neoangiogenesis induced by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (FGF2), with an anti-angiogenic activity greater than that of endostatin or thrombospondin-1 on an equivalent molar basis. It inhibited the growth of endothelial cells (EC) but not fibroblasts or smooth muscle cells and the effect was reversible as removal of *ADAMTS1* led to the resumption of EC proliferation (36). Since this initial demonstration of the anti-angiogenic action of *ADAMTS1*, evidence has surfaced for three distinct mechanisms that may contribute towards it, namely sequestration of VEGF<sub>165</sub> (42), binding to FGF2 (43) and proteolytic cleavage of thrombospondin (TSP) -1 and -2, releasing bioactive anti-angiogenic fragments (44).

*ADAMTS1* has been shown to inhibit EC proliferation induced by both VEGF<sub>165</sub> and FGF2, with a concomitant inhibition of VEGFR2 phosphorylation (42). *ADAMTS1* bound VEGF<sub>165</sub> *in vitro* but not VEGF<sub>121</sub> which lacks the heparin-binding domain of the larger form, however cross-linking analysis indicated the interaction was direct and did not require co-association of heparan sulphate proteoglycans. Further, deletion analysis showed that binding involved only the ancillary domain of *ADAMTS1*. This is interesting since full-length active 87kDa *ADAMTS1* had previously been shown by the same authors to be cleaved by MMP-2, -8 and 15 within its spacer region to generate a 65kDa N-terminal portion and release the two C-terminal TSRs, with a concomitant reduction in its anti-angiogenic function (23). This suggested that at least some of the anti-angiogenic activity of *ADAMTS1* must reside in the C-terminal TSRs. However, additional studies have shown that the metalloproteinase catalytic activity of *ADAMTS1* also plays a role in suppression of angiogenesis. Overexpression of *ADAMTS1* in T47D mammary carcinoma cells reduced growth and vascularization of tumors in nude mice and this

effect was not seen with a catalytically dead (E-A) version of the enzyme (45). In a subsequent study, it was revealed that thrombospondins-1 and -2, which are homotrimeric, ECM-associated molecules in their native state, are cleaved by ADAMTS1 to liberate monomeric, soluble C-terminal fragments that block angiogenesis (44). The anti-angiogenic actions of TSP-1 and -2 are known to involve the three TSRs contained in these molecules, and mass spectrometry confirmed that the ADAMTS1 cleavage site lay just upstream of these repeats (44). Neo-epitope antibodies raised against sequences adjacent to the cleavage site confirmed that this cleavage occurs *in vivo* during skin wound healing and that *Adamts1*<sup>-/-</sup> mice showed reduced levels of TSP cleavage, associated with delayed wound closure and an increased angiogenic response. Taken together these observations provide powerful support for a role for the catalytic activity of ADAMTS1 in regulation of angiogenesis. Recently, the importance of this as a tissue-specific control mechanism has been demonstrated. Using colon and renal carcinoma cells, overexpression of TSP1 reduced the growth of metastases in the liver but not the lungs of recipient mice, which correlated with an increased level of ADAMTS1-mediated processing of TSP1 (to generate the anti-angiogenic fragments) in liver compared to lung (46). Thus, the level of expression of ADAMTS1 in a tissue may be a key factor controlling the suitability of the organ “soil” environment for metastasis formation.

### 4.1.2. Pro-tumorigenic/metastatic actions of ADAMTS1

Given these powerful negative effects of ADAMTS1 on neovascularisation, what might be the molecular mechanisms underlying its pro-metastatic actions? Liu and colleagues observed enhanced shedding of soluble heparin-binding epidermal growth factor (HB-EGF) and amphiregulin along with activation of the EGF Receptor and promotion of cell invasion (39). Further, they showed that the N- and C-terminal ADAMTS1 fragments inhibited Erk pathway activation in response to soluble HB-EGF and amphiregulin, potentially by binding to them and blocking their availability. Shedding of EGFR ligands by ADAMTS1 has also been invoked as the mechanism by which it collaborates with MMP-1 to promote bone metastasis (47). These authors argue that simultaneous expression of these proteases by breast cancer cells leads to enhanced release of amphiregulin, HB-EGF and transforming growth factor- $\alpha$  from the tumor cells which collectively suppress the expression of osteoprotegerin by osteoblasts in the bone environment. This in turn promotes osteoclast differentiation which creates an osteolytic environment that favours bone metastasis. This model is supported by clinical data that indicate a higher risk of bone metastasis in patients whose breast tumors were immunopositive for both ADAMTS1 and MMP1 (47).

Several other possible mechanisms by which ADAMTS1 might facilitate tumor growth and metastasis are emerging from the recent identification of biologically relevant ADAMTS1 substrates. ADAMTS1 sheds the ectodomain of the membrane-bound heparan sulphate proteoglycan syndecan-4 leading to alterations in the cytoskeleton, reduced adhesion and increased cell

migration (48). Semaphorin 3C was also recently identified as an ADAMTS1 substrate by a combination of proteomic approaches (49). Semaphorins are recognized as guidance cues in the development of the nervous system, and intriguingly, ADAMTS1-mediated release of semaphorin 3C from the ECM enhanced breast cancer cell migration (49). A similar proteomic strategy has identified five other ADAMTS1 substrates, namely the basement membrane proteins nidogen-1 and -2, the desmosomal protein desmocollin-3, and dystroglycan and Mac-2 binding protein (50). Thus the generation of novel bioactive fragments from its substrates, together with physical changes in cell behaviour as a result of shedding of adhesion molecules, could underlie the invasion and metastasis promoting effects of ADAMTS1. ADAMTS1 has also been shown to interact with and cleave Tissue Factor Pathway Inhibitor-2 (TFPI-2) an ECM-associated serine protease inhibitor (51). Since TFPI-2 inhibits the activities of several proteases including trypsin and plasmin, which in turn influence the activation of pro-MMPs, its cleavage by ADAMTS1 could profoundly affect the extracellular proteolytic balance. Also, ADAMTS1 associates via its ancillary domain with the ECM protein fibulin-1, which is not cleaved by ADAMTS1, but instead the complex displays enhanced aggrecanase activity (52). These observations add further support to the notion that particular tissue or tumor microenvironments, with the correct presentation of cofactors and substrates, may be necessary for cancer promotion by ADAMTS1.

Two additional aspects are relevant to this discussion, the first concerning the response of stromal cells to tumor-derived ADAMTS1, the second being the effect of ADAMTS1 on tumor cell phenotype. Overexpression of ADAMTS1 in BZR bronchial tumor epithelial cells was shown to have no effect on tumor cell growth *in vitro* but it enhanced *in vivo* growth, which was accompanied by induction of a stromal reaction including production of MMP-13, fibronectin, TGF $\beta$  and IL-1 $\beta$  (53). Catalytic activity was required for the effect. Key factors in conditioned media from ADAMTS1-expressing cells that were responsible for the increased chemotactic response of stromal fibroblasts were TGF $\beta$  and IL-1 $\beta$ . In another recent study using the HT1080 fibrosarcoma model (54), ADAMTS1 was found to increase tumor growth by inducing “vasculogenic mimicry”, whereby the tumor cells themselves were induced to acquire endothelial-like properties, including the expression of endothelial markers such as VE-cadherin. In fact, host vessels were diminished in the ADAMTS1-expressing tumors arguing that the pseudovascular networks became the dominant means of perfusion in these tumors.

### 4.2. ADAMTS8

ADAMTS8 (METH-2) is another key member of the aggrecanase/angioinhibitory ADAMTS clade. It is in general expressed at a lower level than ADAMTS1 and *in situ* hybridisation has shown its expression predominantly in embryonic and adult lung tissues and weak expression in, brain, heart and placenta (36). Expression profiling has shown that ADAMTS8 is downregulated in a number of different types of cancer, including breast (31), NSCLC

(32) and brain cancers (55). In NSCLC, promoter hypermethylation has been shown in 67% of tumors analysed (56), however this does not appear to contribute to the reduced expression seen in gliomas (55).

Although expression of ADAMTS8 mRNA is downregulated in mammary carcinomas when compared to non-neoplastic mammary tissue (31), further analysis in a cohort of 229 patients showed that patients whose tumors had higher ADAMTS8 expression had a decreased overall survival rate compared to those with lower levels (57). These data will be discussed further in the section on ADAMTS15.

### 4.3. ADAMTS9

ADAMTS9 and its relative ADAMTS20 have important roles in melanoblast development (14). These ADAMTSs have a characteristic feature in their ancillary domains that resembles a module in the *C.elegans Gon1* gene that is important for gonadal morphogenesis (reviewed in (5)). *Adamts9*<sup>-/-</sup> mice die prior to gastrulation indicating an essential developmental role for the gene (58). These characteristics suggest that ADAMTS9 may be involved in cell fate specification by influencing cell migration.

There is increasing data indicating that *ADAMTS9* is an important tumor suppressor gene that acts as an angiogenesis inhibitor. When *ADAMTS9* was initially identified, it was noted that its chromosomal location (3p14.3-p21.1) placed it in a region known to be lost in hereditary renal cancers (59). Subsequently, somatic cell hybridization identified *ADAMTS9* as a functional tumor suppressor gene in esophageal cancer (60). In 15/16 esophageal carcinoma cell lines complete loss or down-regulation of *ADAMTS9* expression was observed, which correlated with promoter hypermethylation. A similar strategy has recently identified *ADAMTS9* as a tumor suppressor gene in nasopharyngeal carcinoma (NPC), where loss of the gene was also correlated with increased lymph node metastasis in patients (61). Transfection of *ADAMTS9* reduced colony formation by NPC cell lines. Another study has also pointed to *ADAMTS9* as a locus that is lost in NPC (62).

Exciting recent data have indicated that ADAMTS9 is a cell autonomously acting angiogenesis inhibitor (58). These authors have shown that *Adamts9*<sup>+/-</sup> mice spontaneously develop increased corneal neovascularisation as they age, and tumor xenografts show increased vessel density. They observed expression of ADAMTS9 by endothelial cells in various tissues during development and show that knockdown of ADAMTS9 in human EC led to increased formation of tube-like structures on Matrigel, accompanied by increased spreading and migration in scratch wound assays *in vitro*. Importantly, the proteolytic activity of ADAMTS9 was required for its effects. However ADAMTS9 did not cleave TSP1, and neither did it bind to VEGF<sub>165</sub>, arguing that its anti-angiogenic actions are fundamentally different from those of ADAMTS1 and that it must act via proteolysis of a different target.

At present it is unclear how ADAMTS9 acts and whether its developmental, tumor suppressive and anti-angiogenic roles reflect a single function or multiple distinct activities. This will clearly be a fertile area for further study.

### 4.4. ADAMTS12

ADAMTS12 may be relevant to the pathogenesis of both cancer and arthritis. Like its relative ADAMTS7, ADAMTS12 is a mucin proteoglycan that interacts with and degrades cartilage oligomeric matrix protein (COMP), a major noncollagenous component of cartilage (63). COMP degradation is a hallmark of arthritis and functional data indicate that ADAMTS7/12 are the key enzymes responsible. ADAMTS12 expression is increased in osteoarthritic joints (64) and during chondrogenesis, where ADAMTS12 acts via its metalloproteinase activity to inhibit chondrocyte differentiation (65). However from the cancer standpoint, a tumor suppressor gene function has emerged for ADAMTS12. Ectopic expression of ADAMTS12 in Madin-Darby canine kidney cells (MDCK cells) inhibited the hepatocyte growth factor (HGF) induced epithelial to mesenchymal transition (EMT) (66). A catalytically inactive ADAMTS12 mutant also showed the same effect indicating that metalloproteinase activity is not required, however the presence of the ancillary domain is essential. The inhibition of HGF-induced scattering was shown to be caused by an inactivation of the RAS-MAPK pathway. ADAMTS12 inhibited VEGF-induced tubulogenesis by bovine aortic endothelial cells and *in vivo* tumor formation by A549 lung carcinoma cells (66). In a subsequent study, these authors went on to show that ADAMTS12 is epigenetically silenced by promoter hypermethylation in colon cancer cells (67). However, a higher level of ADAMTS12 was actually observed in tumor samples compared to normal tissues, but further examination showed that this arose from elevated ADAMTS12 expression in stromal fibroblasts. When fibroblasts and tumor cells were co-cultured *in vitro*, expression of ADAMTS12 by the fibroblasts led to suppression of tumor cell proliferation. A tumor suppressive role for ADAMTS12 has received further support recently from work with *Adamts12*<sup>-/-</sup> mice (68). These mice show no apparent developmental defects, but implantation of malignant PDVA keratinocytes resulted in lesions that were more invasive and more highly vascularized. Increased angiogenesis was also seen in Matrigel plug assays in these mice, and *ex vivo* in aortic ring assays. Exogenous ADAMTS12 inhibited angiogenesis in the aortic ring assay and this again occurred with a catalytically inactive version.

Collectively these observations support an important tumor suppressive role for ADAMTS12 via effects on both angiogenesis and tumor cell proliferation, and that these effects are attributable to the ancillary domain of the protein.

### 4.5. ADAMTS13

ADAMTS13 is von Willebrand factor (vWF)-cleaving protease, responsible for processing of large vWF

multimers in the circulation that otherwise cause platelet aggregation and occlusion of vessels. Large multimers of vWF can also increase adhesion of platelets to circulating cancer cells and thus promote metastasis. Thus a decrease in ADAMTS13 activity could potentially be associated with increased susceptibility for metastasis. Analysis of the levels of both vWF and ADAMTS13 in plasma samples from patients with localised solid tumors and patients with disseminated metastases showed a significant increase in the large vWF polymers and corresponding significant decrease in ADAMTS13 activity in the samples from patients with disseminated metastases (69). These data indicate that ADAMTS13 may be important in the formation of emboli of tumor cells and platelets that enhance tumor cell dissemination. Another study also reported an ADAMTS13 deficiency in patients with advanced stage-malignant tumors or grade IV colon cancer compared to patients with limited stage-malignant tumors or stage II colon cancer, further indicating a role for ADAMTS13 in metastasis formation (70). However, conflicting data have been presented (71, 72) indicating that this area needs further investigation. The relevance of ADAMTS13 levels to the detection of circulating tumor cells would seem to be a fertile topic.

### 4.6. ADAMTS15

ADAMTS15 is a member of the hyaluronanase/aggrexanase/angiostatin-inhibitory clade, though to date its activities and substrates are not known. However, evidence is accumulating for a tumor suppressor role for ADAMTS15. In a full survey of the expression of the 19 *ADAMTS* genes in breast cancer, our group showed that although there was no significant difference between the level of expression of the gene in normal mammary tissue and mammary cancers its expression in tumors was inversely correlated with patient survival (31). This was substantiated in a larger cohort of patients where it was shown that higher expression of *ADAMTS15* correlated with improved relapse-free survival suggesting a role as a metastasis suppressor (57). Indeed, patients whose tumors had high *ADAMTS8* and low *ADAMTS15* expression had a particularly poor prognosis with a 5-fold increased risk of relapse and a 3-fold increased risk of death. Intriguingly, during the timecourse of development of tumors in the aggressive MMTV-Polyoma middle T-antigen mouse model of mammary cancer, a similar elevation of *Adams8* and diminution of *Adams15* was observed. Since these genes lie adjacent to each other in a head-to-head arrangement at 11q24.3 in the human genome, it is possible that their expression is coordinated and this control is lost during tumor progression.

Two cancer genome sequencing studies have identified *ADAMTS15* as a candidate cancer (CAN) gene in colorectal and pancreatic cancer (15, 73). In the first study of this type, which involved analysis of over 13,000 genes in 11 breast cancer tumors and 11 colorectal cancer tumors, over 180 genes were identified with a mutation rate higher than expected to be observed by chance when taking into account the background mutation rate. Breast cancers showed an average of 12 mutations whereas colorectal

cancers showed 9 mutations. *ADAMTS15* was identified as a CAN gene in the colorectal cancer series, and again subsequently in a set of 24 pancreatic cancers (74).

A more detailed analysis focussed on *ADAMTS15* in colorectal cancer has confirmed that it is genetically inactivated by mutation in colon cancer (75). A total of three mutations were found in 50 tumors and a fourth in analysis of six colorectal cancer cell lines. The tumor-derived mutations were shown to be somatic in origin by comparison to normal DNA from the same individuals. One mutation was synonymous however the remaining 3 could result in aberrant proteins. Two of the mutations were base deletions resulting in frameshifts and truncated proteins, while a third was a point mutation in the first of the two C-terminal TSRs. One of the frameshift mutations (G849fs) was investigated in greater detail since this gave rise to a protein truncated just short of the C-terminal TSRs. Functional *in vitro* and *in vivo* studies using colon cancer cell lines and SCID mice revealed that wild type *ADAMTS15* is capable of reducing tumor growth and invasion. Ectopic expression of *ADAMTS15* in a colorectal cancer cell line that expressed endogenous *ADAMTS15* at a low level (HCT116) reduced colony formation and invasion compared to control cells but these effects were attenuated with the G849fs mutant. These outcomes were reversed in SW620 cells when the high endogenous *ADAMTS15* level was knocked down using shRNA. These *ADAMTS15* depleted cells showed increased tumor growth in SCID mice compared to control SW620 cells. The clinical relevance of *ADAMTS15* was also evaluated by immunohistochemistry on two tissue arrays with normal and tumor colorectal tissue. *ADAMTS15* expression was detected at high levels in normal tissue and was depleted in all the corresponding tumor samples, and was inversely correlated with histopathologic grade in colorectal cancers (75).

For *ADAMTS15* there was no evidence of epigenetic silencing in colorectal tumors. Moreover, no potential anti-angiogenic effect was noted by Vitoria and colleagues, however these authors did observe that cells expressing wild-type, but not mutant *ADAMTS15* showed reduced basal activity of the Ras-MAPK pathway in the tumor cells (75). They argue that this control of Ras-MAPK signaling, which is an important aspect of EMT, may be critical for the tumor suppressor action of *ADAMTS15* and mislocalization of the protein through loss of ECM association may thus contribute to tumorigenesis.

### 4.7. ADAMTS18

*ADAMTS18* is located at 16q23.1 in a region that is frequently lost during the development of many different tumors. Down-regulation of *ADAMTS18* expression in multiple tumor cell lines of diverse tissue origin has been correlated with promoter hypermethylation (76). Functional evidence for a tumor suppressive role was provided by the observation that ectopic expression of *ADAMTS18* in two carcinoma cell lines (nasopharyngeal HONE1 and esophageal EC109) reduced colony formation and anchorage independent growth. There are no details at present on which region of *ADAMTS18* confers tumor

suppression, however a recent study suggests that the ancillary domain plays an important role in dissolution of platelet thrombi (77). ADAMTS18 is expressed by endothelial cells and its secretion was enhanced by thrombin *in vitro* and *in vivo*. Thrombin was also shown to cleave ADAMTS18 generating a 45kDa C-terminal fragment that was as active as the anti-platelet integrin GPIIb/IIIa49-66 in induction of oxidative fragmentation of platelet aggregates. *In vivo* an antibody against ADAMTS18 shortened bleeding time in mice and the C-terminal fragment protected against postischemic cerebral stroke. These data indicate that ADAMTS18 has a potent role in hemostasis and the C-terminal ancillary domain may inhibit metastasis through modulation of platelet function, possibly by disruption of emboli of tumor cells and platelets, though this remains to be established.

### 5. PERSPECTIVE

In the space of little more than a decade since the discovery of the first ADAMTS, a tremendous amount of information has accumulated about the involvement of these extracellular enzymes in development and diverse pathologies, including inherited human disorders. The links to cancer pathogenesis and metastasis are growing and what is evident from this review of the field is that several ADAMTSs are likely to be functionally important tumor/metastasis suppressors. However, ADAMTS1 epitomizes the complexity of these enzymes with its display of both pro- and anti-cancer actions, which appear to depend on whether the protein is present in its full-length form or is internally cleaved to release all or part of its ancillary domain. A general theme that has emerged is that the C-terminal ancillary domain modules may be the source of much of the inhibitory activity. The ancillary domains contain the TSRs, and as it is the 3TSR regions of TSP-1 and -2 that are principally responsible for the anti-angiogenic actions of the thrombospondins via their association with the membrane glycoprotein CD36, which leads to induction of EC apoptosis (46), it is tempting to speculate that this is also the basis for the negative effects of the ADAMTSs on neovascularisation. This aspect requires further extensive evaluation. It is possible that the TSR domains in different ADAMTSs may have different interaction partners and thus biological effects. Moreover the central TSR may differ from the C-terminal versions, as recently borne out by a study that showed anti-angiogenic activity for the central TSR of ADAMTS5, but not the lone C-terminal module (78).

ADAMTS1 also exemplifies the notion that the actions of the enzymes are highly context dependent: potentially, the catalytic activity of ADAMTSs may create novel bioactive molecules from larger precursors that in turn can influence the nature of the tumor microenvironment or the metastatic niche. Clearly there remains a lot to be established about the functions of the ADAMTSs, but based on what we know already there is strong potential for translation into cancer therapies. For instance, dissection of the anti-angiogenic, anti-tumorigenic actions of ADAMTS ancillary domains may lead to novel clinically useful agents based on the TSRs or other features. Likewise, the possibility of next generation, ADAMTS-selective MPIs becomes realistic based

on what is being learned from the differences in active site topology and mechanisms of the ADAM(TS)s and MMPs. Indeed these two very different types of agents might show synergies when used in combination.

Fundamentally, however, we need to know more about the basic biological roles of these enzymes, which will hopefully accrue from further studies of gene knockout mouse models. It is clear that unlike most of the MMPs, these enzymes have critical developmental functions in determination of cell identity and fate and in organ formation, and it will be exciting to see further unravelling of their *in vivo* roles. We also need to determine the target substrates for these enzymes using new mass spectrometric “degradomic” technologies (79), but a particular challenge will be to decipher the truly biologically relevant molecules in specific cell/tissue/tumor/disease contexts.

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