Published in final edited form as:

Curr Drug Targets. 2008 October ; 9(10): 863–868.

Regulation of Thrombospondin1 by Extracellular Proteases

M. Luisa Iruela-Arispe

Abstract

The contribution of proteases to developmental, physiological and pathological processes has been well accepted. Cleavage of matrix proteins is a key requirement for cell migration and remodeling of the extracellular environment. The constant process of matrix turnover is dependent on the delicate balance between degradation and synthesis. In addition, regulated proteolysis also allows for the release and activation of growth factors and cytokines. Similarly to other extracellular matrix proteins, thrombospondins are also targets of proteolysis. While in some cases enzymatic activity is associated with degradation of the protein; in other situations, targeted and selective cleavage offers the means to release polypeptides with either alternative or enhanced function. Here we provide a summary of the published information related to thrombospondin proteolysis within the context of how proteolysis of extracellular matrix proteins impact diversification of protein function. We also discuss its biological relevance and potential therapeutic value of thrombospondin proteolysis with particular emphasis on angiogenesis.

Keywords

ADAMTS1; angiogenesis; anti-angiogenesis; matrix metalloproteases; matrix degradation; protein processing; proteolysis

Introduction

Regulated extracellular proteolysis play significant roles in developmental, physiological and pathological processes. The most frequent view is that proteolysis mediates destruction of proteins necessary for tissue expansion and cell motility. In reality, there is equal number of examples where proteolysis is required for activation of substrates or for diversification of the functional profile of a specific protein. Today, extracellular proteolysis is viewed as a more specific process; one that facilitates not only migration, but that simultaneously enables the activation of a cohort of proteins associated with tissue remodeling, expansion, and repair.

The cast members responsible for extracellular proteolysis are a large number of membrane-bound and secreted proteases that include two major cohorts of enzymes classified according to their cleavage properties and dependability on zinc for catalytic activity. They include: serine, aspartic and cysteine proteases on the first group and matrix metalloproteases (MMPs, ADAMs and ADAMTS) on the second group.

Matrix Metalloproteinases (MMPs) comprise one of the largest groups of extracellular enzymes whose activity is among the key rate-limiting steps in extracellular matrix

Correspondence should be address to: Luisa Iruela-Arispe, Dept. of Molecular, Cell and Developmental Biology, 615 Charles Young Drive South, BSRB 445F – UCLA, Los Angeles, CA 90095, arispe@mbi.ucla.edu, Telephone # (310) 794-5763, Fax # (310) 793-5766.

Conflict of Interest: There are no conflicts of interest to disclose

degradation [1,2]. These zinc-dependent endopeptidases include 28 distinct family members that collectively are capable of cleaving all matrix molecules. The activity of MMPs are regulated at three main levels: transcriptionally; post-transcriptionally, through activation (intra or extracellular); and by a group of endogenous inhibitors named tissue inhibitors of metalloproteases (TIMPs). The prototypical structure of MMPs include five major regions/ domains: (1) the signal peptide region; (2) the propeptide domain, which confers the status of latency to these enzymes; (3) the catalytic domain, this region houses the conserved Zn⁺² binding region (HE_xGH_{xx}G_{xx}HS/T) and is responsible for the activity of these enzymes; (4) the hemopexin domain, which determines the substrate specificity of MMPs and (5) a hinge region, which facilitates presentation of substrates to the catalytic domain. A small subgroup of MMPs also have a transmembrane domain that includes 20 or so hydrophobic residues and enables the enzymes to remain membrane-bound. These are frequently called MT-MMP, for membrane-type MMPs [3,4]. Much work has been done to ascertain the substrate specificity of MMPs, their mechanisms of action and specific TIMP inhibitors [5]. Collectively these enzymes are able to cleave collagens, glycoproteins and proteoglycans that populate the extracellular matrix of all four major tissue types [1–4]. However, except for TSP5 there is no information as to whether the anti-angiogenic TSPs (1 and 2) are substrates for MMPs.

The ADAM family (A disintegrin and metalloproteinase) includes a group of 29 enzymes with sequence similarity to the reprotolysin family of snake venomases that are also significantly related to matrix metalloproteinases. These enzymes also have members with integral-membrane and secreted forms and a multidomain structure comprised of propeptide, metalloprotease, disintegrin-like, cystein-like and epidermal growth factor-like domains [6–8]. Several ADAMs are expressed in multiple spliced forms with diverse functional properties. Although their expression is broad, many members of the ADAM family of proteases are expressed in the testis and ovary and display functions related to reproduction. Substrates of ADAM enzymes differ from MMPs and include growth factors (eg., TNF, TGF- α , HB-EGF), receptors (Notch, p75, p55, nectin, amphiregulin), and cell-cell adhesion molecules (E-cadherin) [6–8].

ADAMTSs are a subgroup of ADAM proteases characterized by the presence of thrombospondin type I sequence repeats (or TSRs) in the carboxyterminal region of the prototypical ADAM multidomain structure [9]. A total of 19 members of this family have been characterized to date and all are secreted glycoproteins. The presence of TSR repeats facilitates anchorage to the extracellular matrix and, in some cases, binding to substrates. ADAMTSs are involved in the processing of collagen, coagulation and degradation of proteoglycans and extracellular glycoproteins, including TSP1 and 2 [9,10].

ADAMTS1, the first member of the ADAMTS family to be cloned, was initially characterized as an inhibitor of angiogenesis stimulated by FGF-2 and VEGF165 [11,12]. Furthermore, ADAMTS1 was shown to block tumor angiogenesis when overexpressed in cancer cell lines by an anti-angiogenic mechanism [13]. A single point mutation in the catalytic domain of ADAMTS1 was sufficient to abolish its angio-inhibitory activities indicating that the catalytic function of the enzyme was required for this property [13]. Subsequent studies to identify substrates of ADAMTS1, provided insight on the mechanism of action responsible for suppressing angiogenesis. ADAMTS1 cleaves proteoglycans (aggrecan, versican, syndecan4) [14–16] and two glycoproteins present in vascular basement membranes: TSP1 and nidogen [10,17]. The relevance of TSP1 cleavage to the anti-angiogenic properties displayed by ADAMTS1 is discussed later within this review.

Proteolysis as a mean to regulate signaling cascades and provide function diversity

Proteolysis has become an integral part of cell signaling. Either by directly contributing to the activation of growth factors or through the modification of cell surface receptors, proteolytic changes are more frequently than not, part of the cascade of signaling pathways. For example, the cellular effects of thrombin are mediated by a group of four protease-activated receptors (PARs). These are G-protein-coupled receptors that carry their own ligand in a latent, inactive form. The ligand remains masked until proteolytic cleavage occurs by the serine protease thrombin [18,19]. Signaling initiated by thrombin-mediated cleavage of PARs contributes to haemostasis and thrombosis. In endothelial cells, PARs participate in the regulation of vascular permeability, tone, secretion and contraction [19]. They also contribute of the pro-inflammatory phenotype displayed by endothelial cells during atherosclerosis. PARs are a typical example of programmed autologous cleavage in the regulation of receptor signaling. Another classical example is Notch activation. In the case of Notch, ligand binding (by members of the Delta or Jagged families) is only the first step in activation [20]. Subsequent proteolytic events are needed to release the intracellular domain of the Notch receptor from its membrane anchorage and initiate signaling events [20]. Both members of the ADAM family of proteases (ADAM10 or 17) facilitate the initial cleavage and subsequently, presenilin proteases complete secondary processing resulting in the active form of the Notch receptor [20]. Proteolytic processing is essential for Notch activation and for the progression of the signaling cascade, in fact, presenilin inhibitors are frequently used as pharmacological blockers of Notch signaling [21].

Activation of some growth factors also requires proteolytic processing. VEGF-C and D, for example, are cleaved by plasmin releasing the VEGF homology domain region (VHD) that contains the receptor binding sites from a larger precursor [22]. This proteolytic processing generates fragments with a much higher affinity to VEGFR2 and VEGFR3 than the full-length form secreted by cells [23,24].

The proteins described above comprise only a small subset of molecules that exemplifies how shedding or extracellular cleavage provides a mechanism for regulated activation that relies on the presence or activity of a selective group of enzymes.

In addition to their activities in the regulation of signaling events, proteases also contribute to the diversification of extracellular matrix function. Cleavage of plasminogen to give rise to plasmin is a classical example, in which the resulting protein harbors a function not represented in the parental protein template. During the process of angiogenesis, the value of regulated protein processing has been demonstrated in both physiological and pathological conditions. Cleavage of collagen XVIII and plasminogen by MMPs results in the release of Endostatin and Angiostatin, respectively, both recognized inhibitors of angiogenesis [25–28]. Additionally, cleavage of collagen IV by MMP9 unmasks a cryptic site that stimulates migration of endothelial cells and angiogenesis *in vivo*; while the full length protein inhibits migration [29]. More recently, collagen IV and V have been shown to release bioactive anti-angiogenic molecules upon cleavage by a subgroup of matrix metalloproteases [30]. The process offers a regulatory feedback loop where degradation of the basement membrane during neoangiogenesis can possibly control the extent of the vascular response.

Thrombospondin1 as a substrate for extracellular proteases

Thrombospondin-1 is a member of a family of multimeric, calcium-binding extracellular glycoproteins that is secreted by a variety of cell types and is involved in the modulation of

cell-attachment, migration, proliferation, cell-cell and cell-matrix interactions [reviewed by 31–34].

The effects of TSP1 on endothelial cells have been shown to occur, in some cases by direct receptor signaling [35–40], and in other cases, through the ability of TS-1 to interact with other extracellular proteins including growth factors [41–43]. In fact, by nature of its interaction with proteoglycans, extracellular matrix molecules and growth factors, TSP1 directs the assembly of multiprotein complexes that modulate cellular phenotype. TSP1 also directly affects the activity of transforming growth factor beta (TGF- β) and plasmin [44–47], and can, therefore, indirectly work through modulation of these proteins.

Understanding the biological significance of these interactions and multitude of cell signaling pathways has become a challenge for investigators in this field. The generation of TSP1 null mice [48] provided clear support to the concept that TSP1 is required for the regulation of epithelial growth in the lung and lung homeostasis. In the absence of the protein, animals develop multifocal pneumonia and are more prone to inflammatory events [48]. In addition, the animals display hyperplasia of pancreatic islands and show delayed wound healing [44]. Some pathophysiological alterations, but not all, were overcome by treatment of animals with TSP1 peptides that mediate activation of TGF- β [44]. These results underscore the biological relevance of TSP1 for activation of TGF- β in vivo, but also bring to light other functions of this molecule that are independent of TGF- β -activation. Particularly interesting was the observation that TSP1 null animals show a reduced litter number and increased blood vessel profiles in several organs [47]. These defects are possibly related to the role of TSP1 in the modulation of angiogenesis that are independent of TGF- β .

The role of TSP1 as an angiogenesis inhibitor has received considerable attention. In vivo, TSP1 suppresses FGF-2-mediated angiogenesis in the cornea pocket assay and inhibits growth of blood vessels in the CAM assay [43,49]. Experiments in vitro performed by many laboratories have shown that the protein modulates adhesion, suppresses migration, inhibits proliferation, and signals apoptosis of endothelial cells [50,51]. In addition, overexpression of TSP1 by several tumor cell lines is associated with reduced tumor burden of xenografts due to a decrease in vascular density in comparison to control tumors [50,51]. Furthermore, systemic treatment of tumor-bearing mice with TSP1 or peptides derived from the TSP1 type I repeats inhibit tumor growth [52]. Collectively these findings support the concept that TSP1 is an endogenous angiogenesis inhibitor. Experimental search for the mechanism of action still continues. Although in some settings, a particular function of TSP1 is highlighted; it is likely that signaling on cell surface receptors, regulation of enzymatic activity, and sequestration of growth factors combined, rather than individually, are responsible for the effect of TSP1 in the suppression of capillary growth.

Modulation of enzymatic activity by TSP1 is a well documented property attributed to this protein. In vitro, TSP1 has been shown to be slow (rate constant approximately $6.3 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$), tight-binding ($\text{kD}10^{-9} \text{ M}$) inhibitor of plasmin. The effect of TSP1 on plasmin function has been also examined by loss of amidolytic activity, loss of ability to degrade fibrinogen, and decrease lysis zones in fibrin plate assays [47,53]. Stoichiometric titrations indicate that approximately 1 mol of plasmin interacts with 1 mol of TSP1 [53]. Plasmin in a complex with streptokinase or bound to epsilon-aminocaproic acid is protected from inhibition by TSP1, thereby implicating the lysine-binding kringle domains of plasmin in the inhibition process [53]. TSP1 also inhibits urokinase plasminogen activator, but more slowly than plasmin. The effect on uPA is specific, as TSP1 was shown to enhance the amidolytic activity of tPA and also it has no effect on the amidolytic activity of alpha-thrombin or factor Xa [53,54]. In addition, TSP-1 has been shown to be a competitive inhibitor of

neutrophil elastase [55]. In competitive binding assays, neutrophil elastase bound to TSP1 with a dissociation constant of 17nM. Although TSP1 is cleaved by neutrophil elastase, the site(s) of the limited cleavage are independent of the competitive inhibition of elastase activity by TSP1. Through this activity, TSP1 can protect fibronectin from cleavage by neutrophil elastase [55,56].

More recently, it has become evident that TSP1 and 2 regulate the activity of MMP9 and MMP2 respectively [57–59]. These results would be consistent with the reported effects of TSP1 on the suppression of endothelial cell migration. One can envision a scenario in which suppression of MMP-9 activity by TSP1 could hamper the migratory ability of endothelial cells depending upon the nature of the substrate [57]. This property can also contribute to the anti-angiogenic effects of TSP1. The ability of TSP2 to suppress MMP2 activity has been well-documented and is, at least, partially responsible for the alterations in collagen fibrinogenesis present in animals that lack TSP2 [59].

Release of TSP anti-angiogenic peptides by ADAMTS1

As previously discussed, modulation of MMP activity by TSP1 and TSP2 are likely contributors on the overall effect of these proteins in the inhibition of endothelial cell migration and angiogenesis. Like many matrix proteins, TSP1 and TSP2 function contextually as adapters and modulators of cell-matrix interactions, a concept that contributed to their matricellular status [33].

In addition, it is also clear that TSP1 is a large modular protein that interacts with several receptors through its multiple domains to elicit distinct functions. Anti-angiogenic properties in TSP1 have been mostly attributed to a subset of aminoacids located in the second and third type I domains [39,60,61]. Synthetic peptides that mimic the sequence of the second and third type I repeats are able to bind and sequester growth factors, particularly FGF2 and VEGF [42,43]. In addition, these peptides induce apoptosis when presented to endothelial cells, but not mediate cell death of smooth muscle cells or fibroblasts. The molecular mechanisms associated with this property has been elucidated and relate to their ability to bind and activate CD36, a transmembrane protein abundant in macrophages, endothelial and some epithelial cells [39,62]. TSP2, in contrast, does not bind to CD36 and its anti-angiogenic properties are linked to its effects in the suppression of MMP2 and perhaps on a yet to be identified receptor, as its N-terminal globular region has been shown to inhibit endothelial cell migration in vitro and reduce tumor vascularization in vivo [63].

Side by side comparison has shown that fragments from TSP1 and TSP2 are more effective than the whole molecule, when in equal molar basis [49]. In vivo, it has been difficult to ascertain whether TSP1 and 2 work as a whole molecular trimer complex or as fragments in the inhibition of angiogenesis.

As previously mentioned, ADAMTS1 is able to cleave both TSP1 and 2 releasing the C-terminal region from the trimeric amino-terminal domain [10] (Figure 1). The specificity of the cleavage was tested by exposing TSP proteins to a catalytically inactive form of ADAMTS1 generated by a single point mutation (E385A) in the zinc binding region [10]. This mutant form of the enzyme was unable to cleave either TSP1 nor TSP2. The processing site for TSP1 was mapped to aa 311 this single cut releases two fragments of 125kDa and 36kDa. N-terminal Edman degradation sequencing of the 125kD indicated the sequence LRRPPL indicating that the cleavage occurred between glutamic acid 311 and leucine 312 [10]. This site is consistent with the classification of glutamyl endopeptidase attributed to ADAMTS1 and based on the cleavage of other substrates such as aggrecan and versican [16,64]. Susceptibility of murine TSP1 to ADAMTS1 cleavage was also tested. Murine TSP1 shares high sequence identity to the human ortholog and was also expected to be

cleaved. The cleavage site with human TSP1 is mostly identical to the murine TSP1 and the released fragments retains functional similarity [10].

Other ADAMTS enzymes (ADAMTS4, 5, 8, 9) were also used in TSP1 proteolytic assays and so far only ADAMTS1 is able to mediate processing. Other enzymatic groups, however can cleave TSP1 as well. A particularly well known proteolytic cleavage is the one mediated by thrombin. This enzyme is generally used to facilitate the release of TSP1 from platelet alpha granules during the purification of human TSP1. Earlier studies showed that thrombin was able to release a small fragment of TSP1 resulting from a cleavage event within the procollagen-like domain [65]. The resulting fragments (25kDa and 160kDa) are distinct from the 36kDa and 140kDa fragments released by ADAMTS1 and displays distinct properties. In particular, the fragment released by thrombin preserves the trimeric structure of the C-terminal region; while ADAMTS1 cleavage releases the C-terminal tail of the protein as free monomers exposing the type I repeats (Figure 1).

TSP1 is also cleaved by cathepsins, leukocyte elastases and plasmin [44,56,66]. While cleavage of TSP by elastase and plasmin results in degradation of the protein, exposure to cathepsin G results in a single cut located in amino terminal region and releasing a fragment of approximately 28kDa that includes the heparin-binding domain and a larger fragment (165kDa) from the carboxy-terminal region [67]. The specific cleavage site was not mapped, but functionally the larger fragment was able to mediate platelet aggregation similarly to the intact protein. Differently to ADAMTS1 and similarly to thrombin cleavage, cathepsin G generates a trimer that includes the carboxy-terminal region and releases monomeric fragments containing the heparin-binding domains [67]. It is unknown whether TSP2 is cleaved by cathepsin G or thrombin.

TSP1 and TSP2 share similar structure and both proteins form homotrimers in vivo. In addition, they display high sequence homology which increases towards the carboxyterminal region [68]. However, this similarity is particularly low within the region flanking the cleavage site of TSP1 (aa270–aa344) by ADAMTS1. Indeed, while the enzyme also targets TSP2, the site of cleavage differs. Exposure of TSP2 to ADAMTS1 results in the release of a 42kDa fragment, this is about 6kDa more C-terminal to the start of the type I repeats [10]. Native TSP1 and TSP2 exist as homotrimers through the intermolecular disulfide bonds and the coiled-coil domain [69]. Additional intramolecular disulfide bonds exists within the linker region between the coiled-coil domain and the procollagen domain [70]. The ADAMTS1 cleavage site in TSP1 was mapped to this linker region. Thus, as result of the cleavage by ADAMTS1, the amino-terminus of TSPs remains as a trimer, while the carboxyterminal region is released as active monomers that are potentially no longer linked to the extracellular milieu.

Processing of TSP1 by ADAMTS1, enables the molecule to gain access and activate CD36 in invading capillaries and suppress neovascularization by induction of endothelial cell apoptosis [62]. Additionally, TSP1 type I repeats can also sequester angiogenic growth factors [42,43,71]. Studies using the fragments generated by ADAMTS1 proccession of TSP1 and TSP2 have demonstrated anti-migratory, anti-proliferative activity in vitro [10]. In addition, several lines of evidence indicate that this ADAMTS1 produces active TSP1 peptides in vivo. First, skin wounds of ADAMTS1 null mouse heal faster similar to those in TSP1 and 2, and do not show processing of TSP1 in vivo [10]. Second, biochemical analysis of TSP1 within T47D breast carcinoma tumors that overexpress ADAMTS1 is able to cleave TSP1 in vivo. Overall these data indicates that proteolytic processing of TSP1 and 2 by ADAMTS1 enhances their anti-angiogenic properties.

Concluding remarks

The current view of protease function has changed significantly due to the comprehensive evaluation of their substrate specificity, proteolytic activities and non-catalytic functions. Today MMPs are no longer viewed as degrading enzymes, but molecules that actively participate in highly complex processes such as the regulation of cell signaling, tissue morphogenesis, and pathophysiology. Specifically during the process of blood vessel formation, proteolytic function part-takes in the remodeling and expansion of the vasculature. Understanding the spectrum of proteases that target or generate angiogenesis inhibitory proteins will provide valuable information on how to effectively modulate their specific activities to treat disease. The specificities of each protease could be exploited to develop novel therapeutic strategies or to amplify anti- or pro-angiogenic responses that is already in place within tissues. In relation to TSPs is clear that while these molecules are secreted as large trimeric polypeptides, their exposure to specific microenvironmental settings alters their structure and activity in a tissue- and pathophysiological-specific manner. Our challenge is to map the spectrum of fragments and to characterize their biological activities.

Acknowledgments

Luisa Iruela-Arispe is supported by grants from the National Institutes of Health (RO1CA77420, RO1CA126935, RO1HL74455)

REFERENCES

1. Matrisian LM. Trends Genet 1990;6:121–125. [PubMed: 2132731]
2. Nagase H, Woessner JF. J. Biol. Chem 1999;274:21491–21491. [PubMed: 10419448]
3. Page-McCaw A, Ewald AJ, Werb Z. Nat. Rev. Mol. Cell. Biol 2007;8(3):221–233. [PubMed: 17318226]
4. Overall CM, Lopez-Otin C. Nat.Rev. Cancer 2002;2(9):657–672. [PubMed: 12209155]
5. Visse R, Nagase H. Circ. Res 2003;92:827–839. [PubMed: 12730128]
6. Mochizuki S, Okada Y. Cancer. Sci 2007;98(5):621–628. [PubMed: 17355265]
7. Lu X, Lu D, Scully MF, Kakkar VV. Cardiovas. Hematol. Agents Med. Chem 2007;5(1):29–42.
8. Arribas J, Bech-Serra JJ, Santiago-Josefat. Cancer Met. Rev 2006;25(1):57–68.
9. Apte SS. Int. J. Biochem. Cell Biol 2004;36(6):981–985. [PubMed: 15094112]
10. Lee NV, Sato M, Annis DS, Loo JA, Wu L, Mosher DF, Iruela-Arispe ML. EMBO J 2006;25(22):5270–5283. [PubMed: 17082774]
11. Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F, Matsushima K. J. Biol. Chem 1997;272(1):556–562. [PubMed: 8995297]
12. Vazquez F, Hastings G, Ortega MA, Lane TF, Oikemus S, Lombardo M, Iruela-Arispe ML. J. Biol. Chem 1999;274(33):23349–23357. [PubMed: 10438512]
13. Iruela-Arispe ML, Carpizo D, Luque A. Ann. N. Y. Acad. Sci 2003;995:183–190. [PubMed: 12814950]
14. Kuno K, Okada Y, Kawashima H, Nakamura H, Miyasaka M, Ohno H, Matsushima K. FEBS Lett 2000;478(3):241–245. [PubMed: 10930576]
15. Sandy JD, Westling J, Kenagy RD, Iruela-Arispe ML, Verscharen C, Rodriguez-Manzaneque JC, Zimmermann DR, Lemire JM, Fischer JW, Wight TN, Clowes AW. J. Biol. Chem 2001;276(16):13372–13378. [PubMed: 11278559]
16. Rodriguez-Manzaneque JC, Westling J, Thai SN, Luque A, Knauper V, Murphy G, Sandy JD, Iruela-Arispe ML. Biochem. Biophys. Res. Commun 2002;293(1):501–508. [PubMed: 12054629]
17. Canals F, Colome N, Ferrer C, Plaza-Calogne MC, Rodriguez-Manzaneque JC. Proteomics 2006;1:S28–S35. [PubMed: 16511810]
18. Leger AJ, Covic L, Kuliopulos A. Circulation 2006;114(10):1070–1077. [PubMed: 16952995]

19. Coughlin SR. J. Thromb. Haemost 2005;3(8):1800–1814. [PubMed: 16102047]
20. Weinmaster G. Curr. Opin. Genet. Dev 2000;10:363–369. [PubMed: 10889061]
21. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Limdblom P, Alva J, Nilsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C. Nature. 2007
22. McColl BK, Baldwin ME, Roufail S, Freeman C, Moritz RL, Simpson RJ, Alitalo K, Stacker SA, Achen MG. J. Exp. Med 2003;198(6):863–868. [PubMed: 12963694]
23. Joukov V, Sorsa T, Kumar M, Jeltsch L, Claesson-Welsh Y, Cao O, Saksela N, Kalkkinen N, Alitalo K. EMBO J 1997;16:3898–3911. [PubMed: 9233800]
24. Stacker SA, Stevers K, Caesar C, Vitali A, Domagala T, Nice E, Roufail S, Simpson RJ, Moritz R, Karpanen T. J. Biol. Chem 1999;274:32127–32136. [PubMed: 10542248] 24 Stacker SA, Stevers K, Caesar C, Vitali A, Domagala T, Nice E, Roufail S, Simpson RJ, Moritz R, Karpanen T. J. Biol. Chem 1999;274:32127–32136. [PubMed: 10542248]
25. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. Cell 1994;79:315–328. [PubMed: 7525077]
26. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhear JR, Olsen BR, Folkman J. Cell 1997;88:277–285. [PubMed: 9008168]
27. Sasaki T, Fukai N, Mann N, Gohring W, Olsen BR, Timpl R. EMBO J 1998;17(15):4249–4256. [PubMed: 9687493]
28. O'Reilly MS, Wiederschain D, Stetler-Stevenson WG. J. Biol. Chem 1999;274:29568–29571. [PubMed: 10506224]
29. Hangai M, Kitaya N, Xu J, Chan CK, Kim JJ, Werb Z, Ryan SJ, Brooks PC. Am. J. Pathol 2002;161(4):1429–1437. [PubMed: 12368215]
30. Hamano Y, Zeisberg M, Sugimoto H, Lively JC, Maeshima Y, Yang C, Hynes RO, Werb Z, Sudhakar A, Kalluri R. Cancer Cell 2003;3(6):589–601. [PubMed: 12842087]
31. Chen H, Herndon ME, Lawler J. Matrix Biol 2000;19:597–614. [PubMed: 11102749]
32. Adams JC, Lawler J. Int. J. Biochem. Cell Biol 2004;36:961–968. [PubMed: 15094109]
33. Bornstein P. J. Clin. Invest 2001;107:929–934. [PubMed: 11306593]
34. Tucker RP. Int. J. Biochem. Cell Biol 2004;36:969–974. [PubMed: 15094110]
35. Gao AG, Frazier WA. J Biol Chem 1994;269:29650–29657. [PubMed: 7525586]
36. Godyna S, Liao G, Popa I, Stefansson S, Argraves WS. J Cell Biol 1995;129:1403–1410. [PubMed: 7775583]
37. Calzada MJ, Sipes JM, Kruttsch HC, Yurchenco PD, Annis DS, Mosher DF, Roberts DD. J Biol Chem 2003;278:40679–40687. [PubMed: 12909644]
38. Graf R, Freyberg M, Kaiser D, Friedl P. Apoptosis 2002;7:493–498. [PubMed: 12370491]
39. Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Nat Med 2000;6:41–48. [PubMed: 10613822]
40. Kruttsch HC, Choe BJ, Sipes JM, Guo N, Roberts DD. J Biol Chem 1999;274:24080–24086. [PubMed: 10446179]
41. Yang Z, Strickland DK, Bornstein P. J Biol Chem 2001;276:8403–84038. [PubMed: 11113133]
42. Gupta K, Gupta P, Wild R, Ramakrishnan S, Hebbel RP. Angiogenesis 1999;3:147–158. [PubMed: 14517432]
43. Taraboletti G, Belotti D, Borsotti P, Vergani V, Rusnati M, Presta M, Giavazzi R. Cell Growth Differ 1997;8:471–479. [PubMed: 9101093]
44. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, Bouck N. Cell 1998;93:1159–1170. [PubMed: 9657149]
45. Schultz-Cherry S, Chen H, Mosher DF, Misenheimer TM, Kruttsch HC, Roberts DD, Murphy-Ullrich JE. J Biol Chem 1995;270:7304–7310. [PubMed: 7706271]
46. Schultz-Cherry S, Murphy-Ullrich JE. J Cell Biol 1993;122:923–932. [PubMed: 8349738]
47. Anonick PK, Yoo K, Webb DJ, Gonias SL. Biochem J 1993;289:903–909. [PubMed: 7679575]
48. Lawler J, Sunday M, Thibert V, Duquette M, George EL, Rayburn H, Hynes RO. J Clin Invest 1998;101:982–992. [PubMed: 9486968]
49. Iruela-Arispe ML, Lombardo M, Kruttsch HC, Lawler J, Roberts DD. Circ 1999;100 1423–1231.

50. Zhang X, Lawler J. *Microvasc. Res* 2007;74:795–798.
51. Iruela-Arispe ML, Luque A, Lee N. *Int. J. Biochem. Cell Biol* 2004;36:1038–1078. [PubMed: 15094119]
52. Guo N, Krutzsch HC, Inman JK, Roberts DD. *Cancer Res* 1997;57:1735–1742. [PubMed: 9135017]
53. Hogg PJ, Stenflo J, Mosher DF. *Biochem* 1992;31(1):265–269. [PubMed: 1531022]
54. Silverstein RL, Nachman RL, Pannell R, Gurewich V, Harpel PC. *J. Biol. Chem* 1990;265(19): 11289–11294. [PubMed: 2141608]
55. Hogg PJ, Owensby DA, Chesterman CN. *J. Biol. Chem* 1993;268:21811–21818. [PubMed: 8408036]
56. Hogg PJ. *Thromb. Haemost* 1994;72:787–792. [PubMed: 7740442]
57. Rodriguez-Manzanique JC, Lane TF, Ortega MA, Hynes RO, Lawler J, Iruela-Arispe ML. *Proc. Natl. Acad. Sci* 2001;98:12485–12490. [PubMed: 11606713]
58. Bein K, Simons M. *J. Biol. Chem* 2000;275:32167–32173. [PubMed: 10900205]
59. Yang Z, Kyriakides TR, Bornstein P. *Mol. Biol. Cell* 2000;11(11):3353–3364. [PubMed: 11029041]
60. Liu P, Wang Y, Li YH, Yang C, Zhou YL, Li B, Lu SH, Yang RC, Cai YL, Tobelem G, Caen J, Han ZC. *Leuk. Res* 2003;27:701–708. [PubMed: 12801528]
61. Tolsma SS, Volpert OV, Good DJ, Frazier WA, Polverini PJ, Bouck N. *J. Cell Biol* 1993;122:497–511. [PubMed: 7686555]
62. Reiher FK, Volpert OV, Jimenex B, Crawford SE, Dinney CP, Henkin J, Haviv F, Bouck NP, Campbell SC. *Int. J. Cancer* 2002;98(5):682–689. [PubMed: 11920636]
63. Noh YH, Matsuda K, Hong YK, Kunsfeld R, Riccardi L, Koch M, Oura H, Dadras SS, Streit M, Detmar M. *J. Invest. Dermatol* 2003;121:1536–1543. [PubMed: 14675207]
64. Westling J, Gottschall PE, Thompson VP, Cockburn A, Perides G, Zimmermann DR, Sandy JD. *Biochem. J* 2004;377:787–795. [PubMed: 14561220]
65. Lawler J, Connolly JE, Ferro P, Derick LH. *Ann NY Acad. Sci* 1986;485:273–287. [PubMed: 3471148]
66. Bonnefoy A, Legrand C. *Thromb. Res* 2000;98(4):323–332. [PubMed: 10822079]
67. Rabhi-Sabile S, Pidard D, Lawler J, Renesto P, Chignard M, Legrand C. *FEBS Lett* 1996;386(1): 82–86. [PubMed: 8635609]
68. Bornstein P. *FASEB J* 1992;6(14):3290–3299. [PubMed: 1426766]
69. Engel J. *Int. J. Biochem. Cell Biol* 2004;36(6):997–1004. [PubMed: 15094115]
70. Misenheimer TM, Huwiler KG, Annis DS, Mosher DF. *J. Biol. Chem* 2000;275(52):40938–40945. [PubMed: 11016937]
71. Margosio B, Marchetti D, Vergani V, Giavazzi R, Rusnati M, Presta M, Taraboletti G. *Blood* 2003;102(13):4399–4406. [PubMed: 12947001]

FIGURE 1.
Domain structure of thrombospondin 1 and thrombospondin 2 and location of proteolytic sites.