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Zymogen activation, inhibition, and ectodomain shedding of matriptase

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1. ABSTRACT

Matriptase is a member of an expanding group of type II transmembrane serine proteases. Recently, much has been learned about the biochemistry, cellular biology, normal tissue physiology, and human pathology of this protease, and of its inhibitor, termed the hepatocyte growth factor inhibitor-1 (HAI-1). This review examines the recent literature that has characterized the regulation of matriptase and HAI-1 with an emphasis on the molecular mechanisms governing its zymogen activation, inhibition by HAI-1, and ectodomain shedding.

2. INTRODUCTION

The activation of protease zymogens and the inhibition of active proteases are, in general, the two major mechanisms that control proteolysis. Enzymatically active proteases have the potential to cause great harm to cells by cleaving peptide bonds in proteins or peptides in undesirable ways. Therefore, most serine proteases are synthesized by cells as single-chain zymogens which exhibit much weaker proteolytic activity than their active counterparts. As a result, these newly synthesized protease zymogens can undergo posttranslational modification, and intracellular trafficking to the location at which they will execute their function, without the risk of damaging the cell. In order to acquire their full enzymatic activity, these zymogens have to undergo a specific proteolytic cleavage in the canonical activation motif, a process of activation which converts the single-chain zymogens to disulfide-linked, two-chain, active proteases. Since proteolytic activation of protease zymogens occurs just once in the life

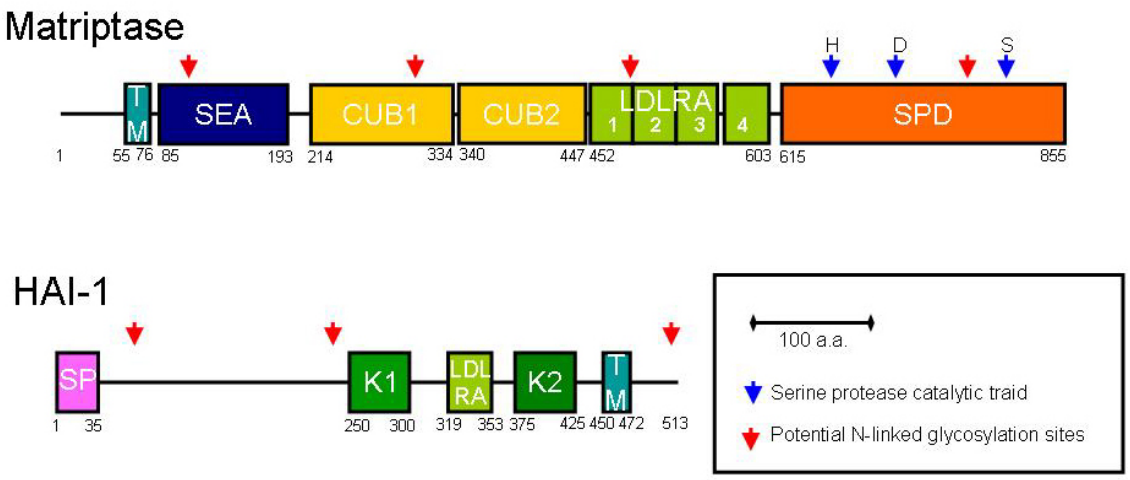


Figure 1. Schematic representation of the domain structures of matriptase and HAI-1**.** See text for details. Numbers listed underneath each domain indicate the initiation and termination number, from left to right, of amino acids of the individual domain. H, D, and S showing above light blue arrow indicate serine protease catalytic triad of matriptase. The putative N-linked glycosylation sites are also indicated by red arrow.

of the enzyme and is an irreversible process, cells must have a way in which to "switch-off" the protease activity when it is no longer required. This is mainly achieved by protease inhibitors, a group of proteins which can bind to active proteases and significantly reduce or destroy their enzymatic activity. Again, since the enzymatic activity of proteases is potentially extremely harmful to cells, if their hydrolytic activity is left unchecked, both the activation and inhibition of these enzymes have to be tightly regulated in order to achieve proteases active at the right time and in the right place. For most proteases, activation of the zymogen and inhibition of the active enzyme are simple events requiring the presence of upstream protease activators and protease inhibitors, respectively. However, for those proteases that are on the top of a protease cascade, activation is a more complex process and often represents the most important checkpoint for the regulation of that entire protease cascade.

Matriptase, also known as membrane-type serine protease-1 (MT-SP1), tumor-associated differentially expressed gene-15 (TADG-15), suppressor of tumorigenicity-14 (ST14), and in the mouse, epithin, is a member of the type II transmembrane serine protease (TTSP) family(1-6). For simplicity, we will use the name matriptase in this review. The cognate inhibitor of matriptase has been identified, and is termed the hepatocyte growth factor inhibitor-1 (HAI-1) (7). This Kunitz-type serine protease inhibitor was named based upon its ability to inhibit the protease, hepatocyte growth factor activator (HGFA) (8). Matriptase and HAI-1 are involved in multiple pathological processes in addition to their actions in normal cellular physiology (9-14). In particular, matriptase and HAI-1 play a role in carcinogenesis and tumor progression. The mechanisms which govern activation, inhibition, and clearance of matriptase are complex and well-coordinated to ensure its tight control in normal cells and, it is likely that derangement of these physiological regulatory mechanisms leads to inappropriate activity of the enzyme and so may contribute to its roles in human cancers.

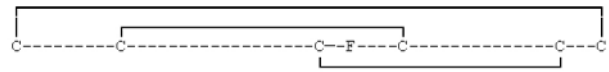
3. THE DOMAIN STRUCTURE AND PROCESSING

3.1. Matriptase structure

Matriptase is a mosaic protein of 855 amino acids with a calculated molecular mass of 94.7kDa (Figure 1). The amino-terminal end of the enzyme contains a transmembrane domain and a 54 amino acid cytoplasmic tail which contains a consensus phosphorylation site for protein kinase C. The cytoplasmic tail of matriptase can interacts with filamins (15), large dimeric actin binding proteins, which organize filamentous cortical actin in networks, and can directly interact with more than 30 proteins with great functional diversity. A SEA (sea urchin sperm protein, enteropeptidase, and agrin) module is found external to the cell membrane. The SEA module is a recently identified extracellular domain of ~ 120 amino acids (16), which undergoes posttranslational self-cleavage (17). A SEA module is present in several TTSPs, including enteropeptidase (18), matriptase, matriptase 3 (19), HAT (20), DESC1 (21), DESC4 (22), and serase-1B (23).

Two tandem CUB (C1r/s, Uegf, and Bone morphogenic protein-1) domains and four tandem low density lipoprotein receptor class A domains (LDLRA) are found external to the membrane. These domains may function in protein-protein interactions. For example, in the complement protease cascade, CUB domains participate in the formation of C1r/C1s tetramers prior to binding of C1q and the activation of the C1r and C1s proteases within the complex (24). In the LDL receptor protein and related proteins, the LDLR class A domains mediate the binding of the LDL receptor or related proteins to ligands such as apolipoproteins B and E, the receptor-associated protein (RAP), and uPA/PAI-1 complexes (25, 26). Four potential N-linked glycosylation sites are also found in the matriptase sequence. The carboxyl-terminal end of the protein contains a serine protease catalytic

Accession Code	Protein Name	Domain		Peptide Sequence	
Homo sapiens					
O43278	HAI-1	(domain 1)	245	QTEDYCLASNK-VGRCRGSFPRWYYDPT---EQICKSFVYGGCLGN--KNNYLREEECILACRGVQG	305
O43278	HAI-1	(domain 2)	370	SDKGHCVDLPD-TGLCKESIPRWYYNPF---SEHCARFTYGGCYGN--KNNFEEEQQCLESCRGISK	430
O43291	HAI-2	(domain 1)	33	SIHDFCLVSKV-VGRCRASMPRWWYNVT---DGSCQLFVYGGCDGN--SNNYLTKEECLKKCATVTE	93
O43291	HAI-2	(domain 2)	128	NYEEYCTANAV-TGPCRASFPRWYFDVE---RNSCNNFIYGGCRGN--KNSYRSEEACMLRCFRQQE	188
P05067	APP		286	VVREVCSEQAE-TGPCRAMISRWYFDVT---EGKCAPFFYGGCGGN--RNNFDTEEYCMAVCGSAMS	346
P02760	AMBP	(domain 1)	226	KKEDSCQLGYS-AGPCMGMTSRYFYNGT---SMACETFQYGGCMGN--GNNFVTEKECLQTCRTVAA	286
P02760	AMBP	(domain 2)	282	RTVAACNLPIV-RGPCRAFIQLWAFDAV---KGKCVLFPYGGCQGN--GNKFYSEKECREYCGVPGD	342
Q06481	APPH		305	DVKAVCSQEAM-TGPCRAVMPRWYFDLS---KGKCVRFIYGGCGGN--RNNFESEDYCMAVCKAMIP	365
Q02388	Collagen α1(VII) chain		2870	DSDDPCSLPLD-EGSCTAYTLRWYHRAVTGSTEACHPFVYGGCGGN--ANRFGTREACERRCPPRVV	2933
P12111	Collagen α3(VI) chain		3106	TETDICKLPKD-EGTCRDFILKWYYDPN---TKSCARFWYGGCGGN--ENKFGSQKECEKVCAPVLA	3166
P10646	TFPI-1	(domain 1)	49	LMHSFCAFKAD-DGPCKAIMKRFFFNIF---TRQCEEFIYGGCEGN--QNRFESLEECKKMCTRDNA	109
P10646	TFPI-1	(domain 2)	120	EKPDFCFLEED-PGICRGYITRYFYNNQ---TKQCERFKYGGCLGN--MNNFETLEECKNICEDGPN	180
P10646	TFPI-1	(domain 3)	212	HGPSWCLTPAD-RGLCRANENRFYYNSV---IGKCRPFKYSGCGGN--ENNFTSKQECLRACKKGFI	272
P48307	TFPI-2	(domain 1)	31	NNAEICLLPLD-YGPCRALLLRYYYDRY---TQSCRQFLYGGCEGN--ANNFYTWEACDDACWRIEK	91
P48307	TFPI-2	(domain 2)	91	KVPKVCRLQVSVDDQCEGSTEKYFFNLS---SMTCEKFFSGGCHRNRIENRFPDEATCMGFCAPKKI	154
P48307	TFPI-2	(domain 3)	153	KIPSFCYSPKD-EGLCSANVTRYYFNPR---YRTCDAFTYTGCGGN--DNNFVSREDCKRACAKALK	213
O95428*	Papilin		687	AYPVRCLLPSA-HGSCADWAARWYFVAS---VGQCNRFWYGGCHGN--ANNFASEQECMSSCQGSLH	737
Q6UXZ9*	WFIKKN-2	(domain 2)	323	FPAAECLKPPD-SEDCGEEQTRWHFDAQ---ANNCLTFTFGHCHRN--LNHFETYEACMLACMSGPL	383
Q6UXZ9*	WFIKKN-2	(domain 3)	381	GPLAACSLPAL-QGPCKAYAPRWAYNSQ---TGQCQSFVYGGCEGN--GNNFESREACEESCPFPRG	441
Q8IUA0*	WAP-8		81	PFQEPCMLPVR-HGNCNHEAQRWHFDFK---NYRCTPFKYRGCEGN--ANNFLNEDACRTACMLIVK	141
Daboia russellii siamensis					
P00990	RVV-II		2	DRPTFCNLAPE-SGRCRGHLRRIYYNLE---SNKCKVFFYGGCGGN-ANNFETRDECRETCGGK	60



* Primary accession numbers based on Swiss-Prot accession numbers system.

Figure 2. Kunutz domain sequence alignment among human and viper Kunitz domain-contain proteins. See text for discussion. Consensus sequences of a Kunitz domain include six cystines, which can form three intradomain disulfide bonds represented by solid lines between two C (cystine) in the lower panel. The active centers (the amino acid residue C-terminal to the second conserved cysteine residue) are indicated in red and mainly are either Arg or Lys. A second Arg residue (in blue) important for inhibitory activity against matriptase is located N-terminal to the second conserved cysteine residue in the Kunitz domain I of HAI-1. The corresponding Arg is only observed in the Kunitz domain 1 of HAI-2 and a Kunitz inhibitor from snake venoms.

domain that includes a conserved histidine/aspartic acid/serine (HDS) catalytic triad essential for proteolytic activity. The crystal structure of the matriptase serine protease domain revealed a trypsin-like substrate binding pocket, a small hydrophobic S2 site, and a second negatively charged binding site (27). The structure suggests a substrate cleavage preference with a basic P1 residue, a small hydrophobic P2 residue and a basic P3/P4 residue. A conserved disulfide bond which links the serine protease domain and the rest of matriptase keeps the two chains of the enzyme together after the generation of the active form of the protease (28).

3.2. HAI-1 structure

The hepatocyte growth factor activator inhibitor-1 (HAI-1) (8) and its splice variant, HAI-1 B, are Kunitz-type, serine protease inhibitors (Figure 1). Both isoforms share the same biochemical features with the exception of an extra 16 amino acid residues in HAI-1B (29), and both forms are likely involved in biological and pathological processes in the same manner. For simplicity, we will use the name HAI-1 in this review. The HAI-1 mRNA encodes a protein of 513 amino acids, although the full-length processed protein contains 478 amino acids, resulting from cleavage of a 35 amino acid signal sequence, and has a calculated molecular mass of 53 kDa (8). HAI-1 is linked to cellular membranes by a transmembrane domain in its carboxyl terminus, and contains a 41 amino acid cytoplasmic tail. Two Kunitz-type serine protease inhibitory domains (Kunitz domains I and II) of approximately sixty amino acids are found external to the membrane. The two Kunitz domains of HAI-1 are separated by a single intervening LDL receptor class A domain. HAI-1 contains three potential N-linked glycosylation sites. Kunitz domains, of which the fifty-eight amino acid inhibitor bovine pancreatic trypsin inhibitor (BPTI) is the prototype, are polypeptide domains that competitively inhibit their target serine proteases, in contrast to serpin-type inhibitors that form covalent, irreversible interactions with their targets (30). The canonical active center (the amino acid residue C-terminal to the second conserved cysteine residue) was identified as Arg-260 for Kunitz domain 1 and Lys-385 for Kunitz domain 2 and predicts their inhibitory specificity against trypsin-like serine proteases. Using molecular modeling of the HAI-1 Kunitz domains I and II, and their predicted interaction with the crystal structure of the active matriptase catalytic domain, it appears that it is Kunitz domain I binds and inhibits matriptase. This molecular modeling also predicted that Arg-258 in Kunitz domain I is essential for matriptase inhibition by interacting with the second negatively charged binding site in the matriptase serine protease domain. These predictions were further confirmed by point mutagenesis of HAI-1 (29, 31). The specificity of HAI-1 against matriptase may be derived from the combination of Arg-258 and Arg-260. By comparing the corresponding amino acid residues in human Kunitz domain-containing proteins, the Arg-258 of HAI-1 is very rare (Figure 2). Although an Arg or Lys residue is at the active centers of most Kunitz domain-containing proteins, only the Kunitz domain I of HAI-2 contains Arg in the position corresponding to Arg-258 of HAI-1. This unique combination of two arginine residues in the active center was also observed in snake venoms (Figure 2).

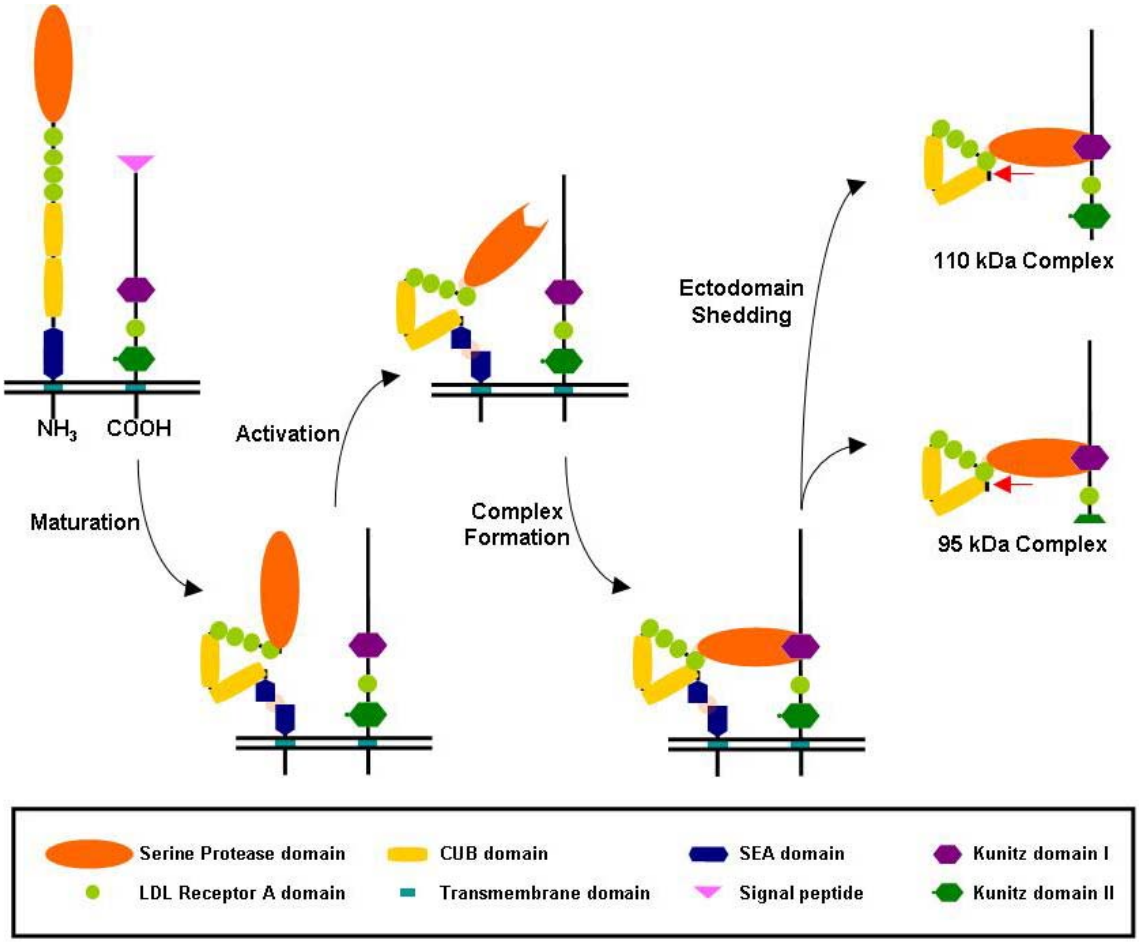


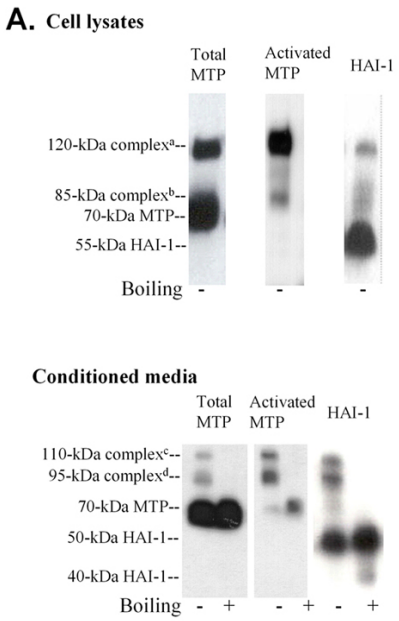
Figure 3. Schematic representation of biological fates of matriptase and HAI-1. See text for discussion. Red arrows indicate the alternative cleavage site for protease(s) involved in ectodomain shedding.

3.3. Proteolytic processing

Matriptase and HAI-1 undergo several of steps of proteolytic processing during maturation, activation, and shedding (Figures 3 and 4). Matriptase is synthesized as a polypeptide with a size of around 95-kDa, slightly greater than its calculated mass due to glycosylation. However, this full length form of matriptase is barely detected in cells that endogenously express the protease. This could be the results of very efficient conversion of full length matriptase to its mature form *via* a cleavage at G-149 within SEA domain (N-terminal processing). As with other SEA domain-containing proteins (17), self-cleavage may be the mechanism for the N-terminal processing of matriptase. It is presumed that no protease is involved in this event. Full length matriptase can constitute up to a quarter of the total matriptase in transfected cell systems, which is likely an artifact resulting from overexpression of the protein (32, 33). While the N-terminal processing splits matriptase into two fragments consisting of amino acids 1 to 149 and 150 to 855, respectively, both matriptase fragments are still bound together *via* an unknown mechanism so that the bulk of matriptase extracellular domains remain attached to the cell membrane *via* a noncovalent interaction with its own membrane-bound N-terminal fragment. This is an interesting mechanism that contrasts with the direct membrane insertion seen for other integral membrane proteins, including HAI-1. After lyses of cells in non-ionic detergents, both matriptase fragments apparently remain bound together, however they are dissociated by SDS, even under non-boiled conditions. Point mutation of matriptase at G-149 blocks this N-terminal processing and cleavage within SEA domain resulting in the detection of the protein as a 95-kDa band by immunoblotting (32, 33). The biosynthesis and maturation of HAI-1 follow a more classic and simple pathway than matriptase. As described above, HAI-1 is synthesized as a polypeptide with 513 amino acids followed by a cleavage of a 35 amino acid signal sequence, resulting in mature HAI-1 with 478 amino acids (the 55-kDa HAI-1).

Activation is the next step in the proteolytic processing of matriptase following its synthesis and maturation. In spite of the apparent complexity of the activation mechanism and how it is regulated (see below), the activation of matriptase zymogen follows a very classic scheme by which a canonical activation motif is cleaved to convert the single-chain zymogen to a disulfide-linked two-chain active enzyme which consists of noncatalytic domain of 45-kDa and a trypsin-like, serine protease domain of 25-kDa (28).

Although matriptase and HAI-1 are membrane-



B.

			MW (kDa)	Anti-Matriptase		Anti-HAI-1
				M32	M69	M19
MTP	Latent Form	Cell	70	+	-	-
		Media	70	+	-	-
	Activated Form	Cell	120[a]	+	+	+
			85[b]	-	+	+
		Media	110[c]	+	+	+
			95[d]	+	+	+
HAI-1	Free Form	Cell	55	-	-	+
		Media	50	-	-	+
			40	-	-	+
	Complex Form	Cell	120[a]	+	+	+
			85[b]	-	+	+
		Media	110[c]	+	+	+
			95[d]	+	+	+

a. 120-kDa complex : a 70-kDa matriptase and a 55-kDa HAI-1

b. 85-kDa complex : a 25-kDa matriptase and a 55-kDa HAI-1

c. 110-kDa complex : a 70-kDa matriptase and a 50-kDa HAI-1

d. 95-kDa complex : a 70-kDa matriptase and a 40-kDa HAI-1

Figure 4. Monoclonal antibodies and the detection of matriptase, HAI-1 and their complexes. See text for details. (A) Cell lysates from 184 A1N4 mammary epithelial cells and condition media harvested form T-47D breast cancer cells were analyzed by western blotting *via* M32, M69, and M19 monoclonal antibodies, which recognize total matriptase, activated matriptase, and HAI-1, respectively. (B) Summary of the various forms of matriptase (MTP) and HAI-1 detected by mAbs.

associated proteins, both proteins were initially identified and purified from the conditioned media (8, 34, 35) of cells and human milk (7). This is a result from ectodomain shedding, a final proteolytic processing of matriptase and HAI-1. Matriptase can be shed by proteolytic cleavage at 189Lys-Ser190 and 204Lys-Thr205 (28), which yields matriptase fragments in the extracellular milieu with slightly smaller sizes than that of the membrane-associated form (15, 36). HAI-1 shedding can also occur by proteolytic cleavage at two different sites: one near the transmembrane domain, which yields a 50-kDa HAI-1 fragment and another likely within the Kunitz II domain, which results in a 40-kDa HAI-1 fragment. In LNCaP prostate cancer cells, shedding of the 50-kDa HAI-1 fragment occurs constitutively, but shedding of the 40-kDa fragment is dependent on androgen stimulation and is closely associated with matriptase zymogen activation (36). In addition to the proteolytic processing of matriptase and HAI-1 associated with their maturation, activation, and shedding, a cleavage at the disulfide linkage, which connects the serine protease domain and the noncatalytic domains of matriptase, occurs only after lysis of cells to convert the 120-kDa matriptase-HAI-1 complex to an 85-kDa complex (37).

3.4. Matriptase-HAI-1 complexes

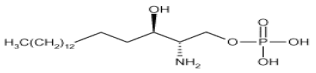
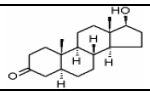
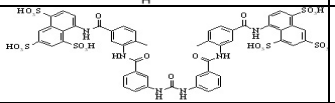
In spite of the coexpression and colocalization of both matriptase and HAI-1 in many epithelial cells, the affinity between latent form of matriptase and HAI-1 is very low. While latent matriptase may interact with HAI-1 during its activation process, there is no stable complex of latent matriptase with HAI-1. In contrast to latent matriptase, active matriptase binds very tightly to HAI-1 forming a very stable complex which is resistant to SDS and can be detected by immunoblotting under nonreduced and nonboiled conditions (37). Formation of the matriptase-HAI-1 complex is an inevitable consequence of activation of matriptase zymogen (see below). The initial matriptase-HAI-1 complex is detected in cell lysates with a size of 120-kDa, and contains 70-kDa activated matriptase and 55-kDa mature, membrane-bound HAI-1 (37).

This 120-kDa membrane-associated matriptase-HAI-1 complex is subsequently shed from cell surfaces into the conditioned media of cells (28, 36). There are two major forms of matriptase-HAI-1 complexes detected in the extracellular milieu: the 95- and 110-kDa matriptase-HAI-1 complexes (36, 38). Both complexes are composed of the same 70-kDa activated matriptase and HAI-1 fragments of different sizes. The 110-kDa form contains the 50-kDa HAI-1fragment and the 95-kDa complex contains the 40-kDa HAI-1 fragment (Figures 3 and 4).

3.5. Monoclonal antibodies

Much of the studies leading to our understanding of zymogen activation and inhibition of matriptase have been based on detection and/or purification of the individual products of proteolytic processing of matriptase

Table 1. Summary and comparison of matriptase activation in different model systems

	Chemical Structure	Cell Type	Subcellular Localization	Signaling Pathway	Time Kinetics	Protein synthesis	HAI-1 Inhibition
S1P-induced		184 A1N4 Immortal mammalian epithelial cells	Cell-cell junctions	S1P Receptor GPCR	10 min	no	Immediately
DHT-induced		LNCaP Prostate cancer cells	Cell surfaces	Androgen Receptor	6 hr	yes	Immediately
Suramin-induced		Various cell types	Intracellular vesicle-like structures	?	5-10 min	no	Immediately
Constitutive	-	Breast cancer cells	Membrane ruffles	?	5-10 min	no	Immediately
In vitro	-	Various cell types	Intracellular pool	spontaneous	5-10 min	no	Immediately

and HAI-1 and the complexes of active matriptase and HAI-1 using anti-matriptase and anti-HAI-1 monoclonal antibodies. In Table 1, we summarize the various forms of matriptase and HAI-1 and their detection by these monoclonal antibodies. Three mAbs have been heavily used in our studies on matriptase. These mAbs include (1) anti-total matriptase M32 which recognizes the third LDL receptor class A domain and can detect both latent and activated forms of matriptase, (2) anti-activated matriptase mAb M69 which is able to distinguish the activated matriptase from its latent counterpart, and (3) anti-HAI-1 mAb M19 which can recognize both complexed and free (uncomplexed) HAI-1 (Figure 4). Among these mAbs, M69 is quite unique and interesting. Though the exact epitope recognized by mAb M69 has not been identified, this epitope is specifically associated with activation of matriptase and is conformation-dependent. Since discrete, highly localized conformation changes are accompanied with activation of serine proteases in order to form the substrate binding pockets, we have proposed that mAb M69 recognizes these activation-associated, conformational changes (28).

4. AUTOACTIVATION AND INHIBITION

4.1. Activational Cleavage

As described above, the activation of serine protease zymogens, including matriptase, requires cleavage at a canonical activation motif. While for most serine proteases, this activational cleavage is facilitated by other upstream active proteases, activation of the matriptase zymogen appears to be initiated by the intrinsic, weak proteolytic activity of its own zymogen form (33). Mutation of any of the amino acids making up the matriptase catalytic triad, results in a protein that is unable to undergo this activational cleavage, even though the catalytic triad is quite distal to the activation cleavage site. Consistent with the hypothesis of zymogen mediated-activation, the noncatalytic domains of matriptase and the posttranslational modifications of the protein are required for the activation of the matriptase zymogen. As described above, matriptase undergoes N-terminal proteolytic processing at G-149 within SEA domain. Point mutation of matriptase at G-149 completely abolishes this N-terminal processing and results in the accumulation of full length matriptase, which does not undergo zymogen activation (33). Matriptase contains four putative N-linked glycosylation sites. Point mutation of matriptase at these sites such that N-linked glycosylation cannot occur has revealed that N-linked glycosylations of the first CUB domain and the serine protease domain are required for matriptase activation (33). The CUB domains and LDL receptor class A domains of matriptase are also involved in its zymogen activation. Point mutations in the LDL receptor class A domains of matriptase, which destroy the calcium cages of these domains, significantly decrease activation of the matriptase zymogen (33). Deletion of either of the matriptase CUB domains individually also significantly affects matriptase zymogen activation. However, deletion of both CUB domains together increases zymogen activation, suggesting a complex role for the CUB domains in zymogen activation (33). Both CUB domains together may serve to prevent premature matriptase activation, but may also provide the structural basis for protein-protein interactions which are believed to be important for matriptase zymogen activation.

Autoactivation, in which one zymogen molecule interacts with another, resulting in transactivation of each zymogen, by virtue of their weak intrinsic proteolytic activity, is believed to be a relevant mechanism for some serine proteases, particularly those at the pinnacle of protease cascades. The best-studied example of this mechanism is in the activation of complement subcomponent C1r protease zymogens, where interaction of C1r/C1s tetramers with the C1q protein induces conformational changes that result in C1r protease transactivation (39). The active site triad of serine proteases already is pre-formed in the zymogen state (40), and so autoactivation probably requires the interaction of two protease zymogen molecules together, and possibly with other proteins, to induce conformational changes in the substrate binding pocket necessary for catalysis. The appropriate posttranslational modifications and functional noncatalytic domains of matriptase probably contribute to the protein-protein interactions required for matriptase zymogen activation.

4.2. Unexpected roles of HAI-1

HAI-1 was initially identified as one of two inhibitors of HGF activator (8), a liver-derived, blood-borne serine protease which is a potent activator of pro-HGF (41). The identification of HAI-1 as a physiologically relevant (cognate) inhibitor of matriptase began with the identification of two matriptase complexes

in the conditioned media of breast cancer cells (35). We subsequently purified one of these matriptase complexes (the 95-kDa form) from human milk (7). When the purified 95-kDa matriptase complex was dissociated by boiling or exposure to low pH, a binding protein was separated from active matriptase and was identified as a 40-kDa fragment of HAI-1 based on N-terminal and internal amino acid sequences (7). In addition to the formation of complexes with matriptase in the lactating human mammary gland and in cultured human epithelial cells (35), the functional linkage of HAI-1 to matriptase has been inferred from their coexpression in the epithelial elements in most human epithelium-containing tissues (42, 43) and in a variety of cultured epithelial cells (44), from their subcellular colocalization within cells and at cell surfaces (38, 45), from the involvement of HAI-1 in biosynthesis, trafficking and zymogen activation of matriptase (31), and from the phenotypes of transgenic mice. Matriptase-induced malignant transformation and its strong oncogenic potential are counteracted by increasing epidermal HAI-1 expression, which prevents both epidermal tumor susceptibility and premalignant progression in the skin of keratin-5-matriptase transgenic mice (11). Keratin-5-matriptase/keratin-5-HAI-1 double-transgenic mice have the phenotype of wild-type or keratin-5-HAI-1 single-transgenic mice. Similarly, functional defects in placental and embryonic development in HAI-1-deficient mice are completely restored by the simultaneous deficiency of matriptase (13). Matriptase/HAI-1 double-knockout embryos are capable of normal placentation whereas knockout of HAI-1 alone leads to disruption of chorionic trophoblast differentiation and labyrinth layer formation. These findings suggest that matriptase is the cognate protease of HAI-1.

HAI-1 is a Kunitz-type serine protease inhibitor and, as such, binds to active matriptase in pH-dependent, reversible and competitive manner, as one would expect (28). What is unexpected is the paradoxical requirement for HAI-1 in matriptase zymogen activation. Activation of the matriptase zymogen does not occur when the protease is co-expressed with HAI-1 that is mutated in its LDL receptor class A domain (33). A role for HAI-1 in matriptase zymogen activation is also supported by the observation that HAI-1 is translocated and accumulates along with matriptase at cell-cell junctions or in the intracellular vesicle-like structures during of matriptase zymogen activation (37). The HAI-1 LDL receptor class A domain, along with its counterparts in the matriptase molecule, may participate in matriptase activation by providing an essential structural basis for the required protein-protein interactions during matriptase zymogen activation.

In addition to its roles in the activation and inhibition of matriptase, the intimate relationship of HAI-1 and matriptase is evident as early in their life history as their synthesis and maturation in the endoplasmic reticulum (ER) and Golgi apparatus. Matriptase is only translated at very low levels and accumulates in the ER/Golgi area, in breast cancer cells that do not naturally express these proteins are transfected with matriptase in the absence of HAI-1 (31). The apparent inability of newly synthesized matriptase to traffic beyond the ER/Golgi area may stem from the potentially toxic effect of its own proteolytic activity. No difficulty in trafficking is seen when cells are transfected with enzymatically dead matriptase mutated at any of the three amino acid residues of active site, even in the absence of HAI-1 (31). When coexpressed with HAI-1, wild-type matriptase is again able to traffic out of ER/Golgi area to cell surfaces. This chaperon-like activity of HAI-1 for the biosynthesis and trafficking of wild-type matriptase may result from its ability to inhibit matriptase proteolytic activity, since a HAI-1 mutant that bears point mutations at the active center (Arg-258 or Arg-260) of Kunitz domain I, loses not only its ability to inhibit matriptase activity, but also the chaperon-like activity (31). Interestingly, both the matriptase inhibitor activity and chaperon-like activity remains intact in HAI-1 mutated at the active center of Kunitz domain II.

The level of HAI-1 expression seems to be an important factor in the regulation of matriptase zymogen activation. Reduced HAI-1 expression by transfection with HAI-1-targeting siRNAs results in the spontaneous activation of matriptase zymogens in immortalized mammary epithelial cells and potentiates sphingosine 1-phosphate (S1P)-stimulated zymogen activation (31). Partial sequestration of HAI-1 by treatment with the anti-HAI-1 mAb M19 significantly increases S1P-induced matriptase zymogen activation (31). Taken together, these data suggest that HAI-1 is not only the cognate inhibitor of matriptase but also participates in almost every aspect of matriptase biochemistry and biology.

The essential role of HAI-1 in matriptase zymogen activation, and the colocalization of HAI-1 and matriptase during the activation process apparently provide rapid access of HAI-1 to the newly activated matriptase, and so inhibition immediately follows protease activation. Indeed activated matriptase was detected only in its HAI-1 complexes.

4.3. Achilles' heel

Matriptase may be a good drug target for the therapy of human carcinomas. Studies from our group and others clearly demonstrate the important role of matriptase in cancer. Increased expression of matriptase has been observed in a wide variety of primary human tumors of epithelial origin, including breast, prostate, colon, stomach, cervical, thyroid, and ovarian carcinomas (5, 6, 44, 46-51). In addition to its increased expression in tumors of an epithelial origin, matriptase is overexpressed in malignant mesothelioma with epithelial phenotype up to several hundred folds (52). In human breast cancer, matriptase is expressed in ductal carcinoma *in situ* (DCIS) and in invasive tumors (44). Furthermore, tight correlation among the expression of matriptase and its substrate, hepatocyte growth factor (HGF) and the c-met receptor was observed in a cohort of 330 node-negative breast carcinomas. More importantly, high-level expression of the c-met receptor, matriptase, and HAI-1 are associated with poor patient outcome (53). Matriptase may be involved in cancer invasion and metastasis by serving as a membrane activator directly on cancer cell surfaces to recruit and activate urokinase type plasminogen activator (uPA), MMP-3, hepatocyte growth factor (HGF), and insulin-like growth factor binding protein-related protein-1 (IGFBP-rP1), all of which are important in various aspects of cancers, including extracellular matrix degradation, adhesion,

cellular motility and tumor vascularization (29, 54-58). The prometastatic role of matriptase was supported by the enhanced lymph node metastasis in nude mice of matriptase-overexpressing gastric cancer cells (50), and by the decreased invasiveness of matriptase anti-sense treated ovarian tumor cells (56). More recently in a transgenic model, mild overexpression of matriptase in the skin, when even partially opposed by endogenous HAI-1, caused spontaneous squamous cell carcinomas and significantly potentiated carcinogen-induced tumor formation (11), suggesting that matriptase possesses strong oncogenic potential. These studies not only clearly demonstrate the role of matriptase in human carcinomas, but also suggest that matriptase may be a useful drug target for the control of epithelial cancers. Indeed, a matriptase-selective inhibitory drug has recently been shown to suppress the growth of human prostate cancer cells as tumors in a xenograft model (59). However, the classical approach to targeting proteases by inhibition of the activity of the catalytic domain often poses serious problems in terms of selectivity for the desired protease target, due to the high structural homology and overlapping specificity among serine protease catalytic domains. This results in problems resulting from the inhibition of other, off target proteases, particularly at higher doses, often leading to unacceptable side-effects. For example, the limited positive outcomes in the clinical trials of MMP inhibitors are in part attributed to the overlapping effects of those MMP inhibitors targeting proteolytic activities (60). In addition, synthetic matriptase inhibitors, which target the catalytic domain of the enzyme, may not efficiently inhibit the biochemical activated matriptase, even if very potent, due to the very low level of "active" matriptase detected in cancer cells. This scarcity of active matriptase in cancer cells does not result from the lack of zymogen activation. Indeed, breast cancer cells constitutively activate matriptase at very high levels, comparing to mammary epithelial cells (38). The lack of active matriptase in cancer cells is due to the intimate coupling of zymogen activation with HAI-1-mediated inhibition. As a result, almost all the active matriptase is sequestered into complexes with HAI-1 immediately following its activation, such that the catalytic site is available for inhibition by synthetic inhibitors only for the shortest time, limiting their potential effectiveness in the blockade of matriptase functions.

The autoactivation of matriptase, which requires interactions between matriptase zymogen molecules and other proteins, provides a unique opportunity to inhibit the actions of this protease. It has already been shown that interference with the protein-protein by anti-matriptase monoclonal antibodies significantly suppresses matriptase activation (45, 61). Such activation-based inhibitors will not depend on interactions with the highly conserved serine protease domain and should therefore, be much more specific than the classical protease inhibitors which target the proteolytic activity of active enzymes.

5. CELLULAR CONTROL

As a protease at the pinnacle of protease cascade, the initiation of matriptase zymogen activation is the most critical step in the regulation of not only matriptase activity, but also that of downstream proteases, substrates, and those physiological functions controlled by this matriptase-initiated protease cascade. Initiation of matriptase zymogen activation is a complex process which is regulated by a series of cellular events induced by exogenous factors. Given the diversity of its physiological functions (62), the exogenous inducers of matriptase zymogen activation may also be cell-type specific. However, the effects of the various exogenous inducers seem to converge on a set of common regulatory mechanisms, which is composed of a soluble cytosolic suppressor and cell membrane anchored activation machinery which is responsible for the activational cleavage.

5.1. Lysophospholipids

Lysophospholipids, including lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), are recognized to be major lipid mediators in serum and possess growth factor-like activities. Activated platelets are the major source of both LPA and S1P in serum (63). The role of lysophospholipids in matriptase zymogen activation was revealed by the identification of an evolutionally conserved factor(s) found in sera from reptilian, avian, and mammalian species, that stimulates the transient activation of matriptase zymogen on the surface of 184 A1N4 immortalized breast epithelial cells, immediately followed by the shedding of the protease and HAI-1 (28). Addition of fresh culture media supplemented with 0.5% serum to serum-starved 184 A1N4 cells, causes a rapid zymogen activation of matriptase (within 10 minutes). This amount of serum (0.5%) can only sustain the zymogen activation for few hours. Investigation of this phenomenon revealed that the activating species was associated with lipoproteins and further study of the phospholipid components of lipoproteins, led to the identification of S1P as the responsible agent, by thin layer chromatography (TLC) (64). Nanomolar concentrations of commercially available S1P can mimic serum and induce the rapid activation of matriptase zymogen on the cell surface of 184 A1N4 cells (64). Other structurally related lysophospholipids, such as LPA and sphingosylphosphorylcholine (SPC) also have similar abilities, but require much higher concentrations.

S1P is known to induce a wide spectrum of biological responses in a variety of cell types, including proliferation, survival, actin cytoskeletal rearrangement, assembly of adherens junctions, cell-shape changes, contraction, and cellular motility (65-67). As far as we know, the functional linkage of S1P and the cell surface proteolysis of matriptase is unprecedented. The potent effects of S1P are exerted through the interaction of S1P with a subfamily of cell surface G protein coupling receptors: the endothelial differentiation gene (EDG) family, which have been renamed S1P receptors by the International Union of Pharmacology Societies (IUPHAR) (68). An array of studies on EDG/S1P receptors indicated that the effects of S1P on cellular responses rely to a great extent on the relative levels of expression of EDG/S1P receptors and that the overlapping as well as distinct expression patterns of S1P receptors vary in different cell types. There are five major EDG/S1P receptors, EDG1/S1P1, EDG5/S1P2, EDG3/S1P3, EDG6/S1P4, EDG8/S1P5 (69). The roles of the S1P receptors and their downstream signaling in zymogen activation of matriptase remain to be further investigated.

5.2. Junctional Protease

While it is to be expected for S1P-induced zymogen activation of matriptase to occur at the cell membrane of mammary epithelial cells, zymogen

activation is in fact confined to strong E-cadherin-based cell-cell junctions (45). In the absence of S1P stimulation, weaker cell-cell junctions were observed in 184 A1N4 cells due to loss of adherens junctions and the subcortical belts of actin filaments. Matriptase was detected in its latent form mainly in its intracellular pool in the ER/Golgi area. Low level latent matriptase was also detected at cell surface. In concert with the S1P-induced formation of subcortical actin filament belts and the assembly of adherens junctions, matriptase translocates, accumulates and becomes activated at these newly formed adherens junctions which seem to be required for the accumulation of matriptase at cell surfaces of 184 A1N4 cells (45). Activated matriptase first appears as small spots at cell-cell contacts, particularly at the focal points where total matriptase is most concentrated. Activation then appears to propagate along cell-cell contacts. Destruction of or blocking the formation of actin filaments and the assembly of adherens junctions totally abolished the accumulation and activation of matriptase at cell surface (45). The transient nature of the induction of matriptase activation by culture media supplemented with 0.5% serum in serum-starved 184 A1N4 cells is the results from consumption and depletion of S1P in culture media, which results in weakened cell-cell junctions and the suppression of matriptase activation. The coupling of matriptase zymogen activation to the assembly of adherens junction and rearrangement of the actin cytoskeletal may serve to ensure tight control of matriptase activity, restricted to cell-cell junctions of mammary epithelial cells (45).

5.3. Deregulation

While breast cancer cells express matriptase as well as S1P receptors, the cellular responses to S1P and matriptase zymogen activation in these cells are quite different from mammary epithelial cells. Breast cancer cells constitutively activate matriptase regardless of the presence of S1P (38). In addition, the subcellular localizations of matriptase are altered in breast cancer cells. Cell-cell junctions are not the only locations where activated matriptase is detected. Instead, both the latent and activated forms of matriptase are misrouted to membrane ruffles on T-47D breast cancer cells, particularly after EGF treatment (38). Interestingly, S1P is no longer the sole factor which regulates assembly of adherens junctions in breast cancer cells. Aberrant responses to S1P in actin filament rearrangement were also observed in breast cancer cells. In spite of the deregulation of matriptase activation, breast cancer cells still seem to maintain the mechanisms governing the inhibition and removal of matriptase. Matriptase is coexpressed with HAI-1 in all breast cancer cells tested so far (44). Both proteins are also colocalized in breast cancer cells. Since activated matriptase is only detected in HAI-1 complexes in breast cancer cells, just as in 184 A1N4 mammary epithelial cells, matriptase zymogen activation is thought to be immediately followed by HAI-1-mediated inhibition. Furthermore, ectodomain shedding of activated matriptase in HAI-1 complexes also occurs robustly in breast cancer cells since the majority of activated matriptase was detected in the conditioned media of breast cancer cells (38). Since breast epithelial cells have the physiological mechanisms for cell-cell adhesion and for the regulation of matriptase as a junctional protease, it is intriguing to speculate that the deregulation of these mechanisms in breast cancer cells may facilitate its malignant phenotypes. Thus, a junctional protease is converted into one of the malignant components of breast cancer cells.

5.4. Steroid Hormones

The role of steroid hormones in the regulation of matriptase proteolysis was established as parts of our research endeavor searching for extracellular stimulators of matriptase zymogen activation in cancer cells in order to understand how the normal physiological S1P-based regulation of activation is deranged in cancer. We established the functional link between androgens and matriptase zymogen activation in prostate cancer cells based on two independent observations: 1) matriptase and HAI-1 were shed into the extracellular milieu from LNCaP prostate cancer cells in response to androgen exposure (70) and 2) matriptase activation was closely followed by shedding of matriptase and HAI-1 (28).

Similar to the dependence on S1P for the activation of matriptase in 184 A1N4 mammary epithelial cells, androgens play a key role in the regulation of matriptase proteolysis in the hormone-dependent LNCaP prostate cancer cells. Hormone starvation of LNCaP cells, similar to the serum-starvation of 184 A1N4 cells, significantly suppresses matriptase zymogen activation, without altering the expression of matriptase mRNA in LNCaP prostate cancer cells (36).

In contrast to S1P which induces matriptase activation, *via* the S1P/EDG receptors, very rapidly (onset within 10 min), treatment with androgens induces robust matriptase zymogen activation, *via* the androgen receptor (AR), much more slowly. The transcription and translation of AR target genes, is required and it takes at least 6 hr after androgen exposure for the effects to be seen. Similar to S1P-induced activation in 184 A1N4 cells, androgen-induced activation of matriptase in LNCaP cells was closely followed by HAI-1-mediated inhibition and shedding of matriptase and HAI-1 into extracellular milieu (36).

To the best of our knowledge, the functional linkage, at the level of zymogen activation, between steroid sex hormones and cell surface proteolysis is unprecedented. Several proteases have been identified to be regulated by androgens and as AR target genes, including prostate specific antigen (PSA), glandular kallikrein (71), a protease with high homology with enamel matrix serine proteinase 1 (72), protease/KLK-L1 (73), TMPRSS2 (74, 75), and MMP-2 (76) in prostate cancer cells. Altered expression of these proteases is the major effects of androgen treatment in prostate cancer cells, and while zymogen activation and release to culture media has also been observed for these proteases in response to androgen exposures (75), this is thought to result from increased gene expression.

In contrast to the striking hormone dependence in prostate cancer cells, hormone-starvation of hormone-dependent breast cancer cells did not alter matriptase activation. There was no obvious increase in activated matriptase following treatment of hormone-starved breast cancer cells with 17 beta-estradiol (36). This may result from the already high basal level of activated matriptase in breast cancer cells. This difference in the hormone

responsiveness of matriptase activation highlights the complexity of the regulatory mechanism. Again, in spite of the differences in the regulation of zymogen activation between mammary epithelial cells, breast cancer cells, and prostate cancer cells, all types of cells share the same mechanisms of HAI-1 mediated inhibition and ectodomain shedding.

6. *IN VITRO* ACTIVATION

In spite of the divergence of the regulation of matriptase activation among different cell types and the commonalities of the subsequent steps of matriptase activation, inhibition and shedding suggest that it is likely that the different activational stimuli converge on an activation mechanism. This hypothesis is supported by: 1) the identification of suramin as a universal inducer of matriptase activation in all cell types, 2) the discovery of a cell membrane anchored activation machinery, and 3) of a cytosloic suppressor of matriptase activation.

6.1. Suramin

Suramin is a sulfide-rich, anionic small molecule. The discovery of suramin as an inducer of matriptase activation was unexpected. Since suramin has been shown to be able to uncouple the interaction between S1P and S1P3 (77), we used this compound to investigate whether S1P3 is involved in S1P-induced activation of matriptase. Instead of suppression of S1P-induced matriptase activation, as we had expected, treatment of cells with suramin alone causes robust activation of matriptase, to greater extent and more rapidly that was seen with S1P (37). The rapid kinetics suggests that altered gene expression is not important for suramin-induced matriptase activation. The role of HAI-1 in suramin-induced matriptase is similar to that in S1P and androgen induced activation, namely all the activated matriptase sequestered in HAI-1 complex.

In spite of these similarities, suramin is different from S1P and androgen by virtue of in its broader cell-type specificity and the subcellular localization of matriptase activation. Suramin can induce matriptase activation in various cell types including mammary epithelial cells, breast cancer cells, and prostate cancer cells. But in contrast to S1P induced activation in 184 A1N4 mammary epithelial cells which occurs at cell junctions, suramin treatment caused the accumulation of matriptase and HAI-1 in vesicle-like, intracellular structures in which activated matriptase was detected (37). It is worthwhile mentioning here that in spite of being an integral membrane protein, the majority of matriptase is detected in the ER/Golgi area (35, 45) and only a small portion of the protease is at cell surface. Activation of matriptase in these intracellular vesicle-like structures is the first line of evidence that the intracellular pool of matriptase is capable of undergoing activation. It was this observation that led to the establishment of a cell-free, *in vitro* assay for matriptase activation (61).

6.2. Biomembrane

The need for an *in vitro* system for matriptase zymogen activation became apparent when it was appreciated that autoactivation was the mechanism to turn on the activation of the matriptase zymogen. An *in vitro* system of matriptase zymogen activation is superior to one based on whole cells since it facilitates the identification and analysis of the individual components in the activation machinery, and the immediate upstream regulatory factors at which the signals of different exogenous inducers of matriptase zymogen activation converge. After fruitless attempts using purified matriptase or soluble cell lysates, we established a cell-free, *in vitro* system of matriptase zymogen activation utilizing cell homogenates which contain the intracellular pool of matriptase residing at the perinuclear region (61). This *in vitro* system exhibits characteristics analogues to what is seen *in vivo* in terms of the rapid kinetics, immediate HAI-1-mediated inhibition, and formation of matriptase-HAI-1 complex. We have principally made use of 184 A1N4 cells, for the *in vitro* system, however, cell homogenates of various breast cancer cells and prostate cancer cells work just as well. The ubiquity of the *in vitro* assay using materials derived from different cell types resembles the action of suramin which induces matriptase zymogen activation in live cells of various cell types, but is in contrast to S1P and androgens which showed cell-type specificity.

Some insights into matriptase zymogen activation have been obtained making use of this newly established *in vitro* system (61). Anchorage at lipid bilayers seems to be required for matriptase and its activation machinery in order to achieve efficient activation, which results in cleavage of half of the latent matriptase within 20 min. Addition of nonionic detergents, such as Triton X-1000, to the cell homogenates suppressed matriptase zymogen activation. Furthermore, simply anchorage at lipid bilayers is not sufficient for activation, because sonication of the cell homogenates inhibited *in vitro* activation, and because matriptase present in membrane vesicles also failed to undergo *in vitro* activation. This suggests that the lipid biomembrane, at which matriptase and its activation machinery are attached, require organization into a higher biomembrane structures in order to provide the proper platform to accommodate all the required components of the activation machinery and to ensure efficient interactions among these components.

The fundamental difference between autoactivation and conventional activation of serine proteases lies in the manner that the activational cleavage is carried out. For conventional activation, other active proteases are involved; whereas autoactivation is more dependent on protein-protein interactions. This fundamental difference results in several intriguing biochemical features of matriptase zymogen activation. Small molecule protease inhibitors fail to inhibit the *in vitro* matriptase zymogen activation; presumably since no active proteases involved in the process (61). Moreover, *in vitro* matriptase activation occurs within a relatively narrow pH range with an asymmetrical profile for activation rate *versus* pH. This is different to conventional protease-catalyzed activation in which a symmetrical, bell-shaped profile would be expected for a plot of activity *versus* pH, because enzymatic activity would drop on either side of the optimal pH, due to ionization or protonation of the amino acids participating in catalysis with the increase or decrease of pH, respectively. This unique pH effect on *in vitro* activation may result from interference with the protein-protein interactions or the higher order structure of the lipid

bilayers rather than simply from interference with the amino acid residues actually participating in the subsequent proteolytic cleavage. The effects of ionic strength and temperature on *in vitro* matriptase activation are also consistent an autoactivation model. A sharp decrease in matriptase activation was observed with increasing sodium chloride concentration from 80 mM to 100 mM. This modest increase in ionic strength again may disrupt protein-protein interactions or the higher order structure of the lipid bilayers. The involvement of a lipid bilayer biomembrane in the activation of the matriptase zymogen dictates a typical temperature dependence on activation due to the physical fluidity of biomembrane in which a phase transition of biomembrane from a gel (frozen) state to a liquid state. This may explain the observation that *in vitro* activation of matriptase occurs very slowly at 12^{o}C, but at much more rapidly at 13^{o}C and beyond. In the gel state, the movement of proteins within the bilayer, including the matriptase zymogen, would be much slower, interfering with the protein-protein interactions required for activation.

6.3. Cytosolic suppressor

The spontaneous activation of the intracellular pools of matriptase zymogen in the cell-free setting indicates that the activation machinery for matriptase coincides intracellularly with matriptase zymogen at the ER/Golgi region and ready to execute its function and activate matriptase under right buffer conditions. This raises the question as to how live cells prevent premature zymogen activation prior to translocation of matriptase to its physiological location for activation. The presence of a cytosolic suppressor(s) could in part be the answer (61). When the soluble cytosolic fractions of cell homogenates are separated from the insoluble fractions which contain matriptase and its activation machinery, the rate of *in vitro* zymogen activation of matriptase significantly increases resulting in the cleavage of about one-third to a half of the matriptase within 10 min at room temperature. In contrast, in the presence of the cytosolic suppressor(s), less than 10% of the matriptase undergoes activation under the same conditions. Therefore, matriptase zymogen activation may be regulated by two independent mechanisms: a soluble, cytosolic suppressor and autonomous, lipid anchored activation machinery at the lipid bilayer biomembrane. The activation machinery is apparently well preserved after homogenization of cells in the absence of detergent. The cytosolic suppressor(s) seems to be diluted enough during homogenization to allow matriptase activation to proceed spontaneously. Removal of the cytosolic suppressor from matriptase activation machinery by centrifugation further allowed matriptase activation to proceed to a much greater extent.

7. CONCLUSION AND PERSPECTIVES

Matriptase and HAI-1 are a cognate pair of membrane-associated proteolyic enzyme and inhibitor. Their close relationship provides an excellent model to study how an important protease is regulated in physiological processes and the impact of deregulation of this regulation in diseases, particularly cancers. While the hallmarks of life history of matriptase, including zymogen activation, protease inhibition, and removal, share the same biochemical features of most other proteases, and have been well maintained even in cancer cells, subtle alterations in where and how zymogen activation of matriptase occurs, and the loss of balance between the protease and the inhibitor could be very important to human cancers. Understanding the molecular mechanisms governing zymogen activation and inhibition also highlights the need for alternative approaches, such as anti-activation of matriptase, to develop therapeutic agents which can control matriptase proteolysis more efficiently than classic protease inhibitors which target the proteolytic activity. Investigation of the components of the matriptase activation machinery and its upstream regulatory mechanisms, including the cytosolic suppressor(s) will hold the key to further understand the biology and pathology of matriptase.

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Abbreviation: AR: androgen receptor; EDG: endothelial differentiation gene; HAI-1: hepatocyte growth factor inhibitor-1; HGFA: hepatocyte growth factor activator; LDLRA: low density lipoprotein receptor class A domains; LPA: lysophosphatatic acid; MMP: matrix metalloproteinase; S1P: shinphingosine 1-phospahte; SDS: sodium dodecyl sulfate

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