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Review

Matriptase and its putative role in cancer

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Abstract. Tumor progression and metastasis are the pathologic effects of uncontrolled or deregulated invasive growth, a process in which proteases play a fundamental role. They mediate the degradation of extracellular matrix components and intercellular cohesive structures to allow migration of the cells into the extracellular environment and activate growth and angiogenic factors. In addition to metalloproteases and the plasminogen activation system, another protease, matriptase, contributes substantially to these processes. Matriptase is a type II transmembrane trypsin-like serine protease that is expressed by cells of epithelial origin and is overexpressed in a variety of human cancers. It has been suggested that this protease not only facilitates cellular invasiveness but may also activate oncogenic pathways. This review summarizes current knowledge about matriptase, its putative role in tumor initiation and progression, and its potential as a novel target in anti-cancer therapy.

Keywords. Matriptase, MT-SP1, protease, cancer, inhibitor.

Matriptase function

Matriptase, also known as MT-SP1 [1], TADG-15 [2], and ST14/SNC19 [3–5], was originally isolated due to its gelatinolytic activity and was therefore thought to be involved in the degradation of the extracellular matrix (ECM) or in tissue remodeling [6, 7]. Subsequently, the substrate preference of matriptase was mapped using a positional scanning synthetic combinatorial library and substrate phage display [8]. The four preferred residues N-terminal to the cleavage site contained arginine or lysine at P4, a non-basic residue at P3, serine at P2, and arginine at P1, directly N-terminal to the cleavage site. An alanine is preferred at P1′, the position C-terminal to the cleavage site (Fig. 1). Based on this profile, three surface-localized protein substrates, protease-activated receptor 2 (PAR-2), the zymogen form of the urokinase-type plasminogen activator (pro-uPA), and the inactive proform of the hepatocyte growth factor, also known as scatter factor (pro-HGF/SF), have been proposed and subsequently verified in *in vitro* experiments [8, 9].

Two of them, pro-HGF/SF and pro-uPA, have been shown to be of major importance for the process of invasive growth, a complex biological program that includes cell proliferation, cell-cell dissociation, migration, crossing of the basal membrane, and colonization of distant sites. Invasive growth occurs in physiological processes such as organ regeneration and maintenance as well as in pathological processes such as tumor growth and metastasis.

Processing of pro-uPA

uPA belongs to the urokinase plasminogen activator system, which is composed of the serine protease (uPA), its cell-surface-associated receptor (uPAR), two plasminogen activator inhibitors (PAI-1 and PAI-2) and the zymogen plasminogen that can be activated by uPA to form active plasmin, a non-specific trypsin-like protease that catalyzes fibrinolysis [10] and also the degradation of the ECM [11], together with the activation of certain growth factors [12–14] as well as the zymogen forms of several matrix metalloproteinases (MMPs) [15, 16]. The

Preferred cleavage sequences of matriptase

			cleavage sites		
P4	**P3**	**P2**	**P1**	↓	**P1´**
R/K	X	S	R	↓	A
or					
X	R/K	S	R	↓	A
(X is a non-basic amino acid)					
Activation sites of					
matriptase	R	Q	A	R	↓ V
pro-uPA	P	R	F	K	↓ I
pro-HGF/ SF	K	Q	L	R	↓ V
PAR-2	S	K	G	R	↓ S
IGFBP-rP1	K	A	L	H	↓ V
profilaggrin	R	K	R	R	↓ G

Figure 1. Preferred cleavage sequences of matriptase and activation sites of its potential protein substrates.

uPA system has a wide range of distinct but overlapping functions in the processes of tissue remodeling, invasiveness, and angiogenesis: First, uPA/uPAR promotes extracellular proteolysis by regulating plasminogen activation. Second, uPA/uPAR regulates cell/ECM interactions as an adhesion receptor for vitronectin and through its capacity to modulate integrin function. Third, uPA/uPAR regulates cell migration as a signal transduction molecule and by its intrinsic chemotactic activity [reviewed in ref. 17]. Components of the uPA system are often up-regulated in cancer, and it is generally accepted that they then promote tumor invasion and metastasis.

uPA consists of two disulfide-linked polypeptide chains, a C-terminal B chain, containing the serine protease domain, and an N-terminal A chain consisting of a growth factor domain, a kringle domain, and an interdomain linker region. The one-chain zymogen form of uPA, pro-uPA, has an activity about 250-fold less than that of two-chain uPA [18]. Conversion of pro-uPA to the more active uPA form occurs by proteolytic cleavage of the peptide bond connecting the A and B chains. This cleavage is catalyzed by plasmin [19, 20] and several other convertases, like plasma kallikrein [21], mast cell tryptase [22] and, as described below, matriptase.

To assess the relevance of matriptase in pro-uPA processing, its efficiency has been compared with that of the other pro-uPA convertases *in vitro*: recombinant matriptase converts pro-uPA as efficiently as plasmin, and these two activate pro-uPA much more efficiently than the other proteases [23].

The relevance of matriptase as a pro-uPA convertase at the cellular level has recently been demonstrated by the findings of Suzuki et al. [24] showing that down-regulation of matriptase by antisense oligonucleotides in HRA human ovarian cancer cells results in the inhibition of the activation of receptor-bound pro-uPA and by our findings, showing that the inhibition of matriptase by either small active-site inhibitors or by selective small inhibitory RNA (siRNA) severely affects the activation of pro-uPA at the surface of PC-3 prostate carcinoma cells [23]. In both studies, the reduced activation of uPA by inhibition of matriptase is associated with a significant reduction in the invasiveness of the cells through a reconstituted ECM (Matrigel).

uPAR concentrates pro-uPA at the cell surface and localizes the protease to specific compartments of the cell surface such as cell/ECM contacts and, in particular, the invasion front of migrating cells [25, 26]. Consistent with the hypothesis that matriptase activates pro-uPA, matriptase co-localizes with pro-uPA [7, 27].

Processing of pro-HGF/SF

HGF/SF is a pleiotropic factor that was identified as a growth factor for hepatocytes (HGF) [28, 29] and as a secretory product of fibroblasts that dissociates epithelial cells and increases their motility (SF) [30–32]. HGF/SF has mitogenic, motogenic, and morphogenic functions in various cell types through its high-affinity receptor tyrosine kinase, Met, that is encoded by the c-met proto-oncogene [33, 34]. Receptor binding induces multiple signaling pathways, including Ras-MAPK, PI3K, Src, and Stat3, which eventually result in the co-ordinated regulation of the expression and/or the activation of gene products required for invasive growth. A number of studies have demonstrated that HGF/SF and Met have important roles in tumorigenesis, invasiveness of tumor cells, differentiation, and tumor angiogenesis [reviewed in ref. 35] and that the inhibition of HGF/SF dramatically reduces tumor growth and metastasis [36–43].

HGF/SF is a mesenchymally derived heparin-binding glycoprotein secreted as an inactive precursor (proHGF/SF). Normally it remains in its precursor form and is distributed ubiquitously as a latent paracrine factor. To exhibit its biological function, an extracellular proteolytic conversion of the single-chain precursor to the two-chain heterodimeric active form is essential [40, 44, 45]. Since this activation process is a critical limiting step in the associated signaling pathway, its regulation is crucial for the pathophysiological roles of HGF/SF *in vivo.* In fact, enhanced activation of HGF/SF has been reported in tumor tissues [46–48].

To date, seven proteases have been suggested to be involved in the activation of HGF/SF: uPA and tissue-type plasminogen activator (tPA) [49], hepatocyte growth factor activator (HGFA), a serum protease homologous to the coagulation factor XIIa (FXIIa) [50–52], FXIIa itself [53], coagulation factor FXIa, plasmakallikrein [54], and matriptase [9]. To assess the relevance of matriptase as

an activator of pro-HGF/SF, we compared the efficiency of matriptase in the processing of HGF/SF with that of the other HGF/SF-activating proteases *in vitro*. Matriptase processed the inactive form of HGF/SF as efficiently as HGFA and these two were much more active than the other pro-HGF/SF convertases. Moreover, we demonstrated that matriptase efficiently controlled pro-HGF/SF conversion, even within a cellular context, and that this matriptase-dependent pro-HGF/SF conversion at the cell surface is relevant for the biological responses triggered by pro-HGF/SF. Inhibition of matriptase with either selective siRNA or with small peptidomimetic active-site inhibitors led to an impaired pro-HGF/SF-induced scattering of the prostate carcinoma-derived cell line PC-3. However, cell scattering was not influenced when induced by mature, active HGF/SF, indicating that the effect of matriptase inhibition occurred at the level of pro-HGF/SF activation [23]. Other findings corroborate the hypothesis that matriptase might have a functional role in the HGF/SF pathway. First, transgenic expression of HGF/SF in the epidermis of mice has the same effect as transgenic epidermal expression of epithin [55], the mouse homologue of matriptase (see below): both increase the incidence of spontaneous and induced squamous cell carcinomas [56, 57], suggesting a linear molecular flowchart along which augmented matriptase activity would lead to enhanced production of mature HGF/SF, which in turn might stimulate keratinocyte transformation [58]. Concomitantly, List et al. [56] showed that epithin activates PI3K-Akt signaling, one of the downstream pathways that can be activated upon ligand binding of HGF/SF to its receptor Met. Second, a tight correlation between the expression of HGF/SF, Met, and matriptase has been found in tissue microarrays of node-negative breast cancer. In this study, high levels of Met, matriptase, and HAI-1 were associated with poor patient outcome, implicating a functional role of matriptase in HGF/SF/Met signaling [59].
By controlling the activity of uPA and HGF/SF, matriptase is a prime constituent in the activation cascade for invasive growth (Fig. 2).

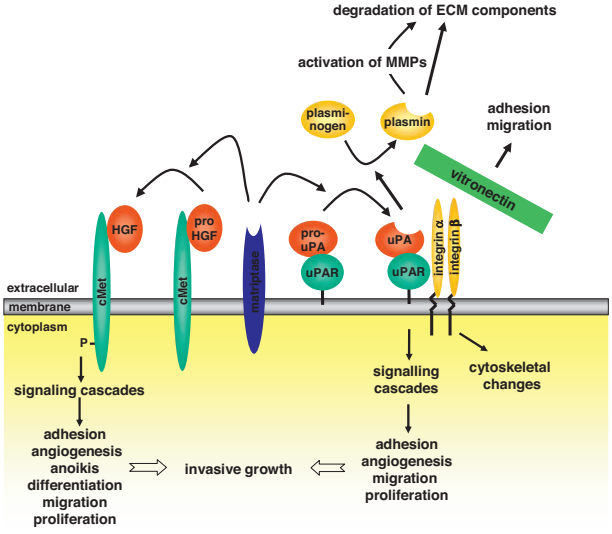

Figure 2. Major proposed functions of matriptase in invasive growth. Schematic drawing of the two major pathways that are hypothesized to mediate the pro-invasive effects of matriptase.

Potential role of matriptase in cell adhesion

In addition to pro-uPA and pro-HGF/SF, matriptase is able to process a number of substrates that are involved in cell adhesion. A secreted form of matriptase has been shown to cleave fibronectin and laminin [60], both components of the ECM that mediate cell attachment and migration. It is also able to activate PAR-2 [8], a G-protein-coupled receptor that plays a pivotal role in cell adhesion and early inflammatory processes. Consistent with its role in regulating cell adhesion, matriptase transfection into colorectal cancer cells has been found to decrease their adherence ability to the ECM and to influence cell cytoskeletal protein (F-actin) organization [61, 62].

Recently, another substrate of matriptase was identified: insulin-like growth factor binding protein-related protein-1 (IGFBP-rP1), also known as angiomodulin (AGM), mac25, or prostacyclin-stimulating factor (PSF) [63]. The IGFBP-rP1 mRNA is expressed in a wide range of normal tissues and the protein has been found in the blood vessels of various human cancer tissues [64] and in invading tumor cells [65]. IGFBP-rP1 exerts a weak cell adhesion activity through low-affinity binding to heparan sulfate proteoglycans, type IV collagen, and syndecan-1 on the cell surface [64, 66, 67] and stimulates cell growth in an insulin- or insulin-like growth factor (IGF)-dependent manner [66]. On the other hand, IGFBP-rP1 was reported to exhibit a tumor-suppressive activity when overexpressed in cancer cells [68–70]. IGFBP-rP1 can be converted from a single-chain form to a two-chain form and this processing notably alters the IGF/insulin-dependent and -independent activities of the protein. For example, IGFBP-rP1 loses its IGF/insulin-binding activity and IGF/insulin-dependent growth-stimulating activity but acquires high syndecan-1-mediated cell adhesion activity [66]. It seems conceivable that matriptase regulates invasive growth in part by modulating biological activities of IGFBP-rP1.

Role of matriptase in development

Recently, matriptase knockout mice showed that the protease is essential for postnatal survival. The postnatal death of the matriptase-depleted mice resulted from dehydration which was caused by a lack of epithelial barrier function in the skin of newborns. These matriptase knockout mice also had abnormal hair follicle develop-

ment and disturbed thymic homeostasis, as indicated by increased lymphocyte apoptosis in the thymuses [71]. Matriptase thus seems to have pleiotropic functions in the development of the epidermis, hair follicles, and cellular immune system.

In an attempt to understand the role of matriptase in these processes, the knockout mice were analyzed for potential substrates: the animals showed profilaggrin accumulation in epidermal tissue. This indicates that matriptase might be involved in the activation of profilaggrin to filaggrin, a major protein that is involved in keratin aggregation and in terminal epidermal differentiation [72]. Matriptase is able to process profilaggrin *in vitro* [72]. However, the two proteins are located in different cellular compartments. Whereas profilaggrin is a cytoplasmic protein, the catalytic domain of matriptase is located in the extracellular space. Therefore, at first glance, a direct activation of profilaggrin by matriptase seems unlikely. However, Pearton et al. [73] found that terminal differentiation of keratinocytes is associated with dramatic changes in intracellular organelles and the plasma membrane that lead to cytoplasmic access of proteins that are normally located in the lumen of intracellular organelles or on the cell surface. Matriptase could thus have access to profilaggrin and cleave it directly. Alternatively, the matriptase could either activate a profilaggrin-processing protease or influence profilaggrin processing indirectly, i.e. by growth factor activation or remodeling of the ECM.

By using enzymatic gene trapping to study the spatiotemporal expression and function of matriptase, List et al. [74] showed very recently that the protease has diverging functions in mouse keratinized tissue development, homeostasis, and malignant transformation. In staged embryos, the onset of epidermal matriptase expression coincided with that of profilaggrin expression and acquisition of the epidermal barrier. However, matriptase expression was also found in profilaggrin-negative cells, such as undifferentiated and rapidly proliferating matrix cells and in a keratin-5-positive proliferative cell compartment during carcinogen-induced malignant progression. The results suggest that the profilaggrin-processing activity of matriptase accounts for its role in the formation of the epithelial barrier function, but that other activities of the protease are responsible for its role in hair follicle growth or tumor progression. Furthermore, the ability of matriptase to degrade or process a variety of extracellular proteins suggests a possible physiological function in the control of invasive growth by regulating ECM degradation, cell-cell and/or cell-substratum adhesion and the activity of growth-regulating proteins. From these functions it is easily understood that matriptase might play an important role in tumor invasion and metastasis, when overexpressed, deregulated, or uncontrolled. The preferred cleavage sequence of matriptase and the activation sites of its potential protein substrates are shown in Figure 1. Proposed physiological functions are listed in Table 1.

Augmented matriptase levels promote cancer initiation and progression

List et al. [56] have generated transgenic mice with increased expression of matriptase in the epidermis. They found that even a modest increase of matriptase activity was sufficient for the induction of spontaneous squamous cell carcinomas and strongly potentiated chemical skin carcinogenesis, possibly through activation of the tumor-promoting PI3K-Akt pathway. In contrast, double transgenic mice co-expressing matriptase and its cognate inhibitor HAI-1 did not develop spontaneous skin cancers and did not show increased susceptibility to chemical carcinogenesis. The cognate matriptase inhibitor is thus able to compensate for the effect of augmented matriptase activity, providing a rationale for the inhibition of matriptase to prevent tumor growth. Likewise, elevated levels of matriptase accelerated the dissemination capacity of a gas-

Table 1. Potential substrates of matriptase and associated functions of matriptase action.

Matriptase substrate	Effect of matriptase	Terminal epithelial differentiation	Proliferation	ECM degradation/ remodeling	Adhesion	Migration/ invasiveness
Profilaggrin	processing	X				
Pro-uPA	activation		X	X	X	X
Pro-HGF	activation		X	X	X	X
PAR-2	activation				X	
IGFBP-rP1	modulation of activity		X		X	
Fibronectin	degradation				X	X
Laminin	degradation				X	X
Gelatin	degradation			X		
Collagen type IV	degradation			X		

tric cancer cell line when injected intraperitoneally into athymic mice. Whereas lymph node metastases were only found in 30% of the mice that obtained mock-transfected control cells, lymph node metastases were detected in 80% of the mice injected with matriptase-transfected cells [75]. Consistently, matriptase overexpression was shown to enhance the invasiveness of colorectal cancer cells significantly *in vitro* [62]. These studies show that elevated levels of matriptase can promote both tumor onset and metastasis formation and suggest a direct functional involvement of the protease in the initiation and progression of cancer. There is extensive evidence that the matriptase level is augmented by either deregulation, stabilization or overexpression in a variety of tumor tissues.

Regulation of matriptase activity and its pathologic deregulation in cancer

Matriptase is essential for postnatal survival [71]. At the same time, matriptase causes cancer when only modestly overexpressed in transgenic mice [56]. Thus, it is obvious that matriptase must be tightly regulated to prevent damage caused by uncontrolled activity. The regulation of matriptase is not yet fully understood. However, it is clear that the multidomain structure and posttranslational modifications of matriptase play a crucial role in its regulation.

Matriptase, 95 kDa, has a multidomain structure that is common for the group of type II transmembrane serine proteases: From the N terminus to the C terminus it consists of a short cytoplasmic domain with unknown function, a transmembrane region, a domain found in sea urchin sperm protein, enterokinase and agrin (SEA domain), two complement sub-component C1r/s, urchin embryonic growth factor and bone morphogenetic protein 1 (CUB) domains, four tandem repeats of a low-density lipoprotein (LDL) receptor class A domain, and a C-terminal trypsin-like serine protease catalytic domain (Fig. 3). Matriptase has four putative N-linked glycosylation sites that are homogeneously distributed over the protein, at amino acid positions 109, 302, 485, and 772.

Matriptase is a zymogen that has to be activated by proteolytic cleavage to accomplish its biological function. The activation site is located directly N-terminal to the catalytic domain. Once processed, the active catalytic domain stays attached to the membrane by a disulfide bond linking the pro-domain to the catalytic domain [8, 76] unless it is shed at either of the shedding sites at residues 190 or 205 [77, 78]. Since matriptase bearing a mutation in its catalytic triad (see Fig. 3 for explanation) is unable to undergo this activational cleavage, matriptase is thought to catalyze its own activation [77], most likely by auto-activation [79]. Using additional point and deletion mutants, it was shown that in addition to the proteolytic activity, activation of matriptase requires glycosylation of

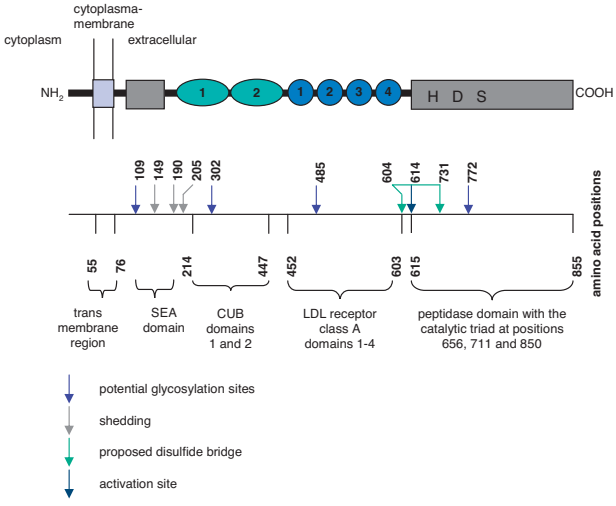

Figure 3. Multidomain structure and posttranslational modifications of matriptase. Schematic drawing of the domain structure of matriptase. The arrows indicate the locations of potential glycosylation sites (blue), shedding sites (gray), a proposed disulfide bridge (green), and the activation site (dark blue). The drawing is not to scale.

the first CUB domain and the serine protease domain, intact LDL receptor class A domains and an initial endoproteolytical cleavage at Gly149 in the SEA domain of the zymogen [77]. As the SEA domain stays intact despite being cleaved, matriptase is membrane bound during its activation.

In mammary epithelial cells, matriptase activation requires a blood-derived factor, the bioactive phospholipid sphingosine-1-phosphate (S1P) [80, 81]. Interestingly, unlike non-transformed mammary epithelial cells, breast cancer cells do not respond to this activator of matriptase. Similar levels of activated matriptase were detected in breast cancer cells grown in the presence or absence of S1P. However, up to fivefold higher levels of activated matriptase were detected in the conditioned media from the cancer cells grown in the absence of serum and S1P, when compared with non-transformed mammary epithelial cells, suggesting that matriptase activity is deregulated in these breast cancer cells [82].

As mentioned above, shed matriptase has been found in a complex with HAI-1 in human milk, indicating that HAI-1 might be a cognate inhibitor of the protease [76]. Paradoxically, HAI-1 has not only inhibitory function, but is also required for matriptase activation [77]. This is thought to ensure that matriptase can be quickly inactivated once it is set free from the membrane to protect the cells from uncontrolled matriptase activity. Recent studies by Oberst et al. [83] suggest that the regulatory role of HAI-1 even goes beyond that of inhibition and activation and that it also regulates the proper expression and intracellular trafficking of matriptase. The ratio of matriptase to HAI-1 has been shown to be shifted towards matriptase in late-stage tumors (see below) and this imbalance

has been proposed to promote the proteolytic activity of matriptase and would, consequently, be important for the development of advanced disease [84].

Both matriptase and HAI-1 were identified by quantitative proteomic analysis as cellular proteins shed into culture media in response to androgen exposure in LNCaP prostate cancer cells [85]. Subsequently in addition to shedding, androgen was also found to stimulate matriptase activation in androgen-sensitive prostate carcinoma cells, and this process is mediated by the androgen receptor. In contrast, treatment of hormone-starved breast cancer cells with 17beta-estradiol had no effect on matriptase activation, in part due to their high constitutive level of activated matriptase [86].

Matriptase protein was shown to be stabilized by beta-1-6 GlcNAc branching, an oligosaccharide modification produced by UDP-GlcNAc alpha-mannoside beta 1-6-N-acetylglucosaminyltransferase (GnT-V) [75]. GnT-V is overexpressed in a variety of cancers, and beta-1-6 GlcNAc branching is associated with malignant transformation [87–90]. A recent study by Siddiqui et al. [91] showed a high degree of correlation of GnT-V activity with matriptase expression in tissue microarrays of breast carcinomas. The authors found that high levels of GnT-V activity and matriptase were predictive of poor outcome and were significantly associated with disease-related survival. Acquired resistance of matriptase to degradation through glycosylation by GnT-V and a corresponding increase in the active form could possibly be the mechanism by which beta 1–6 GlcNAc branching is linked to cancer progression.

Matriptase overexpression in cancer

Matriptase is expressed by the epithelial elements of almost all organs examined so far [60, 78]. The protease has also been shown to be overexpressed in a variety of cancer cell lines and tumor tissues, and high matriptase levels are, in many cases, correlated with poor clinical outcome (see Table 2). These findings corroborate the hypothesis that matriptase may play an important role in cancer progression and suggest that matriptase may also represent a novel prognostic diagnostic marker.

Prevention of tumor growth and metastasis by matriptase inhibition

The previous sections have illustrated that matriptase is very likely involved in the initiation and progression of cancer and that the matriptase level is augmented by either deregulation, stabilization, or overexpression in a variety of tumor entities. Consequently, inhibition of matriptase may be a means to prevent tumor growth and metastasis formation. Indeed, several animal studies now demonstrate the potency of matriptase inhibition in anticancer treatment.

A reduction of tumor growth and metastasis formation by matriptase inhibition was first suggested by Takeuchi et al. [92]. They described that the primary tumor size of nude mice implanted with PC-3 prostate carcinoma cells was significantly smaller in mice that were treated for 7 weeks with either the serine protease inhibitor, ecotin, or the ecotin mutant M84R/M85R, compared with the primary tumor size of vehicle-treated control mice. In addition, the number of metastases derived from the primary tumors was similarly lower in the inhibitor-treated mice than in vehicle-treated mice. Inhibition was not unexpected with ecotin M84R/M85R treatment, because the PC-3 cells are known to express uPA and the ecotin mutant is a good uPA inhibitor (K_i = 1 nM). However, wild-type ecotin is a poor, micromolar inhibitor of uPA. These authors concluded that the decrease in tumor size and metastasis in the mouse model involves the inhibition of additional serine proteases. Since ecotin is a very good inhibitor of matriptase (K_i = 0.78 nM) and the protease is expressed in PC-3 cells, it was proposed that the effect of ecotin on tumor growth and metastasis could be a result of matriptase inhibition.

In another mouse model of prostatic cancer using the human androgen-independent prostate cell lines CWR22R and CWRSA6, matriptase inhibition also reduced tumor growth. In this study, a selective and potent (K_i = 3.3 nM) arginal-derived matriptase inhibitor, CVS-3983, was administered at a dose of 25 mg per kg intraperitoneally twice daily, 7 days per week, for 2–3 weeks to nude mice with established tumors. CVS-3983 reduced the final mean tumor volume by 65.5% and 56.2% in the CWR22R and CWRSA6 model, respectively, compared with vehicle-treated mice. CVS-3983 also reduced the invasion of the cell lines through reconstituted ECM (Matrigel) [93].

In an orthotopic xenograft model of prostate cancer, using the same human prostate carcinoma cell line, PC-3, that was used in the 'ecotin model', we studied the effects of two small-molecule matriptase inhibitors, inhibitor no. 8 and 59, on primary tumor growth and metastasis formation. The inhibitors are bis-basic secondary amides of sulfonylated 3-amidino-phenylalanine that have a high affinity for matriptase (K_i = 46 and 6.7 nM for the inhibitors no. 8 and 59, respectively) and low affinity towards related trypsin-like serine proteases. They were obtained by screening a library of peptidomimetic serine protease inhibitors and subsequent structure-based optimization of the candidates [94]. The inhibitors were administered intraperitoneally at a daily dose of 5 mg per kg, 7 days a week for 4 weeks to nude mice with established orthotopic prostate tumors that were transplanted from the invasive part of human PC-3 tumor tissue pregrown subcutaneously in nude mice. The inhibitors reduced tumor growth

Table 2. Matriptase overexpression in cancer and correlation with clinical outcome.

Cancer	Type of probes/number of samples	Method of analysis	Frequency of matriptase expression in the respective tumor type	Factor of overexpression in malignant tissue compared with normal tissue	Correlation with clinical outcome	Reference
Ovarian cancer	epithelial ovarian cancer/54	IHC	stage I/II: 82%, stage III/IV: 55%	n.d.	correlation of the matriptase: HAI-1 ratio with the clinical outcome of advanced-stage tumors; loss of HAI-1 expression associated with stage III/IV tumors	84
	OSPC cell lines/12, primary cultures/5	GEP	n.d.	>5-fold	n.d.	96
	tissues of various types of ovarian carcinoma/184	TMA	73–96%, depending on tumor type	5- to 18-fold	increased expression of matriptase in serous adenocarcinoma has significant clinicopathological relationship to the aggressiveness seen with this tumor	97
Prostate carcinoma	Prostate cancer/44, benign tissue/23	RT-PCR	n.d.	high overexpression compared with benign species	n.d.	98
	normal tissue/6, benign hyperplasia/6, intraepithelial neoplasia/4 and adenocarcinomas of grades 6, 24 and 44	RT-PCR, IB, IHC	94%	up to 5-fold	increased expression of matriptase and decreased expression of HAI-1 with increasing tumor grade; proposal as a novel biomarker	99
Malignant pleural mesothelioma	cell lines/10, primary tumor tissues/4	GEP, RT-PCR, IB	n.d.	800-fold increase compared with healthy tissue	n.d.	100
Cervical carcinoma	cervical carcinoma cell lines/19, normal cervical keratinocyte control cultures/8	RT-PCR, IHC	cervical carcinoma cell lines: 74%, squamous cell cervical carcinoma: 100%, normal cervical keratinocyte control cultures: 0%	n.d.	n.d.	101
	healthy and various stages of malignant tissue/89, ISCC/26	RT-PCR, IHC	95% in ISCC, 0% in normal squamous epithelia	increased expression of matriptase is associated with histopathologic grades of cervical neoplasia		102
Breast carcinoma	tissue of node-negative breast cancer/330	TMA	tight correlation between the expression of HGF, Met, and matriptase	high-level expression of Met, matriptase, and HAI-1 are associated with poor patient outcome		59
	tissue/670	TMA	n.d.	n.d.	significant association of GnT-V activity with disease-related survival	91
pancreatic cancer	cell lines and tissue	Dd, RT-PCR IHC	n.d.	high overexpression in hypoxic regions of the tumor	n.d.	Büchler, personal communication
papillary thyroid cancer	tissue	GEP, RT-PCR	69%	~2-fold	n.d.	103
kidney cancer	tissue/1	RT-PCR	co-up-regulation with TMEFF1	3-fold	n.d.	104
lung cancer	tissue/1	RT-PCR	n.d.	expression in tumor tissue but not in adjacent normal tissue	n.d.	104
liver cancer	tissue/1	RT-PCR	n.d.	2.5-fold	n.d.	104

IHC, immunohistochemistry; GEP, gene expression profiling; IB, immunoblotting; TMA, Tissue micro array; RT PCR, quantitative real time PCR; Dd, differential display; ISCC, invasive squamous cell carcinoma; n.d., not done.

up to 40% and reduced metastasis formation substantially. Whereas 30% of the control mice had metastases in the thorax, metastases were only found in 14% and 20% of the animals treated with inhibitors 8 or 59, respectively. The effect was even more pronounced with abdominal metastases. Whereas metastases were found in 50% of the abdomens of the control mice, metastasis formation was completely abolished in both of the treatment groups.

One of these inhibitors (no. 8) was also used in *in vitro* studies. Here, it reduced significantly the pro-HGF/SF-induced and HGF/SF-induced invasiveness of PC-3 cells in an *in vitro* invasion assay (Matrigel). In this assay, down-regulation of matriptase expression by selective siRNAs yielded a comparable reduction in invasiveness, demonstrating that the effect seen with the inhibitors is specific for matriptase [23].

Matriptase inhibitor no. 8 was also shown to effectively reduce the growth and prevent metastasis formation in an orthotopic nude mouse xenograft model of pancreatic cancer [unpublished data].

By down-regulating matriptase expression with antisense oligonucleotides in the human ovarian cancer cell line, HRA inhibition of matriptase was shown once again to reduce both cellular invasiveness *in vitro* and intraperitoneal tumor growth in nude mice. Tumors derived from parenteral HRA cells grew 2.5 to 3 times larger than tumors derived from HRA cells transfected with matriptase-specific antisense oligonucleotides [24, 95].

Viability and proliferation of the cells were not affected by matriptase inhibition in any of the *in vitro* studies, nor were any side effects reported from the *in vivo* studies.

Summary

Matriptase is a type II transmembrane serine protease that is expressed in epithelial cells and that has been shown to be overexpressed in a variety of tumor tissues. Matriptase is thought to have pleiotropic functions. It was originally isolated due to its matrix-degrading activity. Studies with matriptase-depleted mice then suggested that it is involved in the processing of profilaggrin, an aberrant protein involved in keratin aggregation and in terminal epidermal differentiation. Slight overexpression of matriptase in keratinocytes of transgenic mice causes squamous cell carcinoma. Correspondingly, the inhibition of matriptase is effective in the reduction of tumor growth and metastasis formation. The oncogenic and metastatic potential of matriptase are suggested to derive from its ability to activate efficiently the pro-oncogenic and pro-metastatic factors pro-uPA and pro-HGF/SF. As matriptase seems to be functionally involved in tumor growth and spread in certain tumor entities, it might represent a potential new target for anti-cancer therapy as well as a novel prognostic diagnostic marker.

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