Journal of Science and Applications:
BioMedicine
Review

An overview of St14: A type II transmembrane serine protease

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Abstract: St14 is a type II transmembrane serine protease that contains a cytoplasm N-terminal domain, a stem domain, and a serine protease catalytic domain. It is primarily synthesized as an inactive zymogen and co-expressed with the cognate inhibitor hepatocyte growth factor inhibitor (HAI) in epithelial cells. Accumulating evidence suggests that it plays critical roles in pre-implant embryonic compaction, placental development, as well as epidermal barrier formation after activation. The homologous ablation of St14 in mouse underlies the neonatal death at 48h for severe deficiency of the epidermal barrier. It has also been considered as a prognostic marker of epithelial cancers as it is involved in cancer metastasis and invasion in multiple ways by activating several kinds of substrates, such as hepatocyte growth factor (HGF), urokinase-type Plasminogen activator (uPA). Notably, the strict regulation of St14 by HAIs is found to play a profound role in all of these activities. However, very little is currently understood about the mechanisms that downregulate St14 gene expression in early neonatal mortality. Herein we summarize the current knowledge of the regulation network and the understanding of the biological functions of St14.

Published by www.inter-use.com. Available online March 5th 2015, Vol. 3 Issue 2 Page 10-17.

Keywords: St14, HAI, Embryonic development, Cancer invasion, Metastasis

1. Introduction

Cell surface-activation induced by transmembrane serine proteases has been considered as a critical mechanism for cross-talk between different cell types. Most important cellular activities are triggered by extracellular signaling molecules, but many of these signaling molecules are usually generated in inactive forms and require cleavage by the transmembrane serine proteinases before mediating the activities of target cells. Thus, these membrane-associated serine proteases serve as "activators" or key intermediates for cooperation between different types of cells. As a type II transmembrane serine protease, St14 has received more and more attention for it has been found to be extensively involved in many kinds of physiological and pathological activities.

St14 is one of the type II transmembrane serine proteases (TTSP) and is co-expressed with the cognate transmembrane inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), in various epithelial cells. It is initially synthesized as an inactive zymogen and requires a series of processes prior to activation, including sequential N- and C-terminal cleavage and transient association with HAI-1. It is reported that St14 plays important roles in placental development, epidermis terminal differentiation, and hair follicle growth, as well as cancer invasion and metastasis. Notably, its proteolytic activity is tightly regulated by HAI-1 and HAI-2 in both of tumorigenesis and embryonic development. St14 that is unopposed by HAIs would place the embryos at high risk of developmental failure and promote tumor metastasis and invasion in epithelial cells. This review delineates the structure features, substrate specificity, activation, inhibition, and functions of St14.

2. The structure of St14

St14, also known as Matriptase, Epithin, MT-SP1, TMPRSS14, or TGAD15, was first reported in 1993 as a novel gelatinolytic protease in human breast cancer cell lines and the homologue in mice was firstly cloned in 1999 from thymic stromal cells [1, 2]. Orthologs of St14 in other vertebrate animal models, including rat, chicken, zebrafish *et al*. have been identified and are functionally conserved. Structurally, it contains a short cytoplasmic N-terminal, a C-terminal serine protease (SP) catalytic domain, and a single cross-transmembrane stem domain which is composed of a SEA (sea urchin sperm protein, enteropeptidase, agrin)

domain, two CUB (complement factor 1R-urchin embryonic growth factor-bone morphogenetic protein) domains, and four LDLRA (low density lipoprotein receptor class A) repeats, as shown in Fig. 1. The understanding of the roles of these domains is summarized as following.

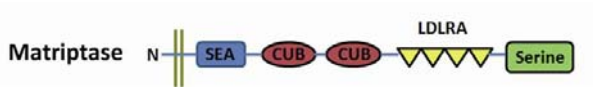

Fig. 1. The structure of St14.

2.1. The cytoplasmic domain

The cytoplasmic domain of St14 is composed of 54 amino acids and orients St14 as a type II transmembrane serine protease. Although the roles of the cytoplasmic domain are not fully understood, it is speculated that this short domain may be involved in binding to cytosolic proteins and regulating their cellular distribution and activities. Indeed, a study reports that the cytoplasmic domain can mediate the interaction between St14 and the cytoskeleton in epithelial cell lines after treatment with phorbol myristate acetate (PMA) [3]. This linkage results in cytoskeleton rearrangement as well as accumulation and release of St14. Similar results can be achieved in mammary epithelial cells by treatment with sphingosine-1-phosphate. Yeast two-hybrid screen recovers that the interaction between St14 and the PMA-induced cytoskeleton is mediated by Filamin-A, which is known to interact with actin to promote the branching of actin filaments. The mechanism underlying the interaction between St14 and filamin is the phosphorylation of the cytoplasmic domain, as there is a consensus phosphorylation site for PKC. Moreover, it has been demonstrated that PKC could be activated by PMA [4]. However, the hypothesis of PMA/PKC/St14 pathway involvement in the interaction between St14 and Filamin-A requires further investigation.

2.2. The stem domain

The four tandem LDLRA repeats within the extracellular stem domain are widely presented in type II transmembrane serine proteases and are homologous to the low density lipoproteins (LDL) receptors. LDLR domains are found to be important for interactions between St14 and other proteins. Tie2, an angiopoietin receptor required for angiogenesis, is found to be associated with St14 by LDLRA domains. Deletion of LDLRA domains would impact this interaction and results in degradation of St14 [5]. In addition, the LDLRA domains are also involved in activation of St14, to be discussed below.

The CUB domains are ubiquitous in extracellular and plasma membrane-associated proteins, and are regarded as the linker between St14 with itself or other proteins. For example, TMEFF1, a transmembrane protein involved in growth factor signaling pathway, is reported to interact with St14 through CUB domains [6]. Particularly, the CUB domains in St14 have been reported to serve as the structural basis for inactive St14 homodimerization process in low level HAI-1-expressing cell lines, which may be the mechanism by which excessive activity of St14 is inhibited [7]. Moreover, the second CUB domain in St14 is reportedly involved in the interaction between St14 and HAI-1 [8].

In all, the stem domain is suggested to mediate interactions of St14 with substrates and inhibitors, as well as other cytosolic proteins. These interactions may potentially regulate the proteolytic activity of St14. However, much of the roles of the stem domain remain unknown.

2.3 The catalytic domain

The catalytic domain is well-studied and highly conserved between serine proteases. The central of the catalytic domain is a catalytic triad which is a coordinated structure consisting of three key amino acids: His, Asp and Ser. The substrate specificity pocket (S1) of St14 includes Asp-627 at the bottom with Gly-655 and Gly-665 at the neck, which determines the preference of St14 to cleave the proteins containing Lys and Arg at P1 position [9]. Moreover, St14 can be inhibited by many serine inhibitors such as HAI-1, aprotinin, and ecotin by binding to the catalytic domain [10]. Besides, there is a proteolytic activation site within the catalytic domain which is a conserved RIVGG motif and important for the activation of St14 [11].

3. The activation and inhibition of ST14

St14 is mainly expressed as an inactive zymogen in various epithelial tissues and requires activation by several processes. The N-terminal cleavage occurs at the conserved motif G-SVIA of the SEA domain, and

the second cleavage occurs within the conserved R-VVGG motif. However, St14 still attaches to the cell surface in spite of the N-terminal cleavage, possibly via non-covalent interaction between SEA domains by forming a complex with HAI-1 [12]. Meanwhile, St14 remains in two-chain form after C-terminal cleavage due to disulfide bonds formed between cysteines within the catalytic and stem domains respectively. The mechanism of activation of St14 is incompletely understood, but it is proposed to be transactivation or auto-activation by the observation that mutations in any of the catalytic triad would inhibit the activation of St14 [11].

Additionally, any or all mutations in the four LDLRA repeats also impair its activation and loss of the fourth LDLRA domain prevents shedding of St14 to the cell surface [13], while entire deletion of all of the LDLRA domains enhances its activation, suggesting that the LDLRA domains may serve a dual function: to facilitate specific activation and as a self-inhibitor to prevent the pre-maturation of St14. Moreover, LDLRA domains are reported to affect the requirement of HAI-1 in activation of St14, as point mutations in the LDLRA and Kunitz domain 1 of HAI-1 prevent St14 activation and cell surface translocation [14]. Interestingly, this is consistent to the discovery that the transient interaction between St14 and HAI-1 is essential for St14 activation, as only HAI-1-complexed but not HAI-1-free active St14 is identified [15].

The glycosylation of asparaginates is also involved in St14 activation. As reported by Oberst *et al* [11], the glycosylation of Asn302 and Asn772 of St14 are critical for zymogen activation in breast cancer cells, whereas the glycosylation of Asn109 and Asn485 is dispensable for this process. In spite of this, it cannot be ruled out that glycosylation of Asn109 and Asn485 may function in other ways, as the mature St14 is highly glycosylated.

The inhibition of St14 by HAI-1 is firstly reported by the identification of the complex from human milk [16]. HAI-1 is a type I transmembrane protein that contains two extracellular Kunitz-type domains separated by a LDLRA domain. The Kunitz-type domains are necessary for inhibition of trypsin-like serine proteases and are found to form stable inhibitor complexes with St14 by binding to the active site of the catalytic domain [17]. The combination of St14 with HAI-1 is not only important for its activation, but also for functional regulation of St14 in both of physiological and pathological progresses. During embryonic development, knock out of HAI-1 would result in placental defects and place the embryos at high risk of development failure [18]. In polarized epithelial cells, the active St14 is inhibited by HAI-1 soon after its synthesis to prevent harmful proteolytic activity; disruption of the balance between St14 and HAI-1 in adult epidermis causes severe hyperplasia, abnormal cellular differentiation and malignant transformation [19]. Moreover, the decreased expression of HAI-1 is correlated with many kinds of cancers, such as epithelial ovarian cancer, human breast cancer and colorectal cancer [20-22].

Additionally, HAI-2, which is homologous of HAI-1 but lacks the LDLRA domain, is another inhibitor of St14 and is also indispensable for mouse embryonic development. The HAI-2-deficient mice present with placental and neural tube defects, but the placental defects could be completely restored by simultaneous inactivation of St14 [23], indicating that the developmental deficiency in HAI-2-deficient mice is largely caused by dysregulated activity of St14. HAI-2 is also found to be involved in tumor aggregation [24], which will be discussed later.

3.1. Specificity of the substrate

The substrate specificity of human St14 was first determined by Takauchi using PS-SCL (positional scanning synthetic combinatorial libraries) and substrate phage display [25]. According to their report, St14 prefers to cleave proteins containing the amino acid sequence P4(Arg/Lys)-P3(X)-P2(Ser)-P1(Arg)-P1'(Ala) as well as P4(X)-P3(Arg/Lys)-P2(Ser)-P1(Arg)-P1'(Ala), where X must be non-basic amino acids. This profile corresponds well with the substrates of St14, which include HGF, uPA, and PAR2 *et al*. Among these substrates, the most well-documented substrates are urokinase-type plasminogen activator (uPA), the G-protein-coupled protease activated receptor-2 (PAR2), and hepatocyte growth factor (HGF), as they are all involved in various activities including normal and malignant morphogenesis [26]. Since the substrates of St14 include several kinds of molecules, they may give rise to distinct consequences in different contexts. For instance, HGF is a pleotropic growth factor and the activation of HGF by St14 could result in activities such as morphogenesis and tumor aggregation.

4. Function of St14

4.1 In early embryonic development and postnatal survival

St14 has been proven to play important roles in both amphibian and rodent embryonic development. Overexpression of XMT-SP1, which is an ortholog in Xenopus laevis, leads to significant embryonic developmental defects and rapid embryonic cell death [27]. In mouse embryonic development, St14 is found crucial for pre-plant embryo compaction as downregulation of St14 at 8-cell stage results in blockade of compaction and subsequent embryo death [28]. Meanwhile, St14 is found to be co-localized with E-cadherin, which is a tight junction molecule that is essential for mouse embryonic development. St14 shows abnormal distribution when E-cadherin is downregulated, suggesting that there might be an interaction between St14 and E-cadherin. Additionally, there is a synergetic reduction of blastulation rate when St14 and E-cadherin are simultaneously knocked down, indicating again the significant roles that St14 and E-cadherin play in regulating mouse embryonic compaction.

In List's study, the $St14^{-/-}$ mice could develop to term but displayed several kinds of defects such as an impaired epidermal barrier, ichthyosis manifested by red, dry, and wrinkled skin, and abnormal hair follicle development. Furthermore, the neonatal $St14^{-/-}$ mice died within 48h from severe dehydration [29]. Moreover, these mice also showed other epidermal defects including swollen appearance of the corneocytes, vibrissal follicle hypoplasia, and poor differentiation of pelage hair follicles. Additionally, abnormal vacuolization in the resorptive epithelia and edema in the small intestine and colon was found. These deficiencies revealed the essential role of St14 in the terminal differentiation of the epidermis, hair follicle development, and postnatal survival. The mechanism underlying the involvement of S14 in epidermal development is thought to be mediated by St14/prostasin proteolytic cascade. Evidence supports that prostasin is a novel substrate of St14, and prostasin-deficient mice showed epidermal defects that were identical to the St14-deficient mice [30].

Besides St14 deficiency, an excess of St14 could also result in abnormal development, as the balance between St14 and HAI-1 is also critical for the integrity of the placenta. It was found that HAI-1 deficient mice presented with placenta labyrinth hypogenesis during embryogenesis due to loss of undifferentiated chorionic trophoblasts. In contrast, the simultaneous knock out of St14 and HAI-1 significantly alleviated these defects [31], indicating that excessive St14 may disrupt the integrity of chorionic trophoblasts and thus impair placental labyrinth morphogenesis. Detailed analysis suggests that the placental deficiency is caused by altered expression of E-cadherin and catenin, as well as disorganized laminin deposition. However, this is somewhat contradictory to the observation in pre-implant embryo compaction, that it is decreased but not excessive St14 that results in degradation of E-cadherin. Whether this is contributed to different substrates and pathways in embryo and placenta is not clear.

Besides abnormal epidermis development, the accelerated $CD4^+CD8^+$ double positive thymocyte apoptosis is also markedly discovered in $St14^{-/-}$ mice. This seems to be specific to $CD4^+CD8^+$ double positive thymocytes since other lymphoid cells are normal. The mechanism by which St14 prevents the thymocyte apoptosis is unclear, as the thymic environment has a negative role in selecting the thymocytes [32]. Moreover, uPA and HGF seems have no effect on promoting the survival and amplification of $CD4^+CD8^+$ double positive thymocyte. Thus, St14 may function in thymocyte apoptosis by alternative pathways that are independent of uPA or HGF.

St14 is also involved in transendothelial migration of active macrophage cells in the inflammatory microenvironment. Upon activation by interferon-r (IFN-r), St14 is over-expressed on the surface as well as in the cytoplasm in several macrophage cell lines and enhances the transendothelial migration of macrophages [33]. Moreover, two STAT1 binding sites are found in the promoter of St14, indicating that the expression of St14 might be regulated by STAT1 directly and the transendothelial migration-induced by St14 might contribute to IFN /STAT1 pathway.

Recently, St14 has also been found to be specifically expressed in neural progenitor cells (NPCs) and neurons, and its expression is increased at the completion of switches from mESCs to NPCs and from NPCs to neurons [34]. It is reported to promote NPCs migration, probably by activating HGF as HGF can induce human neural stem cell migration via PI3K

[35]; thus the PKC/St14/HGF/PI3K pathway may be also involved in mouse NPCs migration. However, inhibition or downregulation of St14 doesn't impair the switch from ES cells to NPCs, and it seems contradictory that the St14 KO mice present a normal phenotype of central nervous system [34]. The possible reason is that the function of St14 is slight in the entire CNS and can be complemented by the redundancy of the serine proteases superfamily.

4.2. In tumor cell invasion and metastasis

Nowadays, increasing attention has been given to the role of St14 in cancers because it is found be consistently dysregulated in various human cancers including breast, ovary, lung, as well as prostate; thus, it has been considered as a prognostic marker of epithelia cancer. Moreover, mice expressing St14 in the epidermis develop skin carcinoma spontaneously, and cancer cells with over-expression of St14 grow faster and produce much larger solid tumors with more vascularization. Conversely, downregulation or inhibition of St14 could reduce tumor size and give rise to impaired metastasis. Further analysis suggests that St14 may act in several different ways in improving the tumor invasion and metastasis, such as angiogenesis and epithelial-mesenchymal transition (EMT).

Angiogenesis is a crucial progress during cancer progression and is found to be affected directly by St14 [36]. Tie2, which is a receptor of the angiopoietin family and plays central roles in regulating vessel remodeling and endothelial permeability, is found to be cleaved and activated by St14 [5]. After activation by St14, Tie2 binds to angiopoietin, resulting in vessel remodeling and endothelial permeability, and this is possibly related to phosphatidylinositol 3-kinase (PI3K) [37].

During tumor progression, EMT - a process by which epithelial cells lose cell polarity and cell adhesion, and become migratory and invasive - provides mechanisms for epithelial cells to overcome the physical barrier imposed by intercellular junctions. Therefore, EMT is often considered as a prerequisite for tumor invasion. It has been reported that upregulation of St14 in MDCK cells results in considerable morphological changes from a polarized epithelial morphology to an elongated fibroblast-like morphology. Moreover, epithelial markers including E-cadherin were degraded whereas the mesenchymal markers were upregulated and the cell-cell junction was disassembled.

EMT in tumors induced by St14 seems convergent with the TGF-β pathway because on one hand, TGF-β is a well-known inducer of EMT in tumor cells [38]; on the other hand, the interaction between St14 and TGF-β pathway has been reported by the observation that Smad2/4 could give rise to upregulation of St14. Furthermore, the TGF-β-induced cell invasion and migration are significantly prevented by knockdown of St14, suggesting that St14 might be a target of TGF-β signaling in tumor cells [39].

As mentioned above, the proteolytic activity of St14 is strictly controlled by HAI-1, and multiple studies have demonstrated that the inhibition of St14 by HAI-1 is involved in tumor aggregation [40]. In human pancreatic cancer cell lines, knock out of HAI-1 significantly promotes EMT, while St14 and HAI-1 double KO cells can reverse these changes. This is similar to the engineered over-expression of HAI-1, suggesting that EMT resulted from HAI downregulation is probably attributed to the deregulated activity of St14. Likewise, inactivation of HAI-1 and HAI-2 in breast cancer cells significantly increase HGF–mediated cell migration and invasion [41]. These studies suggest that inhibition of St14 by HAIs also play important roles in tumor cell invasion and metastasis. Due to the tight interactions between St14 and HAIs, another point of view suggests that the tumorigenesis is not contributed to the absolute dysregulated level of St14, but the ratio or balance between St14 and HAIs [42].

The mechanism underlying tumorigenesis induced by St14 may be related to the substrates of St14 such as uPA, HGF. And MMP3 *et al*., as all of them are implicated in tumor cell invasion. HGF is a well-known inducer of EMT via its receptor c-Met, and MMP3 can trigger the degradation of E-cadherin accompanied by the dissolution of cell-cell junctions [43, 44]. uPA could activate plasminogen and enhance cellular motility and tumor vascularization by depredating the basement membrane and extracellular matrix [45]. This tumor-induced activity of St14 is specific to epithelia origin as St14 appears to be absent in tumors originated from mesenchymal.

5. Perspective

Proteomics and bioinformatics have provided necessary insight into the structural and functional properties of St14. Gene ablation has proved that St14 plays important roles in both physiological and pathological activities. As scientists have obtained more and more knowledge about St14, however, pertinent questions remain obscure, such as: 1) Which substrate is responsible for the indispensability of St14 for epidermal development, hair growth, as well as the formation of epidermal barrier? 2) St14 is involved in tumor EMT, and during embryonic development, embryos also undergo EMT to morphogenesis -does St14 participate in the embryonic EMT? 3) Is there a direct interaction between St14 and E-cadherin in the mouse early embryo? Moreover, it is notable that downregulation but not upregulation of St14 in mouse pre-implant embryos impairs the expression and location of E-cadherin and results in abnormal mouse embryonic development, which is opposite to the results in tumors where St14 promotes degradation of tight junctions; these underlying mechanisms are unknown. 4) What are the functions of St14 in non-epidermal tissues? 5) Although the non-catalytic domains are expected to function in the location, activation, secretion, and inhibition of St14, whether they are responsible for St14 participating in substrates specificity and thus mediating the distinct responses in different context is unknown.

Most studies have shown that increased level of St14 is found to be closely related to the various kinds of tumor malignancy. However, some studies have shown contradictory results that St14 is not positively related to the grade of human breast cancers and has no effect on survival [46]. Moreover, Wang *et al*. even obtained opposite results that demonstrated that in highly invasive human breast cancer cell lines, St14 functions as a tumor repressor but not inducer by reducing tumor proliferation and invasion [47]. This discrepancy in cancer cells suggests that the function of St14 is complicated and might be finely controlled in different cell types.

References

[1] Shi YE, Torri J, Yieh L, Wellstein A, Lippman ME, and Dickson RB. Identification and characterization of a novel matrix-degrading protease from hormone-dependent human breast cancer cells. Cancer Res. 1993;53(6):1409-15.

[2] Kim MG, Chen C, Lyu MS, Cho EG, Park D, Kozak C, and Schwartz RH. Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains. Immunogenetics. 1999; 49(5):420-8.

[3] Kim C, Cho Y, Kang CH, Kim MG, Lee H, Cho EG, and Park D. Filamin is essential for shedding of the transmembrane serine protease, epithin. EMBO Rep. 2005;6(11):1045-51.

[4] Arribas J, Coodly L, Vollmer P, Kishimoto TK, Rose-John S, and Massague J. Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors. J Biol Chem. 1996;271(19):11376-82.

[5] Kim C, Lee HS, Lee D, Lee SD, Cho EG, Yang SJ, Kim SB, Park D, and Kim MG. Epithin/PRSS14 proteolytically regulates angiopoietin receptor Tie2 during transendothelial migration. Blood. 2011;117(4):1415-24.

[6] Ge W, Hu H, Ding K, Sun L, and Zheng S. Protein interaction analysis of ST14 domains and their point and deletion mutants. J Biol Chem. 2006;281(11):7406-12.

[7] Xu H, Xu Z, Tseng IC, Chou FP, Chen YW, Wang JK, Johnson MD, Kataoka H, and Lin CY. Mechanisms for the control of matriptase activity in the absence of sufficient HAI-1. Am J Physiol Cell Physiol. 2012;302(2):C453-62.

[8] Inouye K, Tsuzuki S, Yasumoto M, Kojima K, Mochida S, and Fushiki T. Identification of the matriptase second CUB domain as the secondary site for interaction with hepatocyte growth factor activator inhibitor type-1. J Biol Chem. 2010; 285(43):33394-403.

[9] Lin CY, Anders J, Johnson M, Sang QA, and Dickson RB. Molecular cloning of cDNA for matriptase, a matrix-degrading serine protease with trypsin-like activity. J Biol Chem. 1999;274(26):18231-6.

[10] Yamasaki Y, Satomi S, Murai N, Tsuzuki S, and Fushiki T. Inhibition of membrane-type serine protease 1/matriptase by natural and synthetic protease inhibitors. J Nutr Sci Vitaminol (Tokyo). 2003;49(1):27-32.

[11] Oberst MD, Williams CA, Dickson RB, Johnson MD, and Lin CY. The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. J Biol Chem. 2003;278(29):26773-9.

[12] Cho EG, Kim MG, Kim C, Kim SR, Seong IS, Chung C, Schwartz RH, and Park D. N-terminal processing is essential for release of epithin, a mouse type II membrane serine protease. J Biol Chem. 2001;276(48):44581-9.

[13] Cho EG, Schwartz RH, and Kim MG. Shedding of membrane epithin is blocked without LDLRA4 and its protease activation site. Biochem Biophys Res Commun. 2005;327(1):328-34.

[14] Oberst MD, Chen LY, Kiyomiya K, Williams CA, Lee MS, Johnson MD, Dickson RB, and Lin CY. HAI-1 regulates activation and expression of matriptase, a membrane-bound serine protease. Am J Physiol Cell Physiol. 2005;289(2):C462-70.

[15] List K BT, Szabo R. Matriptase: potent proteolysis on the cell surface. Mol Med. 2006;(1-3)(12):1-7.

[16] Lin CY, Anders J, Johnson M, and Dickson RB. Purification and characterization of a complex containing matriptase and a Kunitz-type serine protease inhibitor from human milk. J Biol Chem. 1999;274(26):18237-42.

[17] Rawlings ND, Tolle DP, and Barrett AJ. Evolutionary families of peptidase inhibitors. Biochem J. 2004;378(Pt

3):705-16.
[18] Fan B, Brennan J, Grant D, Peale F, Rangell L, and Kirchhofer D. Hepatocyte growth factor activator inhibitor-1 (HAI-1) is essential for the integrity of basement membranes in the developing placental labyrinth. Dev Biol. 2007;303(1):222-30.
[19] List K, Szabo R, Molinolo A, Sriuranpong V, Redeye V, Murdock T, Burke B, Nielsen BS, Gutkind JS, and Bugge TH. Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev. 2005;19(16):1934-50.
[20] Parr C, Watkins G, Mansel RE, and Jiang WG. The hepatocyte growth factor regulatory factors in human breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2004.10(1 Pt 1):202-11.
[21] Oberst MD, Johnson MD, Dickson RB, Lin CY, Singh B, Stewart M, Williams A, al-Nafussi A, Smyth JF, Gabra H, et al. Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters. Clinical cancer research : an official journal of the American Association for Cancer Research. 2002;8(4):1101-7.
[22] Zeng L, Cao J, and Zhang X. Expression of serine protease SNC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 in normal and malignant tissues of gastrointestinal tract. World journal of gastroenterology : WJG. 2005;11(39):6202-7.
[23] Szabo R, Hobson JP, Christoph K, Kosa P, List K, and Bugge TH. Regulation of cell surface protease matriptase by HAI2 is essential for placental development, neural tube closure and embryonic survival in mice. Development. 2009;136(15):2653-63.
[24] Kataoka H, Itoh H, Uchino H, Hamasuna R, Kitamura N, Nabeshima K, and Koono M. Conserved expression of hepatocyte growth factor activator inhibitor type-2/placental bikunin in human colorectal carcinomas. Cancer letters. 2000;148(2):127-34.
[25] Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, and Craik CS. Cellular localization of membrane-type serine protease 1 and identification of protease-activated receptor-2 and single-chain urokinase-type plasminogen activator as substrates. J Biol Chem. 2000;275(34):26333-42.
[26] Lee SL, Dickson RB, and Lin CY. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. J Biol Chem. 2000;275(47):36720-5.
[27] Yamada K, Takabatake T, and Takeshima K. Isolation and characterization of three novel serine protease genes from Xenopus laevis. Gene. 2000;252(1-2):209-16.
[28] Khang I, Sonn S, Park JH, Rhee K, Park D, and Kim K. Expression of epithin in mouse preimplantation development: its functional role in compaction. Dev Biol. 2005;281(1):134-44.
[29] List K, Haudenschild CC, Szabo R, Chen W, Wahl SM, Swaim W, Engelholm LH, Behrendt N, and Bugge TH. Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. Oncogene. 2002;21(23):3765-79.
[30] Chen YW, Wang JK, Chou FP, Chen CY, Rorke EA, Chen LM, Chai KX, Eckert RL, Johnson MD, and Lin CY. Regulation of the matriptase-prostasin cell surface proteolytic cascade by hepatocyte growth factor activator inhibitor-1 during epidermal differentiation. J Biol Chem. 2010;285(41):31755-62.
[31] Szabo R, Molinolo A, List K, and Bugge TH. Matriptase inhibition by hepatocyte growth factor activator inhibitor-1 is essential for placental development. Oncogene. 2007;26(11):1546-56.
[32] Sebzda E, Mariathasan S, Ohteki T, Jones R, Bachmann MF, and Ohashi PS. Selection of the T cell repertoire. Annu Rev Immunol. 1999;17(829-74.
[33] Lee D, Lee HS, Yang SJ, Jeong H, Kim DY, Lee SD, Oh JW, Park D, and Kim MG. PRSS14/Epithin is induced in macrophages by the IFN-gamma/JAK/STAT pathway and mediates transendothelial migration. Biochem Biophys Res Commun. 2011;405(4):644-50.
[34] Fang JD, Chou HC, Tung HH, Huang PY, and Lee SL. Endogenous expression of matriptase in neural progenitor cells promotes cell migration and neuron differentiation. J Biol Chem. 2011;286(7):5667-79.
[35] Kendall SE, Najbauer J, Johnston HF, Metz MZ, Li S, Bowers M, Garcia E, Kim SU, Barish ME, Aboody KS, et al. Neural stem cell targeting of glioma is dependent on phosphoinositide 3-kinase signaling. Stem Cells. 2008;26(6):1575-86.
[36] Kim SB, Lee D, Jeong JW, Kim C, Park D, and Kim MG. Soluble epithin/PRSS14 secreted from cancer cells contains active angiogenic potential. Mol Cells. 2010;29(6):617-23.
[37] Kolsch V, Charest PG, and Firtel RA. The regulation of cell motility and chemotaxis by phospholipid signaling. J Cell Sci. 2008;121(Pt 5):551-9.
[38] Derynck R, and Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol. 2007; 9(9):1000-4.
[39] Lee HS, Kim C, Kim SB, Kim MG, and Park D. Epithin, a target of transforming growth factor-beta signaling, mediates epithelial-mesenchymal transition. Biochem Biophys Res Commun. 2010;395(4):553-9.
[40] Cheng H, Fukushima T, Takahashi N, Tanaka H, and Kataoka H. Hepatocyte growth factor activator inhibitor type 1 regulates epithelial to mesenchymal transition through membrane-bound serine proteinases. Cancer Res. 2009;69(5):1828-35.
[41] Parr C, and Jiang WG. Hepatocyte growth factor activation inhibitors (HAI-1 and HAI-2) regulate HGF-induced invasion of human breast cancer cells. Int J Cancer. 2006;119(5):1176-83.
[42] Kojima K, and Inouye K. Activation of matriptase zymogen. J Biochem. 2011;150(2):123-5.
[43] Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, and Bissell MJ. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol. 1997;139(7):1861-72.
[44] Orlichenko LS, and Radisky DC. Matrix metalloproteinases stimulate epithelial-mesenchymal transition during tumor development. Clin Exp Metastasis. 2008;25(6):593-600.
[45] Suzuki M, Kobayashi H, Kanayama N, Saga Y, Lin CY, Dickson RB, and Terao T. Inhibition of tumor invasion by genomic down-regulation of matriptase through suppression of activation of receptor-bound pro-urokinase. J Biol Chem. 2004;279(15):14899-908.
[46] Welman A, Sproul D, Mullen P, Muir M, Kinnaird AR, Harrison DJ, Faratian D, Brunton VG, and Frame MC. Diversity of matriptase expression level and function in breast

cancer. PLoS One. 2012;7(4):e34182.
[47] Wang Y, Rathinam R, Walch A, and Alahari SK. ST14 (suppression of tumorigenicity 14) gene is a target for miR-27b, and the inhibitory effect of ST14 on cell growth is independent of miR-27b regulation. J Biol Chem. 2009;284(34):23094-106.