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JB Commentary

Activation of matriptase zymogen

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Matriptase is a type II transmembrane serine protease expressed abundantly in the epithelial cells and keratinocyte. It plays a key role in the establishment and maintenance of epithelial integrity. Matriptase is considered to be at the most upstream in cellular protease cascade. Activation of its zymogen is the most critical step in regulation of downstream proteases activities and physiological functions. It has recently found that the exposure of matriptase-expressing epithelial cells and its homogenate to mildly acidic pH induces the rapid activation of matriptase zymogen. On the other hand, high ionic strength prevents this activation. The activation of the zymogen is thought to be triggered by the acidification and the lowering of ionic strength in cell-surface microenvironments.

Keywords: activation/HAI-1/inhibition/matriptase/type II transmembrane serine protease.

Abbreviations: HAI-1, hepatocyte growth factor activator inhibitor type-1; HGF, hepatocyte growth factor; S1P, sphingosine 1-phosphate; uPA, urokinase-type plasminogen activator.

Matriptase (also known as membrane-type serine protease 1 and epithin) is a member of type II transmembrane serine protease groups. Matriptase was first described in 1993 as a new gelatinolytic activity in cultured human breast cancer cells (*1*). Immunohistochemistry and *in situ* hybridization analyses revealed that matriptase is expressed in the epithelial component of almost all organs and occurs on the basolateral sides of normal epithelial cells (*2*, *3*). It cleaves a number of proteins, including single-chain urokinase-type plasminogen activator (uPA) and pro-hepatocyte growth factor (HGF) (*4*). Matriptase-deficient mice showed disorders in epidermal barrier formation, hair follicle development and thymic homeostasis (*5*). It has recently been reported that a recombinant rat matriptase causes detachment and apoptosis of small-intestinal epithelial IEC-6 cells *in vitro* (*6*). These findings suggest that matriptase plays a key role in the establishment and maintenance of epithelial integrity.

Matriptase is synthesized as a single-chain zymogen form that consists of catalytic and non-catalytic stem domains. The zymogen is cleaved at its canonical activation motif (between Arg614 and Val615) to generate disulfide-linked-two-chain fully active enzyme (Fig. 1A). Although activation of most serine protease zymogens is facilitated by other upstream proteases, that of matriptase zymogen appears to be initiated through the autoactivation mechanism in which the zymogen possessing the intrinsic weak proteolytic activity cleaves the activation motif of another one (*7*, *8*).

The proteolytic activity of matriptase is strictly regulated through the inhibition by a cognate Kunitz-type serine protease inhibitor, hepatocyte growth factor activator inhibitor type-1 (HAI-1). HAI-1 comprises multiple domains including two Kunitz domains. It inhibits matriptase by the interaction between Kunitz domain I and the catalytic site of matriptase (*9*, *10*). The non-catalytic domain of matriptase enhances the inhibition by HAI-1, and the secondary interaction site in the non-catalytic domain has recently been identified (*11*, *12*). The ratio in the amounts of matriptase and HAI-1 has been shown to increase in late-stage tumours (*13*). This imbalance might lead to the excessive activity of matriptase and would be significant for the development of advanced disease.

Paradoxically, HAI-1 is needed for autoactivation of matriptase zymogen, and a model for the lifecycle of matriptase is proposed (*14*, *15*). In most polarized epithelial cells, matriptase zymogen bio-synthesized is targeted to the basolateral plasma membrane and undergoes autoactivation to generate active matriptase, which is quickly inhibited by HAI-1 to form matiriptase-HAI-1 complex. The complex is either shed from the basal plasma membrane or internalized and trafficked to the apical plasma membrane. The complex on the apical membrane is also shed into the lumen by simultaneous proteolytic cleavages at both matriptase and HAI-1. This transcytosis mechanism is supported by the existence of matriptase-HAI-1 complex in human milk and urine (*16*). HAI-1 is considered to protect cells from the harmful activity of matriptase during the intracellular trafficking of active matriptase.

Sphingosine 1-phosphate (S1P), a major lipid mediator in serum, stimulates the transient activation of the zymogen on the surface of immortalized breast epithelial cells (*17*). Androgens, steroid sex hormone, play a key role in the regulation of the zymogen activation in hormone-starved lymph node prostatic adenocarcinoma LNCaP cells (*18*). Suramin, a sulfide-rich anionic small molecule, was found as a universal inducer of matriptase activation in various cell types (*19*).

It has been shown that the exposure of a variety of matriptase-expressing epithelial cells and its homogenate to mildly acidic pH (pH 5.2–6.8; most optimally at

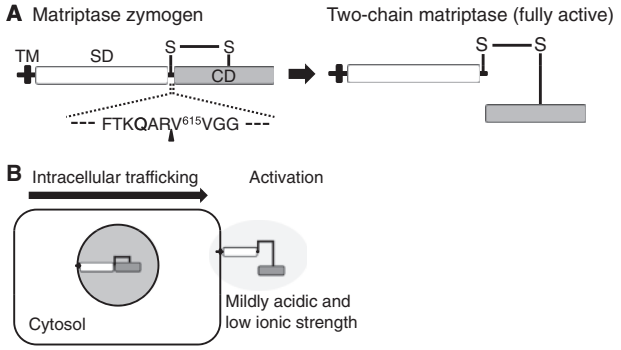

Fig. 1 The activation of matriptase zymogen is considered to occur within cell-surface microenvironments with low ionic strength and mild acidity. Schematic illustrations of the structure of matriptase (A) and activation of matriptase zymogen on the cell surface (B). The activation cleavage site is indicated by arrowhead. TM, transmembrane domain; SD, stem domain; CD, catalytic domain.

pH 6.0) induces rapid activation of matriptase zymogen (*20*). The activity of recombinant matriptase zymogen was observed at pH 5.0–7.0 using a peptide substrate, and the optimal pH was determined to be pH 6.0 (*21*). As the concentration of NaCl increases, the activity decreases and is almost diminished above 75 mM NaCl at pH 6.0. From these lines of evidence, the activation of the zymogen is considered to be triggered by the acidification and the lowering of ionic strength within cell-surface microenvironments. After activation, the optimal pH of matriptase shifts to pH 9 from that (pH 6.0–6.5) given with matriptase pseudozymogen in the hydrolysis of the synthetic substrate employed. Although the pseudozymogen shows no activity towards physiological substrates such as single-chain uPA and proHGF, after activation into mature matriptase, it has been changed to exhibit efficient processing activities towards the physiological substrates at pH 8.5 (*4*, *22*). If it is reasonably assumed that the active site of matriptase was structured similarly to that of trypsin, that the optimal pH should be given primarily by the pK_a value of the catalytic His residue at the active site, but the pK_a value might be influenced by the ionic conditions around the His residue and also by the ionic states of the substrates. The molecular mechanism of the activation of matriptase zymogen and that for the remarkable shift of the optimal pH by the activation as well could be revealed by future studies, which would provide us with the information for regulating the matriptase-related physiological phenomena.

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