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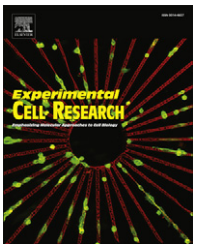

Research Article

Hepatocyte growth factor activator inhibitor-2 prevents shedding of matriptase

Brian R. Larsen[a,1], *Simon D.R. Steffensen*[a,1], *Nis V.L. Nielsen*[b], *Stine Friis*[a], *Sine Godiksen*[a,c], *Jette Bornholdt*[a,c], *Christoffer Soendergaard*[a], *Annika W. Nonboe*[a], *Martin N. Andersen*[d], *Steen S. Poulsen*[d], *Roman Szabo*[e], *Thomas H. Bugge*[e], *Chen-Yong Lin*[f], *Hanne Skovbjerg*[a,g], *Jan K. Jensen*[b], *Lotte K. Vogel*[a,*]

[a]*Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark*

[b]*Danish–Chinese Centre for Proteases and Cancer, Department of Molecular Biology and Genetics, Faculty of Science, Aarhus University, Gustav Wieds vej 10, DK-8000 Aarhus C, Denmark*

[c]*Department of Biology, Faculty of Science, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark*

[d]*Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark*

[e]*Proteases and Tissue Remodeling Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA*

[f]*Department of Biochemistry and Molecular Biology, Greenebaum Cancer Centre, University of Maryland, Baltimore, MD 21201, USA*

[g]*Medical Department, Amager Hospital, DK-2300, Copenhagen S, Denmark*

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ABSTRACT

Hepatocyte growth factor activator inhibitor-2 (HAI-2) is an inhibitor of many proteases *in vitro*, including the membrane-bound serine protease, matriptase. Studies of knock-out mice have shown that HAI-2 is essential for placental development only in mice expressing matriptase, suggesting that HAI-2 is important for regulation of matriptase. Previous studies have shown that recombinant expression of matriptase was unsuccessful unless co-expressed with another HAI, HAI-1. In the present study we show that when human matriptase is recombinantly expressed alone in the canine cell line MDCK, then human matriptase mRNA can be detected and the human matriptase ectodomain is shed to the media, suggesting that matriptase expressed alone is rapidly transported through the secretory pathway and shed. Whereas matriptase expressed together with HAI-1 or HAI-2 accumulates on the plasma membrane where it is activated, as judged by cleavage at Arg614 and increased peptidolytic activity of the cell extracts. Mutagenesis of Kunitz domain 1 but not Kunitz domain 2 abolished this function of HAI-2. HAI-2 seems to carry out its function intracellularly as this is where the vast majority of HAI-2 is located and since HAI-2 could not be detected on the basolateral plasma membrane

Abbreviations: CHO, Chinese hamster ovary; ECL®, enhanced chemiluminescence; ER, endoplasmic reticulum; EYFP, enhanced yellow fluorescent protein; FT, flow through fraction; HAI-1, hepatocyte growth factor activator inhibitor-1; HAI-2, hepatocyte growth factor activator inhibitor-2; MDCK, Madin-Darby canine kidney; RT, room temperature

*Correspondence to: Lotte K. Vogel, Ph.D., Blegdamsvej 3, Bldg. 18.4, 2200 Copenhagen N, Denmark, +45 35 32 74 46. Fax: +45 35 36 79 80.
E-mail address: vogel@sund.ku.dk (L.K. Vogel).

[1] These authors contributed equally.

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where matriptase resides. However, minor amounts of HAI-2 not undergoing endocytosis could be detected on the apical plasma membrane. Our results suggest that Kunitz domain 1 of HAI-2 cause matriptase to accumulate in a membrane-bound form on the basolateral plasma membrane.

Introduction

Hepatocyte growth factor activator inhibitor-2 (HAI-2), also known as placental bikunin [1,2], has emerged as a potential inhibitor of the type II transmembrane serine protease, matriptase [3,4]. HAI-2 is encoded by the *SPINT2* gene and its function is essential for life as HAI-2 knock-out mice die during mid-gestation with signs of dysfunctional placental development and missing neural tube closure [5]. HAI-2 and matriptase double knock-out mice survive until birth with normal placental development showing that HAI-2 is unnecessary for placental development if matriptase is absent [3]. In contrast, the neural tube closure defects seem to be the result of lack of a different function of HAI-2, most likely inhibition of an unknown protease, as matriptase and HAI-2 double knock-out mice still carry this feature [3]. A recent publication clearly shows that matriptase and the serine protease, prostasin, are both components of a proteolytic cascade regulated by HAI-1 and HAI-2 important for development of the placenta [6].

Humans homozygous or compound heterozygous for an assortment of *SPINT2* autosomal recessive mutations, suffer from congenital sodium diarrhea [7]. These individuals also display a broad range of developmental abnormalities that include duplication of internal organs, duplication and abnormal location of digits, craniofacial dysmorphisms, anal and choanal atresia, fistulas and hamartoma [7]. Together this suggests that HAI-2 plays an important role during tissue morphogenesis.

Matriptase plays a crucial role in maintaining functional epithelial barriers as matriptase knock-out mice have lethal epithelial barrier defects of the skin [8] and conditional knock-out of the protease in adult mice leads to lethal barrier defects in the intestine [9]. In humans, dysfunctional matriptase has been linked to an autosomal recessive form of ichthyosis characterized by a missense mutation (G827R) in the catalytic domain of matriptase [10]. Matriptase has also been coupled to cancer development as transgenic mice with a modest overexpression of the protease in the skin all developed dysplasia of which 70% progressed into carcinomas, whereas control littermates were unaffected [11].

The enzymatic activity of matriptase is not controlled by HAI-2 alone, as hepatocyte growth factor activator inhibitor-1 (HAI-1) encoded by the *SPINT1* gene, also acts as an inhibitor of matriptase [11–14]. In the above mentioned study of matriptase-overexpressing mice, co-expression of matriptase together with HAI-1 completely abolished tumor formation suggesting that the enzymatic activity of matriptase can cause cancer [11]. Lack of HAI-1 is also lethal as the knock-out mice die during gestation [13].

Both HAI-2 and HAI-1 are type I transmembrane serine protease inhibitors belonging to the Kunitz family with a short intracellular tail and a larger extracellular part containing two inhibitory Kunitz domains (KD). HAI-2 consists of 252 amino acids adding up to a calculated molecular weight of approximately 28 kDa containing two potential N-glycosylation sites [1,2].

Matriptase, HAI-2 and HAI-1 are expressed in most epithelial cells [4,15–17]. Under normal physiological conditions many epithelial cells maintain a polarized state, in which the plasma membrane of differentiated cells is divided into an apical and a basolateral domain separated by tight-junctions, controlling paracellular flow and preventing diffusion of outer leaflet proteins between the apical and the basolateral plasma membrane domains. Matriptase is synthesized as a single-chain inactive protein, which is initially cleaved at Gly149 in its SEA domain [18]. The N-terminal fragment (1–149) remains non-covalently associated with the rest of the protein but dissociates away during western blotting sample preparation. Matriptase is subsequently activated by a proteolytic cleavage following Arg614 in the linker to the C-terminal serine protease domain. The serine protease domain remains covalently linked to the rest of the protein by a disulfide bridge [18]. Matriptase can be detected as a 70 kDa form under non-reducing conditions by western blotting. This is referred to as total matriptase, and represents both the zymogen and fully activated matriptase that are indiscernible under these conditions. However, under reducing conditions, the catalytic domain of activated matriptase becomes detectable on western blots as a separate 30 kDa band.

It is at present unclear where and how HAI-2 interacts with matriptase. In contrast matriptase and HAI-1 form a complex on the plasma membrane after activation of matriptase has taken place [19,20]. HAI-1 thus acts as a bonafide inhibitor of matriptase. However, the role of HAI-1 is not fully understood as it appears that matriptase cannot be recombinantly expressed in the absence of co-expression with HAI-1 [21,22]. In the present study we have investigated the subcellular localization and function of HAI-2. HAI-2 is smaller and less complex than HAI-1 and may therefore shed light on the function of HAIs during expression of matriptase. Our results suggest that matriptase recombinantly expressed without HAI-2 or HAI-1 is rapidly transported through the secretory pathway and shed, whereas matriptase expressed together with HAI-2 accumulate on the plasma membrane as a result of a process dependent on Kunitz domain 1 of HAI-2. Matriptase accumulated at the plasma membrane is activated, as judged by peptidolytic activity of the cell lysate and cleavage at Arg614.

Materials and methods

Cell culture: The human colon epithelial cell line Caco-2 was grown in minimal essential medium supplemented with 2 mM l-glutamine, 10% fetal bovine serum, 1 × non-essential amino acids, 100 units/ml penicillin and 100 μg/ml streptomycin at