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Short Communication

Loss of Matriptase Suppression Underlies *Spint1* Mutation-Associated Ichthyosis and Postnatal Lethality

Roman Szabo,* Peter Kosa,* Karin List,† and Thomas H. Bugge*

From the Proteases and Tissue Remodeling Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland; and the Department of Pharmacology,† Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan*

Hepatocyte growth factor activator inhibitor-1 (HAI)-1 is an epithelial Kunitz-type transmembrane serine protease inhibitor that is encoded by the *SPINT1* gene. HAI-1 displays potent inhibitory activity toward a large number of trypsin-like serine proteases. HAI-1 was recently shown to play an essential role in postnatal epithelial homeostasis. Thus, *Spint1*-deficient mice were found to display severe growth retardation and are unable to survive beyond postnatal day 16. The mice present histologically with overt hyperkeratosis of the forestomach, hyperkeratosis and acanthosis of the epidermis, and hypotrichosis associated with abnormal cuticle development. In this study, we show that loss of inhibition of a proteolytic pathway that is dependent on the type II transmembrane serine protease, matriptase, underlies the detrimental effects of postnatal *Spint1* deficiency. Matriptase and HAI-1 precisely co-localize in all tissues that are affected by the *Spint1* disruption. *Spint1*-deficient mice that have low matriptase levels, caused by a hypomorphic mutation in the *St14* gene that encodes matriptase, not only survived the neonatal period but were healthy and displayed normal long-term survival. Furthermore, a detailed histological analysis of neonatal, young adult, as well as aged mice did not reveal any abnormalities in *Spint1*-deficent mice that have low matriptase levels. This study identifies matriptase suppression as an essential function of HAI-1 in postnatal tissue homeostasis. *(Am J Pathol 2009, 174:2015–2022; DOI: 10.2353/ajpath.2009.090053)*

Trypsin-like serine proteases constitute a large family of secreted and membrane-bound proteolytic enzymes that operate in the pericellular environment to facilitate embryonic development tissue homeostasis, tissue remodeling, and tissue repair. These enzymes are synthesized as inactive precursors, or zymogens, that are converted to their catalytically active form by endoproteolytic cleavage within a highly conserved activation site. This activation step is irreversible, and proteolysis is terminated by specific macromolecular protease inhibitors that bind directly to the active site of their cognate proteases.[1–3] Inhibition of trypsin-like serine proteases is mediated by a large number of serine protease inhibitors belonging to one of three structurally and functionally distinct families: serpin-type inhibitors, Kazal-type inhibitors, and Kunitz-type inhibitors.[1–3] Hepatocyte growth factor activator-1 (HAI-1) is a recently discovered membrane-associated Kunitz-type serine protease inhibitor. This peculiar serine protease inhibitor, which is structurally distinct from other previously described Kunitz-type inhibitors, is a type I transmembrane glycoprotein that consists of an N-terminal MANEC-domain followed by two extracellular Kunitz-type inhibitory domains that are separated by a single low-density lipoprotein receptor type a repeat, and a C-terminal transmembrane/cytoplasmic tail domain.[4–8] HAI-1 is widely expressed in embryonic and extra-embryonic epithelia of the developing embryo, as well as in simple, stratified and pseudo-stratified epithelia of the adult organs of humans and mice.[9–13] HAI-1 was originally identified as the cognate inhibitor of the trypsin-like serine protease, hepatocyte growth factor activator. However, the isolated Kunitz-type inhibitor domains of HAI-1 were subsequently shown to display potent inhibitory activity toward a large number of other trypsin-like serine proteases when analyzed *in vitro*, suggesting that HAI-1

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Address reprint requests to Thomas H. Bugge, Ph.D., Proteases and Tissue Remodeling Section, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive, Room 211, Bethesda, MD 20892. E-mail: thomas.bugge@nih.gov.

could have multiple inhibitory targets and be a key player in many physiological processes.[14–20]

The widespread epithelial expression of HAI-1 in embryonic and adult epithelial tissues would also predict an essential function of the transmembrane serine protease inhibitor in mammalian development and adult tissue homeostasis. Indeed, inactivation of the *Spint1* gene encoding HAI-1 in mice resulted in embryonic lethality at mid-gestation due to placental failure caused by agenesis of the placental labyrinth.[13,21,22] This uniform lethality of *Spint1* null embryos at mid-gestation has complicated the analysis of the function of HAI-1 in adult tissue homeostasis. However, a recent elegant study by Nagaike and co-workers bypassed the embryonic lethality imposed by the placental requirement for HAI-1 by injection of *Spint1* null embryonic stem cells into wild-type blastocysts, leading to the generation of chimeric mice with high-grade deletion of *Spint1* in the embryo proper, but sufficient placental *Spint1* to support development to term.[23] These high-grade *Spint1*$^{-/-}$ chimeric mice were unremarkable at birth. However, they uniformly suffered from severe growth retardation and were incapable of surviving beyond postnatal day 16. Histopathological examination revealed that *Spint1*$^{-/-}$ mice displayed a range of abnormalities of keratinized epithelium, including hyperplasia of the forestomach, severe ichthyosis of the skin with inflammatory cell infiltration, and abnormal hair development. However, the molecular basis for these dramatic manifestations of HAI-1 ablation was not determined, and several possible explanations were proposed by the authors of the study.[23]

Matriptase (also known as MT-SP1, epithin, and TADG15) is a modular trypsin-like serine protease encoded by the *ST14* gene that belongs to the recently established type II transmembrane serine protease family.[24–30] Like HAI-1, matriptase is widely expressed in both embryonic and adult epithelia of mice and humans.[9–11,13,22,31] Homozygosity for mutations in the *ST14* gene was recently shown to be the etiological origin of two human syndromes termed congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis and autosomal recessive ichthyosis with hypotrichosis.[32–36] Mice with wholesale deletion of the *St14* gene complete embryonic development, but they die shortly after birth as a consequence of compromised epidermal barrier function.[37,38] However, an *St14* hypomorphic mouse strain, generated by insertion of a retroviral targeting vector into intron 1 of the *St14* gene, which displays 1% residual matriptase mRNA levels in the epidermis and up to 18% residual mRNA levels in other tissues, has normal postnatal and long-term survival, revealing that low matriptase suffices to maintain epithelial homeostasis.[39]

In the present study, we hypothesized that the detrimental effects of postnatal loss of HAI-1 were caused by excess matriptase activity. Indeed, using a genetic approach, we demonstrate in this study that the requirement of HAI-1 for postnatal mouse development and epithelial homeostasis is mechanistically linked to the inhibition of matriptase. Thus, whereas *Spint1*$^{-/-}$ mice with one or two wild-type *St14* alleles died *in utero*, *Spint1*$^{-/-}$ mice on the *St14* hypomorphic mouse background not only completed embryonic development, but were viable, healthy, and displayed normal long-term survival. This study identifies matriptase as a critical inhibitory target for HAI-1 in keratinized squamous epithelium, shows that HAI-1 inhibition of protease targets besides matriptase is not essential for mouse health and long-term survival, and reveals that a delicate balance between matriptase and HAI-1 maintains homeostasis of adult mammalian tissues.

Materials and Methods

Mice and Tissue Acquisition

All procedures involving live animals were performed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited vivarium, following Institutional Guidelines and standard operating procedures. The generation and genotyping of the *Spint1* null mice and β-galactosidase-tagged *St14* hypomorphic mice have been described.[13,39,40] Histological analysis, immunohistochemistry and whole mount X-gal staining was performed exactly as described.[10,13,31,38,40]

Blood Chemistry Analysis

To isolate serum, blood was collected from the periportal vein of freshly euthanized animals, clotted for 2 hours at room temperature, and centrifuged at 800 × *g* for 10 minutes at room temperature. The clear upper phase was transferred into a new tube and immediately used for the analysis or stored at −80°C. The biochemical analysis of mouse sera from at least three animals of each genotype and sex was performed by the Department of Laboratory Medicine at the National Institutes of Health Clinical Center (Bethesda, MD).

Intestinal Epithelial Permeability

Ten μl per gram of body weight of 22 mg/ml fluorescein isothiocyanate (FITC)-labeled dextran (average 4,000 g/mol, Sigma, St. Louis, MO) in PBS was injected directly into the stomach of 6 month-old animals using a 1.5-inch long, bulb-tipped gastric gavage needle (Roboz, Gaithersburg, MD) attached to a 1-ml syringe. After four h, the animals were euthanized by CO_2 inhalation, blood was extracted, and serum was prepared as described above. Fifty μl of serum was then diluted 1:3 in 1× PBS (Gibco-Invitrogen, Carlsbad, CA) and the concentration of FITC-dextran was determined by reading the fluorescence at 535 nm after excitation at 485 nm using a Victor3V spectrophotometer (PerkinElmer, Waltham, MA).

Detection of Matriptase Protein in Mouse Skin

Whole skins from newborn mice were homogenized in glass homogenizers in ice-cold RIPA buffer (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 1% NP-40, 0.1% SDS)