Cell Tissue Res (2013) 351:245–253
DOI 10.1007/s00441-012-1348-1

REVIEW

The matriptase-prostasin proteolytic cascade in epithelial development and pathology

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Received: 9 November 2011 / Accepted: 20 January 2012 / Published online: 18 February 2012

Abstract The type II transmembrane serine protease matriptase has an essential role in the integrity and function of multiple epithelial tissues. In the epidermis, matriptase activates the glycosylphosphatidylinositol (GPI) anchored membrane serine protease prostasin to initiate a proteolytic cascade that is required for the development of the stratum corneum barrier function. Accordingly, mice deficient for matriptase phenocopy mice deficient for epidermal prostasin and present with impaired corneocyte differentiation, imparied lipid matrix formation, loss of profilaggrin processing and loss of tight junction formation and function. Together, these defects lead to a compromised epidermal barrier and result in fatal dehydration during the neonatal period. Proteolytic activity of the matriptase-prostasin cascade is regulated in the epidermis via inhibition by the Kunitz-type serine protease inhibitor hepatocyte growth factor activator inhibitor-1 (HAI-1). Importantly, targeted postnatal ablation of matriptase in mice perturbs the function of multiple adult tissues, indicating an ongoing requirement for matriptase proteolysis in the maintenance of diverse types of epithelia. Impaired matriptase proteolytic activity has been linked to human Autosomal Recessive Icthyosis with Hypotrichosis (ARIH), whereas aberrant matriptase activity has been implicated in Netherton's Syndrome. This review will summarize information pertaining to the role of matriptase in epithelial biology and will discuss recent advancements in our understanding of how matriptase activity is regulated and the down-stream effectors of matriptase proteolysis.

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Keywords Matriptase · Prostasin · Epithelial barrier · Extracellular proteolysis

Matriptase is a type II transmembrane serine protease

Serine proteases represent a significant portion of the mammalian degradome, encoding over 175 predicted proteins within the human genome (Puente et al. 2005). The vast majority of these proteases are secreted; however, a burgeoning sub-class has recently been characterized that localizes to the plasma membrane, indicating a potential role in cell surface and pericellular proteolytic signaling. These membrane serine proteases are attached to the cell membrane via three different mechanisms; (1) a carboxy terminal GPI anchor, (2) a carboxy-terminal transmembrane domain (Type I), or (3) an amino terminal transmembrane domain (Type II transmembrane serine proteases, or TTSPs). There are 17 TTSPs conserved between mice and humans, all containing a serine protease domain with a highly conserved chymotrypsin (S1) fold that is found in the majority of secreted serine proteases (Di Cera 2009; Hooper et al. 2001). TTSPs are synthesized as inactive single chain zymogens and are subsequently cleaved into a two-chain active enzyme during a process that allows the protease domain to remain tethered to the cell surface by a disulfide bridge (Bugge et al. 2009). The serine protease domain of TTSPs is located at the C-terminus and all contains a variable "stem region" between the N-terminal transmembrane domain and the protease domain that is critical to the demarcation of four subfamilies: the HAT/DESC subfamily, Hepsin/TMPRSS subfamily, Corin subfamily and matriptase subfamily (Bugge et al. 2009; Hooper et al. 2001).

Matriptase is a member of the eponymous matriptase subfamily, which includes matriptase 1–3 and polyserase-1. Following from the transmembrane domain, the stem

region of matriptase comprises a single SEA (sea urchin sperm protein, enteropeptidase, agrin) domain, two CUB (Cls/Clr urchin embryonic growth factor, bone morphogenic protein-1) domains and four LDLA (low density lipoprotein receptor class A) domains. At the C-terminal end of the stem region is an essential activation motif conserved in all TTSPs where proteolytic cleavage activates the serine protease domain. The unique stem region of individual TTSPs is critical for the regulation of expression, activation and interactions with substrates and inhibitors (Szabo and Bugge 2008; Szabo et al. 2003). Among TTSPs, matriptase proteolytic activity is relatively discriminating in vitro, perhaps owing to structural motifs in the stem region and a protease domain that confer substrate specificity (Beliveau et al. 2009). Matriptase expression is found in a variety of epithelial tissues, where it has been shown to have pleiotropic effects on development, cell–cell adhesions and homeostasis.

In nearly all murine epithelial tissues, matriptase is co-expressed with the membrane serine protease prostasin, one of only two known mammalian proteins attached to the membrane via a GPI-anchor (Chen et al. 2001a; Hooper et al. 1999; List et al. 2007b). Prostasin is also termed channel-activating protease 1 (CAP-1), following from its ability to activate epithelial sodium channels (ENaCs) and effect the sodium current across the plasma membrane in vitro (Adachi et al. 2001; Tong et al. 2004; Vallet et al. 1997; Vuagniaux et al. 2002). Prostasin contains an N-terminal secretion signal that is cleaved during intracellular transport in the ER and a GPI-anchor is attached at the C-terminal (Chen et al. 2001b; Yu et al. 1994, 1995). From the N-terminus, prostasin comprises a pro-domain and a serine protease domain with trypsin-like activity. Subsequent to surface localization, prostasin is cleaved at a conserved activation site in the pro-domain and remains attached to the serine protease domain via a disulfide bridge (Chen et al. 2001b). Unlike matriptase (see below), prostasin zymogen is incapable of auto-activation and thus requires proteolytic processing by a second protease for conversion into an active protease (Shipway et al. 2004).

Matriptase activation

In order to become catalytically active, matriptase must undergo two sequential proteolytic processing events that occur near the termini of the stem region. The first cleavage occurs after Gly149 within a conserved GSVIA motif in the SEA domain and may occur spontaneously during intracellular transport as the result of conformation-induced hydrolysis (Kim et al. 2005; Oberst et al. 2003). While this severs the link between the subsequent peptide and the signal anchor, matriptase remains attached to the cell surface, possibly through non-covalent interactions within the protein itself or through complex formation with the Kunitz-type serine protease inhibitor HAI-1 (Oberst et al. 2005, 2003). The second cleavage occurs after Arg614 within the highly conserved RVVGG activation motif and requires both the initial SEA domain cleavage event and the catalytic amino acids of the matriptase serine protease domain (Oberst et al. 2003). Matriptase undergoes rapid auto-activation in vitro, which is believed to occur through trans-activation following oligomerization, a process that requires the presence of a biomembrane (Lee et al. 2007; Oberst et al. 2003; Takeuchi et al. 2000).

Interestingly, matriptase activation appears to require physical interaction with its related inhibitor, HAI-1, which may serve to protect against aberrant matriptase proteolysis. In the absence of HAI-1, nascent matriptase constructs are sequestered in the Golgi in cell culture models, while deletion or mutation of the LDLA domain of HAI-1 results in both a reduction in surface matriptase expression and abolishment of matriptase activity (Oberst et al. 2005, 2003). Consistent with a role in matriptase activation, HAI-1 co-localizes with matriptase in a variety of epithelial tissues and is found in a complex with the protease in instances where matriptase is released from the cell surface (Lin et al. 1999; Szabo et al. 2007; Wang et al. 2009). The fact that HAI-1 is an extremely effective inhibitor of matriptase in vitro, has a high affinity for matriptase binding and is typically co-expressed with matriptase in vivo has led to the hypothesis that matriptase is only active for a brief interval at the cell surface prior to its inactivation by HAI-1 (Chen et al. 2010b; Tseng et al. 2010). Matriptase can be activated in cell culture systems through the addition of reactive oxygen species (ROS) or through subtle acidification of the extracellular pH (Chen et al. 2010a; Tseng et al. 2010). The ability of matriptase to become activated at an acidic pH may have physiological relevance, as the epidermal matrix becomes acidic at the transitional layer between the stratum granulosum and stratum corneum where matriptase is active (see below). In vitro substrates of matriptase proteolysis include urokinase-type plasminogen activator (uPA), the G-protein-coupled protease activated receptor-2 (PAR2), platelet-derived growth factor-D (PDGF-D) and human acid-sensing ion channel 1, while known in vivo substrates include hepatocyte growth factor (HGF) and the serine protease prostasin (Clark et al. 2010; Lee et al. 2000; Netzel-Arnett et al. 2006; Szabo et al. 2011; Takeuchi et al. 2000, Ustach et al. 2010).

Matriptase in epithelial biology

The crucial function of the epidermis is to form a protective barrier between underlying tissue and the external

environment. The epidermal barrier provides a bulwark against environmental toxins and pathogens, while concurrently preventing the loss of vital fluids and dissipation of thermal energy. Critical to barrier function is the formation of a water impermeable network of dead cells imbedded within a lipid-enriched extracellular matrix that comprises the outer layer of the skin. In order to form this layer (the stratum corneum, or cornified layer), keratinocytes proliferate from the basal lamina and undergo several differentiation events while traversing through the strata that constitute the living epidermis (Ovaere et al. 2009) (Fig. 1a). At the periphery of these strata, a transitional layer between the stratum granulosum and stratum corneum separates the living epidermis from the non-living epidermis and is the location where keratinocytes undergo terminal differentiation into corneocytes. Corneocyte differentiation is a specialized form of cell death that results in the loss of cellular organelles, morphological flattening and the formation of a dense complex of keratin filaments that is surrounded by a specialized cornified envelope. As corneocytes are connected by corneodesmosome adhesions and become embedded in the lipid matrix of the stratum corneum, epidermal barrier function is conferred. In the transitional layer, matriptase initiates a proteolytic cascade that is required for normal corneocyte differentiation, essential to the integrity of the epidermal barrier and obligatory to post-natal survival.

Evidence implicating matriptase in epidermal development was garnered from transgenic mice containing a null mutation lacking all but a small fragment of the matriptase protein (List et al. 2002, 2003). Matriptase-deficient mice present with a variety of epidermal defects; including a generalized disruption of the stratum corneum architecture, loss of vesicular bodies that generate intercorneocyte lipids and hypoplasia and dysgenesis of hair follicles (List et al. 2002, 2003). Importantly, these defects result in compromised epidermal

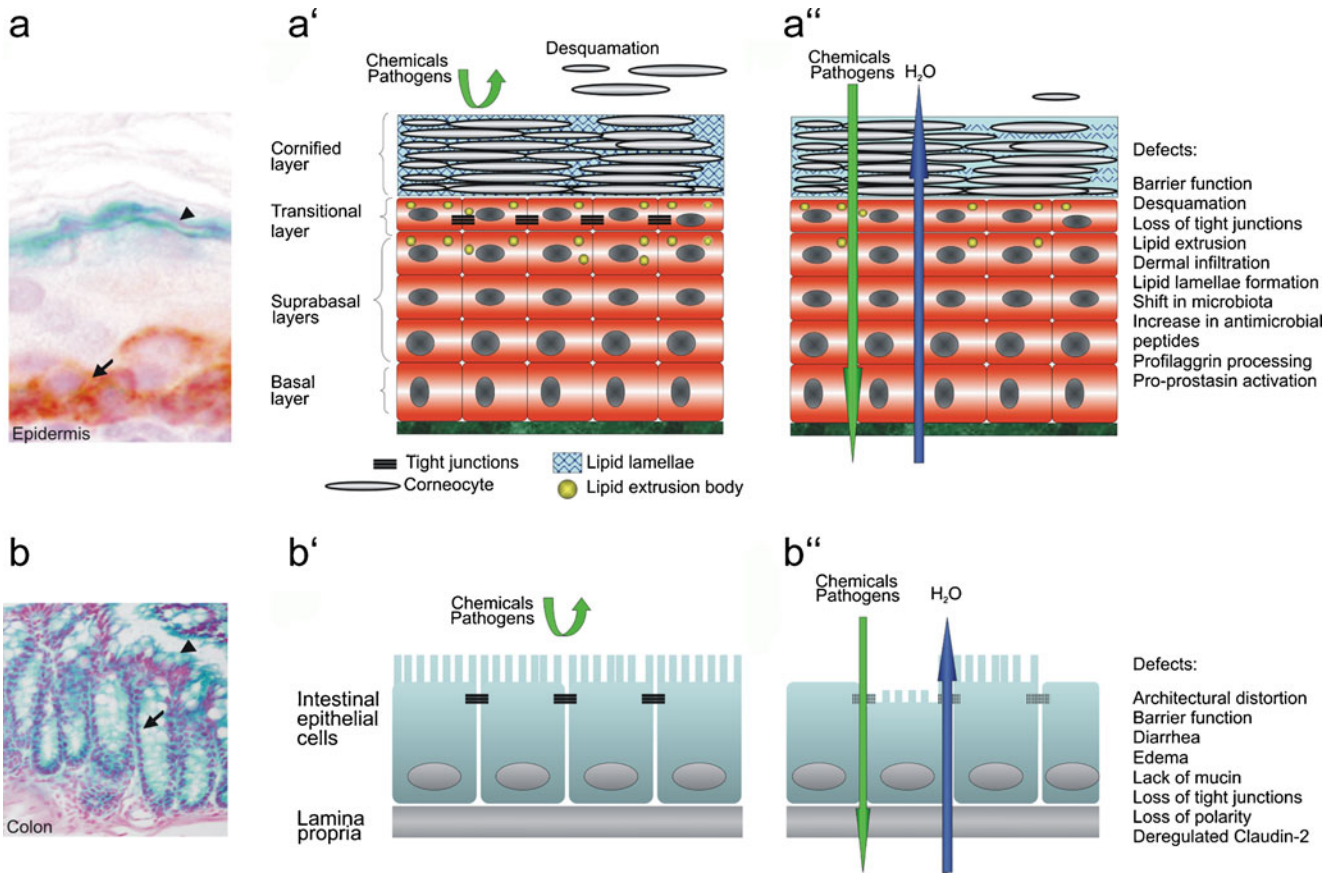

Fig. 1 To visualize the localization of endogenous matriptase (*blue color*), a knock-in mouse with a promoterless β-galactosidase marker gene inserted into the endogenous matriptase gene was used (List et al. 2006). **a** In the epidermis, matriptase is expressed in the transitional layer (*arrowhead*). The basal layer was visualized using a keratin-5 antibody (*red color*, *arrow*). Deficiency in epidermal matriptase or its target prostasin results in a plethora of defects (compare **a‘** to **a“**), which together result in impaired barrier function. These defects include the loss of tight junctions, lipid extrusion and impaired processing of profilaggrin. **b** In the intestine, matriptase is expressed in goblet cells (*arrow*) and in surface mucosal cells (*arrowhead*). Continual matriptase expression is required for tissue maintenance and results in defects in tight junctions and cell polarity (compare **b‘** to **b“**). Ablation of matriptase in the large intestine causes architectural distortion and compromised barrier function resulting in edema and diarrhea and causing premature death

barrier function that leads to fatal dehydration within 48 h of birth. Matriptase hypomorphic mice containing approximately 1% matriptase mRNA in the epidermis have a shift in the phylogeny of bacterial colonization, an increase in antimicrobial peptides and enhanced transepidermal water loss, indicating that both *outward* and *inward* barrier function require matriptase activity (List et al. 2002, 2007a; Scharschmidt et al. 2009). Unlike matriptase null animals, however, these animals are able to survive through the neonatal period, ostensibly owing to less severe impairment of the epidermal barrier function (List et al. 2007a). Mice deficient in epidermal prostasin display defects that are identical to matriptase-deficient mice, suggesting that the two proteases reside in the same proteolytic pathway (Leyvraz et al. 2005). In support of this hypothesis, enzymatic gene trapping of matriptase combined with immunohistochemistry of prostasin revealed that the two proteases co-localize in the transitional layer of the epidermis, where keratinocytes undergo terminal differentiation (List et al. 2007b; Netzel-Arnett et al. 2006). Furthermore, the developmental onset of expression of matriptase and prostasin is synchronized and correlates with the acquisition of epidermal barrier function during embryonic development in mice (Netzel-Arnett et al. 2006). Importantly, matriptase is an efficient activator of prostasin zymogen in vitro and epidermal tissue from matriptase deficient mice does not contain the activated prostasin found in wild-type epidermis (Netzel-Arnett et al. 2006). Taken together, this indicates that matriptase activates prostasin in vivo and may act as the initiator of a proteolytic cascade in the epidermis, following from the ability of matriptase to autoactivate.

Precise spatial and temporal co-expression of matriptase and prostasin would appear to be a prerequisite for proteolytic cross-talk, as the window for matriptase activity is considerably diminished by the inhibitor HAI-1. Indeed, HAI-1 is capable of rapidly inactivating both matriptase and prostasin and upon experimental induction of matriptase activity, complexes of matriptase and HAI-1, as well as prostasin and HAI-1, are readily detected in a skin organotypic model (Chen et al. 2010b). Recent work has established that, in polarized epithelium, matriptase and prostasin co-localize briefly at the basolateral plasma membrane prior to HAI-1-mediated matriptase endocytosis (Friis et al. 2011). Basolateral prostasin is also endocytosed, however, not before being activated by matriptase and is subsequently transcytosed to the apical plasma membrane where it accumulates (Friis et al. 2011). Thus, complex mechanisms involving matriptase, prostasin, HAI-1 and intracellular transport may all be involved in regulating the matriptase-prostasin proteolytic cascade.

One effect of matriptase or prostasin deficiency in the epidermis is the loss of proteolytically processed filaggrin, indicating that this proprotein may be a downstream target of the matriptase-prostasin cascade (List et al. 2003). Processing of profilaggrin into mature filaggrin monomers and secondary products is a critical component of corneocyte differentiation and aids keratin aggregation and cornified envelope formation and may affect gene transcription that regulates stratum corneum development (Dale et al. 1978; Pearton et al. 2002; Steinert et al. 1994; Zhang et al. 2002). Loss of function mutations in the human filaggrin gene are associated with ichthyotic skin phenotypes similar to those observed under conditions of matriptase deficiency and filaggrin siRNA interferes with barrier function in a human skin organotypic model (Mildner et al. 2010; Smith et al. 2006). The exact mechanism by which matriptase/prostasin proteolytic activity regulates profillagrin processing remains to be elucidated; however, it may involve ion transport. Prostasin is a proteolytic activator of epithelial sodium channels (ENaCs) in several experimental systems, which regulate sodium current in epithelial cells (Vuagniaux et al. 2000, 2002; Bruns et al. 2007). Following proteolytic processing by the proprotein convertase furin, ENaCs are transferred to the plasma membrane, where further processing by prostasin may result in a high probability open configuration (Bruns et al. 2007; Ovaere et al. 2009). Open ENaC conformation is associated with Na^+ influx that results in membrane depolarization, causing an influx of calcium ions through voltage-gated calcium channels that are necessary for profilaggrin processing in the cytoplasm (Markova et al. 1993; Resing et al. 1993). When prostasin is inactive, a reduction in Na^+ conductance causes hyperpolarization of the plasma membrane that impairs the opening of voltage-gated calcium channels (Vuagniaux et al. 2002). Thus, matriptase activation of prostasin may cause a downstream Ca^{2+} influx resulting in profillagrin processing and contributing to corneocyte differentiation and stratum corneum formation.

In addition to the absence of profilaggrin processing, both matriptase- and prostasin-deficient mice display defects in the formation and function of epithelial tight junctions. Immunohistochemical detection of tight junction proteins (TJPs) in matriptase- or prostasin-deficient animals reveals varying degrees of abnormality, ranging from decreased staining to a complete lack of focal expression of the TJPs claudin-1, ZO-1 and occludin (Leyvraz et al. 2005; List et al. 2009). In addition to aberrant protein expression, a tracer injected into the dermis of matriptase- and prostasin-deficient mice diffuses through the stratum granulosum and into the stratum corneum, indicating a concomitant defect in epidermal tight junction function (Leyvraz et al. 2005; List et al. 2009). While the role of prostasin in tight junction formation outside the epidermis has not been addressed, matriptase deficiency also disrupts tight junctions in intestinal epithelium (List et al. 2009). In Caco-2 cell monolayers, which model intestinal epithelium, matriptase knockdown or specific peptide-mediated inhibition of matriptase activity causes an increase in macromolecular paracellular

permeability and a decrease in transepithelial electrical resistance (TEER) (Buzza et al. 2010; Sambuy et al. 2005). Matriptase-deficient Caco-2 monolayers as well as matriptase hypomorphic mice were shown to express abnormally high levels of the TJP claudin-2, which is associated with the formation of ion channels that decrease the cohesion between adjacent epithelial cells (Van Itallie and Anderson 2006). Claudin-2 is incorporated into intercellular junctions in matriptase-silenced Caco-2 monolayers and has deregulated expression in matriptase hypomorphic mice as the result of impaired protein turnover (Buzza et al. 2010). This may contribute to the observation that continual matriptase expression is required throughout the lifespan of mice for the maintenance of integrity in multiple adult epithelia. Postnatal ablation of matriptase from the large intestine through conditional gene knockout results in persistent edema and increased epithelial permeability, likely caused by the loss of function of tight junctions (List et al. 2009) (Fig. 1b). Because matriptase has not yet been proven to proteolytically process TJPs, additional experimentation is required to elucidate the downstream effectors of matriptase activity that contribute to tight junction formation and maintenance. A summary of the defects in the epidermis and large intestinal epithelium associated with the loss of matriptase is shown in Fig. 1.

Matriptase pathobiology

In mice, matriptase deficiency as well as the deficiency of its cognate inhibitor HAI-1 result in postnatal lethality, indicating that matriptase activity may require precise regulation during the development and growth of epithelial tissues. HAI-1 null mice perish during embryogenesis due to defects in placental development, a phenotype that is reversed in matriptase/HAI-1 double-deficient animals (Fan et al. 2007; Szabo et al. 2007). Chimeric mice with HAI-1 expressed only in the trophoblast survive through birth but develop severe icthyosis and perish by day 16 (Nagaike et al. 2008). Histological examination of these HAI-1 chimeric null animals reveals hyperkeratosis of the forestomach and hyperkeratosis and acanthosis of the epidermis. These phenotypes are reversed when HAI-1 is eliminated in the genetic background of matriptase hypomorphic mice and normal lifespan is restored, indicating that matriptase suppression via HAI-1 is essential to postnatal epithelial function (Szabo et al. 2009). In humans, hypomorphic matriptase activity is at the etiology of Autosomal Recessive Ichthyosis with Hypotrichosis (ARIH), a congenital disorder that manifests in individuals with a homozygous point mutation in the matriptase serine protease domain (Basel-Vanagaite et al. 2007; Alef et al. 2009). This G827R mutant form of matriptase has dramatically reduced proteolytic activity toward several synthetic peptides as well as recombinant prostasin in vitro (List et al. 2007a). In order to assess the contribution of reduced matriptase activity to ARIH symptoms, hypomorphic mice were characterized in detail (List et al. 2007a). Interestingly, hypomorphic matriptase mice phenocopy all of the major symptoms of human ARIH; including icthyosis and hypertrichosis as well as abnormalities in tooth enamel. Importantly, hypomorphic matriptase mice also display hyperproliferation of basal keratinocytes and impaired desquamation in the stratum corneum, which are microscopic hallmarks of the disease (Basel-Vanagaite et al. 2007; List et al. 2007a). From a functional standpoint, the proteolytic processing of prostasin as well as profilaggrin is greatly reduced in matriptase hypomorphic mice as well as in human ARIH patients (Alef et al. 2009; List et al. 2007a),

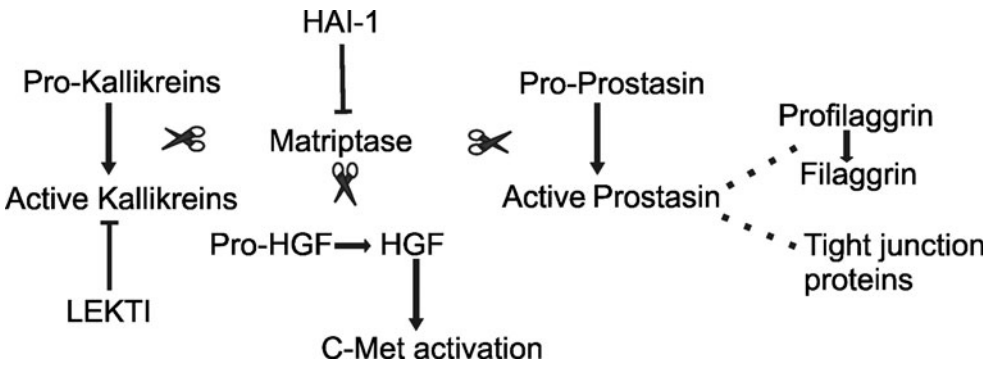

Fig. 2 Outline of the matriptase-prostasin proteolytic axis in the epidermis based on data from studies using genetic mouse models. The auto-activating type II transmembrane serine protease matriptase activates the GPI-anchored serine protease pro-prostasin in the epidermis. The activity of matriptase is inhibited by hepatocyte growth factor activator inhibitor -1 (HAI-1). Matriptase cleaves and activates the pro-form of hepatocyte growth factor (HGF) that activates c-Met. Furthermore, matriptase is capable of activating epidermal kallikreins. Matriptase and prostasin null mice have identical phenotypes, which include impaired proteolysis of epidermal profilaggrin to filaggrin monomers and loss of epidermal tight junctions. The underlying mechanisms are not fully understood. Thus, it is unclear whether matriptase/prostasin mediated proteolysis is directly involved in profilaggrin processing and degradation of tight junction proteins (indicated by *dotted lines*)

indicating that the matriptase-prostasin proteolytic cascade may be deregulated in human ARIH.

Derestricted matriptase activity has recently been implicated in the disease Netherton's syndrome, which is characterized by the detachment of the stratum corneum, exposing the living surface of the epidermis to the external environment and leading to chronic inflammation (Descargues et al. 2005; Sales et al. 2010; Smith et al. 1995a, b). Netherton's syndrome is caused by the loss of the serine protease inhibitor LEKTI, which is a critical regulator of kallikrein proteases in the epidermis. Kallikrein proteases are synthesized and activated in the granular layer but are prevented from performing proteolysis by forming complexes with LEKTI (Bitoun et al. 2002; Deraison et al. 2007; Sales et al. 2010; Schechter et al. 2005). In the upper regions of the stratum corneum, decreased pH causes these complexes to disassociate, resulting in kallikrein-mediated corneodesmosome degradation ultimately leading to desquamation. In the absence of LEKTI, ectopic desquamation occurs at the boundary between the granular and transitional layer, resulting in loss of the stratum corneum. LEKTI-deficient mice phenocopy the symptoms associated with human Netherton's syndrome (Descargues et al. 2005; Hewett et al. 2005; Yang et al. 2004). When LEKTI deficiency is combined with matriptase deficiency, aberrant protease activity in the epidermis is abolished, stratum corneum function is improved and corneodesmosome function in the lower epidermis is restored (Sales et al. 2010). While LEKTI does not directly inhibit matriptase, matriptase is capable of activating prokallikreins, indicating that, when LEKTI/kallikrein inhibitory complexes are absent, matriptase acts to initiate the ectopic activation of kallikreins (Sales et al. 2010). Thus, deregulated matriptase activity may be involved in multiple epithelial pathologies and precise regulation of the downstream targets of matriptase proteolysis is essential to the proper formation of the epidermal strata.

Matriptase has garnered significant attention as a potential oncogene, as its expression correlates with the severity of tumors in the breast and prostate and de novo matriptase expression has been found in both ovarian and cervical carcinomas (Lee et al. 2005; Saleem et al. 2006; Tanimoto et al. 2005; Tsai et al. 2008). The ratio of matriptase/HAI-1 mRNA is increased in ovarian and colorectal cancer, indicating that deregulated matriptase proteolysis may contribute to tumor formation or metastasis (Oberst et al. 2002; Vogel et al. 2006). Interestingly, while matriptase expression in normal mouse epidermis is confined to the post-mitotic transitional layer, matriptase spatially translocates to the proliferative basal layer during premalignant progression and is highly expressed in rapidly dividing tumor cells in squamous cell carcinoma (SCC) (List et al. 2006). Transgenic mice engineered to express matriptase in the basal layer of the epidermis develop spontaneous SCC, confirming the ability of matriptase to initiate tumor formation when expressed in proliferative cells (List et al. 2005). Importantly, when these mice also express the inhibitor HAI-1 transgene in the basal layer, the oncogenic properties of matriptase are completely abolished (List et al. 2005). Recent work has demonstrated that matriptase-induced SCC in mice requires c-Met and that matriptase increases the processing of pro-HGF in primary keratinocytes, resulting in the initiation of the c-Met/mTor signaling axis and increased cell proliferation and migration (Szabo et al. 2011).

Concluding remarks

In summary, matriptase is a type II transmembrane serine protease localizing to the plasma membrane, where it is involved in cell surface proteolysis including activation of prostasin. The matriptase-prostasin proteolytic cascade (summarized in Fig. 2.) is crucial to the formation of the epidermal barrier, terminal differentiation of keratinocytes and the proper formation of tight junctions. Matriptase activity is under tight control by the kunitz-type serine protease inhibitor HAI-1 and deficiency of either of these two proteins results in lethality in mice, highlighting the importance of both expression and regulation of matriptase protein during development. Postnatal ablation of matriptase from epithelial tissue results in severe defects in multiple epithelia, indicating an ongoing requirement for matriptase activity in tissue maintenance and homeostasis. Mutations resulting in decreased matriptase activity have been implicated as the etiological origin of Autosomal Recessive Icthyosis and Hypotrichosis (ARIH) in humans, while ectopic matriptase activity contributes to Netherton's syndrome in a mouse model of the disease. Future research should seek to unveil the mechanisms of matriptase/prostasin transcriptional regulation and identify the molecular mechanisms by which matriptase/prostasin exerts downstream effects such as the maintenance of tight junctions. In addition, as matriptase is one of several mammalian TTSPs, many of which remain relatively uncharacterized, there is strong impetus to continue to explore this unique class of cell surface proteases.

Acknowledgments This work was supported by start-up funds for KL from the Department of Pharmacology, Wayne State School of Medicine and the Barbara Ann Karmanos Cancer Institute.

References

Adachi M, Kitamura K, Miyoshi T, Narikiyo T, Iwashita K, Shiraishi N, Nonoguchi H, Tomita K (2001) Activation of epithelial sodium channels by prostasin in Xenopus Oocytes. J Am Soc Nephrol 12:1114–21

Alef T, Torres S, Hausser I, Metze D, Tursen U, Lestringant GG, Hennies HC (2009) Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in ST14 is associated with impaired profilaggrin processing. J Invest Dermatol 129:862–9

Basel-Vanagaite L, Attia R, Ishida-Yamamoto A, Rainshtein L, Ben Amitai D, Lurie R, Pasmanik-Chor M, Indelman M, Zvulunov A, Saban S, Magal N, Sprecher E, Shohat M (2007) Autosomal recessive ichthyosis with hypotrichosis caused by a mutation in ST14, encoding type II transmembrane serine protease matriptase. Am J Hum Genet 80(3):467–77

Beliveau F, Desilets A, Leduc R (2009) Probing the substrate specificities of matriptase, matriptase-2, hepsin and DESC1 with internally quenched fluorescent peptides. FEBS J 276:2213–26

Bitoun E, Chavanas S, Irvine AD, Lonie L, Bodemer C, Paradisi M, Hamel-Teillac D, Ansai S, Mitsuhashi Y, Taieb A, de Prost Y, Zambruno G, Harper JI, Hovnanian A (2002) Netherton syndrome: disease expression and spectrum of SPINK5 mutations in 21 families. J Invest Dermatol 118:352–61

Bruns JB, Carattino MD, Sheng S, Maarouf AB, Weisz OA, Pilewski JM, Hughey RP, Kleyman TR (2007) Epithelial Na+ channels are fully activated by furin- and prostasin-dependent release of an inhibitory peptide from the gamma-subunit. J Biol Chem 282 (9):6153–60

Bugge TH, Antalis TM, Wu Q (2009) Type II transmembrane serine proteases. J Biol Chem 284:23177–81

Buzza MS, Netzel-Arnett S, Shea-Donohue T, Zhao A, Lin CY, List K, Szabo R, Fasano A, Bugge TH, Antalis TM (2010) Membrane-anchored serine protease matriptase regulates epithelial barrier formation and permeability in the intestine. Proc Natl Acad Sci USA 107:4200–5

Chen LM, Hodge GB, Guarda LA, Welch JL, Greenberg NM, Chai KX (2001a) Down-regulation of prostasin serine protease: a potential invasion suppressor in prostate cancer. Prostate 48:93–103

Chen LM, Skinner ML, Kauffman SW, Chao J, Chao L, Thaler CD, Chai KX (2001b) Prostasin is a glycosylphosphatidylinositol-anchored active serine protease. J Biol Chem 276:21434–42

Chen CJ, Wu BY, Tsao PI, Chen CY, Wu MH, Chan YL, Lee HS, Johnson MD, Eckert RL, Chen YW, Chou F, Wang JK, Lin CY (2010a) Increased matriptase zymogen activation in inflammatory skin disorders. Am J Physiol Cell Physiol

Chen YW, Wang JK, Chou FP, Chen CY, Rorke EA, Chen LM, Chai KX, Eckert RL, Johnson MD, Lin CY (2010b) Regulation of the matriptase-prostasin cell surface proteolytic cascade by hepatocyte growth factor activator inhibitor-1 during epidermal differentiation. J Biol Chem 285:31755–62

Clark EB, Jovov B, Rooj AK, Fuller CM, Benos DJ (2010) Proteolytic cleavage of human acid-sensing ion channel 1 by the serine protease matriptase. J Biol Chem 285:27130–43

Dale BA, Holbrook KA, Steinert PM (1978) Assembly of stratum corneum basic protein and keratin filaments in macrofibrils. Nature 276:729–31

Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A, Wagberg F, Brattsand M, Hachem JP, Leonardsson G, Hovnanian A (2007) LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. Mol Biol Cell 18:3607–19

Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, Elias P, Barrandon Y, Zambruno G, Sonnenberg A, Hovnanian A (2005) Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 37:56–65

Di Cera E (2009) Serine proteases. IUBMB Life 61:510–5

Fan B, Brennan J, Grant D, Peale F, Rangell L, Kirchhofer D (2007) Hepatocyte growth factor activator inhibitor-1 (HAI-1) is essential for the integrity of basement membranes in the developing placental labyrinth. Dev Biol 303:222–30

Friis S, Godiksen S, Bornholdt J, Selzer-Plon J, Rasmussen HB, Bugge TH, Lin CY, Vogel LK (2011) Transport via the transcytotic pathway makes prostasin available as a substrate for matriptase. J Biol Chem 286:5793–802

Hewett DR, Simons AL, Mangan NE, Jolin HE, Green SM, Fallon PG, McKenzie AN (2005) Lethal, neonatal ichthyosis with increased proteolytic processing of filaggrin in a mouse model of Netherton syndrome. Hum Mol Genet 14:335–46

Hooper JD, Nicol DL, Dickinson JL, Eyre HJ, Scarman AL, Normyle JF, Stuttgen MA, Douglas ML, Loveland KA, Sutherland GR, Antalis TM (1999) Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Res 59:3199–205

Hooper JD, Clements JA, Quigley JP, Antalis TM (2001) Type II transmembrane serine proteases. Insights into an emerging class of cell surface proteolytic enzymes. J Biol Chem 276:857–60

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

Lee SL, Dickson RB, Lin CY (2000) Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. J Biol Chem 275:36720–5

Lee JW, Yong Song S, Choi JJ, Lee SJ, Kim BG, Park CS, Lee JH, Lin CY, Dickson RB, Bae DS (2005) Increased expression of matriptase is associated with histopathologic grades of cervical neoplasia. Hum Pathol 36:626–33

Lee MS, Tseng IC, Wang Y, Kiyomiya K, Johnson MD, Dickson RB, Lin CY (2007) Autoactivation of matriptase in vitro: requirement for biomembrane and LDL receptor domain. Am J Physiol Cell Physiol 293:C95–105

Leyvraz C, Charles RP, Rubera I, Guitard M, Rotman S, Breiden B, Sandhoff K, Hummler E (2005) The epidermal barrier function is dependent on the serine protease CAP1/Prss8. J Cell Biol 170:487–96

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

List K, Haudenschild CC, Szabo R, Chen W, Wahl SM, Swaim W, Engelholm LH, Behrendt N, Bugge TH (2002) Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. Oncogene 21:3765–79

List K, Szabo R, Wertz PW, Segre J, Haudenschild CC, Kim SY, Bugge TH (2003) Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. J Cell Biol 163(4):901–10

List K, Szabo R, Molinolo A, Sriuranpong V, Redeye V, Murdock T, Burke B, Nielsen BS, Gutkind JS, Bugge TH (2005) Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev 19 (16):1934–50

List K, Szabo R, Molinolo A, Nielsen BS, Bugge TH (2006) Delineation of matriptase protein expression by enzymatic gene trapping suggests diverging roles in barrier function, hair formation, and squamous cell carcinogenesis. Am J Pathol 168:1513–25

List K, Currie B, Scharschmidt TC, Szabo R, Shireman J, Molinolo A, Cravatt BF, Segre J, Bugge TH (2007a) Autosomal ichthyosis with hypotrichosis syndrome displays low matriptase proteolytic activity and is phenocopied in ST14 hypomorphic mice. J Biol Chem 282:36714–23

List K, Hobson JP, Molinolo A, Bugge TH (2007b) Co-localization of the channel activating protease prostasin/(CAP1/PRSS8) with its candidate activator, matriptase. J Cell Physiol 213:237–45

List K, Kosa P, Szabo R, Bey AL, Wang CB, Molinolo A, Bugge TH (2009) Epithelial integrity is maintained by a matriptase-dependent proteolytic pathway. Am J Pathol 175:1453–63

Markova NG, Marekov LN, Chipev CC, Gan SQ, Idler WW, Steinert PM (1993) Profilaggrin is a major epidermal calcium-binding protein. Mol Cell Biol 13:613–25

Mildner M, Jin J, Eckhart L, Kezic S, Gruber F, Barresi C, Stremnitzer C, Buchberger M, Mlitz V, Ballaun C, Sterniczky B, Fodinger D, Tschachler E (2010) Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol 130:2286–94

Nagaike K, Kawaguchi M, Takeda N, Fukoshima T, Sawaguch IA, Kohama K, Setoyama M, Kataoka H (2008) Defect of hepatocyte growth factor activator inhibitor type 1/serine protease inhibior. Kunitz type 1 (Hai-1/Spint1) leads to ichthyosis-like condition and abnormal hair development in mice. Am J Pathol 173:1–12

Netzel-Arnett S, Currie BM, Szabo R, Lin CY, Chen LM, Chai KX, Antalis TM, Bugge TH, List K (2006) Evidence for a matriptase-prostasin proteolytic cascade regulating terminal epidermal differentiation. J Biol Chem 281:32941–5

Oberst MD, Johnson MD, Dickson RB, Lin CY, Singh B, Stewart M, Williams A, al-Nafussi A, Smyth JF, Gabra H, Sellar GC (2002) Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters. Clin Cancer Res 8:1101–7

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

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

Ovaere P, Lippens S, Vandenabeele P, Declercq W (2009) The emerging roles of serine protease cascades in the epidermis. Trends Biochem Sci 34:453–63

Pearton DJ, Dale BA, Presland RB (2002) Functional analysis of the profilaggrin N-terminal peptide: identification of domains that regulate nuclear and cytoplasmic distribution. J Invest Dermatol 119:661–9

Puente XS, Gutierrez-Fernandez A, Ordonez GR, Hillier LW, Lopez-Otin C (2005) Comparative genomic analysis of human and chimpanzee proteases. Genomics 86:638–47

Resing KA, Johnson RS, Walsh KA (1993) Characterization of protease processing sites during conversion of rat profilaggrin to filaggrin. Biochemistry 32:10036–45

Saleem M, Adhami VM, Zhong W, Longley BJ, Lin CY, Dickson RB, Reagan-Shaw S, Jarrard DF, Mukhtar H (2006) A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. Cancer Epidemiol Biomarkers Prev 15:217–27

Sales KU, Masedunskas A, Bey AL, Rasmussen AL, Weigert R, List K, Szabo R, Overbeek PA, Bugge TH (2010) Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. Nat Genet 42:676–83

Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stammati A, Zucco F (2005) The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol Toxicol 21:1–26

Scharschmidt TC, List K, Grice EA, Szabo R, Renaud G, Lee CC, Wolfsberg TG, Bugge TH, Segre JA (2009) Matriptase-deficient mice exhibit ichthyotic skin with a selective shift in skin microbiota. J Invest Dermatol 129:2435–42

Schechter NM, Choi EJ, Wang ZM, Hanakawa Y, Stanley JR, Kang Y, Clayman GL, Jayakumar A (2005) Inhibition of human kallikreins 5 and 7 by the serine protease inhibitor lympho-epithelial Kazal-type inhibitor (LEKTI). Biol Chem 386:1173–84

Shipway A, Danahay H, Williams JA, Tully DC, Backes BJ, Harris JL (2004) Biochemical characterization of prostasin, a channel activating protease. Biochem Biophys Res Commun 324:953–63

Smith DL, Smith JG, Wong SW, deShazo RD (1995a) Netherton's syndrome. Br J Dermatol 133:153–4

Smith DL, Smith JG, Wong SW, deShazo RD (1995b) Netherton's syndrome: a syndrome of elevated IgE and characteristic skin and hair findings. J Allergy Clin Immunol 95:116–23

Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-Jones S, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WH (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 38:337–42

Steinert PM, North AC, Parry DA (1994) Structural features of keratin intermediate filaments. J Invest Dermatol 103:19S–24S

Szabo R, Bugge TH (2008) Type II transmembrane serine proteases in development and disease. Int J Biochem Cell Biol 40:1297–316

Szabo R, Wu Q, Dickson RB, Netzel-Arnett S, Antalis TM, Bugge TH (2003) Type II transmembrane serine proteases. Thromb Haemost 90:185–93

Szabo R, Molinolo A, List K, Bugge TH (2007) Matriptase inhibition by hepatocyte growth factor activator inhibitor-1 is essential for placental development. Oncogene 26:1546–56

Szabo R, Kosa P, List K, Bugge TH (2009) Loss of matriptase suppression underlies spint1 mutation-associated ichthyosis and postnatal lethality. Am J Pathol 174:2015–22

Szabo R, Rasmussen AL, Moyer AB, Kosa P, Schafer JM, Molinolo AA, Gutkind JS, Bugge TH (2011) c-Met-induced epithelial carcinogenesis is initiated by the serine protease matriptase. Oncogene (in press)

Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, Craik CS (2000) 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 275:26333–42

Tanimoto H, Shigemasa K, Tian X, Gu L, Beard JB, Sawasaki T, O'Brien TJ (2005) Transmembrane serine protease TADG-15 (ST14/Matriptase/MT-SP1): expression and prognostic value in ovarian cancer. Br J Cancer 92:278–83

Tong Z, Illek B, Bhagwandin VJ, Verghese GM, Caughey GH (2004) Prostasin, a membrane-anchored serine peptidase, regulates sodium currents in JME/CF15 cells, a cystic fibrosis airway epithelial cell line. Am J Physiol Lung Cell Mol Physiol 287:L928–35

Tsai WC, Chu CH, Yu CP, Sheu LF, Chen A, Chiang H, Jin JS (2008) Matriptase and survivin expression associated with tumor progression and malignant potential in breast cancer of Chinese women: tissue microarray analysis of immunostaining scores with clinicopathological parameters. Dis Markers 24:89–99

Tseng IC, Xu H, Chou FP, Li G, Vazzano AP, Kao JP, Johnson MD, Lin CY (2010) Matriptase activation, an early cellular response to acidosis. J Biol Chem 285:3261–70

Ustach CV, Huang W, Conley-LaComb MK, Lin CY, Che M, Abrams J, Kim HR (2010) A novel signaling axis of matriptase/PDGF-D/ß-PDGFR in human prostate cancer. Cancer Res 70(23):9631–40

Vallet V, Chraibi A, Gaeggeler HP, Horisberger JD, Rossier BC (1997) An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature 389:607–10

Van Itallie CM, Anderson JM (2006) Claudins and epithelial paracellular transport. Annu Rev Physiol 68:403–429

Vogel LK, Saebo M, Skjelbred CF, Abell K, Pedersen ED, Vogel U, Kure EH (2006) The ratio of Matriptase/HAI-1 mRNA is higher

in colorectal cancer adenomas and carcinomas than corresponding tissue from control individuals. BMC Cancer 6:176

Vuagniaux G, Vallet V, Jaeger NF, Pfister C, Bens M, Farman N, Courtois-Coutry N, Vandewalle A, Rossier BC, Hummler E (2000) Activation of the amiloride-sensitive epithelial sodium channel by the serine protease mCAP1 expressed in a mouse cortical collecting duct cell line. J Am Soc Nephrol 11 (5):828–34

Vuagniaux G, Vallet V, Jaeger NF, Hummler E, Rossier BC (2002) Synergistic activation of ENaC by three membrane-bound channel-activating serine proteases (mCAP1, mCAP2, and mCAP3) and serum- and glucocorticoid-regulated kinase (Sgk1) in Xenopus Oocytes. J Gen Physiol 120:191–201

Wang JK, Lee MS, Tseng IC, Chou FP, Chen YW, Fulton A, Lee HS, Chen CJ, Johnson MD, Lin CY (2009) Polarized epithelial cells secrete matriptase as a consequence of zymogen activation and HAI-1-mediated inhibition. Am J Physiol Cell Physiol 297:C459–70

Yang T, Liang D, Koch PJ, Hohl D, Kheradmand F, Overbeek PA (2004) Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/- mice. Genes Dev 18:2354–8

Yu G, Swiston J, Young D (1994) Comparison of human CAP and CAP2, homologs of the yeast adenylyl cyclase-associated proteins. J Cell Sci 107(Pt 6):1671–8

Yu JX, Chao L, Chao J (1995) Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. J Biol Chem 270:13483–9

Zhang D, Karunaratne S, Kessler M, Mahony D, Rothnagel JA (2002) Characterization of mouse profilaggrin: evidence for nuclear engulfment and translocation of the profilaggrin B-domain during epidermal differentiation. J Invest Dermatol 119:905–12