Mutation G827R in Matriptase Causing Autosomal Recessive Ichthyosis Yields an Inactive Protease

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Matriptase is a member of the novel family of type II transmembrane serine proteases. It was recently shown that a rare genetic disorder, autosomal recessive ichthyosis with hypotrichosis, is caused by a mutation in the coding region of matriptase. However, the biochemical and functional consequences of the G827R mutation in the catalytic domain of the enzyme have not been reported. Here we expressed the G827R-matriptase mutant in bacterial cells and found that it did not undergo autocatalytic cleavage from its zymogen to its active form as did the wild-type matriptase. Enzymatic activity measurements showed that the G827R mutant was catalytically inactive. When expressed in HEK293 cells, G827R-matriptase remained inactive but was shed as a soluble form, suggesting that another protease cleaved the full-length mature form of matriptase. Molecular modeling based on the crystal structure of matriptase showed that replacing Gly827 by Arg blocks access to the binding/catalytic cleft of the enzyme thereby preventing autocatalysis of the zymogen form. Our study, thus, provides direct evidence that the G827R mutation in patients with autosomal recessive ichthyosis with hypotrichosis leads to the expression of an inactive protease.

Type II transmembrane serine proteases (TTSPs) belong to a novel family of proteolytic enzymes anchored to cell membranes with extracellular catalytic domains (1, 2). There are currently upwards of 20 known members of this family that are involved in the maintenance of normal homeostasis but that cause disease when deregulated or mutated (1). TTSPs are mosaic proteins containing multiple domains involved in cellular targeting, protein-protein interactions, and enzymatic catalysis. Like many other proteolytic enzymes, they undergo post-translational processing events to generate active proteases from inactive zymogens.

The most studied TTSP at both the physiological and biochemical levels is matriptase (3). Although matriptase deficiency-mice develop to term, they die within 48 h due to the lack of epidermal barrier function and thymic homeostasis (4). Matriptase expressed in keratinocytes is a key regulator of epidermal differentiation, which is an essential component of the pro-fillagrin processing pathway (5). Fillagrin is an important protein that is involved in epidermal barrier function (6) and late stratum corneum differentiation (7). It has also been suggested that matriptase is a proteolytic activator of prostatin (8), a serine protease that is crucial for epidermal permeability and postnatal survival (9), and that it may play important roles in tumor cell metastasis and invasiveness (10–15). Indeed, matriptase causes malignant transformations when orthotopically overexpressed in the skin of mice, suggesting a causal role in human carcinogenesis (15). Moreover, it is overexpressed in many cancer tissues, including primary breast carcinomas, ovarian tumors of epithelial origin, and colon tumors (10), and has potential as a prognostic marker in ovarian cancer (16).

Matriptase has a complex biosynthetic profile (3). Like many other enzymes, matriptase is initially synthesized as an inactive zymogen that must be converted to an active protease by proteolytic cleavage. It has been proposed that the first cleavage occurs at Gly149 early in the secretory pathway, releasing the enzyme from its transmembrane anchor (17). However, interactions with the cleaved domain and/or its cognate inhibitor, hepatocyte growth factor activator inhibitor 1 (HAI-1), would enable matriptase to remain associated with membranes. Another cleavage, which appears to be autocatalytic, occurs at the junction between the stem region and the catalytic domain at Arg614 (R↓VVGG), converting the inactive zymogen into the active two-chain form. Last, the active enzyme is shed from the cell surface into the extracellular environment. In fact, three isoforms have been detected extracellularly; the SEA domain isoform cleaved at Gly149 (12) and two isoforms cleaved at Lys189 and Lys205, respectively (18).

A recent mutational analysis of human individuals with marked skin hyperkeratosis revealed a missense mutation (G827R) in the catalytic domain of matriptase (19). To investigate the biochemical consequences of this mutation on matriptase, we expressed and characterized G827R-matriptase and compared it to the wild-type enzyme.

EXPERIMENTAL PROCEDURES

Cell Culture—Human embryonic kidney HEK293 cells were cultured in high glucose Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 2 mm glutamine, 10 IU/ml penicillin, and 50 μg/ml streptomycin.

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*This work was supported by the Canadian Institutes for Health Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 The abbreviations used are: TTSP, type II transmembrane serine protease; ARIH, autosomal recessive ichthyosis with hypotrichosis; HAI-1, hepatocyte growth factor activator inhibitor 1; AMC, 7-amino-4-methylcoumarin; Boc, t-butoxycarbonyl.
**Mutation G827R Yields an Inactive Matriptase**

**Plasmid Constructions and Site-directed Mutagenesis**—The Mat(596–855) construction used for bacterial expression (pQE30 vector, Qiagen, Mississauga, ON, Canada) has been described previously (20). The plasmids containing human matriptase and HAI-1 cDNA used for expression in mammalian cells (cloned in pcDNA3.1, Invitrogen) were generous gifts from Dr. Chen-Yong Lin (Georgetown University, Washington, D. C.). G827R-Mat(596–855)-pQE30 and G827R-matriptase-pcDNA3.1 were generated by substituting Gly\(^{827}\) by Arg and S805A-matriptase-pcDNA3.1 by substituting Ser\(^{805}\) by Ala using the QuikChange II XL mutagenesis kit (Stratagene, La Jolla, CA) as described by the manufacturer.

**Expression of Matriptase in Escherichia coli**—Purification of enzymes expressed in E. coli was performed as previously described (20). Proteins were separated by SDS-PAGE on a 12% polyacrylamide gel and visualized using GelCode blue stain reagent (Pierce).

**General Kinetic Methods**—Enzymatic assays were performed in 100 mM Tris-HCl, pH 9, containing 500 μg/ml bovine serum albumin. Enzyme activities were monitored by measuring the release of fluorescence (excitation, 360 nm; emission, 441 nm) from Boc-Gln-Ala-Arg-AMC (50 μM) (Bachem Biosciences, King of Prussia, PA) for 20 min at 37 °C in a FLX-800 TBE microplate reader (Bio-Tek Instruments, Winooski, VT).

**Circular Dichroism Analysis**—Circular dichroism measurements were performed using a Jasco I-810 spectropolarimeter equipped with a Jasco Peltier-type thermostat. The instrument was calibrated with an aqueous solution of d-10- (+) -camphorsulfonic acid at 290.5 nm. Samples were loaded into quartz cells with a path length of 0.1 cm. Far-UV CD spectra were recorded at the desired temperature from 200 to 250 nm by averaging three scans at 0.1-nm intervals. Protein samples were diluted to 5 μM in 50 mM Tris, pH 9, containing 400 mM NaCl, 10% glycerol, and 1 mM β-mercaptoethanol. Temperature denaturations were performed from 5 to 95 °C with a heating rate of 1 °C/min, and unfolding was recorded at 227 nm.

**Expression and Detection of Matriptase in Mammalian Cells**—HEK293 cells were transfected with 6 μg of plasmids using Lipofectamine 2000 reagent (Invitrogen) in 10-cm plates. After a 24-h transfection, the medium was replaced by serum-free 293 SFM II (Invitrogen). After an additional 24-h incubation, cells were centrifuged, conditioned medium was collected, and cell fractions (membrane and soluble proteins) were prepared using the ProteoExtract Native Membrane Protein Extraction kit (EMD Biosciences Inc., La Jolla, CA). Proteins in the conditioned medium were precipitated with five volumes of acetone for 2 h at −80 °C and resuspended in water. Proteins were then separated by SDS-PAGE on 12% polyacrylamide gels and transferred to Polyscreen polyvinylidene difluoride membranes (PerkinElmer Life Sciences). Matriptase was detected using polyclonal rabbit anti-human matriptase antibody (Bethyl Laboratories, Montgomery, TX) and horseradish peroxidase-coupled donkey anti-rabbit antibody (GE Healthcare). HAI-1 was detected using polyclonal goat anti-human HAI-1 antibody (R&D Systems, Minneapolis, MN) and horseradish peroxidase-coupled donkey anti-goat antibody (Jackson Immunoresearch, West Grove, PA). Immunoreactive bands were visualized using Western Lightning Plus Chemiluminescent reagent (PerkinElmer Life Sciences) and x-ray films (Kodak X-Omat Blue, PerkinElmer Life Sciences).

**Cell Surface Biotinylation**—Three 10-cm plates of confluent HEK293 cells were transfected for 24 h. Biotinylation with sulfo-NHS-SS-biotin (Pierce) and isolation of cell surface proteins were performed with cells on ice according to the manufacturer’s instructions. Before biotinylation, selected cell plates were incubated with a solution of 0.25% trypsin for 5 min at 37 °C.

**Molecular Modeling**—The pdb file of the structure of the catalytic domain of matriptase (21) was used to model the structural effects of the G827R mutation. All the molecular mechanics and modifications of the pdb file were performed with the Insight II-2000 suite (acceleris) running on an SGI Octane 2 computer. Briefly, the Biopolymer module was used to introduce the G827R mutation into the wild-type structure. Molecular dynamic simulations were then performed to explore the conformations that the Arg\(^{827}\) side chain could adopt in the catalytic domain. This was done using the Discover module and the consistent valence force field. The simulation was preceded by an equilibration step and lasted for 10 ps (10,000-fs steps) at 300 K. Three different simulations were performed with different initial conformations of the Arg\(^{827}\) side chain. All three converged to the same final conformation where the guanidino moiety of the Arg side chains formed H-bonds with the carbonyl of Ser\(^{825}\) and the hydroxyl of the catalytic Ser\(^{805}\).

**RESULTS**

**Characterization of Recombinant G827R-matriptase**—To examine the effect of the G827R amino acid substitution on matriptase activity, we expressed and purified a truncated (amino acids 596–855) recombinant form in E. coli. This construct consisted of the C-terminal end of the fourth LDLRA domain, the activation domain, and the catalytic domain. Matriptase accumulates in inclusion bodies of bacteria using this system and must be refolded to obtain full activity (11, 20). The refolded wild-type zymogen, which migrates at 29 kDa, was capable of autoactivation as demonstrated by a shift of molecular mass from 29 to 26 kDa (Fig. 1A). Unlike the native enzyme, the G827R zymogen did not undergo autoactivation and remained as a 29-kDa protein (Fig. 1B). This was also the case for a mutant matriptase where the serine of the catalytic triad (Ser\(^{805}\)) was replaced by alanine (data not shown), in support of previously published data (11). We also directly measured the enzymatic activities of these protein preparations during the refolding process. After a 6-h dialysis, proteolytic activity was detected with wild-type matriptase but not with G827R-matriptase (Fig. 1C) nor with S805A-matriptase (data not shown). Proteolytic activity associated with the wild-type matriptase was greatly enhanced after 24 h of dialysis, but the G827R mutant remained inactive. To further characterize the enzymes, we used circular dichroism (CD) to verify whether the purification procedure yielded folded proteins. Both wild-type matriptase and G827R-matriptase recombinant proteins had similar far-UV CD spectra (Fig. 1D), indicating that the two proteins were similarly folded with comparable secondary structure content. The spectra were also typical of stably folded proteins with negligible random coil content. To ascertain the fact that both pro-
Mutation G827R Yields an Inactive Matriptase

Protein expression was induced in BL21(DE3)pLys cells transformed either with Matrip(596–855)-pQE30 construct (A) or G827R-matrip(596–855)-pQE30 construct (B) with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 37 °C. Samples from each purification step (1, total proteins; 2, inclusion bodies; 3, IMAC chromatography; 4, dialysis; 5, anion exchange chromatography) were resolved by SDS-PAGE and stained with GelCode Blue Stain reagent. Molecular weights are in kDa.

When matriptase was transiently expressed alone, immunoreactive bands were not readily detected in the membrane and the soluble fractions (Fig. 3, A and B, lanes 2). However, the 26-kDa catalytic domain and a processed form migrating at 80 kDa could be observed in the conditioned medium (Fig. 3C, lane 2). In contrast, expression of G827R-matriptase led to its detection in all three fractions. An intense doublet of 95 and 80 kDa was observed in the membrane fraction (Fig. 3A, lane 3). Interestingly, only the 80-kDa, but not the 26-kDa band, was detected in the soluble fraction and the conditioned medium of the mutant expressing cells (Fig. 3B and C, lanes 3). These findings suggest that, like wild-type matriptase, G827R-matriptase is processed at the Gly149 site and remains associated with the membrane. However, the autocatalytic activation cleavage at Arg114 is prevented by the G827R mutation.

To analyze the effect of mutating the Ser residue of the catalytic triad on matriptase biosynthesis, we examined processing of S805A-matriptase. As previously mentioned, in bacterial and mammalian expression systems this mutant did not exhibit proteolytic activity nor did it undergo autoactivation. Like G827R-matriptase, S805A-matriptase levels are elevated when compared with the wild-type enzyme, and it is detected in all three fractions (Fig. 3, A–C, lanes 4). In membrane fractions it is detected as a 95/80-kDa doublet (Fig. 3A, lane 4). Processed forms could also be detected in the soluble fraction (Fig. 3B, lane 4) and in the conditioned medium (Fig. 3C, lane 4). Surprisingly, a very faint and reproducible 26-kDa band corresponding to the matriptase catalytic domain could also be detected in the membrane fraction and the conditioned medium of S805A-matriptase-transfected cells (Fig. 3, A and C, lanes 4), indicating that a cleavage at Arg114 had occurred. This suggests that, under
Mutation G827R Yields an Inactive Matriptase

The membrane-associated HAI-1 regulates the activity and expression of matriptase (10). To verify the effect of HAI-1 on matriptase expression, we first looked for the presence of HAI-1 in untransfected HEK293 cells. We did not detect any endogenous levels of this protein in these cells (Fig. 3, A–C, lanes 1–4). Next, co-transfection of HAI-1 and matriptase led to a significant increase in matriptase protein levels in all three fractions (Fig. 3, A–C, lanes 6) when compared with cells transfected with matriptase alone. In fact, the levels of matriptase protein were now comparable with those obtained in cells transfected with G827R-matriptase or S805A-matriptase (Fig. 3, A–C, lanes 3 and 4). Co-transfection of HAI-1 did not influence the expression levels of G827R-matriptase and S805A-matriptase (Fig. 3, A–C, lanes 7 and 8). When co-expressed with HAI-1, the SDS-PAGE migration patterns of matriptase and the two mutants in the membrane and soluble fractions were identical. However, although the 26-kDa band was detected in the conditioned medium of HAI-1/matriptase co-transfected cells (Fig. 3C, lane 6), it was not detected in the HAI-1/G827R-matriptase nor HAI-1/S805A-matriptase extracellular media (Fig. 3C, lanes 7 and 8). This could indicate that wild-type matriptase activation was partially inhibited by HAI-1, since only a portion of the 80-kDa form of matriptase was converted to the 26-kDa active enzyme when co-expressed with the inhibitor. This is in contrast to the near complete processing of the 80-kDa form into the 26-kDa form when matriptase was expressed alone (Fig. 3C, lane 2). An alternative explanation to the higher levels of the 80-kDa form observed in Fig. 3C, lane 6, would be that HAI-1 would protect it from degradation. Finally, the results also demonstrate that the cleavage of S805A-matriptase is HAI-1 inhibited because the cleaved catalytic domain is not present when the mutant is co-expressed with HAI-1 (Fig. 3, A and C, lanes 8).

All these findings suggest that the initial biosynthetic steps (cleavage at the Gly149 site) of G827R-matriptase occur normally but that conversion from the processed to the active enzyme does not and that without HAI-1 active matriptase may become unstable, leading to its degradation and low protein expression. Inhibiting the catalytic activity of matriptase would prevent this effect (10), allowing the enzyme to accumulate in cells. Indeed, inhibition of matriptase by HAI-1 restored protein levels, similar to levels achieved when the enzyme is catalytically inactive (S805A and G827R substitutions).

HAI-1 has been detected as a 66-kDa full-length protein and shorter shed forms (22). We performed Western blotting to confirm that HAI-1 was expressed in our experiments. The full-length 66-kDa protein was detected mainly in the membrane fractions (Fig. 3A, lanes 5–8) and to a lesser extent in the soluble fractions (Fig. 3B, lanes 5–8). The detection of full-length HAI-1 in the soluble fractions may be due to small amounts of membrane-associated proteins in these samples. Like matriptase, the inhibitor is released into the conditioned medium (Fig. 3C, lanes 5–8). Shedding was independent of the presence of active matriptase, indicating that another protease is involved in this processing event. Finally, lower levels of free HAI-1 detected in samples where either G827R-matriptase or S805A-matriptase was co-expressed with the inhibitor (compare lanes 5 with 6–8) may signify that a proportion of expressed HAI-1 remained associated to the enzymes.

G827R-matriptase Is Catalytically Inactive in HEK293 Cells—To confirm that G827R-matriptase did not undergo activation in eukaryotic cells, we directly measured the proteolytic activity of the membrane and soluble conditioned medium fractions of cells transfected with either wild-type or G827R-matriptase. As shown in Fig. 4A, activity measurements using the fluorogenic substrate Boc-Gln-Ala-Arg-AMC in membrane fractions revealed that proteolytically active matriptase was present even though matriptase remained undetected by Western blotting (Fig. 3A, lane 2). The activity of matriptase was abolished when it was co-expressed with HAI-1. Conversely, no activity could be detected in the membrane fractions of G827R-matriptase-expressing cells despite the fact that it was expressed at much higher levels than wild-type matriptase (Fig. 3A, lane 3). We detected proteolytic activity in the soluble fractions of cells expressing wild-type matriptase, which once again was inhibited when matriptase was co-expressed with HAI-1 (Fig. 4B). No significant activity was detected in cells expressing G827R matriptase (Fig. 4B). Last, Boc-Gln-Ala-Arg-
AMC activity was found in the conditioned medium of cells expressing wild-type matriptase but not in the conditioned medium of cells expressing G827R-matriptase (Fig. 4C). These findings clearly show that matriptase was active and that the G827R mutation completely abolished its catalytic activity in mammalian cells.

**G827R-matriptase Is Present at the Cell Surface**—Matriptase is a transmembrane cell surface protein, and its expression and localization are regulated by HAI-1 (10). To determine whether the G827R mutation affected trafficking to the plasma membrane, we performed cell surface protein biotinylation assays. Under the conditions of biotinylation, we were unable to detect matriptase at the cell surface when the wild-type enzyme was expressed alone (Fig. 5A, lane 3). However, when it was co-expressed with HAI-1, both the full-length and the Gly149 processed forms were detected at the cell surface (Fig. 5A, lane 4). The two forms of G827R-matriptase were also observed at the cell surface, but their presence was independent of the co-expression with HAI-1 (Fig. 5A, lanes 5 and 6). Full-length HAI-1 was also detected at the cell surface whether it was expressed alone (Fig. 5B, lane 2) or co-expressed with either wild-type matriptase or the G827R mutant (Fig. 5B, lanes 4 and 6). However, when cells were treated with trypsin before cell surface biotinylation, the signals were abolished or greatly reduced (Figs. 5, A and B, lanes 7–9). Therefore, the G827R substitution did not impair the ability of the protease to localize at the cell surface. Moreover, unlike wild-type matriptase, the presence of HAI-1 was not essential for G827R-matriptase to localize at the cell surface.
Mutation G827R Yields an Inactive Matriptase

**A**

![Figure A](image)

**B**

![Figure B](image)

**FIGURE 5.** Expression of matriptase at the cell surface. HEK293 cells were co-transfected with combinations of pcDNA3.1 (Mock), HAI-1-pcDNA3.1 (HAI-1), matriptase-pcDNA3.1 (Matrip), and G827R-matriptase-pcDNA3.1 (G827R) as indicated. Cell surface proteins were biotinylated, purified, and analyzed by Western blotting (A) using an antibody directed against the catalytic domain of matriptase and the HAI-1 ectodomain (B). Samples were trypsinized before biotinylation where indicated. Molecular masses are in kDa. Results shown are representative of three independent experiments.

**Molecular Modeling of G827R-matriptase**—To gain insight into the molecular basis of the inactivation of the G827R mutant, we performed molecular modeling using the crystal structure of the catalytic domain of wild-type matriptase co-crystallized with the competitive inhibitor benzamidine (Fig. 6A) (21). As observed, benzamidine (Ben) makes H-Bonds with Asp⁷⁹⁹, which lines the S1 pocket that recognizes the P1 position (Arg or Lys) of the substrate. Benzamidine prevents the recognition of the substrate by occupying the volume usually taken by the P1 residue. In Fig. 6B, we show the same region of a model of the G827R mutant. In the model, Arg⁸²⁷ is found to make H-Bonds with many residues, including the side chain of Asp⁷⁹⁹. Furthermore, the position of benzamidine and Arg⁸²⁷ overlap. This strongly suggests that Arg⁸²⁷ prevents the putative substrate from being recognized by the mutant enzyme, hence explaining the fact that it is inactive.

**DISCUSSION**

The regulation of matriptase expression is a crucial factor in determining the role the enzyme plays in physiological and pathological processes. The loss of matriptase function in mouse models provides strong evidence that matriptase plays a major role in epidermal barrier function (4, 5, 23). Conversely, the overexpression of matriptase has shown that it is involved in epithelial carcinogenesis (15). After analyzing single nucleotide polymorphisms in patients with congenital ichthyosis associated with abnormal hair, Basel-Vanagaite et al. (19) have linked a human physiological disorder, autosomal recessive ichthyosis with hypotrichosis syndrome (ARIH), to a matriptase missense mutation. The mutation replaces a conserved glycine residue in the catalytic domain of the enzyme with an arginine.

Using a biochemical approach combining in vitro and in cellulo experiments, we show that the G827R mutation yields an enzymatically inactive matriptase. Unlike numerous serine proteases, the activation cleavage site of matriptase is autocatalytic (24), which suggests that matriptase can act upstream of a zymogen activation cascade. We show that for the G827R mutant this cleavage does not occur and that no enzymatic activity could be detected either with purified recombinant protein or when the mutant enzyme was expressed in a human epithelial cell line. In fact, the expression, processing, and trafficking to the cell membrane of G827R-matriptase was very similar to HAI-1-complexed matriptase. Interestingly, similar results were obtained when an inactive matriptase with a mutation in the crucial serine residue of the catalytic triad (S805A) was transfected in the BT549 breast cancer cell line (10). This mutant matriptase was expressed at much higher levels than wild-type matriptase but at comparable levels to those obtained for the HAI-1-inhibited matriptase (10). Our observation in transfected mammalian cells that high levels of the inactive S805A-matriptase are similar to those of G827R-matriptase and that S805A-matriptase undergoes a dissimilar processing pattern when compared with G827R-matriptase is intriguing. Production of a cleaved S805A-matriptase serine protease domain could signify that generation of the 26-kDa soluble form in matriptase expressing cells is not strictly autocatalytic and may involve other proteases. It is tempting to speculate that other TTSPs with specificities similar to that of matriptase could cleave at the Arg⁶¹⁴-Val⁶¹⁵ bond. Non-autocatalytic cleavage of S805A-matriptase would be mediated through HAI-1 since co-transfection of HAI-1 abolished production of the 26-kDa form. Indeed, the Kunitz domain-1 of HAI-1 has been shown to potentially inhibit the activity of a variety of serine proteases involved in cancer metastasis including TTSPs (matriptase and hepsin), plasma kallikrein, and trypsin (25). Another possibility for the detection of the 26-kDa form is that, under these conditions, cleavage would be due to a very low catalytic activity of the S805A-matriptase. Our data, thus, demonstrate that S805A-matriptase is more susceptible to cleavage at Arg⁶¹⁴ than G827R-matriptase.

Furthermore, our experimental data are supported by molecular modeling results which show that Arg⁸²⁷ prevents the entry of the basic P1-residue side chain of the activation sequence of matriptase (residue Arg⁶¹⁴) into the acidic catalytic pocket. Our results also demonstrate for the first time that protease activation is not essential for cell surface shedding and that at least one other enzyme would be involved in this processing event. Identifying this “sheddase” could provide a better understanding of the complex processing of matriptase and its role in processing extracellular substrates.

ARIH syndrome has relatively mild manifestations that include skin and hair abnormalities (19). This is in sharp contrast to the phenotype reported for matriptase knock-out mice, which die shortly after birth. ARIH patients have intact cor-
neodesmosomes in the upper cornified layers of affected epidermis, unlike the normal degradation process in these structures in unaffected skin (19). This is also the case with the matriptase knock-out mice phenotype (4). However, unlike what is predicted from the mouse model, there are no significant differences between ARIH patients and normal individuals in the expression or profile of filaggrin, an important element of the cornified envelope. Furthermore, ARIH patients do not display any signs of the T cell-related immunological defect (19) found in matriptase knock-out mice.

There are several possible explanations for these differences. First, the catalytic function of matriptase may be partially redundant in humans but not in mice. Type II transmembrane serine proteases make up a large family with more than 20 identified members so far in humans (1, 2, 26). The proteolytic domains of these enzymes share a high degree of amino acid sequence identity based on the alignment of the catalytic domains and the corresponding phylogenetic tree (1). Furthermore, the fact that knock-out mice for TMPRSS2, a member of the TTSP family, lack a phenotype (27) suggests that these serine proteases may be functionally redundant. It is also tempting to speculate that physiological functions of matriptase not only depend on its catalytic domain but also on protein-protein interactions with domains of the stem region and/or on signaling through its cytoplasmic domain. Last, we cannot rule out the possibility that G827R-matriptase may have very minimal catalytic activity in vivo and that this activity may be sufficient to avoid a lethal phenotype. Nevertheless, using fluorogenic peptides with different amino acids in the P1 position, we were unable to detect any proteolytic activity (data not shown).

The natural substrates of matriptase in a non-pathological setting have not been clearly identified. One possibility is that matriptase processes kallikreins, which are present in normal stratum corneum and which are desquamation-related proteases (28). Like other serine proteases, kallikreins are initially synthesized as inactive zymogens that undergo activation after processing at specific sites. Interestingly, many of the processing sites of kallikreins \((XXX\text{K/R}) \downarrow (I/LV/IGG)\) resemble the autocatalytic site of matriptase \((RQAR \downarrow VVGG)\). Kallikrein 5, which degrades corneodesmosome proteins such as corneodesmosin, desmoglein 1, and desmocollin 1 in the normal desquamation process, is thus an attractive candidate substrate for matriptase (29). The abolished matriptase activity would significantly diminish the levels of active kallikrein 5 and, in doing so, would affect the adhesion functions of corneodesmosome proteins. The marked skin hyperkeratosis observed in ARIH individuals might, thus, be due to the impaired degradation of stratum corneum proteins.

During the course of the revision of this manuscript, another study reported that a mouse model with greatly reduced matriptase expression presented similar phenotypic manifestations to those found in individuals with ARIH (30) underscoring the importance of matriptase in skin physiology. In these mice it was argued that reduced prostasin activation and profillagrin processing were a direct consequence of lowered levels of matriptase, suggesting a role of these proteins in epidermal homeostasis. Despite the existence of this and other mouse models with dysregulated matriptase expression and a matriptase-related human syndrome, future investigations involving structure-function analysis and substrate identification will be necessary to elucidate the exact role of this protein. Our study on G827R-matriptase point to the possibility that TTSPs may be functionally redundant and provide insights into
the regulation of matriptase processing. However, more information on the biochemical properties and physiological roles of other TTSPs is essential to better understand the specific functions of matriptase.

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