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Membrane-Anchored Serine Proteases in Health and Disease

TONI M. ANTALIS,^{*} THOMAS H.
BUGGE,[†] AND QINGYU WU[‡]

^{*}*Center for Vascular and Inflammatory
Diseases, University of Maryland School of
Medicine, Baltimore, Maryland, USA*

[†]*Proteases and Tissue Remodeling Section,
Oral and Pharyngeal Cancer Branch,
National Institute of Dental and
Craniofacial Research, National Institutes of
Health, Bethesda, Maryland, USA*

[‡]*Molecular Cardiology/Nephrology &
Hypertension, Lerner Research Institute/
NB20, Cleveland Clinic, Cleveland, Ohio,
USA*

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Serine proteases of the trypsin-like family have long been recognized to be critical effectors of biological processes as diverse as digestion, blood coagulation, fibrinolysis, and immunity. In recent years, a subgroup of these enzymes has been identified that are anchored directly to plasma membranes, either by a carboxy-terminal transmembrane domain (Type I), an amino-terminal transmembrane domain with a cytoplasmic extension (Type II or TTSP), or through

a glycosylphosphatidylinositol (GPI) linkage. Recent biochemical, cellular, and *in vivo* analyses have now established that membrane-anchored serine proteases are key pericellular contributors to processes vital for development and the maintenance of homeostasis. This chapter reviews our current knowledge of the biological and physiological functions of these proteases, their molecular substrates, and their contributions to disease.

I. Introduction

Proteolytic enzymes comprise over 2% of the known proteome, and their participation in many essential biological processes is well established. The serine proteases constitute one of the largest families of proteolytic enzymes and are well recognized for their pivotal roles in physiological processes as diverse as development, digestion, coagulation, inflammation, and immunity. These enzymes share a common catalytic mechanism for selective cleavage of specific substrates and are frequently involved in consecutive proteolytic reactions or protease cascades, where one protease precursor or zymogen is the substrate for an active protease. This shared mechanism confers the advantage that a single signal may be specifically and irreversibly amplified every time a downstream zymogen is activated, providing the capacity for unleashing a burst of proteolytic potential.

Most of the well-characterized members of the S1 family of serine proteases are either secreted enzymes or exocytosed from secretory vesicles into the extracellular environment. Trypsin and chymotrypsin, the main intestinal digestive enzymes, are prototype members of the S1 family. Over the past 10 years, a structurally distinct group of S1 serine proteases, termed broadly as the membrane-anchored serine proteases, has emerged that are synthesized with amino- or carboxy-terminal extensions that serve to anchor their serine protease catalytic domains directly at the plasma membrane^{1,2} (Fig. 1).

The largest group of membrane-anchored serine proteases is the Type II transmembrane serine proteases or TTSPs.¹ These proteases are synthesized with an amino-terminal signal anchor that is not removed during synthesis, but serves as a transmembrane domain that positions the protease in the plasma membrane with a cytoplasmic amino-terminal domain of variable length (20–160 amino acids) and the catalytic serine protease domain at the carboxy-terminus.¹ These serine proteases are synthesized as single-chain precursors or zymogens; activation produces a two-chain form with the chains linked by a disulfide bridge, so that the active enzyme remains membrane bound. Nineteen human TTSPs have been identified and may be categorized into four subfamilies: Hepsin/TMPRSS, Matriptase, HAT/DESC, and Corin^{3,4} (Fig. 1).

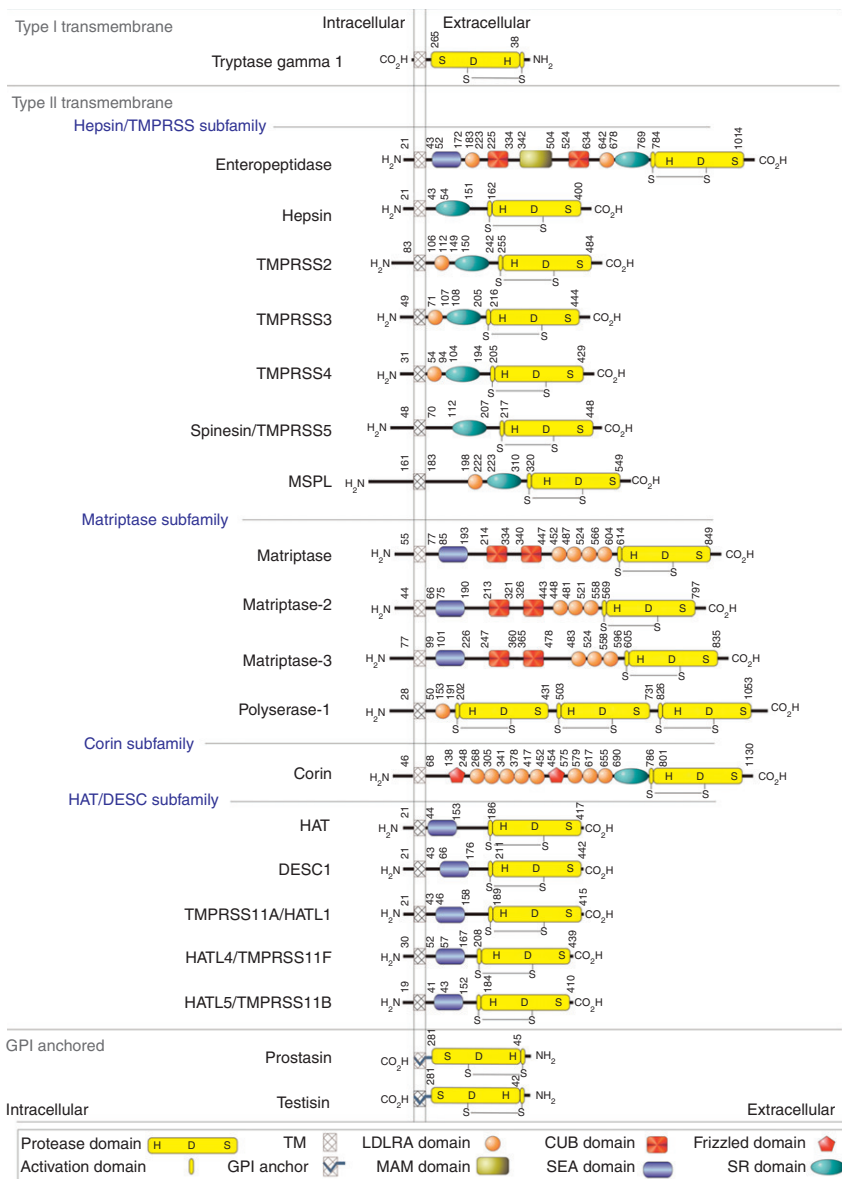


FIG. 1. Domain structures of the human membrane-anchored serine proteases. Structures are grouped according to similarity in domain structure to each other. Consensus domains are as indicated at the bottom of the figure. The location of each protein domain (amino acid numbering) is indicated above the domain. Amino and carboxy termini are as indicated. Protease domain: serine protease domain; activation domain: pro-domain; TM: transmembrane domain; GPI anchor: glycosylphosphatidylinositol linkage domain; LDLRA: LDL receptor class A domain; MAM: meprin, A5 antigen, and receptor protein phosphatase μ domain; CUB: Cls/Clr, urchin embryonic growth factor and bone morphogenetic protein-1; SEA: sea urchin sperm protein, enteropeptidase, agrin domain; Fz: frizzled domain; SR: Group A scavenger receptor domain. Modified from Ref. 2.

Mammalian orthologs, as well as different isoforms between humans and rodents, exist for many if not all of the TTSPs.⁴⁻⁶ There also exist two nonmammalian TTSPs, *Drosophila* stubble-stubbloid (st-sb)⁷ and corin.⁸

In contrast to the TTSPs, additional membrane-anchored serine proteases of the S1 family each possess an amino-terminal signal peptide and enter the secretory pathway. These enzymes are also synthesized as zymogens, with an amino-terminal extension that acts as a pro-peptide, requiring proteolytic cleavage to generate the active enzyme. The Type I transmembrane serine protease, tryptase γ 1, is the only human membrane-anchored serine protease synthesized with a carboxy-terminal hydrophobic extension that serves as a transmembrane domain.^{9,10} The carboxy-terminal extensions of prostasin and testisin are modified posttranscriptionally with a glycosylphosphatidylinositol (GPI) linkage that anchors these proteases in the plasma membrane.¹¹⁻¹⁵

The membrane-anchored serine proteases are proving to be key components of the cell machinery for activation of precursor molecules in the pericellular microenvironment, with several playing vital roles during development and the maintenance of homeostasis. There is also growing evidence for their participation in the pathogenesis of inflammatory and neoplastic diseases. Endogenous protein substrates targeted by membrane-anchored serine proteases include peptide hormones, growth and differentiation factors, receptors, enzymes, adhesion molecules, and viral coat proteins.¹⁶ A number of insights into our understanding of the unique physiological functions of the membrane-anchored serine proteases and their involvement in human pathology have come from a combination of biochemical analyses, animal models, and human patient studies. However, our current understanding of the impact of the membrane-anchored serine proteases on many biological, physiological, and pathological processes is far from complete. This chapter provides a historical perspective on the discovery of these enzymes, current knowledge of their activities and their regulation, and the functional consequences of the activities of these proteases in mammalian physiology and disease. For the interested reader, several other reviews have focused on different aspects of their nomenclature, classifications into subgroups, gene structure and chromosomal localization, tissue- and cell-specific distribution, and biochemical properties.^{1-4,16,17}

II. Structural Features

All of the membrane-anchored serine proteases have membrane-anchoring domains and structurally conserved serine protease catalytic domains. The TTSPs have additional extracellular stem regions that separate the catalytic domains from their transmembrane domains. The extracellular regions of the membrane-anchored serine proteases are believed to be essential to the biological and physiological functions ascribed to these enzymes.

A. Catalytic Domains

The zymogen forms of the membrane-anchored serine proteases are activated by proteolytic cleavage following an arginine or lysine amino acid present in a highly conserved activation motif separating the pro- and catalytic domains. The catalytic mechanism of the membrane serine proteases involves a catalytic triad of three amino acids, serine (nucleophile), aspartate (electrophile), and histidine (base), present in highly conserved sequence motifs. While the geometric orientations of these amino acid residues are similar, the protein folds are variable, which contribute to their selective substrate specificities. The catalytic reaction follows a two-step mechanism for hydrolysis of substrates in which a covalently linked enzyme-peptide intermediate is formed, with the loss of a peptide fragment.¹⁸ This acylation step is followed by a deacylation step which occurs by a nucleophilic attack on the intermediate by water, resulting in hydrolysis of the peptide.

Some insights into the structural features that contribute to the unique catalytic and substrate specificities of the membrane-anchored serine proteases have been obtained through comparative analyses of amino acid sequences^{1,2,19} combined with tertiary structural analyses.²⁰⁻²⁶ Consistent with the family of S1 serine proteases, each catalytic domain possesses two adjacent, six-stranded β -barrel domains that are connected by three trans-domain segments. The catalytic triad amino acids are located along the junction between the two barrels, with the active site cleft running perpendicular to this junction.²⁷ The size, shape, and charge distribution within the formed binding pocket of the active enzyme are determinants of substrate specificity. These pockets are defined by differing substrate-binding subsites (e.g., S_4 - S_2') and loop regions that surround the active site cleft.²³

The specificity for cleavage of substrates with the positively charged amino acid residues, lysine or arginine, in the P_1 position (the position directly preceding the cleaved peptide bond) is conferred by the presence of a conserved aspartate residue at the bottom of the binding pocket of all of the activated enzymes.² The rate of cleavage is influenced by the amino acid residues surrounding the P_1 residue, numbered P_1 to P_n , counting outward from the amino-terminal side of the peptide bond that is cleaved during hydrolysis, and numbered P_1' through P_n' from the carboxy-terminal side.²⁸ In recent years, recombinant catalytic domains of several of the membrane-anchored serine proteases have been produced in various laboratories and peptide screening assays applied to quantitatively identify cleavage preferences for the P_1 and P_1' amino acids, and surrounding amino acid positions.²⁹⁻³⁵

Several of the biochemically purified TTSPs rapidly undergo autocatalytic activation *in vitro* (matrptase^{29,36} matrptase-2,³⁷ hepsin,³⁸ TMPRSS2,³⁹ TMPRSS3,⁴⁰ TMPRSS4,⁴¹ HAT-like 3/TMPRSS11C⁴²). Thus, mutation of

active site catalytic triad residues of several of these TTSPs prevents activation site cleavage.^{3,36,42,43} It has been speculated that autoactivation of these TTSPs could contribute to the initiation of proteolytic cascades at the cell membrane, although the signals that induce internal cleavage or autoactivation *in vivo* have not yet been defined.

B. Extracellular “Stem” Regions

While the catalytic domains of the Type I and GPI-anchored serine proteases lie directly proximal to the membrane anchoring domain, the TTSPs are characterized by the presence of up to 11 extracellular structural domains present between their catalytic domains and the transmembrane domain (Fig. 1). These “stem” regions appear to serve as regulatory and/or binding interaction domains,¹ and are believed to variously contribute to the cellular localization, activation, inhibition, and/or substrate specificity of these enzymes.⁴⁴ The most common stem region structural domain is the low-density lipoprotein (LDL) receptor class A domain (LDLRA): corin contains eight, matriptase contains four, enteropeptidase two, and TMPRSS2 and TMPRSS4 one each of these domains (Fig. 1). In addition to the LDLRA domains, there are Group A scavenger receptor (SR) domains, frizzled domains, Cls/Clr, urchin embryonic growth factor and bone morphogenic protein 1 (CUB) domains, sea urchin sperm protein, enterokinase, agrin (SEA) domains, and meprin, A5 antigen, and receptor protein phosphatase μ (MAM) domains. The specific contributions of each stem domain to TTSP proteolytic activities have not yet been well defined, although for several of the TTSPs, the stem domain is required for efficient cleavage of their physiological substrates.^{45,46}

Targeted release of the extracellular domain, or ectodomain shedding, occurs for several of the membrane-anchored serine proteases, enabling the release of the soluble catalytic domain into the extracellular space. Soluble shed protease forms have been reported for enteropeptidase, HAT, matriptase, matriptase-2, and prostasin.^{11,47–49}

C. Membrane-Anchoring Domains

Surface localization studies demonstrate that membrane-anchored serine proteases normally localize to the cell surface and are differentially distributed on apical or basolateral surfaces of polarized cells in patterns unique for each protease.^{3,4} Studies indicate that the TTSP transmembrane domains are dispensable for the catalytic activities of many of the TTSPs, including enteropeptidase, matriptase, hepsin, corin, and MSPL.⁴ It appears that the function of the transmembrane domain in TTSPs is not to enhance their catalytic activities but to target the TTSPs to plasma membranes and localize their activities at specific sites. Similarly, the GPI-anchored serine proteases are

found to associate with lipid raft microdomains at cell surfaces, but do not require these anchors for catalytic activity. Loss of membrane polarity, such as that in tumors, has been associated with redistribution and/or mislocalization of several of the membrane-anchored serine proteases.^{50,51} In this regard, the significance of cell membrane association for the membrane-anchored serine proteases differs from that for blood-clotting proteases.

III. Regulation by Endogenous Inhibitors

As is the case with all serine proteases, enzyme activities must be tightly regulated in order to prevent inappropriate and frequently destructive proteolysis. The catalytic activities of the membrane-anchored serine proteases are regulated by endogenous protease inhibitors, specifically the Kunitz-type transmembrane serine protease inhibitors, hepatocyte growth factor (HGF) activator inhibitor (HAI)-1/SPINT1 and HAI-2/SPINT2, and several of the serine protease inhibitor family known as serpins. Kunitz-type inhibitors are a class of serine protease inhibitors present in all metazoa, whose prototype is the bovine pancreatic trypsin inhibitor (BPTI). They are competitive inhibitors acting in a substrate-like manner and form very stable complexes of 1:1 stoichiometry with their target enzymes, inhibiting their activity.⁵² Matrilysin,^{53–56} hepsin,⁵⁷ and prostasin^{31,48,57,58} are regulated by the Kunitz inhibitors, HAI-1 and/or HAI-2.

Serpin-type inhibitors utilize an irreversible conformational mechanism presenting a “pseudo-substrate” exposed binding loop to the protease which, upon cleavage, forms a covalent complex with the target protease.^{59,60} The serpins α 1-antitrypsin, α 2-antiplasmin, antithrombin III, protein C inhibitor, PAI-1, and protease nexin I are effective inhibitors of various recombinant catalytic domains of the membrane-anchored serine proteases (reviewed in Ref. 16).

IV. The Type I Transmembrane Serine Protease

A. Trypsin γ 1

Trypsin γ 1 or transmembrane trypsin (TMT) was identified as a major granule constituent of numerous populations of human and mouse mast cells.^{9,10,61,62} The gene that encodes trypsin γ 1 lies on human chromosome 16p13.3, a large locus that contains 14 serine protease-like genes.^{9,63} Expression of trypsin γ 1 is restricted to cells of hematopoietic origin and has been studied in mast cells.⁶² The Type I transmembrane domain at the

carboxy-terminus of tryptase $\gamma 1$ causes its cellular retention when mast cells are induced to release the contents of their secretory granules, at which time it reaches the external face of the plasma membrane.⁶²

The physiological functions of tryptase $\gamma 1$ remain unknown. Mast cells play beneficial immunosurveillance roles in host defense, particularly during parasitic infections, and are also implicated in asthma and other pathological conditions.^{64,65} Administration of recombinant tryptase $\gamma 1$ into the trachea of mice promotes airway hyperresponsiveness and increased expression of interleukin-13 in bronchial alveolar lavage fluids of these animals. This effect was not obtained in STAT6- and IL-4R α -null mice, indicating that tryptase $\gamma 1$ exerts its adverse effect in the lung in this animal model primarily by inducing the expression of IL-13, a central mediator of allergic asthma.

Tryptase $\gamma 1$ has been expressed as a recombinant catalytic domain and its substrate specificity characterized using combinatorial peptide substrate libraries.³³ Tryptase $\gamma 1$ prefers multibasic residues in the P₄-P₁ positions and has a strong preference for aromatic residues in the P₂ position.³³ It has also been shown to be rapidly inactivated by the human plasma serpin α_1 -antitrypsin⁶² and a submicromolar synthetic inhibitor,³³ *in vitro*.

V. The Type II Transmembrane Serine Proteases

A. HEP SIN/TMPRSS Subfamily

The HEP SIN/TMPRSS (transmembrane protease/serine) subfamily has seven members comprising hepsin, TMPRSS2, TMPRSS3, TMPRSS4, TMPRSS5/spinesin, MSPL (mosaic serine protease large-form), and enteropeptidase.⁴ All of these enzymes possess a Group A SR domain in their stem region, preceded by a single LDLRA domain in TMPRSS2, TMPRSS3, TMPRSS4, and MSPL or by an array of SEA, LDLRA, CUB, and MAM domains in enteropeptidase (Fig. 1).

1. ENTEROPEPTIDASE

Enteropeptidase is an intestinal protease discovered by Ivan Pavlov in Russia.⁶⁶ Using surgically modified dogs, Pavlov and colleagues studied the digestive system and found that pancreatic proteases were made as inactive forms. Upon entering the gut, latent pancreatic proteases were activated by another enzyme in the upper section of the intestine. Such an activation mechanism, which occurs outside of the pancreas, is important to prevent autoactivation of pancreatic proteases, thereby avoiding harmful tissue

damage. Pavlov named the intestinal enzyme enterokinase for its transformative activation activity. Currently, the enzyme is called enteropeptidase to reflect its proteolytic, but not kinase, activity.

Initial biochemical studies on enteropeptidase were carried out in the early 1900s, notably by Ernst Waldschmidt-Leitz and colleagues at the Biochemical Institute of the German Technical College in Prague.⁶⁷ Porcine enteropeptidase was partially purified from duodenal fluids and shown to activate trypsinogen from pancreatic extracts. More kinetic studies were done by Moses Kunitz at the Rockefeller Institute for Medical Research in Princeton, New Jersey, demonstrating enzymatic conversion of crystalline trypsinogen to trypsin using purified porcine enteropeptidase.^{68,69} By the 1970s, more advanced methods were used to purify enteropeptidase from porcine, bovine, and human intestinal mucosa.^{70,71} The protein was found to have a heavy chain of approximately 120 kDa and a light chain of approximately 35 kDa connected by a disulfide bond. Such a two-chain structure was known for chymotrypsin and other serine proteases. Enteropeptidase cleaves trypsinogen at the activation site, DDDDK↓I. The activity is inhibited by Kunitz pancreatic trypsin inhibitor, BPTI, and small molecule serine protease inhibitors, such as diisopropyl fluorophosphate (DFP), *p*-aminobenzamidine, and benzamidine, but not by chicken ovomucoid, soybean trypsin inhibitor, chymostatin, pepstatin A, or bestatin.⁶⁶

The partial amino-terminal sequence of the bovine enteropeptidase light chain was determined by Edman degradation, revealing its homology to other trypsin-like serine proteases.⁷² This sequence information helped to clone human, bovine, and porcine enteropeptidase cDNAs.^{73–76} The open reading frame of human enteropeptidase cDNA encodes a Type II transmembrane protein of 1019 amino acids with a calculated mass of 113 kDa and 17 potential N-linked glycosylation sites. The overall protein domain structure is shown in Fig. 1. Within the protease domain, the activation cleavage site, the catalytic triad, and the key cysteine residues are well conserved, indicating that enteropeptidase and other trypsin-like proteases have similar three-dimensional structures and comparable activation and catalytic mechanisms.

The human *PRSS7* gene, encoding enteropeptidase, is located on chromosome 21q21.⁷³ The gene has 25 exons and spans approximately 90-kb in length. Enteropeptidase mRNA is expressed mostly in the duodenum and, at lower levels, in the proximal segment of jejunum, consistent with Pavlov's finding that the enzyme was in the upper part of the small intestine. In these tissues, enteropeptidase mRNA is expressed in the enterocytes and goblet cells of the villus.⁷⁷ As a transmembrane protein, enteropeptidase is expected to remain at the site of expression, consistent with the previous observation that its activity was associated with the brush border of enterocytes.^{78,79} Low levels of

enteropeptidase mRNA were detected in other tissues, including stomach, colon, brain, and skin. The biological significance, if any, of its expression outside the digestive system remains unknown.

Enteropeptidase is anchored on the cell surface through its amino-terminal transmembrane domain. Other structural elements also play a role in its membrane targeting. The mucin-like repeats in the SEA domain and *N*-glycans in the protease domain have been found necessary for apical sorting.^{80,81} Like many membrane proteins, enteropeptidase is shed from the cell surface. Hadorn *et al.* showed that enteropeptidase was released into the small intestine lumen when brush-border membranes were treated with bile-acids or cholecystokinin-pancreozymin.⁸² To date, enzymes responsible for enteropeptidase shedding have not been characterized. It remains to be determined whether the shedding represents a physiological mechanism regulating enteropeptidase activity in the gut.

The mechanism responsible for the activation of the enteropeptidase zymogen is unclear. In transfected cells, enteropeptidase was expressed as a single-chain molecule and no autoactivation was observed, indicating that other proteases are required to activate enteropeptidase.⁸⁰ Trypsin activates enteropeptidase efficiently, suggesting a possible mechanism of reciprocal activation between enteropeptidase and trypsin.⁶⁶ For such an activation cycle to start, however, at least a fraction of enteropeptidase needs to be activated before trypsinogen enters the gut. Zamolodchikova *et al.* identified a novel serine protease, duodenase, from bovine duodenal mucosa.⁸³ The enzyme, which is approximately 30 kDa and consists of 226 amino acids, is expressed in the secretory epithelial cells of Brunner's glands in the proximal segment of the duodenum.⁸⁴ Duodenase has a preferred cleavage sequence with Lys at P₁ and Pro at P₂. It activated recombinant enteropeptidase, although the rate of the activation was approximately 100 times slower than that of trypsin.⁸⁵ It remains to be firmly established whether duodenase is a physiological enteropeptidase activator. More recently, other proteases have been shown to modulate enteropeptidase activity. For example, beta-site APP-cleaving enzyme1 (BACE1) was detected in pancreatic islets and shown to inactivate enteropeptidase by cleavage of its light chain.⁸⁶ This inactivation may represent a defense mechanism to inhibit enteropeptidase that enters the pancreatic duct accidentally, thereby preventing trypsinogen activation in the pancreas.

One of the remarkable features of enteropeptidase is its unique substrate specificity, which recognizes Lys at P₁ and a cluster of four Asp residues at P₂-P₅. Within this recognition sequence, a Lys or an Arg residue at P₁ and Lys residues at P₂ and P₃ appear to be most important for efficient cleavage.⁸⁷ The structural determinants for enteropeptidase substrate specificity have been localized in its protease domain. There is a group of four conserved basic residues, R/KRRK at positions 96-99, which were suspected to interact with

the acidic P₂-P₅ residues in the trypsinogen activation site.⁷⁴ In a crystal structure of bovine enteropeptidase light chain, Lys99 residue was found to have extensive contacts with the P₂ and P₄ Asp residues.²² Lys99 residue is conserved in enteropeptidase from many species. Substitution of Lys99 with Ala by mutagenesis prevented enteropeptidase from activating trypsinogen. In contrast, substituting Lys96, Arg97, and Arg98 residues for bovine enteropeptidase activity had less significant effects.²² The unique enteropeptidase substrate specificity has been exploited in protein engineering. The DDDDK↓I sequence is frequently used in recombinant proteins that require specific cleavage.

Enteropeptidase deficiency impairs food digestion and absorption.⁸⁸ To date, a number of patients who had low or undetectable enteropeptidase activity in intestinal biopsies or duodenal fluid samples have been identified. These patients suffer from diarrhea, vomiting, edema, anemia, and hypoproteinemia. Consequently, the patients fail to gain weight in early infancy. Genetic studies have shown deletion or nonsense mutations in the *PRSS7* gene in enteropeptidase-deficient patients.⁶⁶ For these patients, treatment with pancreatic extracts, which contain active trypsin, helps to initiate proteolytic reactions in the gut. Usually, the therapy is effective for the patients to absorb nutrients and gain weight. Apparently, the digestive enzyme reactions, once started, are self-sustained. Pancreatic extracts can be discontinued over time without causing gastrointestinal symptoms in these patients.⁸⁹

2. HEP SIN

Hepsin was originally cloned in Earl Davie's Laboratory at the University of Washington. Leytus *et al.* screened human liver cDNA libraries, which were rich in serine proteases.⁹⁰ A novel cDNA encoding a trypsin-like serine protease was identified by degenerate oligonucleotides. The protease was named hepsin for its hepatic expression. Rat and mouse hepsin cDNAs were isolated subsequently, which share high sequence homology with human hepsin.^{91,92} The full-length human hepsin cDNA, approximately 1.8-kb in length, encodes a polypeptide of 417 amino acids with a calculated mass of 45 kDa. Its protein domain structure is shown in Fig. 1. There is one potential N-linked glycosylation site at residue 112 in the SR-like domain.

The human *HPN* gene, encoding hepsin, is on chromosome 19 at q11-13.2 and has 14 exons. Hepsin mRNA expression is abundant in the liver.⁹³ Other tissues such as the kidney, pancreas, stomach, prostate, and thyroid express low levels of hepsin mRNA. The promoter sequence of the mouse *Hpn* gene has been characterized.⁹² In a 274-bp sequence upstream of the transcription initiation site, several potential binding sites for transcription factors such as

SP1, AP2, C/EBP, LF-A1, and E-box have been identified. This sequence showed strong promoter activities when tested in human HepG2 cells, indicating that it retains the essential elements required for hepatic expression.

In hepatocytes, hepsin is synthesized as a single-chain zymogen. Human hepsin purified from hepatocytes had an apparent mass of approximately 51 kDa on Western blots.⁹³ A similar molecular mass was found for native rat and recombinant human hepsin. During protein purification, human and rat hepsin were readily activated to become a two-chain molecule. It is unclear whether the activation was mediated by autocleavage or other proteases in hepatocytes. The membrane association and topology of hepsin have been confirmed by immunostaining and proteolytic digestion.

In peptide substrate-based assays, hepsin favors basic residues at the P₁ position. Thr/Leu/Asn, Gln/Lys, and Pro/Lys residues are favored at the P₂, P₃, and P₄ positions, respectively.³¹ This substrate profile does not appear to be unique. Somoza *et al.* have solved the crystal structure of a soluble human hepsin that included the SR-like and protease domains.²¹ As expected, hepsin protease domain has an architecture of two six-stranded β barrels. There are several structurally distinct loops, including an especially large one between residues 241 and 256, which may interact with its substrates. The activity of hepsin is inhibited by nonspecific inhibitors such as leupeptin, aprotinin, antipain, 4-amidophenylmethylsulfonyl fluoride, N $^{\alpha}$ -tosyl-L-lysine chloromethyl ketone, soybean trypsin inhibitor, and antithrombin III.⁹⁴ No inhibition was detected with EDTA, indicating that divalent ions are not required for hepsin activity toward peptide substrates. More recently, HAI-1 and HAI-2 have been reported to be potent hepsin inhibitors.^{31,95}

While many proteins are activated or cleaved by hepsin *in vitro*,⁹⁶⁻⁹⁹ the physiological function of hepsin remains unclear. These proteins include blood clotting factors VII, IX, and XII; pro-urokinase; pro-HGF; liver microsomal glutathione transferase; matriptase; prostasin; epidermal growth factor receptor; and laminin. It is unknown how many of them are hepsin substrates *in vivo*. Studies have suggested that hepsin may play a role in blood coagulation, hepatocyte proliferation/growth, and embryonic development.^{43,100,101} Such functions, however, have not been validated *in vivo*, as hepsin null mice are fertile, grow normally, and exhibit no spontaneous bleeding.^{102,103} Unexpectedly, however, hepsin appears to be important in normal hearing. Guipponi *et al.* detected hepsin mRNA expression in the inner ear.¹⁰⁴ Hepsin null mice have been found to have abnormal cochlea, reduced myelin protein expression in the auditory nerve, and severely impaired hearing. The biochemical basis for hepsin function in cochlear development has not been determined. Apparently, hepsin null mice have low levels of plasma thyroxine, a hormone important for cochlear development.¹⁰⁴ It is unknown whether impaired hearing in hepsin null mice is a result of thyroid hormone deficiency.

Multiple lines of evidence suggest a potential role of hepsin in human prostate cancer growth and progression. Microarray studies detected high levels of hepsin mRNA in human prostate cancers.^{105–108} Elevated hepsin mRNA expression appears to correlate with the disease severity. Single nucleotide polymorphisms (SNPs) in or near the human *HPN* gene are associated with prostate cancer susceptibility and tumor aggressiveness.¹⁰⁹ In functional studies, Xuan *et al.* showed that antibodies neutralizing hepsin protease activity did not block prostate or ovarian cancer cell growth in culture but inhibited their invasion in a Matrigel basement membrane.¹¹⁰ In other studies, however, prostate or other cancer cell lines stably transfected with hepsin cDNA were less invasive in culture or nude mice. Because stably transfected cells often undergo phenotypic changes during selection, it is difficult to determine whether observed differences were related to different levels of hepsin expression. To circumvent this problem, Klezovitch *et al.* created transgenic mice with high levels of hepsin in prostate epithelium, mimicking hepsin overexpression in prostate cancer patients.¹¹¹ The results show that hepsin overexpression did not affect cell proliferation but altered basement membrane structures in prostates in these mice. When the mice were crossed with a nonmetastasizing prostate cancer model, prostate cancer cells became more invasive, causing metastasis in the liver, lung, and bone. Thus, hepsin promotes prostate cancer progression and metastasis *in vivo*, suggesting that hepsin inhibitors may be developed to treat prostate cancer in patients.

3. TMPRSS2

TMPRSS2 cDNA was originally cloned by exon trapping in a gene mapping study of human chromosome 21.¹¹² The *TMPRSS2* gene, located at human chromosome 21q22.3,¹¹² is approximately 44 kb in length and includes 14 exons.¹¹³ The full-length TMPRSS2 cDNA encodes a polypeptide of 492 amino acids, with a domain structure shown in Fig. 1. Mouse TMPRSS2, also called epitheliasin, was isolated from a kidney cDNA library.¹¹⁴ Human TMPRSS2 mRNA is expressed in many tissues, including the prostate, breast, bile duct, kidney, colon, small intestine, pancreas, ovary, salivary gland, stomach, and lung.^{114,115} In these tissues, TMPRSS2 protein and mRNA are mostly in the epithelial cells. In human prostate cancers, TMPRSS2 protein is localized on the apical membrane of secretory epithelia and in the lumen of the glands.³⁹ Similar luminal staining patterns are found in human colon cancers. Many human prostate- and colon-derived cells, including APC-4, LNCaP, LoVo, T84, and Colo-205, also express TMPRSS2 mRNA.³⁹

In vitro-translated TMPRSS2 protein is detected as a zymogen of approximately 54 kDa,³⁹ whereas native and recombinant human TMPRSS2 proteins have a higher molecular mass (~60–70 kDa) due to N-linked glycosylation.^{39,116}

In human prostate cancer tissues or cells, approximately 32-kDa TMPRSS2 protease domain may also be detected, suggesting that the protease is partially activated. This cleavage appears to be due to TMPRSS2 autoactivation, as active site mutation prevented the cleavage of TMPRSS2 in transfected HEK 293T cells.³⁹ To date, TMPRSS2 substrate specificity and catalytic properties have not been well characterized.

The physiological roles of TMPRSS2 are currently unknown. In a *Xenopus* oocyte expression system, TMPRSS2 expression reduced epithelial sodium channel (ENaC) current and protein levels.¹¹⁷ In addition, TMPRSS2 activates influenza virus by cleaving hemagglutinin, suggesting that the enzyme may contribute to virus invasion of human airways.^{118,119} Secreted forms of TMPRSS2 have recently been found in human seminal prostasomes, suggesting its potential role in regulating sperm function.¹¹⁶ In prostate cancer cells, TMPRSS2 was shown to activate PAR-2.¹²⁰ It is unclear whether these activities are critical *in vivo*. TMPRSS2 null mice are viable and fertile with no reported abnormalities.¹²¹

The human *TMPRSS2* gene promoter has a 15-bp androgen response element at position -148 relative to the putative transcription start site. Consistently, TMPRSS2 mRNA expression is elevated in androgen-stimulated prostate cancer (LNCaP) cells.¹¹⁵ The upregulation of TMPRSS2 mRNA by androgen appears to be mediated by the androgen receptor.³⁹ Androgen treatment is also reported to increase TMPRSS2 zymogen activation in cell culture and in a mouse xenograft model, suggesting that TMPRSS2 may contribute to prostate cancer development and progression in an androgen-dependent manner.

Frequent gene fusions between *TMPRSS2* and members of the E26 transformation specific (ETS) transcription factor family have been identified in many prostate cancer patients.¹²² In these patients, the 5'-untranslated region of *TMPRSS2* is fused with the transcription factor *ERG* or *ETV* genes. As a result, the androgen-responsive promoter elements of *TMPRSS2* drive the expression of the ETS family transcription factors to promote prostate cancer progression and invasion. Among these chromosomal rearrangements, *TMPRSS2-ERG* gene fusions are most frequent, accounting for approximately 50% of prostate cancers. Molecular studies show that abnormal ERG expression disrupts normal androgen receptor signaling and activates epigenetic programs, thereby inducing tumorigenesis.¹²³ Although ERG enhances the expression of many proteases, including urokinase-type plasminogen activator (uPA), matrix metalloproteinase (MMP)-3, and MMP9, TMPRSS2 protease activity is unlikely to have a direct role in ERG downstream events, because the *TMPRSS2* protease domain coding sequence is never included in the fusion genes.¹²⁴ Studies have shown that in prostate cancers, ETS transcription factor genes are also fused to other genes that contain androgen-responsive

elements.¹²⁵ These rearrangements have been attributed recently to androgen-induced co-recruitment of the DNA-processing enzyme topoisomerase 2B to sites of genomic breakpoints.¹²⁶

4. TMPRSS3

TMPRSS3 was identified as a novel serine protease overexpressed in ovarian cancers and was originally named TADG-12 (tumor-associated differentially expressed gene-12).¹²⁷ Subsequent reports showed that this protease was also overexpressed in pancreatic and breast cancers. Independently, the gene encoding TMPRSS3 was identified as a candidate gene located within the disease locus (*DFNB10*) for autosomal recessive deafness,¹²⁸ previously mapped by linkage analysis to chromosome 21q22.¹²⁹ The human *TMPRSS3* is approximately 24 kb in length and contains 13 exons.¹³⁰ The approximately 2.4-kb *TMPRSS3* cDNA encodes a polypeptide of 454 amino acids, with an overall domain structure similar to TMPRSS2 (Fig. 1). In fact, these two proteins share approximately 63% sequence similarities. *TMPRSS3* mRNA is expressed in a variety of tissues, including the kidney, lung, colon, thymus, stomach, and cochlea.¹³⁰

TMPRSS3 mutations have been found in patients with congenital and childhood onset autosomal recessive deafness. Scott *et al.* first identified an 8-bp deletion and insertion of 18 β -satellite tandem repeats in the *TMPRSS3* gene in a Palestinian family with congenital deafness.¹²⁸ In the same study, a second splice site mutation in the *TMPRSS3* gene causing a frameshift was found in a Pakistani family with childhood-onset deafness. To date, deletion, insertion, frameshift, and nonsense *TMPRSS3* gene mutations have been reported in over 20 patient families with nonsyndromic autosomal recessive deafness.¹³⁰ Most mutations occur in exons coding for the LDL receptor repeat, SR repeat, or protease domain, resulting in complete or near complete loss of the protease activity. These studies demonstrate that defects in the *TMPRSS3* gene are responsible for hearing loss in these patient families.

Biochemical mechanisms by which *TMPRSS3* regulates normal hearing remain unknown. Analogous to hepsin, *TMPRSS3* mRNA is expressed in the cochlea.¹³¹ Considering that hepsin null mice have severely impaired hearing, it is possible that the TTSPs participate in a proteolytic pathway required for normal cochlear structure and/or hearing function. ENaC is known to regulate Na^+ levels in the perilymph of the inner ear. Guipponi *et al.* have shown that *TMPRSS3* activates ENaC *in vitro* and that most naturally occurring mutations prevented *TMPRSS3* activation in cell-based experiments.⁴⁰ It is unclear, however, whether ENaC is a physiological *TMPRSS3* substrate in the inner ear. It has been reported that pseudohypoaldosteronism type 1 patients, who are homozygous for mutations in the ENaC α subunit, do not suffer hearing loss,¹³² suggesting that *TMPRSS3* may act on other substrates *in vivo*.

5. TMPRSS4

TMPRSS4, initially called TMPRSS3 and also known as CAP2, was originally identified in pancreatic cancers.¹³³ The gene encoding TMPRSS4 is located on human chromosome 11q23.3. Human TMPRSS4 protein consists of 437 amino acids and a domain structure, as shown in Fig. 1. TMPRSS4 mRNA is expressed in the pancreas, lung, stomach, colon, kidney, skin, and eye.^{131,133} The biological function of TMPRSS4 in these tissues is unknown. Like TMPRSS2, TMPRSS4 is able to activate ENaC when coexpressed in *Xenopus* oocytes^{40,134}, and TMPRSS4 was recently shown to cleave in the inhibitory ENaC γ subunit.¹³⁵ TMPRSS4 activates influenza virus hemagglutinin, suggesting that it may facilitate viral infection in lung tissues.¹³⁶

TMPRSS4 mRNA overexpression has been documented in many cancers, including pancreatic, gastric, colorectal, lung, and thyroid.^{133,137} In lung and colon cancer cells, knockdown of TMPRSS4 expression by siRNA reduced cell proliferation and invasion, whereas TMPRSS4 overexpression promoted epithelial–mesenchymal-like transition in culture and metastasis in nude mice.^{138,139} The effects of TMPRSS4 in cancer cells appeared to be mediated by upregulating integrin $\alpha 5$ and its downstream molecules.¹⁴⁰ These data suggest a role of TMPRSS4 in cancer progression and metastasis, although its proteolytic targets remain unknown.

6. SPINESIN

Spinesin, also known as TMPRSS5, was cloned from a human spinal cord library,¹⁴¹ and named spinesin for spinal cord-enriched trypsin-like protease. The human *TMPRSS5* gene is located on human chromosome 11q23. The spinesin protein domain structure is shown in Fig. 1. On Western blots, human spinesin appeared as a major band of approximately 52 kDa, representing its zymogen form.¹⁴¹ In cerebrospinal fluids, a smaller spinesin band of approximately 50 kDa was detected, suggesting that spinesin may be shed from the cell surface.¹⁴² Recombinant soluble spinesin is active toward peptide substrates with an optimal pH of approximately 10.¹⁴² The functions of spinesin are unknown, although its predominant expression in the brain and spinal cord may suggest that its primary function will be in the central nervous system. More recently, spinesin mRNA was detected in rat inner ear tissues, indicating a possible role in the auditory system.¹³¹

7. MSPL

Mosaic serine protease long-form (MSPL), also known as TMPRSS13, was isolated from a human lung cDNA library.¹⁴³ The human *TMPRSS13* gene has 12 exons and is located on chromosome 11q23.2. Several alternatively spliced forms from this gene have been identified.³⁴ MSPL mRNA is expressed in the

lung, placenta, pancreas, and prostate, and encodes a protease of 581 amino acids (Fig. 1). The recombinant human MSPL migrated at approximately 60 kDa and prefers synthetic peptide substrates with Arg at the P₁ position. MSPL activity is inhibited by aprotinin, benzamidine, and soybean trypsin inhibitor.³⁴ The physiological functions of MSPL remain unknown. Similar to other TTSPs, MSPL was recently shown to induce avian influenza virus replication by activating the viral hemagglutinin, suggesting a potential role in human airway viral infections.¹⁴⁴

B. Matriptase Subfamily

The matriptase subfamily consists of the three highly homologous proteases: matriptase, matriptase-2, and matriptase-3, and a protein with an atypical mosaic structure, polyserase-1 (Fig. 1). All matriptases exhibit a similar stem region with one SEA, two CUB, and three (matriptase-2 and matriptase-3) or four (matriptase) LDLRA domains. In polyserase-1, the transmembrane domain is followed by a single LDLRA domain and a tandem repeat of three serine protease catalytic domains, referred to as serase-1, -2, and -3. Although structurally similar, the members of this TTSP subfamily appear to have quite divergent biological functions.

1. MATRIPTASE

In 1993, Matriptase (also known as MT-SPI, TADG-15, CAP3, epithin, and ST14) was identified as a new gelatinolytic activity in conditioned medium from cultured breast cancer cells.¹⁴⁵ It was molecularly cloned by several groups at the turn of the millennium.^{53,146,147} The matriptase gene, *ST14*, is located on human chromosome 11q24-25 and encodes a polypeptide of 855 amino acids. Orthologs of matriptase are present in all vertebrate genomes examined to date, indicating conserved evolutionary functions.³ Matriptase is the most widely expressed member of the matriptase subfamily and is found in the epithelial compartments of most embryonic and adult tissues.¹⁴⁸⁻¹⁵³ A number of studies in different laboratories have revealed insights into the catalytic properties and unique physiological functions of matriptase.

Matriptase is an 80- to 90-kDa cell surface glycoprotein, with the modular structure illustrated in Fig. 1. Matriptase is synthesized as an inactive, single-chain zymogen and its activation is extraordinarily complex (reviewed in⁴⁴). Matriptase activation requires two sequential endoproteolytic cleavages, the first in the amino-terminal SEA domain and the second within the highly conserved activation cleavage site R↓VVG, in the serine protease domain. Proteolytic autoactivation of matriptase appears to be controlled by the stem region, posttranslational modifications, and the cellular localization of the protease⁴⁴; however, the specific mechanisms that trigger the activation of

matriptase are incompletely understood.¹⁵⁴ The inhibition of activated matriptase by HAI-1 was first documented by the identification of matriptase/HAI-1 complexes in human milk, in conditioned medium of cultured mammary epithelial cells, and in a number of cancer cell lines.⁵⁰

Matriptase is widely expressed in various epithelial cells during mouse development. Matriptase can first be detected on embryonic day (E)10 in the epithelial lining of several tissues of the embryo proper, such as the forming olfactory placode, oral cavity and foregut, intestine, inner ear, and apical ectodermal regions of the limbs.¹⁴⁹ However, matriptase may be expressed even earlier in development, possibly already at the pre- or peri-implantation stage.¹⁵⁵ At E14.5 and E16.5, respectively, matriptase becomes expressed in the developing hair follicles and the interfollicular epidermis.¹⁴⁸ Starting on E8.5, matriptase is also present in chorionic trophoblasts of the mouse placenta, and a similar pattern of expression is also observed in the human placenta.^{149,151} During postnatal life, the highest levels of matriptase expression are found in the epithelium of the gastrointestinal tract, matrix cells of the hair follicles, and the distal and collecting tubules of the kidney, while lower levels of matriptase are present in the granular/transitional layer of the epidermis and in the epithelial compartments of developing teeth, vomeronasal cavity, trachea, bronchioles, thymus, inner ear, gall bladder, urinary bladder, ureter, prostate, seminal vesicle, epididymis, uterus, and oviduct.^{148,152,153} In addition to epithelial tissues, matriptase is also expressed by certain leukocyte populations in humans, including monocytoïd cells and mast cells.^{152,156,157}

Matriptase has emerged to play a critical role in skin formation, in epidermal differentiation and skin function (Fig. 2). Initial analyses of matriptase null mice uncovered a critical function for matriptase in the development of epidermal tissues.^{158,159} Matriptase-ablated mice die shortly after birth, due to a severe dehydration that results from an impaired epidermal barrier function. Follicular structures are also affected by matriptase loss, as shown by the absence of whiskers and generalized hypoplasia of pelage hair follicles of null mice. These phenotypes were linked to defects in the initiation of caspase-14-calpain I, bleomycin hydrolase-mediated processing of the epidermal polyprotein, profilaggrin, into filaggrin monomer units and subsequently, into free hygroscopic amino acids, which partake in the formation of the cornified envelope of the uppermost layers of the epidermis and serve as a source of water binding free amino acids that contribute to skin hydration.¹⁶⁰ Matriptase-deficient epidermis also exhibits a defect in the formation of lamellar granules, specialized secretory vesicles that contain lipid material required for the formation of extracellular lipid lamellae within the cornified layer, and display impaired formation of epidermal tight junctions within the granular layer.^{158,161}

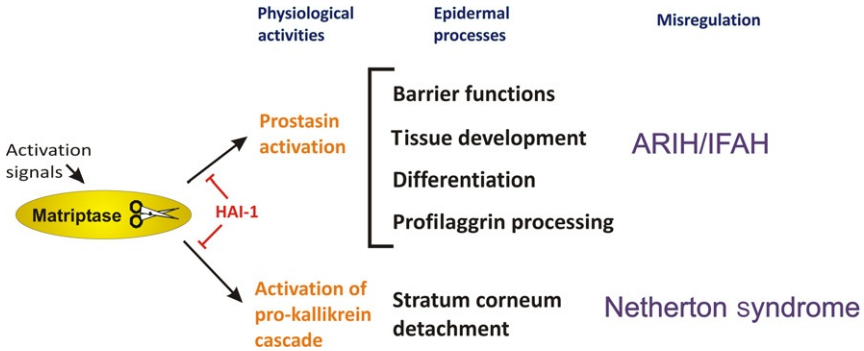


FIG. 2. *Matriptase* is critical for the development and functions of the epidermis. Matriptase activation, triggered by unknown mechanisms in skin, activates the GPI-anchored prostatic zymogen. Prostasin activation is required for epidermal tight junction formation, epidermal lipid synthesis, and induction of caspase-14-calpain I, and bleomycin hydrolase-mediated processing of the epidermal polyprotein, profilaggrin, into filaggrin monomer units, and subsequently, into free hygroscopic amino acids, which participate in the formation of the cornified envelope and contribute to skin hydration. Matriptase deficiency is linked to a rare form of skin disease, referred to as autosomal recessive ichthyosis with hypotrichosis (ARIH), or ichthyosis, follicular atrophoderma, and hypotrichosis (IFAH). Matriptase is also capable of activating proinflammatory pro-kallikrein-related peptidases that are associated with stratum corneum detachment and are responsible for the runaway kallikrein proteolytic cascade associated with LEKTI-deficiency/Netherton syndrome. HAI-1 regulates matriptase in the epidermis.

More recent studies suggest that matriptase is part of an epidermal proteolytic cascade and enables epidermal differentiation through the activation of the GPI-anchored membrane serine protease, prostasin. This is evidenced in part by the identical phenotype of matriptase- and prostasin-deficient mouse skin, and by the absence of active prostasin in matriptase-deficient epidermis.^{150,162} Matriptase and prostasin are found together in a variety of other simple, stratified, and pseudo-stratified epithelia,¹⁶³ suggesting that matriptase may be a candidate activator of the prostasin zymogen in additional physiological settings.

Several recent reports have linked a rare form of skin disease, referred to, respectively, as autosomal recessive ichthyosis with hypotrichosis (ARIH) or ichthyosis, follicular atrophoderma, and hypotrichosis (IFAH), to homozygosity or compound heterozygosity for an assortment of mutations in the human *ST14* gene, encoding matriptase.^{164–166} ARIH/IFAH patients present with mild to moderate ichthyosis, indicative of impaired barrier function, and hair follicle hypoplasia associated with fragile, brittle, dry, and slow-growing scalp hair. All these symptoms resemble transplanted skin from matriptase null mice or skin from matriptase hypomorphic mice.^{158,167} Importantly, prostasin activation is

impaired in ARIH/IFAH epidermis,¹⁶⁵ further supporting the belief that activation of the proastatin zymogen may be a principal function for matriptase during epidermal differentiation.

Matriptase is also linked to the initiation of the Netherton syndrome in a LEKTI-deficient mouse model through the premature activation of an epidermal pro-kallikrein protease cascade.¹⁶⁸ Deficiency in the serine protease inhibitor, LEKTI, is the etiological origin of the Netherton syndrome, and causes detachment of the stratum corneum and chronic inflammation due to excessive kallikrein-related protease activity. Matriptase was demonstrated to initiate activation of proinflammatory pro-kallikrein-related peptidases that are associated with stratum corneum detachment. Moreover, ablation of matriptase from LEKTI-deficient mice dampened inflammation, eliminated aberrant protease activity, prevented detachment of the stratum corneum, and improved the barrier function of the epidermis, demonstrating that the runaway kallikrein proteolytic cascade associated with LEKTI-deficiency was dependent on matriptase activity.

The wide expression of matriptase in epithelial tissues, including simple epithelia of most organs, suggested that matriptase could have functions in epithelial biology beyond the epidermis, but attempts to investigate this notion were initially hampered by the perinatal lethality of matriptase null mice. The subsequent generation of viable matriptase hypomorphic mice¹⁶⁷ and matriptase conditional knockout mice¹⁶¹ afforded the opportunity to explore the function of matriptase in global epithelial biology. Tissue-specific embryonic and postnatal ablation of matriptase from a variety of epithelial tissues, including orofacial epithelium, salivary gland epithelium, lacrimal gland epithelium, and the epithelium of stomach, and small and large intestine, in all cases were associated with severe epithelial dysfunction, with two distinct phenotypes emerging: (a) loss of epithelial function, such as barrier and secretory capacity, but preservation of epithelial anatomy (salivary glands, tear glands, stomach, small intestine) and (b) loss of epithelial function, followed by loss of anatomical integrity (skin, orofacial, and colonic epithelium). In all epithelia, the absence of matriptase uniformly was associated with the rapid loss of epithelial tight junction function, compatible with the localization of matriptase on the lateral membrane of polarized epithelial cells.^{161,169} In polarized monolayers of epithelial cells cultured *ex vivo*, down-regulation of matriptase decreased transepithelial electrical resistance and increased paracellular diffusion of macromolecules. Decreased matriptase was associated with enhanced expression and incorporation of the permeability-associated, “leaky” tight junction protein, claudin-2, at intercellular junctions, suggesting that the reduced barrier integrity was caused, at least in part, by an inability to regulate claudin-2 expression and incorporation into tight junctions.¹⁶⁹

Matriptase is nearly ubiquitously coexpressed with the Kunitz-type transmembrane serine protease inhibitors, HAI-1 and HAI-2, in adult and embryonic tissues^{56,149,151,155,170–172}, and both HAI-1 and HAI-2 display potent matriptase inhibitory activity in purified systems.^{36,56,170,173,174} Knockout studies have revealed that HAI-1 and HAI-2 are both required for the completion of embryonic development in mice.^{149,151,155,175} However, both HAI-1/matriptase double-deficient and HAI-2/matriptase double-deficient mice complete embryonic development, thereby revealing matriptase as a principal inhibitory target for both protease inhibitors.^{149,155} Loss of HAI-1 inhibition of matriptase in mice leads to failure of the chorionic epithelium to differentiate into the placental labyrinth, which prevents embryonic development beyond mid-gestation,¹⁴⁹ whereas other developmental processes proceed normally. HAI-2 inhibition of matriptase, however, is essential at three distinct developmental processes: (a) early (< E8.5) embryonic development, which proceeds normally in HAI-2 null embryos in the absence, but not in the presence of matriptase; (b) placental development, where the failure of the placental labyrinth to undergo branching morphogenesis in HAI-2 null embryos can be prevented by loss of matriptase; and (c) neural tube closure, in which the development of exencephaly and spina bifida is partially rescued by the matriptase deficiency.¹⁵⁵ The functional interactions between matriptase, HAI-1, and HAI-2 during development appear to be uniquely complex for a protease–protease inhibitor system. Thus, not only the complete loss of HAI-1 or HAI-2 but also the combined haploinsufficiency for both inhibitors causes embryonic lethality (nonallelic noncomplementation), which can be prevented by haploinsufficiency for matriptase, suggesting that the two inhibitors functionally cooperate in regulating the activity of matriptase.¹⁵⁵

The requirement for matriptase regulation by HAI-1 is not restricted to development. Thus, the loss of HAI-1 from adult tissues is associated with fatal epithelial dysfunction in mice,¹⁷⁶ but HAI-1-deficient mice in which the level of matriptase is genetically reduced by 85–99% are fertile, healthy, have normal long-term survival, and possess histologically unremarkable epithelia. Similar findings were obtained from studies in zebrafish, where the combined loss of function of the two HAI-1 encoding genes compromises the integrity of embryonic epidermal keratinocytes and causes skin inflammation and embryonic death 18–26 h postfertilization. All of these defects can be fully rescued by a simultaneous downregulation of matriptase.¹⁷⁷

Matriptase has received significant attention in the field of cancer biology due to its remarkably consistent expression in tumors of epithelial origin. Following the first description of matriptase as a major gelatinolytic activity in breast carcinoma,^{145,178} the protease was reported to be expressed in a wide variety of other benign and malignant tumors of epithelial origin, including prostate, ovarian, cervical, gastric, colon, renal cell, esophageal, oral squamous

cell carcinoma (SCC), and malignant pleural mesothelioma (reviewed in Ref. 179). In most carcinomas, malignant progression is associated with a significant increase in matrilysin mRNA or protein expression. For example, in ductal mammary carcinoma, elevated levels of matrilysin correlate with both tumor and nodal staging.¹⁸⁰ A clinical study in node-negative breast cancer patients showed a tight correlation between the expression of matrilysin, cMet, and HAI-1, and poor patient outcome.¹⁸¹ Likewise, more than fivefold overexpression of matrilysin was detected in a study of ovarian cancer, compared to normal ovarian tissues, and this increased expression of matrilysin correlated with tumor aggressiveness.^{182,183} Furthermore, although matrilysin was more frequently expressed in stage I/II tumors than in more advanced-stage III/IV tumors, an increased matrilysin/HAI-1 ratio was indicative of the poor clinical outcome of advanced-stage tumors, suggesting that loss of protease inhibition may play a role in the late stages of the disease.¹⁸⁴ In prostate and cervical carcinoma, a several-fold increase in matrilysin expression was reported for malignant versus nonmalignant tissues, and this correlated with Gleason score and histopathological grade, respectively, of the tumors.^{185,186} Other common human cancers with significantly elevated matrilysin levels include carcinomas of the pancreas, lung, kidney, and liver.¹⁷⁹ In contrast, significant downregulation of matrilysin and HAI-1 has been detected in the samples of gastric and colorectal carcinoma.¹⁷² However, an analysis of the clinicopathological parameters in colorectal adenoma and carcinoma tumors showed an increased matrilysin/HAI-1 ratio in the tumors compared to the corresponding tissue from control individuals.¹⁸⁷ This finding may suggest that in tissues of the gastrointestinal tract, which exhibit high expression of matrilysin and HAI-1,^{152,188,189} the tumorigenic process does not involve further elevation in the expression of the two proteins, but rather may be associated with altered levels of an HAI-1-free, uninhibited form of matrilysin. SCC of the skin represents a second example of matrilysin being dysregulated, not by overexpression, but rather by the translocation of the expression of the protease from a differentiated postmitotic compartment in the epidermis to a basal compartment with high tumorigenic potential.¹⁴⁸

Matrilysin is unique among pericellular proteases in that it possesses a strong oncogenic potential when misexpressed in an epithelial tissue.¹⁷³ Even low-level matrilysin expression in basal keratinocytes of transgenic mice suffices to induce severe hyperproliferation of epidermal keratinocytes, which gradually progresses to invasive SCC. Both the epidermal hyperproliferation and the formation of matrilysin-induced skin tumors are completely abolished by coexpression of HAI-1, demonstrating that the proteolytic activity of matrilysin is critical to its oncogenic potential. The molecular mechanisms of matrilysin-induced epithelial carcinogenesis remain to be determined. In cell-free or cell-based assays, matrilysin can activate several proteins that have been previously associated with malignant progression, including pro-hepatocyte

growth factor/scatter factor (pro-HGF),^{29,190} pro-macrophage stimulating protein-1 (pro-MSP-1),¹⁹¹ pro-urokinase plasminogen activator (pro-uPA),^{29,190} protease activated receptor-2 (PAR₂),^{29,192} and the src-associated transmembrane protein SIMA135/CDCP1.^{193,194}

2. MATRIPTASE-2

Matriptase-2, also called *TMPRSS6*, was independently cloned from liver tissues by two groups in 2002 and 2003 and shown to express a membrane serine protease with homology to matriptase that displayed proteolytic activity toward various macromolecular substrates.^{37,195} Matriptase-2 is encoded by the *TMPRSS6* gene located on human chromosome 22q12.3. Matriptase-2 is approximately 90-kDa cell surface glycoprotein with a modular structure (Fig. 1) and is synthesized as an inactive, single-chain zymogen. Cell surface matriptase-2 is efficiently shed into the conditioned medium of transfected cells in an active two-chain form by proteolytic cleavage within the second CUB domain of the noncatalytic stem region.¹⁹⁶ Whereas matriptase is expressed in a large number of embryonic and adult epithelia, matriptase-2 expression is largely confined to adult and fetal liver in humans and mice, with minor expression in the kidney, uterus, and nasal cavity.^{37,195}

A breakthrough in the understanding of the physiological function of matriptase-2 was enabled by the generation of matriptase-2 knockout mice and by the identification of loss of function mutations in the *TMPRSS6* gene as a cause of the human autosomal recessive disorder, iron-refractory iron deficiency anemia (IRIDA). Both mice and humans with matriptase-2 deficiency suffer from very low iron levels and severe microcytic anemia.¹⁹⁷⁻¹⁹⁹ Matriptase-2 expressed by liver cells functions as a suppressor of the hepatic hormone, hepcidin, which in turn internalizes the iron export protein, ferroportin, on enterocytes and macrophages to reduce iron uptake. Thus, matriptase-2 is a key regulator of systemic iron hemostasis. Hepcidin suppression by matriptase-2 appears to occur at the transcriptional level, as hepcidin mRNA levels are elevated in both matriptase-2-deficient humans and mice.¹⁹⁷⁻¹⁹⁹ This suppression of hepcidin gene transcription has been linked to matriptase-2-mediated degradation of hemojuvelin, a cofactor for bone morphogenetic protein, and a key regulator of hepcidin gene activation.^{200,201}

3. MATRIPTASE-3

Matriptase-3 was identified by bioinformatic analysis in 2005 as a protease with high homology to matriptase.²⁰² The *TMPRSS7* gene encoding matriptase-3 is located on human chromosome 3q13.2 and encodes an approximately 90-kDa glycosylated protein detected on the cell surface.²⁰² Orthologs of the matriptase-3 gene are present in all vertebrates analysed to date, including fish,

birds, rodents, canines, and primates. The pattern of expression of matriptase-3 is conserved in mice and humans, with the highest levels of mRNA expression in the brain, skin, eye, salivary gland, and the reproductive tissues, including prostate, testis, epididymis, ovary, and uterus. The recombinant matriptase-3 catalytic domain can hydrolyse synthetic peptide substrates with a strong preference for arginine in the P₁ position, and shows proteolytic activity toward several macromolecular substrates, including gelatin, casein, and albumin.²⁰² The activated matriptase-3 catalytic domain forms stable inhibitor complexes with an array of serpins, including PAI-1, PCI, α 1-proteinase inhibitor, α 2-antiplasmin, and antithrombin III *in vitro*. Matriptase-3 loss of function studies in animals or humans with matriptase-3 deficiency has not been reported, and the physiological function of the protease remains to be determined.⁴

4. POLYSERASE-1/SERASE1B

Polyserase was cloned from a human liver cDNA as a unique type II transmembrane serine protease with three serine protease domains, two of which display catalytic activity.²⁰³ mRNA capable of encoding full-length human polyserase-1 is detected predominantly in the skeletal muscle, heart, kidney, liver, placenta, and the brain. In addition, a shorter splice variant, termed serase-1B, which contains only the first of the three serine protease domains of polyserase-1, has been described in mice and humans, with its highest expression detected in the liver, small intestine, pancreas, testes, and the peripheral blood CD14+ and CD8+ cells.^{35,203} Recombinant serase-1B shows proteolytic activity toward synthetic peptide substrates, converted pro-uPA into active uPA, and could be inhibited by the serpins, aprotinin, α 2-antiplasmin, and PAI-1.³⁵

The human polyserase gene, called *TMPRSS9*, is located on human chromosome 19p13.3. The 5' promoter region of the mouse polyserase-1 gene has been characterized and contains a GATA motif, a glucocorticoid responsible element, and an E-box sequence required for maximal promoter activity.²⁰⁴ The physiological function of polyserase has not been elucidated.

C. Corin Subfamily

Corin, the single member of the corin subfamily, is characterized by a complex stem region composed of two frizzled domains, eight LDLRA domains, and one Group A SR domain (Fig. 1).

1. CORIN

Human corin cDNA was originally cloned from the heart. In a search for novel serine proteases in the cardiovascular system, Yan *et al.* identified a partially expressed sequence in a genomic database and subsequently cloned

the full-length cDNA encoding corin.²⁰⁵ The protease was named corin for its abundant cardiac expression. Independently, Hooper *et al.* isolated human corin cDNA from HeLa-derived cells that were resistant to tumor necrosis factor- α -induced apoptosis.²⁰⁶ Corin was also cloned from a mouse heart library as a novel protein containing LDL receptor-like repeats, and was named LRP4 (LDL receptor-related protein 4).²⁰⁷

The human *CORIN* gene is present on chromosome 4p12-13, consists of 22 exons, and spans approximately 200 kb.²⁰⁸ Corin is highly expressed in fetal and adult cardiomyocytes. Low levels of corin mRNA are detected in the kidney, bone, brain, skin, and pregnant uterus.¹⁰⁹ In both human and mouse corin genes, there is a 5'-flanking region that contains conserved binding sites for TBX5, GATA, NKX2.5, NF-AT, and Kruppel-like transcription factors.²⁰⁸ Among them, a conserved GATA-binding site is critical for interacting with GATA-4 transcription factor that is important for heart-specific gene expression. GATA-4 protein is known to act downstream of the calcineurin/NF-ATc3 signaling pathway in cardiomyocytes. Most likely, this signaling pathway is involved in regulating corin expression in the heart.

The full-length human corin cDNA encodes a polypeptide of 1042 amino acids with a calculated molecular mass of approximately 116 kDa and a domain structure shown in Fig. 1. The presence of two frizzled-like cysteine-rich domains in the corin extracellular region is unique among the TTSPs.⁴ Native and recombinant human, rat, and mouse corin proteins appear as bands of approximately 150–200 kDa on immunoblots, largely due to N-glycosylation which has been confirmed by glycosidase digestion.⁹⁹ Human corin has 19 predicted N-linked glycosylation sites in its extracellular region, whereas rat and mouse corin has 17 N-glycosylation sites. Corin does not contain detectable amounts of O-glycans or sialic acids.^{209,210}

Corin is synthesized as a zymogen with no detectable activity in functional assays, indicating that cleavage at Arg⁸⁰¹ within the conserved activation site, R↓ILGG is necessary for its activity.²¹¹ A small fraction of activated corin is detected in transfected HEK 293 cells and cardiomyocytes²¹⁰; however, the enzyme(s) responsible for corin activation in these cells have not been determined. Recombinant corin with an engineered activation site has a trypsin-like catalytic activity, favoring peptide substrates with Arg/Lys residues at P₁, Pro/Phe/Gly at P₂, and small neutral amino acids at the P₃ position.²¹¹ The activity is inhibited by leupeptin, aprotinin, and soybean trypsin inhibitor and, unlike many serine proteases, corin activity is not inhibited in the presence of human plasma.²¹¹

The topology of corin on the cell membrane has been confirmed by cell surface protein labeling and protease digestion.^{99,209} In addition to the transmembrane domain, N-glycans on corin also play a role in its cell surface targeting and activation.^{210,212} Inhibition of N-glycosylation or removal of

certain N-glycosylation sites by site-directed mutagenesis impaired corin cell surface expression and zymogen activation. The transmembrane domain is not necessary for corin enzyme activity, since soluble corin without the transmembrane domain exhibited similar activities toward small peptide and biological substrates when compared to that of wild-type corin.²¹¹

Wu *et al.* identified corin as the physiological pro-atrial natriuretic peptide (pro-ANP) convertase.²¹³ ANP, also called atrial natriuretic factor (ANF), is a cardiac hormone that regulates blood pressure and cardiac function by promoting natriuresis, diuresis, and vasodilation. The function of ANP is mediated by its receptor, NPR-A, which promotes intracellular cGMP production. In mice, deficiency in ANP or its receptor causes spontaneous hypertension, demonstrating the importance of the ANP pathway in regulating blood pressure.²¹³ In humans, SNPs and mutations in the *NPPA* gene encoding ANP have been reported in patients with hypertension and heart disease.²¹⁴

In cardiomyocytes, ANP is made as a precursor molecule, pro-ANP. Upon secretion under high blood volume or pressure, pro-ANP is converted to active ANP by proteolytic cleavage. This activation mechanism was recognized for many years, but the responsible enzyme remained unidentified. In cell-based studies, corin activated pro-ANP in a sequence-specific manner.²¹⁵ This activity was confirmed in cardiomyocytes and with purified recombinant corin. Chan *et al.* made corin null mice, which were viable and fertile.²¹⁶ In these mice, lack of corin prevents pro-ANP processing in the heart and causes salt-sensitive hypertension. Corin null mice develop cardiac hypertrophy and exhibit impaired cardiac function. This hypertrophic heart phenotype has been confirmed in a naturally occurring mutant mouse strain, *Kit*^{W-sh}, in which the *corin* gene is disrupted by genetic inversion.²¹⁷ These data show that corin-mediated pro-ANP processing is critical for regulating blood pressure and cardiac function (Fig. 3). Curiously, corin null mice appear to have a lighter coat color, and this phenotype depends on the *agouti* gene.²¹⁸ As corin mRNA and protein are expressed in the dermal papilla in mice, corin may act in the skin to regulate hair color formation by an agouti pathway-dependent mechanism.

In addition to ANP, BNP (B-type natriuretic peptide) and CNP (C-type natriuretic peptide) are additional members of the human natriuretic peptide family, which share similar protein sequences and structures. The function of BNP is to promote natriuresis and diuresis, whereas CNP is involved in endothelial cell growth and chondrocyte differentiation. In cell-based studies, corin also cleaved pro-BNP, although the reaction was less efficient than that for pro-ANP.²¹⁵ Recent studies indicate that both furin and corin convert pro-BNP to BNP.²¹⁹ In contrast, pro-CNP is processed by furin but not corin.²²⁰ It is interesting that these natriuretic peptides have evolved from an ancestor gene, but their activation processing requires different enzymes.

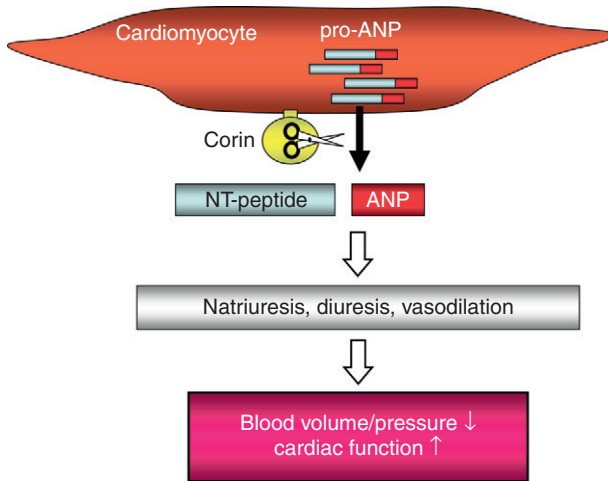


FIG. 3. *Corin-mediated pro-ANP processing in the heart.* In cardiomyocytes, ANP is made as a precursor, pro-ANP. Upon secretion, pro-ANP is cleaved by corin, generating an inactive N-terminal (NT) peptide and an active ANP. ANP promotes natriuresis, diuresis, and vasodilation, thereby reducing blood volume/pressure and improving cardiac function.

Corin variants exist in hypertensive patients. Dries *et al.* have identified two SNPs (T555I/Q568P) in the human *CORIN* gene.²²¹ These SNPs are located on a minor corin gene allele that is present primarily in African-Americans with hypertension. It is known that African-Americans have a high prevalence of cardiovascular disease, but the underlying mechanisms remain unclear. Rame *et al.* have shown that individuals with these corin SNPs have an enhanced cardiac response to high blood pressure, which leads to severe left ventricular hypertrophy.²²² Under pathological conditions, cardiac hypertrophy impairs heart functions. In patients with heart failure, the presence of this corin variant allele is associated with worse clinical outcomes such as hospitalization and death.²²³ Changes in the amino acids, T555I/Q568P, encoded by the SNPs occur within the second frizzled-like domain, an extracellular region demonstrated to be functionally important.⁴⁶ Examination of the effect of these amino acid substitutions on corin function in cell-based studies showed that the corin variant, T555I/Q568P, exhibited markedly reduced activity in processing pro-ANP and pro-BNP.²²⁴ Apparently, the reduced activity was caused by impaired corin zymogen activation but not cell surface expression.²²⁴ The data indicate that these polymorphisms alter corin structure and function, which may contribute to elevated blood pressure and cardiac hypertrophy in patients. Thus, corin defects may present an important mechanism underlying cardiovascular disease.

Soluble corin has been detected in human plasma, suggesting that corin is shed from the cell surface and subsequently circulates in the blood.²²⁵ Interestingly, plasma corin levels were significantly lower in patients with heart failure and the reduction correlated with the disease severity.²²⁶ In contrast, patients with acute myocardial infarction had similar levels of plasma corin compared to that of normal controls, suggesting that low plasma corin levels are associated more closely with pathological changes in heart failure than that in acute ischemic cardiac injury. The protease(s) responsible for corin shedding have not been determined. Low levels of plasma soluble corin in patients with heart failure may reflect reduced corin production, accelerated clearance of plasma corin, and/or downregulation of corin shedding in failing hearts. Additional studies will be required to elucidate corin shedding mechanisms under physiological and pathological conditions, and whether decreased plasma corin could be used as a biomarker for the diagnosis of heart failure.

D. HAT/DESC Subfamily

The HAT/DESC (human airway trypsin-like protease/differentially expressed in squamous cell carcinoma) subfamily comprises five members in humans, HAT, DESC1, TMPRSS11A, HAT-like 4/TMPRSS11F, and HAT-like 5, and two additional members in rodents, HAT-like 2/DESC4 and HAT-like 3.^{3,4} All genes encoding members of this subfamily are located within a single gene cluster on chromosome 4q in humans (5 E1 in mice), suggesting that they originated by gene duplication from a common ancestor.²²⁷ Of all TTSPs, members of the HAT/DESC subfamily exhibit the simplest modular structure of the stem region, which consists of a single SEA domain located adjacent to the cell surface. SEA domains are structurally homologous protein modules that have been identified in many mucin-like proteins. These domains undergo spontaneous posttranslational confirmation-driven hydrolysis of the glycine-serine peptide bond within a conserved GSVVV sequence, although the two peptides remain tightly associated.²²⁸ Little is known about most of the members of the HAT/DESC subfamily, with HAT, DESC1, and TMPRSS11A being the best characterized.

1. HAT

HAT was originally characterized as a novel 28-kDa protease from the sputum of patients with chronic airway diseases.²²⁹ The cDNA was subsequently cloned and the protease was named HAT for human airway trypsin-like protease.²³⁰ HAT cDNA encodes an integral membrane protein of 418 amino acids, with a predicted molecular mass of approximately 46-kDa, which is released in a soluble form from the cell surface.²³⁰ Hence, significant HAT-related activity can be detected in the extracellular airway fluids of patients with

chronic airway inflammatory diseases, particularly in patients with asthma.^{229,231,232} Within human tissues, the airways and the skin have been shown to express HAT.^{233,234} In the airways, HAT is expressed in the apical cilia layer of tracheobronchial epithelial cells.^{234,235}

There is little information regarding the physiological functions and candidate proteolytic targets of HAT; however, HAT upregulation is found to be associated with inflammatory environments.²³³ The available evidence, obtained from the study of *in vitro* cleavage reactions and cell-based assays, supports a potential role for HAT in respiratory homeostasis during inflammatory responses through a capacity to increase mucin gene expression, control fibrin deposition, and stimulate bronchial fibroblast proliferation in airway epithelial cells *in vitro*.^{231,235,236} HAT has been demonstrated to have the capacity to degrade fibrinogen, to activate pro-uPA,^{232,235} to activate membrane receptors such as PAR-2,^{232,235,236} and to cleave the D1–D2 linker sequence of human uPAR (h-uPAR), which prevents interaction with the extracellular matrix component, vitronectin.²³⁷ The physiological relevance of these cleavages, if any, remains untested.

HAT is one of the several TTSPs found to cleave the surface glycoprotein hemagglutinin (HA) of the influenza virus.^{118,238} HA is responsible for initiating influenza virus replication by mediating binding to sialic acid-containing cell surface receptors and fusion of the viral envelope with the endosomal membrane. HA precursor proteins require cleavage at a single arginine amino acid to trigger membrane fusion. Membrane-bound HAT was shown to cleave newly synthesized HA before or during the release of progeny virions as well as HA of incoming viruses prior to endocytosis at the cell surface, supporting its contribution to the facilitation of virus activation and spread.

2. DESC1

Differentially expressed in squamous cell carcinoma (DESC1) was originally identified by representational difference comparative analysis of RNA from SCC of the head and neck compared with matched normal tissue.²³⁹ DESC1 is expressed in normal epithelial cells of prostate, skin, testes, head, and neck, whereas it is downregulated in 11/12 SCCs,²³⁹ suggesting that it may serve as a tumor marker. The human *DESC1* gene encodes a polypeptide of 442 amino acids. The mouse ortholog of DESC1 shares 72% identity with human DESC1.²²⁷ Both proteases are expressed in similar anatomical locations and are predicted to have common functions in the development and maintenance of oral epidermal tissues and the male reproductive tract.²²⁷

The physiological functions of DESC1 are not known. DESC1 is upregulated during the induction of terminal keratinocyte differentiation.²⁴⁰ Madin-Darby canine kidney (MDCK) cells expressing exogenous human DESC1 display enhanced motility and an increase of tubular forms in a

three-dimensional collagen lattice following HGF treatment.²⁴¹ Kinetic studies using internally quenched peptides^{227,242} and structural analyses of the DESC1 catalytic domain²³ reveal DESC1 substrate preference for large hydrophobic residues in P₄/P₃, for small residues in P₂, Arg or Lys in P₁, and hydrophobic residues in P₁' and P₃'. Mouse DESC1 forms stable inhibitory complexes with PAI-1 and PCI, suggesting that these serpins might be regulators of DESC1 proteolytic activities in DESC1 expressing tissues.²²⁷

3. Tmprss11a

Most of the remaining members of the HAT/DESC family have not yet been well characterized. HATL1, also known as Tmprss11a, is expressed in the upper respiratory tract (pharynx and trachea) and has been shown to have the capability to cleave recombinant, native, full-length S-protein trimer (triS-pike) of the severe acute respiratory syndrome, coronavirus (SARS-CoV) *in vitro*.²⁴³

VI. The GPI-Anchored Serine Proteases

A. Prostasin

Prostasin, also known as PRSS8 and channel-activating protease (CAP)-1, was originally identified and characterized from human seminal fluid.¹¹ Subsequent studies using a *Xenopus* oocyte expression system and mammalian epithelial cell cultures independently showed that prostasin expression increased amiloride-sensitive ENaC current.^{134,244–246} As a result, prostasin became the first membrane-anchored serine protease to be implicated in the modulation of fluid and electrolyte regulation via proteolysis of ENaCs.

The *PRSS8* gene encoding prostasin belongs to the family of genes located on the syntenic regions of human chromosome 16p13.3 and mouse chromosome 17^{14,247–249}, and this family includes genes encoding both testisin and tryptase γ 1.⁹ The mouse frizzy (*fr*) and rat “hairless” (*fr*(CR)) mutations are natural variants of the murine prostasin gene (*Prss8*). The deduced amino acid sequence of *PRSS8* predicts a preproenzyme consisting of 343 amino acids, which shows 34–42% identity to human acrosin, plasma kallikrein, and hepsin. Prostasin is posttranscriptionally modified with GPI-anchor^{11–13} and associates with lipid rafts.¹³ In polarized epithelia, prostasin localizes to apical membranes.^{12,250,251} Soluble forms of prostasin are found in human urine^{252,253} and are elevated in hypertensive patients.²⁵⁴ Prostasin has been found to be released from the cell surface by an endogenous GPI-specific phospholipase D1¹³ or, alternatively, may be shed via a tryptic-like proteolytic cleavage in its hydrophobic C-terminal domain.^{11,255}

Tertiary structures of prostaticin^{24–26} and analyses for substrate preference using positional scanning combinatorial substrate libraries³² have revealed insights into prostaticin's unique catalytic activity. Prostaticin was found to have a substrate preference for polybasic substrates: in position P₄, preference was for arginine or lysine; in P₃, for histidine, lysine, or arginine; in P₂, for basic or large hydrophobic amino acids; and in P₁, for arginine and lysine.³² Prostaticin showed no activity with substrates containing isoleucine in position P₁', providing an explanation for the inability of prostaticin to autoactivate. Prostaticin activity was also highly influenced by mono- and divalent metal ions, which were potent inhibitors and substrate specific modulators of enzymatic activity. Interestingly, structural analyses showed that the S₁ subsite loop of prostaticin exhibits a large degree of conformational variation and directly binds the divalent cation, Ca²⁺, being able to move to block or to expose the S₁ subsite.²⁴

The prostaticin zymogen is proteolytically cleaved to an active enzyme by matriptase *in vitro* and *in vivo* in skin¹⁵⁰ and when coexpressed with hepsin in cultured cells.⁹⁶ Low levels of plasmin found in urine from patients with nephritic syndrome may also activate prostaticin in the context of ENaC activation.²⁵⁶ Intriguingly, the zymogen form of matriptase was also able to be converted to an active protease by the addition of active prostaticin, suggesting that, in certain cellular contexts, prostaticin might function both upstream and downstream of matriptase.¹⁹² Comparison of the skin phenotypes of conditional prostaticin-deficient mice with matriptase-deficient mice supports the participation of both serine proteases in the same protease-signaling cascade in the skin.¹⁶² Antithrombin III and protease nexin I are inhibitors of cell surface-associated activities of prostaticin²⁵⁷, and protease nexin I is an effective inhibitor of prostaticin-mediated activation of ENaC.²⁵⁷ Prostaticin is also inhibited by the Kunitz-type inhibitors, HAI-1/SPINT1^{31,48,57,58} and HAI-2/SPINT2,^{32,258} similar to matriptase.

The data suggest that the "open probability" of ENaCs is increased by prostaticin, resulting in an increased cellular uptake of sodium (Na⁺) which can, in turn, regulate the homeostasis of extracellular fluid volume, blood pressure, and Na⁺ reabsorption.²⁵⁹ Prostaticin cleaves the ENaC γ subunit in the extracellular loop at a site (K186) distal to a furin cleavage site, and releases a 43-amino acid inhibitory peptide that results in increased open probability of the channel and full activation.^{260,261} Recent studies have localized this inhibitory peptide to a key 11-mer tract, R158-F168 (RFLNLIPLLVF), which is capable of inhibiting wild-type ENaC expressed in *Xenopus* oocytes and endogenous channels in airway epithelial cells.²⁶² The channel-activating activity of prostaticin is likely to be of pathophysiological significance. In the lung, adequate levels of ENaC activity are essential for controlling airway surface liquid volumes. Mice with targeted disruption of the prostaticin gene in lung epithelium show impaired ENaC-mediated alveolar fluid clearance.²⁶³ In addition, increased ENaC activation in the airway of cystic fibrosis patients has been linked to

excessive prostatic activity on the surface of lung epithelium.^{264,265} Prostatic has been identified as a candidate gene for the development of hypertension in youths,²⁶⁶ which may also be associated with increased ENaC activation.²⁶⁷ Increased levels of urinary prostatic are found in hypertensive patients,²⁵² and inhibition of prostatic in a rat model of hypertension significantly reduced blood pressure.²⁶⁸ Inhibition of prostatic-dependent activation of ENaCs is considered a potential therapeutic strategy for modulating surface liquid volume in cystic fibrosis and salt-sensitive hypertension.

Prostatic also exhibits pleiotropic activities associated with the activation of growth factors, G protein-coupled receptors, and the activation of proteolytic cascades.^{269–271} Less well-characterized potential targets of prostatic include PAR₂ and the epidermal growth factor receptor.²⁶⁹ Soluble recombinant prostatic has also been reported to activate PAR₂ indirectly, through a matriptase-dependent mechanism.¹⁹² Prostatic activity is further implicated in the regulation of epithelial barrier function,¹³ which is believed to be unrelated to ENaC processing activity, suggesting that prostatic may also target as yet unidentified substrates involved in the regulation of epithelial barrier integrity and permeability. Downregulation of prostatic is associated with tumor progression^{269,272,273} through as yet unknown mechanisms.

B. Testisin

Testisin was originally cloned from human eosinophils and from HeLa cervical carcinoma cells.^{15,274} Human testisin has been known as eosinophil serine protease 1 (esp-1)²⁷⁴ and murine testisin²⁴⁷ has been called tryptase 4²⁴⁸ and TESP5.¹⁴ The testisin gene (*PRSS21*) belongs to the family of genes on the syntenic regions of human chromosome 16p13.3 and mouse chromosome 17.^{9,14,247–249} Several isoforms of human testisin have been identified, which are believed to be generated by alternate intron–exon junctional sliding.^{249,275} Testisin is abundantly expressed by male germ cells and sperm,^{15,247,276} and is also present in capillary endothelial cells²⁷⁷ and in eosinophils.^{248,274}

Analyses of the promoter sequences of the human and mouse genes encoding testisin have revealed that the region surrounding the transcription initiation site lacks a TATA consensus sequence, but contains a CCAAT sequence and includes a 385-bp 5'-CpG island.^{247,249} There is a strong correlation with DNA hypermethylation of the 5'-CpG rich island and silencing of testisin gene expression.²⁷⁸ Furthermore, the 5'-flanking region of the testisin gene contains several consensus response elements, including Sp1, AP1, and several testis-specific elements.^{247,249,275}

Testisin is posttranscriptionally modified by the addition of a carboxy-terminal GPI-membrane anchor and has been demonstrated to be compartmentalized at plasma membranes within the dynamic microenvironment of cholesterol-rich membrane microdomains or lipid rafts.¹⁴ Naturally occurring

and recombinant testisin proteins are cell-associated,^{14,15,248} but may be released from cell membranes by exposure to exogenous bacterial phosphatidylinositol-specific phospholipase C (PI-PLC).¹⁴

Developmental evidence links testisin to the maturation and function of mammalian sperm. Testisin is detected on spermatogenic cells throughout spermatogenesis and on mature sperm in humans,²⁷⁶ mice,^{14,247,276} and rats,²⁷⁹ which suggests a similar function among these mammalian species. Mice deficient in testisin display several functional abnormalities associated with epididymal sperm maturation and fertilizing ability.^{276,280} Epididymal sperm lacking testisin show an increased tendency toward decapitation, heterogeneity in sperm form and angulated flagella, decreased numbers of motile sperm, and abnormal sperm volume regulation. Although testisin-deficient mice exhibit normal fertility when bred by continuous mating, these functional sperm defects result in a decreased ability to fertilize oocytes in short-term mating studies.²⁷⁶ The data suggest an essential requirement for testisin during sperm cell maturation processes which occur during epididymal transit and are required for normal fertilizing ability. Functional overlap between testisin and the related fertilization serine protease, acrosin, may compensate for respective functional fertilizing abilities, since acrosin-deficient mice exhibit normal fertility, but acrosin/testisin double knockout mice are subfertile.²⁸¹ It has been proposed that aberrant regulation of testisin may contribute to certain secondary male infertility syndromes such as “easily decapitated” spermatozoa in humans.²⁷⁶

Absence of testisin expression is associated with testicular tumor formation and progression¹⁵, and overexpression of testisin promotes advanced-stage disease in ovarian carcinomas.²⁸² Epigenetic gene silencing may contribute to the downregulation of both prostatic and testisin expression in cancers.^{272,278,282–284}

VII. Perspectives

Over the past few decades, membrane-anchored serine proteases have emerged to play key roles in many diverse aspects of mammalian physiology, including food digestion, fluid balance, blood pressure regulation, hearing and inner ear development, epithelial barrier function, sperm maturation, and regulation of iron homeostasis (Table I). The associations of mutant alleles of the membrane-anchored serine proteases with disease, the misregulation of these enzymes in malignant growth, and the hijacking of their activities by viruses to facilitate infection and propagation, all highlight the pathological impact of misregulation of their activities. Despite remarkable progress in the past few years, many questions remain unanswered. How these proteases

TABLE I
MEMBRANE-ANCHORED SERINE PROTEASE PHYSIOLOGICAL FUNCTIONS IN MAMMALIAN DEVELOPMENT
AND TISSUE HOMEOSTASIS

Enteropeptidase	Proteolytic activation cascade in intestine
Hepsin	Cochlear development and hearing (mice)
TMPRSS3	Hearing
TMPRSS5	Hearing
Matriptase	Epidermal and hair follicle development and epithelial barrier functions
Matriptase-2	Regulates iron hemostasis; deficiency or mutation increases hepcidin levels, leading to iron-refractory iron deficiency anemia (IRIDA)
Corin	Processes critical cardiac pronatriotic peptides involved in the regulation of blood pressure
Prostasin	Epidermal and hair follicle development and epithelial barrier functions; regulation of ENaC-mediated alveolar sodium and water transport (mice)
Testisin	Subfertility and aberrant sperm morphology (mice)

intersect and modulate the protease cascades in the pericellular microenvironment, the signals that trigger their activation, the repertoire of endogenous substrates, and the inhibitors that modulate their activities, are important issues to address. As suitable molecular tools, such as antibodies, activity-based probes, animal models, and human mutational analyses, are refined and developed, we will look forward to gaining a better understanding of the critical roles of these enzymes both in mammalian physiology and in disease.

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REFERENCES

1. Hooper JD, Clements JA, Quigley JP, Antalis TM. Type II transmembrane serine proteases. Insights into an emerging class of cell surface proteolytic enzymes. *J Biol Chem* 2001;**276**:857–60.
2. Netzel-Arnett S, Hooper JD, Szabo R, Madison EL, Quigley JP, Bugge TH, et al. Membrane anchored serine proteases, a rapidly expanding group of cell surface proteolytic enzymes with potential roles in cancer. *Cancer Metastasis Rev* 2003;**22**:237–58.
3. Szabo R, Bugge TH. Type II transmembrane serine proteases in development and disease. *Int J Biochem Cell Biol* 2008;**40**:1297–316.
4. Bugge TH, Antalis TM, Wu Q. Type II transmembrane serine proteases. *J Biol Chem* 2009;**284**:23177–81.

5. Hansen IA, Fassnacht M, Hahner S, Hammer F, Schammann M, Meyer SR, et al. The adrenal secretory serine protease AsP is a short secretory isoform of the transmembrane airway trypsin-like protease. *Endocrinology* 2004;**145**:1898–905.
6. Bhagwandin VJ, Hau LW, Mallen-St Clair J, Wolters PJ, Caughey GH. Structure and activity of human pancreasin, a novel tryptic serine peptidase expressed primarily by the pancreas. *J Biol Chem* 2003;**278**:3363–71.
7. Appel LF, Prout M, Abu-Shumays R, Hammonds A, Garbe JC, Fristrom D, et al. The *Drosophila* Stubble-stubblويد gene encodes an apparent transmembrane serine protease required for epithelial morphogenesis. *Proc Natl Acad Sci USA* 1993;**90**:4937–41.
8. Irving P, Troxler L, Heuer TS, Belvin M, Kopczynski C, Reichhart JM, et al. A genome-wide analysis of immune responses in *Drosophila*. *Proc Natl Acad Sci USA* 2001;**98**:15119–24.
9. Caughey GH, Raymond WW, Blount JL, Hau LW, Pallaoro M, Wolters PJ, et al. Characterization of human gamma-tryptases, novel members of the chromosome 16p mast cell tryptase and prostatic gene families. *J Immunol* 2000;**164**:6566–75.
10. Wong GW, Tang Y, Feyfant E, Sali A, Li L, Li Y, et al. Identification of a new member of the tryptase family of mouse and human mast cell proteases which possesses a novel COOH-terminal hydrophobic extension. *J Biol Chem* 1999;**274**:30784–93.
11. Yu JX, Chao L, Chao J. Prostaticin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. *J Biol Chem* 1994;**269**:18843–8.
12. Chen LM, Skinner ML, Kauffman SW, Chao J, Chao L, Thaler CD, et al. Prostaticin is a glycosylphosphatidylinositol-anchored active serine protease. *J Biol Chem* 2001;**276**:21434–42.
13. Verghese GM, Gutknecht MF, Caughey GH. Prostaticin regulates epithelial monolayer function, cell-specific Gp1d1-mediated secretion and functional role for GPI anchor. *Am J Physiol Cell Physiol* 2006;**291**:C1258–70.
14. Honda A, Yamagata K, Sugiyama S, Watanabe K, Baba T. A mouse serine protease TESP5 is selectively included into lipid rafts of sperm membrane presumably as a glycosylphosphatidylinositol-anchored protein. *J Biol Chem* 2002;**277**:16976–84.
15. Hooper JD, Nicol DL, Dickinson JL, Eyre HJ, Scarman AL, Normyle JF, et al. Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. *Cancer Res* 1999;**59**:3199–205.
16. Antalis TM, Buzza MS, Hodge KM, Hooper JD, Netzel-Arnett S. The cutting edge, membrane-anchored serine protease activities in the pericellular microenvironment. *Biochem J* 2010;**428**:325–46.
17. Puente XS, Sanchez LM, Overall CM, Lopez-Otin C. Human and mouse proteases, a comparative genomic approach. *Nat Rev Genet* 2003;**4**:544–58.
18. Fastrez J, Fersht AR. Demonstration of the acyl-enzyme mechanism for the hydrolysis of peptides and anilides by chymotrypsin. *Biochemistry* 1973;**12**:2025–34.
19. Szabo R, Wu Q, Dickson RB, Netzel-Arnett S, Antalis TM, Bugge TH. Type II transmembrane serine proteases. *Thromb Haemost* 2003;**90**:185–93.
20. Friedrich R, Fuentes-Prior P, Ong E, Coombs G, Hunter M, Oehler R, et al. Catalytic domain structures of MT-SPI/matriptase, a matrix-degrading transmembrane serine proteinase. *J Biol Chem* 2002;**277**:2160–8.
21. Somoza JR, Ho JD, Luong C, Ghate M, Sprengeler PA, Mortara K, et al. The structure of the extracellular region of human hepsin reveals a serine protease domain and a novel scavenger receptor cysteine-rich (SRCR) domain. *Structure* 2003;**11**:1123–31.
22. Lu D, Futterer K, Korolev S, Zheng X, Tan K, Waksman G, et al. Crystal structure of enteropeptidase light chain complexed with an analog of the trypsinogen activation peptide. *J Mol Biol* 1999;**292**:361–73.

23. Kyrieleis OJ, Huber R, Ong E, Oehler R, Hunter M, Madison EL, et al. Crystal structure of the catalytic domain of DESC1, a new member of the type II transmembrane serine proteinase family. *FEBS J* 2007;**274**:2148–60.
24. Spraggon G, Hornsby M, Shipway A, Tully DC, Bursulaya B, Danahay H, et al. Active site conformational changes of prostaticin provide a new mechanism of protease regulation by divalent cations. *Protein Sci* 2009;**18**:1081–94.
25. Tully DC, Vidal A, Chatterjee AK, Williams JA, Roberts MJ, Petrassi HM, et al. Discovery of inhibitors of the channel-activating protease prostaticin (CAP1/PRSS5) utilizing structure-based design. *Bioorg Med Chem Lett* 2008;**18**:5895–9.
26. Rickert KW, Kelley P, Byrne NJ, Diehl RE, Hall DL, Montalvo AM, et al. Structure of human prostaticin, a target for the regulation of hypertension. *J Biol Chem* 2008;**283**:34864–72.
27. Rawlings ND, Barrett AJ. Introduction, serine peptidases and their clans. In: Barrett AJ, Rawlings ND, Woessner JF, editors. *Handbook of proteolytic enzymes*. 2nd ed. London, UK: Elsevier Ltd.; 2004. p. 1417–39.
28. Schechter I, Berger A. On the size of the active site in proteases. I. Papain. *Biochem Biophys Res Commun* 1967;**27**:157–62.
29. Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, Craik CS. 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* 2000;**275**:26333–42.
30. Boulware KT, Daugherty PS. Protease specificity determination by using cellular libraries of peptide substrates (CLiPS). *Proc Natl Acad Sci USA* 2006;**103**:7583–8.
31. Herter S, Piper DE, Aaron W, Gabriele T, Cutler G, Cao P, et al. Hepatocyte growth factor is a preferred in vitro substrate for human hepsin, a membrane-anchored serine protease implicated in prostate and ovarian cancers. *Biochem J* 2005;**390**:125–36.
32. Shipway A, Danahay H, Williams JA, Tully DC, Backes BJ, Harris JL. Biochemical characterization of prostaticin, a channel activating protease. *Biochem Biophys Res Commun* 2004;**324**:953–63.
33. Yuan J, Beltman J, Gjerstad E, Nguyen MT, Sampang J, Chan H, et al. Expression and characterization of recombinant gamma-tryptase. *Protein Expr Purif* 2006;**49**:47–54.
34. Kido H, Okumura Y. MSPL/TMPRSS13. *Front Biosci* 2008;**13**:754–8.
35. Okumura Y, Hayama M, Takahashi E, Fujiuchi M, Shimabukuro A, Yano M, et al. Serase-1B, a new splice variant of polyserase-1/TMPRSS9, activates urokinase-type plasminogen activator and the proteolytic activation is negatively regulated by glycosaminoglycans. *Biochem J* 2006;**400**:551–61.
36. Oberst MD, Williams CA, Dickson RB, Johnson MD, Lin CY. The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. *J Biol Chem* 2003;**278**:26773–9.
37. Velasco G, Cal S, Quesada V, Sanchez LM, Lopez-Otin C. Matriptase-2, a membrane-bound mosaic serine proteinase predominantly expressed in human liver and showing degrading activity against extracellular matrix proteins. *J Biol Chem* 2002;**277**:37637–46.
38. Qiu D, Owen K, Gray K, Bass R, Ellis V. Roles and regulation of membrane-associated serine proteases. *Biochem Soc Trans* 2007;**35**:583–7.
39. Afar DE, Vivanco I, Hubert RS, Kuo J, Chen E, Saffran DC, et al. Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia. *Cancer Res* 2001;**61**:1686–92.
40. Guipponi M, Vuagniaux G, Wattenhofer M, Shibuya K, Vazquez M, Dougherty L, et al. The transmembrane serine protease (TMPRSS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. *Hum Mol Genet* 2002;**11**:2829–36.

41. Andreasen D, Vuagniaux G, Fowler-Jaeger N, Hummler E, Rossier BC. Activation of epithelial sodium channels by mouse channel activating proteases (mCAP) expressed in *Xenopus* oocytes requires catalytic activity of mCAP3 and mCAP2 but not mCAP1. *J Am Soc Nephrol* 2006;**17**:968–76.
42. Stallmach R, Gloor SM. Neurobin/TMPRSS11c, a novel type II transmembrane serine protease that cleaves fibroblast growth factor-2 *in vitro*. *Biochem J* 2008;**412**:81–91.
43. Vu TK, Liu RW, Haakma CJ, Tomasek JJ, Howard EW. Identification and cloning of the membrane-associated serine protease, hepsin, from mouse preimplantation embryos. *J Biol Chem* 1997;**272**:31315–20.
44. List K, Bugge TH, Szabo R. Matriptase, potent proteolysis on the cell surface. *Mol Med* 2006;**12**:1–7.
45. Lu D, Yuan X, Zheng X, Sadler JE. Bovine proenteropeptidase is activated by trypsin, and the specificity of enteropeptidase depends on the heavy chain. *J Biol Chem* 1997;**272**:31293–300.
46. Knappe S, Wu F, Madlansacay MR, Wu Q. Identification of domain structures in the propeptide of corin essential for the processing of proatrial natriuretic peptide. *J Biol Chem* 2004;**279**:34464–71.
47. Desilets A, Beliveau F, Vandal G, McDuff FO, Lavigne P, Leduc R. Mutation G827R in matriptase causing autosomal recessive ichthyosis with hypotrichosis yields an inactive protease. *J Biol Chem* 2008;**283**:10535–42.
48. Lin CY, Tseng IC, Chou FP, Su SF, Chen YW, Johnson MD, et al. Zymogen activation, inhibition, and ectodomain shedding of matriptase. *Front Biosci* 2008;**13**:621–35.
49. Jin X, Hirotsaki T, Lin CY, Dickson RB, Higashi S, Kitamura H, et al. Production of soluble matriptase by human cancer cell lines and cell surface activation of its zymogen by trypsin. *J Cell Biochem* 2005;**95**:632–47.
50. Benaud CM, Oberst M, Dickson RB, Lin CY. Deregulated activation of matriptase in breast cancer cells. *Clin Exp Metastasis* 2002;**19**:639–49.
51. Lucas JM, True L, Hawley S, Matsumura M, Morrissey C, Vessella R, et al. The androgen-regulated type II serine protease TMPRSS2 is differentially expressed and mislocalized in prostate adenocarcinoma. *J Pathol* 2008;**215**:118–25.
52. Laskowski Jr. M, Kato I. Protein inhibitors of proteinases. *Annu Rev Biochem* 1980;**49**:593–626.
53. Lin CY, Anders J, Johnson M, Sang QA, Dickson RB. Molecular cloning of cDNA for matriptase, a matrix-degrading serine protease with trypsin-like activity. *J Biol Chem* 1999;**274**:18231–6.
54. Oberst MD, Chen LY, Kiyomiya K, Williams CA, Lee MS, Johnson MD, et al. HAI-1 regulates activation and expression of matriptase, a membrane-bound serine protease. *Am J Physiol Cell Physiol* 2005;**289**:C462–70.
55. Kojima K, Tsuzuki S, Fushiki T, Inouye K. Roles of functional and structural domains of hepatocyte growth factor activator inhibitor type 1 in the inhibition of matriptase. *J Biol Chem* 2008;**283**:2478–87.
56. Szabo R, Hobson JP, List K, Molinolo A, Lin CY, Bugge TH. Potent inhibition and global colocalization implicate the transmembrane Kunitz-type serine protease inhibitor hepatocyte growth factor activator inhibitor-2 in the regulation of epithelial matriptase activity. *J Biol Chem* 2008;**283**:29495–504.
57. Kirchhofer D, Peek M, Lipari MT, Billeci K, Fan B, Moran P. Hepsin activates pro-hepatocyte growth factor and is inhibited by hepatocyte growth factor activator inhibitor-1B (HAI-1B) and HAI-2. *FEBS Lett* 2005;**579**:1945–50.
58. Fan B, Wu TD, Li W, Kirchhofer D. Identification of hepatocyte growth factor activator inhibitor-1B as a potential physiological inhibitor of prostaticin. *J Biol Chem* 2005;**280**:34513–20.

59. Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, et al. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem* 2001;**276**:33293–6.
60. Gettins PG. Serpin structure, mechanism, and function. *Chem Rev* 2002;**102**:4751–804.
61. Wong GW, Tang Y, Stevens RL. Cloning of the human homolog of mouse transmembrane tryptase. *Int Arch Allergy Immunol* 1999;**118**:419–21.
62. Wong GW, Foster PS, Yasuda S, Qi JC, Mahalingam S, Mellor EA, et al. Biochemical and functional characterization of human transmembrane tryptase (TMT)/tryptase gamma. TMT is an exocytosed mast cell protease that induces airway hyperresponsiveness in vivo via an interleukin-13/interleukin-4 receptor alpha/signal transducer and activator of transcription (STAT) 6-dependent pathway. *J Biol Chem* 2002;**277**:41906–15.
63. Reimer JM, Samollow PB, Hellman L. High degree of conservation of the multigene tryptase locus over the past 150-200 million years of mammalian evolution. *Immunogenetics* 2010;**62**:369–82.
64. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997;**77**:1033–79.
65. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 2007;**217**:141–54.
66. Zheng XL, Kitamoto Y, Sadler JE. Enteropeptidase, a type II transmembrane serine protease. *Front Biosci (Elite Ed)* 2009;**1**:242–9.
67. Waldschmidt-Leitz E. The chemical nature of enzymes. *Science* 1933;**78**:189–90.
68. Kunitz M. Formation of trypsin from crystalline trypsinogen by means of enterokinase. *J Gen Physiol* 1939;**22**:429–46.
69. Kunitz M. Purification and concentration of enterokinase. *J Gen Physiol* 1939;**22**:447–50.
70. Light A, Janska H. Enterokinase (enteropeptidase), comparative aspects. *Trends Biochem Sci* 1989;**14**:110–2.
71. Mann NS, Mann SK. Enterokinase. *Proc Soc Exp Biol Med* 1994;**206**:114–8.
72. Light A, Janska H. The amino-terminal sequence of the catalytic subunit of bovine enterokinase. *J Protein Chem* 1991;**10**:475–80.
73. Kitamoto Y, Veile RA, Donis-Keller H, Sadler JE. cDNA sequence and chromosomal localization of human enterokinase, the proteolytic activator of trypsinogen. *Biochemistry* 1995;**34**:4562–8.
74. Kitamoto Y, Yuan X, Wu Q, McCourt DW, Sadler JE. Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. *Proc Natl Acad Sci USA* 1994;**91**:7588–92.
75. LaVallie ER, Rehemtulla A, Racie LA, DiBlasio EA, Ferenz C, Grant KL, et al. Cloning and functional expression of a cDNA encoding the catalytic subunit of bovine enterokinase. *J Biol Chem* 1993;**268**:23311–7.
76. Matsushima M, Ichinose M, Yahagi N, Kakei N, Tsukada S, Miki K, et al. Structural characterization of porcine enteropeptidase. *J Biol Chem* 1994;**269**:19976–82.
77. Yuan X, Zheng X, Lu D, Rubin DC, Pung CY, Sadler JE. Structure of murine enterokinase (enteropeptidase) and expression in small intestine during development. *Am J Physiol* 1998;**274**:C342–9.
78. Hermon-Taylor J, Perrin J, Grant DA, Appleyard A, Bubel M, Magee AI. Immunofluorescent localisation of enterokinase in human small intestine. *Gut* 1977;**18**:259–65.
79. Lojda Z, Gossrau R. Histochemical demonstration of enteropeptidase activity. New method with a synthetic substrate and its comparison with the trypsinogen procedure. *Histochemistry* 1983;**78**:251–70.

80. Zheng X, Lu D, Sadler JE. Apical sorting of bovine enteropeptidase does not involve detergent-resistant association with sphingolipid-cholesterol rafts. *J Biol Chem* 1999;**274**:1596–605.
81. Zheng X, Sadler JE. Mucin-like domain of enteropeptidase directs apical targeting in Madin-Darby canine kidney cells. *J Biol Chem* 2002;**277**:6858–63.
82. Hadorn B, Steiner N, Sumida C, Peters TJ. Intestinal enterokinase. Mechanisms of its “secretion” into the lumen of the small intestine. *Lancet* 1971;**1**:165–6.
83. Zamolodchikova TS, Vorotyntseva TI, Antonov VK. Duodenase, a new serine protease of unusual specificity from bovine duodenal mucosa. Purification and properties. *Eur J Biochem* 1995;**227**:866–72.
84. Zamolodchikova TS, Sokolova EA, Alexandrov SL, Mikhaleva II, Prudchenko IA, Morozov IA, et al. Subcellular localization, substrate specificity and crystallization of duodenase, a potential activator of enteropeptidase. *Eur J Biochem* 1997;**249**:612–21.
85. Zamolodchikova TS, Sokolova EA, Lu D, Sadler JE. Activation of recombinant proenteropeptidase by duodenase. *FEBS Lett* 2000;**466**:295–9.
86. Hoffmeister A, Dietz G, Zeitschel U, Mossner J, Rossner S, Stahl T. BACE1 is a newly discovered protein secreted by the pancreas which cleaves enteropeptidase in vitro. *JOP* 2009;**10**:501–6.
87. Kim YT, Nishii W, Matsushima M, Inoue H, Ito H, Park SJ, et al. Substrate specificities of porcine and bovine enteropeptidases toward the peptide Val-(Asp)4-Lys-Ile-Val-Gly and its analogs. *Biosci Biotechnol Biochem* 2008;**72**:905–8.
88. Hadorn B, Tarlow MJ, Lloyd JK, Wolff OH. Intestinal enterokinase deficiency. *Lancet* 1969;**1**:812–3.
89. Hadorn B, Haworth JC, Gourley B, Prasad A, Troesch V. Intestinal enterokinase deficiency. Occurrence in two sibs and age dependency of clinical expression. *Arch Dis Child* 1975;**50**:277–82.
90. Leytys SP, Loeb KR, Hagen FS, Kurachi K, Davie EW. A novel trypsin-like serine protease (hepsin) with a putative transmembrane domain expressed by human liver and hepatoma cells. *Biochemistry* 1988;**27**:1067–74.
91. Farley D, Reymond F, Nick H. Cloning and sequence analysis of rat hepsin, a cell surface serine proteinase. *Biochim Biophys Acta* 1993;**1173**:350–2.
92. Kawamura S, Kurachi S, Deyashiki Y, Kurachi K. Complete nucleotide sequence, origin of isoform and functional characterization of the mouse hepsin gene. *Eur J Biochem* 1999;**262**:755–64.
93. Kurachi K, Torres-Rosado A, Tsuji A. Hepsin. *Methods Enzymol* 1994;**244**:100–14.
94. Wu Q. Gene targeting in hemostasis. Hepsin. *Front Biosci* 2001;**6**:D192–200.
95. Li W, Wang BE, Moran P, Lipari T, Ganesan R, Corpuz R, et al. Pegylated kunitz domain inhibitor suppresses hepsin-mediated invasive tumor growth and metastasis. *Cancer Res* 2009;**69**:8395–402.
96. Chen M, Chen LM, Lin CY, Chai KX. Hepsin activates prostaticin and cleaves the extracellular domain of the epidermal growth factor receptor. *Mol Cell Biochem* 2010;**337**:259–66.
97. Nakama S, Oshiro N, Aniya Y. Activation of rat liver microsomal glutathione transferase by hepsin. *Biol Pharm Bull* 2010;**33**:561–7.
98. Tripathi M, Nandana S, Yamashita H, Ganesan R, Kirchofer D, Quaranta V. Laminin-332 is a substrate for hepsin, a protease associated with prostate cancer progression. *J Biol Chem* 2008;**283**:30576–84.
99. Wu Q. The serine protease corin in cardiovascular biology and disease. *Front Biosci* 2007;**12**:4179–90.

100. Kazama Y, Hamamoto T, Foster DC, Kiesel W. Hepsin, a putative membrane-associated serine protease, activates human factor VII and initiates a pathway of blood coagulation on the cell surface leading to thrombin formation. *J Biol Chem* 1995;**270**:66–72.
101. Torres-Rosado A, O'Shea KS, Tsuji A, Chou SH, Kurachi K. Hepsin, a putative cell-surface serine protease, is required for mammalian cell growth. *Proc Natl Acad Sci USA* 1993;**90**:7181–5.
102. Wu Q, Yu D, Post J, Halks-Miller M, Sadler JE, Morser J. Generation and characterization of mice deficient in hepsin, a hepatic transmembrane serine protease. *J Clin Invest* 1998;**101**:321–6.
103. Yu IS, Chen HJ, Lee YS, Huang PH, Lin SR, Tsai TW, et al. Mice deficient in hepsin, a serine protease, exhibit normal embryogenesis and unchanged hepatocyte regeneration ability. *Thromb Haemost* 2000;**84**:865–70.
104. Guipponi M, Tan J, Cannon PZ, Donley L, Crewther P, Clarke M, et al. Mice deficient for the type II transmembrane serine protease, TMPRSS1/hepsin, exhibit profound hearing loss. *Am J Pathol* 2007;**171**:608–16.
105. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, et al. Delineation of prognostic biomarkers in prostate cancer. *Nature* 2001;**412**:822–6.
106. Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, et al. Expression profiling reveals hepsin overexpression in prostate cancer. *Cancer Res* 2001;**61**:5692–6.
107. Stamey TA, Warrington JA, Caldwell MC, Chen Z, Fan Z, Mahadevappa M, et al. Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign prostatic hyperplasia. *J Urol* 2001;**166**:2171–7.
108. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001;**61**:5974–8.
109. Wu Q, Parry G. Hepsin and prostate cancer. *Front Biosci* 2007;**12**:5052–9.
110. Xuan JA, Schneider D, Toy P, Lin R, Newton A, Zhu Y, et al. Antibodies neutralizing hepsin protease activity do not impact cell growth but inhibit invasion of prostate and ovarian tumor cells in culture. *Cancer Res* 2006;**66**:3611–9.
111. Klezovitch O, Chevillet J, Mirosevich J, Roberts RL, Matusik RJ, Vasioukhin V. Hepsin promotes prostate cancer progression and metastasis. *Cancer Cell* 2004;**6**:185–95.
112. Paoloni-Giacobino A, Chen H, Peitsch MC, Rossier C, Antonarakis SE. Cloning of the TMPRSS2 gene, which encodes a novel serine protease with transmembrane, LDLRA, and SRCR domains and maps to 21q22.3. *Genomics* 1997;**44**:309–20.
113. Jacquinet E, Rao NV, Rao GV, Zhengming W, Albertine KH, Hoidal JR. Cloning and characterization of the cDNA and gene for human epitheliasin. *Eur J Biochem* 2001;**268**:2687–99.
114. Jacquinet E, Rao NV, Rao GV, Hoidal JR. Cloning, genomic organization, chromosomal assignment and expression of a novel mosaic serine proteinase, epitheliasin. *FEBS Lett* 2000;**468**:93–100.
115. Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res* 1999;**59**:4180–4.
116. Chen YW, Lee MS, Lucht A, Chou FP, Huang W, Havighurst TC, et al. TMPRSS2, a serine protease expressed in the prostate on the apical surface of luminal epithelial cells and released into semen in prostasomes, is misregulated in prostate cancer cells. *Am J Pathol* 2010;**176**:2986–96.
117. Donaldson SH, Hirsh A, Li DC, Holloway G, Chao J, Boucher RC, et al. Regulation of the epithelial sodium channel by serine proteases in human airways. *J Biol Chem* 2002;**277**:8338–45.

118. Bottcher-Friebertshauer E, Freuer C, Sielaff F, Schmidt S, Eickmann M, Uhlendorff J, et al. Cleavage of influenza virus hemagglutinin by airway proteases TMPRSS2 and HAT differs in subcellular localization and susceptibility to protease inhibitors. *J Virol* 2010;**84**:5605–14.
119. Bottcher E, Matrosovich T, Beyerle M, Klenk HD, Garten W, Matrosovich M. Proteolytic activation of influenza viruses by serine proteases TMPRSS2 and HAT from human airway epithelium. *J Virol* 2006;**80**:9896–8.
120. Wilson S, Greer B, Hooper J, Zijlstra A, Walker B, Quigley J, et al. The membrane-anchored serine protease, TMPRSS2, activates PAR-2 in prostate cancer cells. *Biochem J* 2005;**388**:967–72.
121. Kim TS, Heinlein C, Hackman RC, Nelson PS. Phenotypic analysis of mice lacking the Tmprss2-encoded protease. *Mol Cell Biol* 2006;**26**:965–75.
122. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005;**310**:644–8.
123. Yu J, Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, et al. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell* 2010;**17**:443–54.
124. Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, et al. Role of the TMPRSS2-ERG gene fusion in prostate cancer. *Neoplasia* 2008;**10**:177–88.
125. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* 2007;**448**:595–9.
126. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat Genet* 2010;**42**:668–75.
127. Underwood LJ, Shigemasa K, Tanimoto H, Beard JB, Schneider EN, Wang Y, et al. Ovarian tumor cells express a novel multi-domain cell surface serine protease. *Biochim Biophys Acta* 2000;**1502**:337–50.
128. Scott HS, Kudoh J, Wattenhofer M, Shibuya K, Berry A, Chrast R, et al. Insertion of beta-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset autosomal recessive deafness. *Nat Genet* 2001;**27**:59–63.
129. Bonne-Tamir B, DeStefano AL, Briggs CE, Adair R, Franklyn B, Weiss S, et al. Linkage of congenital recessive deafness (gene DFNB10) to chromosome 21q22.3. *Am J Hum Genet* 1996;**58**:1254–9.
130. Guipponi M, Antonarakis SE, Scott HS. TMPRSS3, a type II transmembrane serine protease mutated in non-syndromic autosomal recessive deafness. *Front Biosci* 2008;**13**:1557–67.
131. Guipponi M, Toh MY, Tan J, Park D, Hanson K, Ballana E, et al. An integrated genetic and functional analysis of the role of type II transmembrane serine proteases (TMPRSSs) in hearing loss. *Hum Mutat* 2008;**29**:130–41.
132. Peters TA, Levchenko E, Cremers CW, Curfs JH, Monnens LA. No evidence of hearing loss in pseudohypoadosteronism type 1 patients. *Acta Otolaryngol* 2006;**126**:237–9.
133. Wallrapp C, Hahnel S, Muller-Pillasch F, Burghardt B, Iwamura T, Ruthenburger M, et al. A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. *Cancer Res* 2000;**60**:2602–6.
134. Vuagniaux G, Vallet V, Jaeger NF, Hummler E, Rossier BC. Synergistic activation of ENaC by three membrane-bound channel-activating serine proteases (mCAP1, mCAP2, and mCAP3) and S. *J Gen Physiol* 2002;**120**:191–201.
135. Garcia-Caballero A, Dang Y, He H, Stutts MJ. ENaC proteolytic regulation by channel-activating protease 2. *J Gen Physiol* 2008;**132**:521–35.

136. Chaipan C, Kobasa D, Bertram S, Glowacka I, Steffen I, Tsegaye TS, et al. Proteolytic activation of the 1918 influenza virus hemagglutinin. *J Virol* 2009;**83**:3200–11.
137. Kebebew E, Peng M, Reiff E, Duh QY, Clark OH, McMillan A. ECM1 and TMPRSS4 are diagnostic markers of malignant thyroid neoplasms and improve the accuracy of fine needle aspiration biopsy. *Ann Surg* 2005;**242**:353–61.
138. Cheng H, Fukushima T, Takahashi N, Tanaka H, Kataoka H. Hepatocyte growth factor activator inhibitor type 1 regulates epithelial to mesenchymal transition through membrane-bound serine proteinases. *Cancer Res* 2009;**69**:1828–35.
139. Jung H, Lee KP, Park SJ, Park JH, Jang YS, Choi SY, et al. TMPRSS4 promotes invasion, migration and metastasis of human tumor cells by facilitating an epithelial-mesenchymal transition. *Oncogene* 2008;**27**:2635–47.
140. Kim S, Kang HY, Nam EH, Choi MS, Zhao XF, Hong CS, et al. TMPRSS4 induces invasion and epithelial-mesenchymal transition through upregulation of integrin alpha5 and its signaling pathways. *Carcinogenesis* 2010;**31**:597–606.
141. Yamaguchi N, Okui A, Yamada T, Nakazato H, Mitsui S. Spinesin/TMPRSS5, a novel transmembrane serine protease, cloned from human spinal cord. *J Biol Chem* 2002;**277**:6806–12.
142. Mitsui S, Watanabe Y, Yamaguchi T, Yamaguchi N. Mosaic serine proteases in the mammalian central nervous system. *Front Biosci* 2008;**13**:1991–2000.
143. Kim DR, Sharmin S, Inoue M, Kido H. Cloning and expression of novel mosaic serine proteases with and without a transmembrane domain from human lung. *Biochim Biophys Acta* 2001;**1518**:204–9.
144. Okumura Y, Takahashi E, Yano M, Ohuchi M, Daidoji T, Nakaya T, et al. Novel type II transmembrane serine proteases, MSPL and TMPRSS13, proteolytically activate membrane fusion activity of the hemagglutinin of highly pathogenic avian influenza viruses and induce their multicycle replication. *J Virol* 2010;**84**:5089–96.
145. Shi YE, Torri J, Yieh L, Wellstein A, Lippman ME, Dickson RB. Identification and characterization of a novel matrix-degrading protease from hormone-dependent human breast cancer cells. *Cancer Res* 1993;**53**:1409–15.
146. Tanimoto H, Shigemasa K, Tian X, Gu L, Beard JB, Sawasaki T, et al. Transmembrane serine protease TADG-15 (ST14/Matriptase/MT-SP1), expression and prognostic value in ovarian cancer. *Br J Cancer* 2005;**92**:278–83.
147. Kim MG, Chen C, Lyu MS, Cho EG, Park D, Kozak C, et al. Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains. *Immunogenetics* 1999;**49**:420–8.
148. List K, Szabo R, Molinolo A, Nielsen BS, Bugge TH. Delineation of matriptase protein expression by enzymatic gene trapping suggests diverging roles in barrier function, hair formation, and squamous cell carcinogenesis. *Am J Pathol* 2006;**168**:1513–25.
149. Szabo R, Molinolo A, List K, Bugge TH. Matriptase inhibition by hepatocyte growth factor activator inhibitor-1 is essential for placental development. *Oncogene* 2007;**26**:1546–56.
150. Netzel-Arnett S, Currie BM, Szabo R, Lin CY, Chen LM, Chai KX, et al. Evidence for a matriptase-prostasin proteolytic cascade regulating terminal epidermal differentiation. *J Biol Chem* 2006;**281**:32941–5.
151. Fan B, Brennan J, Grant D, Peale F, Rangell L, Kirchhofer D. Hepatocyte growth factor activator inhibitor-1 (HAI-1) is essential for the integrity of basement membranes in the developing placental labyrinth. *Dev Biol* 2007;**303**:222–30.
152. Oberst MD, Singh B, Ozdemirli M, Dickson RB, Johnson MD, Lin CY. Characterization of matriptase expression in normal human tissues. *J Histochem Cytochem* 2003;**51**:1017–25.

153. Takeuchi T, Shuman MA, Craik CS. Reverse biochemistry, use of macromolecular protease inhibitors to dissect complex biological processes and identify a membrane-type serine protease in epithelial cancer and normal tissue. *Proc Natl Acad Sci USA* 1999;**96**:11054–61.
154. Benaud C, Oberst M, Hobson JP, Spiegel S, Dickson RB, Lin CY. Sphingosine 1-phosphate, present in serum-derived lipoproteins, activates matriptase. *J Biol Chem* 2002;**277**:10539–46.
155. Szabo R, Hobson JP, Christoph K, Kosa P, List K, Bugge TH. Regulation of cell surface protease matriptase by HAI2 is essential for placental development, neural tube closure and embryonic survival in mice. *Development* 2009;**136**:2653–63.
156. Kilpatrick LM, Harris RL, Owen KA, Bass R, Ghorayeb C, Bar-Or A, et al. Initiation of plasminogen activation on the surface of monocytes expressing the type II transmembrane serine protease matriptase. *Blood* 2006;**108**:2616–23.
157. Cheng MF, Jin JS, Wu HW, Chiang PC, Sheu LF, Lee HS. Matriptase expression in the normal and neoplastic mast cells. *Eur J Dermatol* 2007;**17**:375–80.
158. List K, Szabo R, Wertz PW, Segre J, Haudenschild CC, Kim SY, et al. Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. *J Cell Biol* 2003;**163**:901–10.
159. List K, Haudenschild CC, Szabo R, Chen W, Wahl SM, Swaim W, et al. Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. *Oncogene* 2002;**21**:3765–79.
160. Kamata Y, Taniguchi A, Yamamoto M, Nomura J, Ishihara K, Takahara H, et al. Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids. *J Biol Chem* 2009;**284**:12829–36.
161. List K, Kosa P, Szabo R, Bey AL, Wang CB, Molinolo A, et al. Epithelial integrity is maintained by a matriptase-dependent proteolytic pathway. *Am J Pathol* 2009;**175**:1453–63.
162. Leyvraz C, Charles RP, Rubera I, Guitard M, Rotman S, Breiden B, et al. The epidermal barrier function is dependent on the serine protease CAPI/Prss8. *J Cell Biol* 2005;**170**:487–96.
163. List K, Hobson JP, Molinolo A, Bugge TH. Co-localization of the channel activating protease prostaticin/(CAPI/PRSS8) with its candidate activator, matriptase. *J Cell Physiol* 2007;**213**:237–45.
164. Basel-Vanagaite L, Attia R, Ishida-Yamamoto A, Rainshtein L, Ben Amitai D, Lurie R, et al. Autosomal recessive ichthyosis with hypotrichosis caused by a mutation in ST14, encoding type II transmembrane serine protease matriptase. *Am J Hum Genet* 2007;**80**:467–77.
165. Alef T, Torres S, Hausser I, Metze D, Tursen U, Lestringant GG, et al. Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in ST14 is associated with impaired profilaggrin processing. *J Invest Dermatol* 2009;**129**:862–9.
166. Avrahami L, Maas S, Pasmanik-Chor M, Rainshtein L, Magal N, Smitt J, et al. Autosomal recessive ichthyosis with hypotrichosis syndrome, further delineation of the phenotype. *Clin Genet* 2008;**74**:47–53.
167. List K, Currie B, Scharschmidt TC, Szabo R, Shireman J, Molinolo A, et al. Autosomal ichthyosis with hypotrichosis syndrome displays low matriptase proteolytic activity and is phenocopied in ST14 hypomorphic mice. *J Biol Chem* 2007;**282**:36714–23.
168. Sales KU, Masedunskas A, Bey AL, Rasmussen AL, Weigert R, List K, et al. Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. *Nat Genet* 2010;**42**:676–83.
169. Buzza MS, Netzel-Arnett S, Shea-Donohue T, Zhao A, Lin CY, List K, et al. Membrane-anchored serine protease matriptase regulates epithelial barrier formation and permeability in the intestine. *Proc Natl Acad Sci USA* 2010;**107**:4200–5.

170. Lin CY, Anders J, Johnson M, Dickson RB. Purification and characterization of a complex containing matriptase and a Kunitz-type serine protease inhibitor from human milk. *J Biol Chem* 1999;**274**:18237–42.
171. Oberst M, Anders J, Xie B, Singh B, Ossandon M, Johnson M, et al. Matriptase and HAI-1 are expressed by normal and malignant epithelial cells *in vitro* and *in vivo*. *Am J Pathol* 2001;**158**:1301–11.
172. Zeng L, Cao J, Zhang X. Expression of serine protease SNC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 in normal and malignant tissues of gastrointestinal tract. *World J Gastroenterol* 2005;**11**:6202–7.
173. List K, Szabo R, Molinolo A, Sriuranpong V, Redeye V, Murdock T, et al. Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. *Genes Dev* 2005;**19**:1934–50.
174. Kirchhofer D, Peek M, Li W, Stamos J, Eigenbrot C, Kadkhodayan S, et al. Tissue expression, protease specificity, and Kunitz domain functions of hepatocyte growth factor activator inhibitor-1B (HAI-1B), a new splice variant of HAI-1. *J Biol Chem* 2003;**278**:36341–9.
175. Tanaka H, Nagaike K, Takeda N, Itoh H, Kohama K, Fukushima T, et al. Hepatocyte growth factor activator inhibitor type 1 (HAI-1) is required for branching morphogenesis in the chorioallantoic placenta. *Mol Cell Biol* 2005;**25**:5687–98.
176. Nagaike K, Kawaguchi M, Takeda N, Fukushima T, Sawaguchi A, Kohama K, et al. Defect of hepatocyte growth factor activator inhibitor type 1/serine protease inhibitor, Kunitz type 1 (Hai-1/Spint1) leads to ichthyosis-like condition and abnormal hair development in mice. *Am J Pathol* 2008;**173**:1464–75.
177. Carney TJ, von der Hardt S, Sonntag C, Amsterdam A, Topczewski J, Hopkins N, et al. Inactivation of serine protease Matriptase1a by its inhibitor Hail is required for epithelial integrity of the zebrafish epidermis. *Development* 2007;**134**:3461–71.
178. Lin CY, Wang JK, Torri J, Dou L, Sang QA, Dickson RB. Characterization of a novel, membrane-bound, 80-kDa matrix-degrading protease from human breast cancer cells. Monoclonal antibody production, isolation, and localization. *J Biol Chem* 1997;**272**:9147–52.
179. List K. Matriptase, a culprit in cancer? *Future Oncol* 2009;**5**:97–104.
180. Jin JS, Cheng TF, Tsai WC, Sheu LF, Chiang H, Yu CP. Expression of the serine protease, matriptase, in breast ductal carcinoma of Chinese women, correlation with clinicopathological parameters. *Histol Histopathol* 2007;**22**:305–9.
181. Kang JY, Dolled-Filhart M, Ocal IT, Singh B, Lin CY, Dickson RB, et al. Tissue microarray analysis of hepatocyte growth factor/Met pathway components reveals a role for Met, matriptase, and hepatocyte growth factor activator inhibitor 1 in the progression of node-negative breast cancer. *Cancer Res* 2003;**63**:1101–5.
182. Jin JS, Hsieh DS, Loh SH, Chen A, Yao CW, Yen CY. Increasing expression of serine protease matriptase in ovarian tumors, tissue microarray analysis of immunostaining score with clinicopathological parameters. *Mod Pathol* 2006;**19**:447–52.
183. Santin AD, Cane' S, Bellone S, Bignotti E, Palmieri M, Las Casas LE, et al. The novel serine protease tumor-associated differentially expressed gene-15 (matriptase/MT-SP1) is highly overexpressed in cervical carcinoma. *Cancer* 2003;**98**:1898–904.
184. Oberst MD, Johnson MD, Dickson RB, Lin CY, Singh B, Stewart M, et al. 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* 2002;**8**:1101–7.
185. Saleem M, Adhami VM, Zhong W, Longley BJ, Lin CY, Dickson RB, et al. 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* 2006;**15**:217–27.

186. Lee JW, Yong Song S, Choi JJ, Lee SJ, Kim BG, Park CS, et al. Increased expression of matriptase is associated with histopathologic grades of cervical neoplasia. *Hum Pathol* 2005;**36**:626–33.
187. Vogel LK, Saebo M, Skjelbred CF, Abell K, Pedersen ED, Vogel U, et al. The ratio of Matriptase/HAI-1 mRNA is higher in colorectal cancer adenomas and carcinomas than corresponding tissue from control individuals. *BMC Cancer* 2006;**6**:176.
188. Kishi K, Yamazaki K, Yasuda I, Yahagi N, Ichinose M, Tsuchiya Y, et al. Characterization of a membrane-bound arginine-specific serine protease from rat intestinal mucosa. *J Biochem* 2001;**130**:425–30.
189. Satomi S, Yamasaki Y, Tsuzuki S, Hitomi Y, Iwanaga T, Fushiki T. A role for membrane-type serine protease (MT-SP1) in intestinal epithelial turnover. *Biochem Biophys Res Commun* 2001;**287**:995–1002.
190. Lee SL, Dickson RB, Lin CY. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. *J Biol Chem* 2000;**275**:36720–5.
191. Bhatt AS, Welm A, Farady CJ, Vasquez M, Wilson K, Craik CS. Coordinate expression and functional profiling identify an extracellular proteolytic signaling pathway. *Proc Natl Acad Sci USA* 2007;**104**:5771–6.
192. Camerer E, Barker A, Duong DN, Ganesan R, Kataoka H, Cornelissen I, et al. Local protease signaling contributes to neural tube closure in the mouse embryo. *Dev Cell* 2010;**18**:25–38.
193. Bhatt AS, Erdjument-Bromage H, Tempst P, Craik CS, Moasser MM. Adhesion signaling by a novel mitotic substrate of src kinases. *Oncogene* 2005;**24**:5333–43.
194. He Y, Wortmann A, Burke LJ, Reid JC, Adams MN, Abdul-Jabbar I, et al. Proteolysis induced amino terminal ectodomain shedding of the integral membrane glycoprotein CUB domain containing protein 1 (CDCP1) is accompanied by tyrosine phosphorylation of its carboxy terminal domain and recruitment of Src and PKC[delta]. *J Biol Chem* 2010;**285**:26162–73.
195. Hooper JD, Campagnolo L, Goodarzi G, Truong TN, Stuhlmann H, Quigley JP. Mouse matriptase-2, identification, characterization and comparative mRNA expression analysis with mouse hepsin in adult and embryonic tissues. *Biochem J* 2003;**373**:689–702.
196. Stirnberg M, Maurer E, Horstmeyer A, Kolp S, Frank S, Bald T, et al. Proteolytic processing of the serine protease matriptase-2, identification of the cleavage sites required for its autocatalytic release from the cell surface. *Biochem J* 2010;**430**:87–95.
197. Folgueras AR, de Lara FM, Pendas AM, Garabaya C, Rodriguez F, Astudillo A, et al. Membrane-bound serine protease matriptase-2 (Tmprss6) is an essential regulator of iron homeostasis. *Blood* 2008;**112**:2539–45.
198. Du X, She E, Gelbart T, Truksa J, Lee P, Xia Y, et al. The serine protease TMPRSS6 is required to sense iron deficiency. *Science* 2008;**320**:1088–92.
199. Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nat Genet* 2008;**40**:569–71.
200. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. *Cell Metab* 2008;**8**:502–11.
201. Silvestri L, Guillem F, Pagani A, Nai A, Oudin C, Silva M, et al. Molecular mechanisms of the defective hepcidin inhibition in TMPRSS6 mutations associated with iron-refractory iron deficiency anemia. *Blood* 2009;**113**:5605–8.
202. Szabo R, Netzel-Arnett S, Hobson JP, Antalis TM, Bugge TH. Matriptase-3 is a novel phylogenetically preserved membrane-anchored serine protease with broad serpin reactivity. *Biochem J* 2005;**390**:231–42.

203. Cal S, Quesada V, Garabaya C, Lopez-Otin C. Polyserase-I, a human polyprotease with the ability to generate independent serine protease domains from a single translation product. *Proc Natl Acad Sci USA* 2003;**100**:9185–90.
204. Hayama M, Okumura Y, Takahashi E, Shimabukuro A, Tamura M, Takeda N, et al. Identification and analysis of the promoter region of the type II transmembrane serine protease polyserase-1 and its transcript variants. *Biol Chem* 2007;**388**:853–8.
205. Yan W, Sheng N, Seto M, Morser J, Wu Q. Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *J Biol Chem* 1999;**274**:14926–35.
206. Hooper JD, Scarman AL, Clarke BE, Normyle JF, Antalis TM. Localization of the mosaic transmembrane serine protease corin to heart myocytes. *Eur J Biochem* 2000;**267**:6931–7.
207. Tomita Y, Kim DH, Magoori K, Fujino T, Yamamoto TT. A novel low-density lipoprotein receptor-related protein with type II membrane protein-like structure is abundant in heart. *J Biochem* 1998;**124**:784–9.
208. Pan J, Hinzmann B, Yan W, Wu F, Morser J, Wu Q. Genomic structures of the human and murine corin genes and functional GATA elements in their promoters. *J Biol Chem* 2002;**277**:38390–8.
209. Gladysheva IP, Robinson BR, Houg AK, Kovats T, King SM. Corin is co-expressed with pro-ANP and localized on the cardiomyocyte surface in both zymogen and catalytically active forms. *J Mol Cell Cardiol* 2008;**44**:131–42.
210. Liao X, Wang W, Chen S, Wu Q. Role of glycosylation in corin zymogen activation. *J Biol Chem* 2007;**282**:27728–35.
211. Knappe S, Wu F, Masikat MR, Morser J, Wu Q. Functional analysis of the transmembrane domain and activation cleavage of human corin, design and characterization of a soluble corin. *J Biol Chem* 2003;**278**:52363–70.
212. Kohn EC, Liotta LA. Molecular insights into cancer invasion, strategies for prevention and intervention. *Cancer Res* 1995;**55**:1856–62.
213. Wu Q, Xu-Cai YO, Chen S, Wang W. Corin, new insights into the natriuretic peptide system. *Kidney Int* 2009;**75**:142–6.
214. Hodgson-Zingman DM, Karst ML, Zingman LV, Heublein DM, Darbar D, Herron KJ, et al. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. *N Engl J Med* 2008;**359**:158–65.
215. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a proatrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci USA* 2000;**97**:8525–9.
216. Chan JC, Knudson O, Wu F, Morser J, Dole WP, Wu Q. Hypertension in mice lacking the proatrial natriuretic peptide convertase corin. *Proc Natl Acad Sci USA* 2005;**102**:785–90.
217. Nigrovic PA, Gray DH, Jones T, Hallgren J, Kuo FC, Chaletzky B, et al. Genetic inversion in mast cell-deficient (W^{sh}) mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol* 2008;**173**:1693–701.
218. Enshell-Seiffers D, Lindon C, Morgan BA. The serine protease Corin is a novel modifier of the Agouti pathway. *Development* 2008;**135**:217–25.
219. Semenov AG, Tamm NN, Seferian KR, Postnikov AB, Karpova NS, Serebryanaya DV, et al. Processing of pro-B-type natriuretic peptide, furin and corin as candidate convertases. *Clin Chem* 2010;**56**:1166–76.
220. Wu C, Wu F, Pan J, Morser J, Wu Q. Furin-mediated processing of Pro-C-type natriuretic peptide. *J Biol Chem* 2003;**278**:25847–52.
221. Dries DL, Victor RG, Rame JE, Cooper RS, Wu X, Zhu X, et al. Corin gene minor allele defined by 2 missense mutations is common in blacks and associated with high blood pressure and hypertension. *Circulation* 2005;**112**:2403–10.

222. Rame JE, Drazner MH, Post W, Peshock R, Lima J, Cooper RS, et al. Corin I555(P568) allele is associated with enhanced cardiac hypertrophic response to increased systemic afterload. *Hypertension* 2007;**49**:857–64.
223. Rame JE, Tam SW, McNamara D, Worcel M, Sabolinski ML, Wu AH, et al. Dysfunctional corin I555(P568) allele is associated with impaired brain natriuretic peptide processing and adverse outcomes in blacks with systolic heart failure, results from the genetic risk assessment in heart failure substudy. *Circ Heart Fail* 2009;**2**:541–8.
224. Wang W, Liao X, Fukuda K, Knappe S, Wu F, Dries DL, et al. Corin variant associated with hypertension and cardiac hypertrophy exhibits impaired zymogen activation and natriuretic peptide processing activity. *Circ Res* 2008;**103**:502–8.
225. Peleg A, Jaffe AS, Hasin Y. Enzyme-linked immunoabsorbent assay for detection of human serine protease corin in blood. *Clin Chim Acta* 2009;**409**:85–9.
226. Dong N, Chen S, Yang J, He L, Liu P, Zheng D, et al. Plasma soluble corin in patients with heart failure. *Circ Heart Fail* 2010;**3**:207–11.
227. Hobson JP, Netzel-Arnett S, Szabo R, Rehault SM, Church FC, Strickland DK, et al. Mouse DESC1 is located within a cluster of seven DESC1-like genes and encodes a type II transmembrane serine protease that forms serpin inhibitory complexes. *J Biol Chem* 2004;**279**:46981–94.
228. Macao B, Johansson DG, Hansson GC, Hard T. Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin. *Nat Struct Mol Biol* 2006;**13**:71–6.
229. Yasuoka S, Ohnishi T, Kawano S, Tsuchihashi S, Ogawara M, Masuda K, et al. Purification, characterization, and localization of a novel trypsin-like protease found in the human airway. *Am J Respir Cell Mol Biol* 1997;**16**:300–8.
230. Yamaoka K, Masuda K, Ogawa H, Takagi K, Umemoto N, Yasuoka S. Cloning and characterization of the cDNA for human airway trypsin-like protease. *J Biol Chem* 1998;**273**:11895–901.
231. Matsushima R, Takahashi A, Nakaya Y, Maezawa H, Miki M, Nakamura Y, et al. Human airway trypsin-like protease stimulates human bronchial fibroblast proliferation in a protease-activated receptor-2-dependent pathway. *Am J Physiol Lung Cell Mol Physiol* 2006;**290**:L385–95.
232. Yoshinaga S, Nakahori Y, Yasuoka S. Fibrinogenolytic activity of a novel trypsin-like enzyme found in human airway. *J Med Invest* 1998;**45**:77–86.
233. Iwakiri K, Ghazizadeh M, Jin E, Fujiwara M, Takemura T, Takezaki S, et al. Human airway trypsin-like protease induces PAR-2-mediated IL-8 release in psoriasis vulgaris. *J Invest Dermatol* 2004;**122**:937–44.
234. Takahashi M, Sano T, Yamaoka K, Kamimura T, Umemoto N, Nishitani H, et al. Localization of human airway trypsin-like protease in the airway; an immunohistochemical study. *Histochem Cell Biol* 2001;**115**:181–7.
235. Miki M, Nakamura Y, Takahashi A, Nakaya Y, Eguchi H, Masegi T, et al. Effect of human airway trypsin-like protease on intracellular free Ca²⁺ concentration in human bronchial epithelial cells. *J Med Invest* 2003;**50**:95–107.
236. Chokki M, Eguchi H, Hamamura I, Mitsuhashi H, Kamimura T. Human airway trypsin-like protease induces amphiregulin release through a mechanism involving protease-activated receptor-2-mediated ERK activation and TNF alpha-converting enzyme activity in airway epithelial cells. *FEBS J* 2005;**272**:6387–99.
237. Beaufort N, Leduc D, Eguchi H, Mengele K, Hellmann D, Masegi T, et al. The human airway trypsin-like protease modulates the urokinase receptor (uPAR, CD87) structure and functions. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L1263–72.
238. Bertram S, Glowacka I, Steffen I, Kuhl A, Pohlmann S. Novel insights into proteolytic cleavage of influenza virus hemagglutinin. *Rev Med Virol.* 2010;**20**(5):298–310.

239. Lang JC, Schuller DE. Differential expression of a novel serine protease homologue in squamous cell carcinoma of the head and neck. *Br J Cancer* 2001;**84**:237–43.
240. Sedghizadeh PP, Mallery SR, Thompson SJ, Kresty L, Beck FM, Parkinson EK, et al. Expression of the serine protease DESC1 correlates directly with normal keratinocyte differentiation and inversely with head and neck squamous cell carcinoma progression. *Head Neck* 2006;**28**:432–40.
241. Vilorio CG, Peinado JR, Astudillo A, Garcia-Suarez O, Gonzalez MV, Suarez C, et al. Human DESC1 serine protease confers tumorigenic properties to MDCK cells and it is upregulated in tumours of different origin. *Br J Cancer* 2007;**97**:201–9.
242. Beliveau F, Desilets A, Leduc R. Probing the substrate specificities of matriptase, matriptase-2, hepsin and DESC1 with internally quenched fluorescent peptides. *FEBS J* 2009;**276**:2213–26.
243. Kam YW, Okumura Y, Kido H, Ng LF, Bruzzone R, Altmeyer R. Cleavage of the SARS coronavirus spike glycoprotein by airway proteases enhances virus entry into human bronchial epithelial cells in vitro. *PLoS One* 2009;**4**:e7870.
244. Adachi M, Kitamura K, Miyoshi T, Narikiyo T, Iwashita K, Shiraiishi N, et al. Activation of epithelial sodium channels by prostaticin in *Xenopus* oocytes. *J Am Soc Nephrol* 2001;**12**:1114–21.
245. Vuagniaux G, Vallet V, Jaeger NF, Pfister C, Bens M, Farman N, et al. 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* 2000;**11**:828–34.
246. Tong Z, Illek B, Bhagvandin VJ, Verghese GM, Caughey GH. Prostaticin, 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* 2004;**287**:L928–35.
247. Scarman AL, Hooper JD, Boucaut KJ, Sit ML, Webb GC, Normyle JF, et al. Organization and chromosomal localization of the murine Testisin gene encoding a serine protease temporally expressed during spermatogenesis. *Eur J Biochem* 2001;**268**:1250–8.
248. Wong GW, Li L, Madhusudhan MS, Krilis SA, Gurish MF, Rothenberg ME, et al. Trypsin 4, a new member of the chromosome 17 family of mouse serine proteases. *J Biol Chem* 2001;**276**:20648–58.
249. Hooper JD, Bowen N, Marshall H, Cullen LM, Sood R, Daniels R, et al. Localization, expression and genomic structure of the gene encoding the human serine protease testisin. *Biochim Biophys Acta* 2000;**1492**:63–71.
250. Vallet V, Chraïbi A, Gaeggeler HP, Horisberger JD, Rossier BC. An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature* 1997;**389**:607–10.
251. Selzer-Plon J, Bornholdt J, Friis S, Bisgaard HC, Lothe IM, Tveit KM, et al. Expression of prostaticin and its inhibitors during colorectal cancer carcinogenesis. *BMC Cancer* 2009;**9**:201.
252. Koda A, Wakida N, Toriyama K, Yamamoto K, Iijima H, Tomita K, et al. Urinary prostaticin in humans, relationships among prostaticin, aldosterone and epithelial sodium channel activity. *Hypertens Res* 2009;**32**:276–81.
253. Zhu H, Chao J, Guo D, Li K, Huang Y, Hawkins K, et al. Urinary prostaticin, a possible biomarker for renal pressure natriuresis in black adolescents. *Pediatr Res* 2009;**65**:443–6.
254. Olivieri O, Castagna A, Guarini P, Chiecchi L, Sabaini G, Pizzolo F, et al. Urinary prostaticin, a candidate marker of epithelial sodium channel activation in humans. *Hypertension* 2005;**46**:683–8.
255. Iwashita K, Kitamura K, Narikiyo T, Adachi M, Shiraiishi N, Miyoshi T, et al. Inhibition of prostaticin secretion by serine protease inhibitors in the kidney. *J Am Soc Nephrol* 2003;**14**:11–6.

256. Svenningsen P, Uhrenholt TR, Palarasah Y, Skjoedt K, Jensen BL, Skott O. Prostatein-dependent activation of epithelial Na⁺ channels by low plasmin concentrations. *Am J Physiol Regul Integr Comp Physiol* 2009;**297**:R1733–41.
257. Wakida N, Kitamura K, Tuyen DG, Maekawa A, Miyoshi T, Adachi M, et al. Inhibition of prostatein-induced ENaC activities by PN-1 and regulation of PN-1 expression by TGF-beta1 and aldosterone. *Kidney Int* 2006;**70**:1432–8.
258. Coote K, Atherton-Watson HC, Sugar R, Young A, MacKenzie-Beevor A, Gosling M, et al. Camostat attenuates airway epithelial sodium channel function in vivo through the inhibition of a channel-activating protease. *J Pharmacol Exp Ther* 2009;**329**:764–74.
259. Planes C, Caughey GH. Regulation of the epithelial Na⁺ channel by peptidases. *Curr Top Dev Biol* 2007;**78**:23–46.
260. Bruns JB, Carattino MD, Sheng S, Maarouf AB, Weisz OA, Pilewski JM, et al. Epithelial Na⁺ channels are fully activated by furin- and prostatein-dependent release of an inhibitory peptide from the gamma-subunit. *J Biol Chem* 2007;**282**:6153–60.
261. Carattino MD, Hughey RP, Kleyman TR. Proteolytic processing of the epithelial sodium channel gamma subunit has a dominant role in channel activation. *J Biol Chem* 2008;**283**:25290–5.
262. Passero CJ, Carattino MD, Kashlan OB, Myerburg MM, Hughey RP, Kleyman TR. Defining an inhibitory domain in the gamma subunit of the epithelial sodium channel. *Am J Physiol Renal Physiol*. 2010;**299**(4):F854–61.
263. Planes C, Randrianarison NH, Charles RP, Frateschi S, Cluzeaud F, Vuagniaux G, et al. ENaC-mediated alveolar fluid clearance and lung fluid balance depend on the channel-activating protease 1. *EMBO Mol Med* 2010;**2**:26–37.
264. Donaldson SH, Boucher RC. Sodium channels and cystic fibrosis. *Chest* 2007;**132**:1631–6.
265. Myerburg MM, McKenna EE, Luke CJ, Frizzell RA, Kleyman TR, Pilewski JM. Prostatein expression is regulated by airway surface liquid volume and is increased in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;**294**:L932–41.
266. Zhu H, Guo D, Li K, Yan W, Tan Y, Wang X, et al. Prostatein, a possible candidate gene for human hypertension. *Am J Hypertens* 2008;**21**:1028–33.
267. Hummler E. Implication of ENaC in salt-sensitive hypertension. *J Steroid Biochem Mol Biol* 1999;**69**:385–90.
268. Maekawa A, Kakizoe Y, Miyoshi T, Wakida N, Ko T, Shiraishi N, et al. Camostat mesilate inhibits prostatein activity and reduces blood pressure and renal injury in salt-sensitive hypertension. *J Hypertens* 2009;**27**:181–9.
269. Chen LM, Hatfield ML, Fu YY, Chai KX. Prostatein regulates iNOS and cyclin D1 expression by modulating protease-activated receptor-2 signaling in prostate epithelial cells. *Prostate* 2009;**69**:1790–801.
270. Chen M, Fu YY, Lin CY, Chen LM, Chai KX. Prostatein induces protease-dependent and independent molecular changes in the human prostate carcinoma cell line PC-3. *Biochim Biophys Acta* 2007;**1773**:1133–40.
271. Fu YY, Gao WL, Chen M, Chai KX, Wang YL, Chen LM. Prostatein regulates human placental trophoblast cell proliferation via the epidermal growth factor receptor signaling pathway. *Hum Reprod* 2010;**25**:623–32.
272. Sakashita K, Mimori K, Tanaka F, Tahara K, Inoue H, Sawada T, et al. Clinical significance of low expression of Prostatein mRNA in human gastric cancer. *J Surg Oncol* 2008;**98**:559–64.
273. Ma XJ, Fu YY, Li YX, Chen LM, Chai K, Wang YL. Prostatein inhibits cell invasion in human choriocarcinoma JEG-3 cells. *Histochem Cell Biol* 2009;**132**:639–46.
274. Inoue M, Kanbe N, Kurosawa M, Kido H. Cloning and tissue distribution of a novel serine protease esp-1 from human eosinophils. *Biochem Biophys Res Commun* 1998;**252**:307–12.

275. Inoue M, Isobe M, Itoyama T, Kido H. Structural analysis of esp-1 gene (PRSS 21). *Biochem Biophys Res Commun* 1999;**266**:564–8.
276. Netzel-Arnett S, Bugge TH, Hess RA, Carnes K, Stringer BW, Scarman AL, et al. The glycosylphosphatidylinositol-anchored serine protease PRSS21 (testisin) imparts murine epididymal sperm cell maturation and fertilizing ability. *Biol Reprod* 2009;**81**:921–32.
277. Aimes RT, Zijlstra A, Hooper JD, Ogbourne SM, Sit ML, Fuchs S, et al. Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. *Thromb Haemost* 2003;**89**:561–72.
278. Manton KJ, Douglas ML, Netzel-Arnett S, Fitzpatrick DR, Nicol DL, Boyd AW, et al. Hypermethylation of the 5' CpG island of the gene encoding the serine protease Testisin promotes its loss in testicular tumorigenesis. *Br J Cancer* 2005;**92**:760–9.
279. Nakamura Y, Inoue M, Okumura Y, Shiota M, Nishikawa M, Arase S, et al. Cloning, expression analysis, and tissue distribution of esp-1/testisin, a membrane-type serine protease from the rat. *J Med Invest* 2003;**50**:78–86.
280. Yamashita M, Honda A, Ogura A, Kashiwabara S, Fukami K, Baba T. Reduced fertility of mouse epididymal sperm lacking Prss21/Tesp5 is rescued by sperm exposure to uterine microenvironment. *Genes Cells* 2008;**13**:1001–13.
281. Kawano N, Kang W, Yamashita M, Koga Y, Yamazaki T, Hata T. Mice lacking two sperm serine proteases, ACR and PRSS21, are subfertile, but the mutant sperm are infertile in vitro. *Biol Reprod* 2010;**83**(3):359–69.
282. Shigemasa K, Underwood LJ, Beard J, Tanimoto H, Ohama K, Parmley TH, et al. Overexpression of testisin, a serine protease expressed by testicular germ cells, in epithelial ovarian tumor cells. *J Soc Gynecol Investig* 2000;**7**:358–62.
283. Tang T, Kmet M, Corral L, Vartanian S, Tobler A, Papkoff J. Testisin, a glycosyl-phosphatidylinositol-linked serine protease, promotes malignant transformation in vitro and in vivo. *Cancer Res* 2005;**65**:868–78.
284. Kempkensteffen C, Christoph F, Weikert S, Krause H, Kolleremann J, Schostak M, et al. Epigenetic silencing of the putative tumor suppressor gene testisin in testicular germ cell tumors. *J Cancer Res Clin Oncol* 2006;**132**:765–70.