REVIEW ARTICLE

Matriptase-2 (TMPRSS6): a proteolytic regulator of iron homeostasis

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ABSTRACT

Maintaining the body's levels of iron within precise boundaries is essential for normal physiological function. Alterations of these levels below or above the healthy limit lead to a systemic deficiency or overload in iron. The type-two transmembrane serine protease (TTSP), matriptase-2 (also known as TMPRSS6), is attracting significant amounts of interest due to its recently described role in iron homeostasis. The finding of this regulatory role for matriptase-2 was originally derived from the observation that mice deficient in this protease present with anemia due to elevated levels of hepcidin and impaired intestinal iron absorption. Further *in vitro* analysis has demonstrated that matriptase-2 functions to suppress bone morphogenetic protein stimulation of hepcidin transcription through cell surface proteolytic processing of the bone morphogenetic protein co-receptor hemojuvelin. Consistently, the anemic phenotype of matriptase-2 knockout mice is mirrored in humans with matripase-2 mutations. Currently, 14 patients with iron-refractory iron deficiency anemia (IRIDA) have been reported to harbor various genetic mutations that abrogate matriptase-2 proteolytic activity. In this review, after overviewing the membrane anchored serine proteases, in particular the TTSP family, we summarize the identification and characterization of matriptase-2 and describe its functional relevance in iron metabolism.

Key words: Matriptase-2, proteolytic regulator, iron homeostasis.

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Introduction

Proteolytic enzymes (proteases) are effectors of numerous biological events either as non-specific catalysts of protein degradation or highly selective mediators involved in tightly regulated physiological events.[1] The current classes of proteases are recognized on the basis of their catalytic mechanisms and include serine, cysteine, aspartic, glutamic, metallo- and threonine proteases.[2] Serine proteases are the largest of the classes, displaying ubiquitous organism expression, being found in viruses, bacteria and eukaryotes.[3] Over 20 families (denoted S1-S66) of serine proteases have been identified, these being grouped into clans on the basis of structural similarity and other functional evidence.[4] Serine proteases belonging to clan PA are one of the most well described groups of enzymes to date.[4] The majority of serine proteases in clan PA belong to the S1 family, which encompasses two distinct subfamilies, S1A and S1B. S1A and S1B are phylogenetically distinct groups of enzymes that share common structural architecture. The S1B proteases are ubiquitously expressed in all cellular life and are responsible for intracellular turnover, while S1A proteases mediate a variety of extracellular processes and display a limited distribution in plants, prokaryotes and the archea.[4] The S1A serine proteases, which encompass as many as 140 of the total 569 human degradome (the complete human protease genes complement),[5] modulate a variety of cellular processes by selective cleavage of specific substrates to influence cell behavior. Well known physiological examples include the proteases of the blood coagulation (e.g. thrombin), digestive (e.g. trypsin) and wound healing (e.g. plasmin) cascades. Until 20 years ago, S1A serine proteases had been predominately viewed as secreted enzymes. The identification and characterization of the transmembrane domain containing serine protease, hepsin,[6] catalyzed the emergence of a structurally distinct group termed broadly, the membrane anchored serine proteases. This family of enzymes is increasingly being acknowledged as having critical physiological functions, exemplified recently by the discovery of the iron regulatory role of matriptase-2.[7,8] Matriptase-2 through its proteolytic processing of cell surface hemojuvelin suppresses the

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transcription of hepcidin and as a consequence indirectly regulates systemic iron levels.[9]

Membrane anchored serine proteases

Due to their cell surface localization, the membrane anchored serine proteases differ in biological function to their secreted counterparts.[10] As opposed to participation in distant extracellular catabolic processes, these enzymes regulate key events at the plasma membrane. Peri-cellular proteolysis via cell surface localized proteases is increasingly recognized as an essential pathway through which cells interact with their immediate microenvironment. Cell surface proteolysis regulates the transduction of extracellular stimuli across the cell membrane,[11,12] the release of bioactive growth factors, cytokines and peptide hormones, in addition to facilitating interactions with neighboring cells and proteins of the basement membrane and extracellular matrix.[10] Plasma membrane localization of these serine proteases is mediated by the inclusion in their synthesis of an amino- or carboxyl-terminal hydrophobic extension that is threaded through the lipid bi-layer to allow the extracellular orientation of their protease domains. Although there are significant homologies within their protease domains, variations exist in the anchoring domains utilized by these enzymes. The membrane anchoring sequences have predicted that these proteases are bound by carboxyl-terminal transmembrane domains (type I serine protease), amino-terminal transmembrane domains (type II transmembrane serine protease) or glycosyl-phosphatidylinositol linkages (GPI-anchored serine protease). The largest of these families are the TTSPs,[13,14] which in reality, were first discovered over a century ago when enteropeptidase was demonstrated to be an essential enzyme for the activation of pancreatic digestive proteases. When cloning studies in 1994 identified that enteropeptidase, like the previously described hepsin, contained a type II transmembrane domain,[15] a new family of serine proteases emerged. Over the next five years the family rapidly expanded with identification of TMPRSS2,[16] human airway trypsin-like protease (HAT),[17] corin,[18] and matriptase.[19,20] To date, 20 TTSPs have been identified in humans (Figure 1) with several of these proteases now attributed with regulating critical physiological processes.

The type II transmembrane serine proteases

In addition to their characteristic type II transmembrane spanning region, the TTSPs share a number of common structural features, including a serine protease domain, a variable length stem region consisting of a mosaic of structural domains, and a short cytoplasmic tail (Figure 1). As is the case for the wider S1A family, TTSPs contain a conserved catalytic motif consisting of the triad of residues histidine, aspartate and serine. Based on their amino acid sequences, TTSPs are likely synthesized as single chain zymogens before proteolytic activation following an arginine or lysine residue present in their highly conserved activation domains. The proteases involved in TTSP activation are currently unknown, although due to their substrate preference (arginine or lysine) inter-family activation by those members demonstrating overlapping tissue expression is possible. Further, biochemical experiments have demonstrated the occurrence of auto-activation events for several human members of this family, including, TMPRSS2,[21] matriptase[20] and matriptase-2.[22]

Activated TTSPs are predicted to remain membrane-bound through a conserved disulphide bond linking the pro- and catalytic domains. Interestingly, soluble forms of enteropeptidase,[23] HAT,[24] TMPRSS2,[21] and matriptase[19] have been isolated *in vivo*. Shedding from the cell surface for porcine enteropeptidase and murine matriptase is mediated through proteolytic processing at the respective cleavage sites, ^{117}GSVIV and ^{149}GSVIA, within the SEA (Sea urchin sperm protein, Enteropeptidase, Agrin) domains of their stem regions.[25,26] In other SEA domain-containing membrane proteins, auto-proteolysis after a glycine residue within a conserved motif (e.g. GSVVV) releases these molecules from the cell surface.[27] In addition to SEA domains, the stem regions of the TTSPs may contain additional domains that likely play regulatory and/or protein interaction roles. These include LDLa (Low Density lipoprotein receptor class A) domains; SR (group A Scavenger Receptor) domains; frizzled domains; CUB (Cls/Clr, Urchin embryonic growth factor and Bone morphogenic protein 1) domains; and MAM (a Meprin, A5 antigen, and receptor protein phosphatase μ) domains. The importance of stem regions for normal TTSP biochemical function is illustrated by studies demonstrating their roles in zymogen conversion, substrate recognition and proteolytic activity.[28-31] Additionally, comparisons with similar domain containing proteins allow some speculation on the function of individual domains. The most common TTSP structural domain, LDLa, mediates cellular internalization of macromolecules[32,33] and ligand-stimulated cAMP signaling.[34] Group A scavenger receptor domains are present in soluble and membrane-bound receptors that function in the binding of modified lipoproteins, adhesion and bacterial binding.[35] In several protease classes, CUB domains have been demonstrated to be crucial for substrate recognition and/or cleavage.[36,37] A domain present only in enteropeptidase, MAM, is responsible for protein oligomerization in meprin proteases[38] and tyrosine phosphatase mu,[39] while Frizzled domains function as receptors for Wnt proteins during embryonic development and stem cell self-renewal.[40]

Utilizing the domain composition of the stem region in tandem with phylogenetic analysis of the serine protease domain of the TTSPs has enabled sub-familial classification.[14] The largest of the sub-families, HAT/DESC (Differentially Expressed in Squamous cell Carcinoma) consists of the proteases HAT, DESC1-4[10,41,42] and HAT-like 3-5.[43] HAT was originally purified from the sputum of patients with chronic airway diseases[24] and is proposed to be associated with mucus production[44] and fibrin deposition within airways.[45] Further, elevated levels of HAT are present in the epidermis of psoriasis patients, suggesting an inflammatory role for this enzyme.[46] Physiological roles for other HAT/DESC proteases have not yet been ascertained, however, DESC1 is hypothesized to have tumor suppressive properties in squamous cell carcinoma due to its significant reduction in expres-

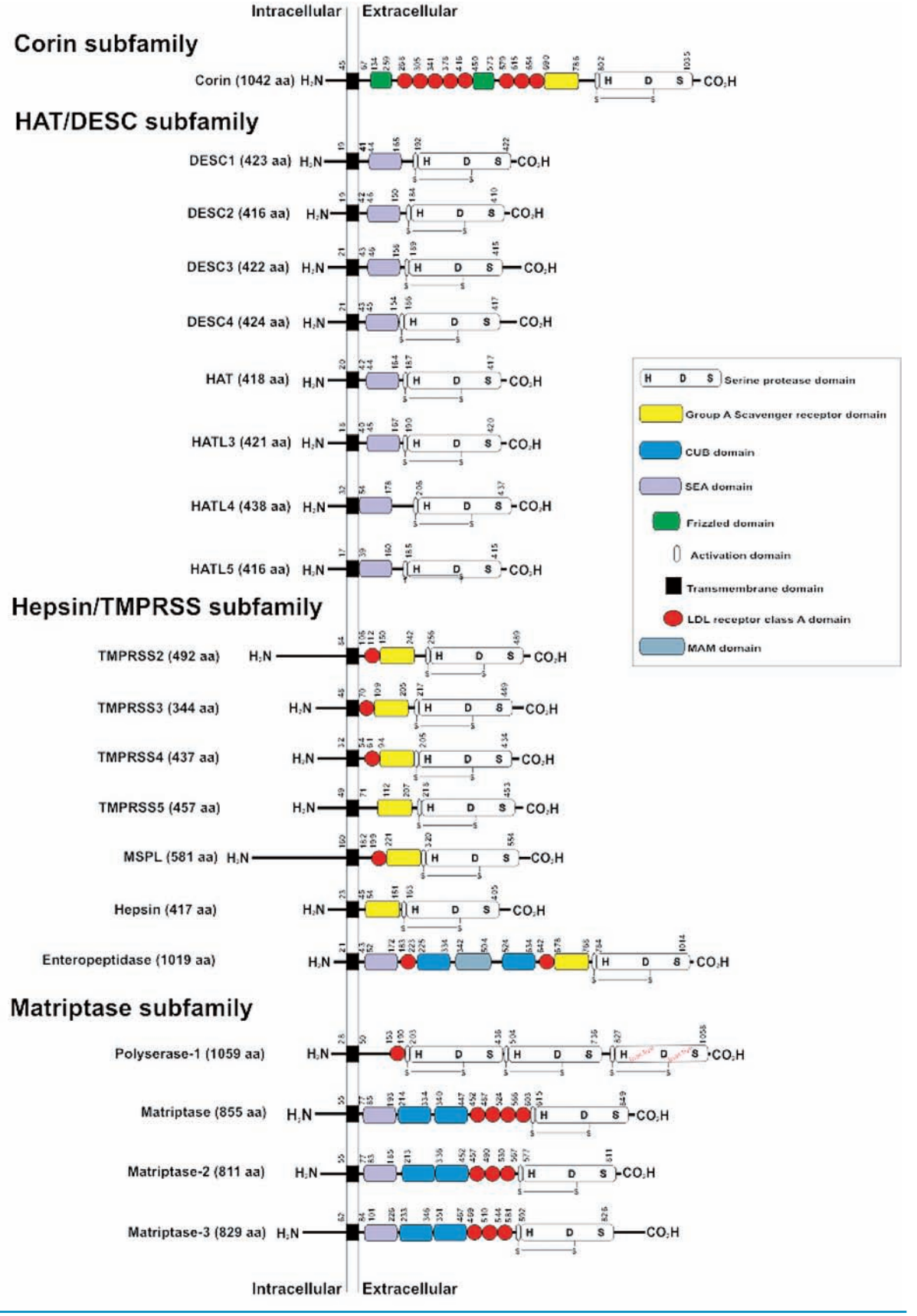

Figure 1. The domain structures of the human type II transmembrane serine proteases. TTSPs are grouped into subfamilies based on stem domain composition and phylogenetic analysis of serine protease domains.[14] A consensus domain legend is provided on the right side of the figure. Domain predictions were generated by scanning the respective amino acid sequences with the SMART algorithm (ExPASy Proteomics Tools website). Assigned numbers refer to the location of each domain in the preproenzyme.

sion during carcinoma progression.[41] Interestingly, overexpression of DESC1 has been documented in tumors derived from kidney, brain and breast tissues, suggesting a pro-tumorigenic function depending on the tissue of origin.[47]

The second largest of the sub-families, hepsin/TMPRSS/enteropeptidase contains TMPRSS 2-5,[48-50] MSPL,[51] hepsin and enteropeptidase. The most comprehensively described member of this sub-family, enteropeptidase, functions near the apex of a proteolytic cascade of digestive enzymes through its conversion of trypsinogen to trypsin.[15] Interestingly, hepsin-deficient mice and human TMPRSS3 mutation studies have demonstrated the requirement of these enzymes for normal auditory function.[52,53] Further, TMPRSS5 is co-expressed with hepsin and TMPRSS3 in spiral ganglions of the mouse cochlear, where it is proposed to influence inner ear function.[54] Loss of TMPRSS2 in mice has no effect on development, fertility, survival or organ pathology.[55] However, expression of the androgen regulated *TMPRSS2* is up-regulated in high-grade prostate cancer, where it is mislocalized, being expressed in the cytoplasm as well as in the cell membrane.[56] It is remarkable that fusions between the promoter of TMPRSS2 and genes of the ETS transcription factor family have been identified in prostate cancer and are potential prognostic biomarkers.[57] There is currently no information on the physiological roles for the remaining members, TMPRSS4 and MSPL, although TMPRSS4 is overexpressed in pancreatic, gastric and colorectal cancers and its overexpression facilitates a cellular, epithelial to mesenchymal transition *in vitro*.[58]

The solitary sub-familial classification given to corin is due to the enzyme's similarity in protease domain structure with non-TTSPs, and unparalleled stem region architecture. In adults corin is expressed exclusively in the heart by cardiac myocytes,[59] where it has been demonstrated to convert pro-atrial natriuretic peptide (pro-ANP) to active ANP in a sequence-specific manner.[60,61] Mice deficient in corin display disrupted pro-ANP conversion, resulting in salt-sensitive hypertension.[62] The hypertensive phenotype is exacerbated when the mice become pregnant. In the human population two non-conserved mutations within the second frizzled domain are associated with higher systolic blood pressure and an increased risk for chronic hypertension.[63,64] Biochemically, the mutations cause reduced pro-ANP activation due to an impaired corin zymogen conversion.[65] Interestingly, the frequency of the mutant allele within the Afro-American population is three times higher than in the American Caucasian population, potentially correlating to the increased risk of hypertension and heart disease in Afro-Americans.[63] The matriptase sub-family contains the highly similar matriptase, matriptase-2,[22,66] matriptase-3[67] and the mosaic poly-protease, polyserase-1.[68] Cloned in our laboratory, polyserase-1 undergoes a series of post-translational processing events to generate three distinct and independent serine protease domains termed serase-1, -2 and the catalytically inactive serase-3.[68] Further, two additional secreted poly-proteases, polyserase-2 and polyserase-3,[69,70] have been recently identified by our profiling studies of the human degradome.[71] The most studied member of the sub-family, matriptase, has been demonstrated by knockout mice studies to be required for postnatal survival, epidermal barrier function, hair follicle development and thymic homeostasis.[72] Potentially, the activation of prostasin by matriptase is an important event for epidermal barrier formation, attested to by a deficiency of active prostasin in mice lacking matriptase, and an overlapping phenotype of mice deficient in each of the enzymes.[73,74] Further, constraint of matriptase activation and inhibition by the transmembrane Kunitz-type serine protease inhibitor, hepatocyte growth factor activator inhibitor (HAI)-1, appears to be a critical regulatory point in embryogenesis[75] and postnatal skin and hair development.[76] While physiological events mediated by matriptase-3 are currently unknown, several very recent studies by us and others have demonstrated matriptase-2 to be a critical regulator of iron homeostasis through its proteolytic processing of membrane hemojuvelin.

Matriptase-2

The matriptase-2 cDNA was identified in human and mouse using in silico approaches.[22,66] The complete human cDNA was cloned from fetal liver and named on the basis of its significant structural similarity to the TTSP matriptase. The mouse coding sequence was identified from an expressed sequence tag clone generated from adult liver. The mouse and rat encoded proteins were originally designated Tmprss6 on the basis of TTSP nomenclature. However, for consistency the matriptase-2 designation is now applied across all species.

The matriptase-2 gene is highly conserved across mammalian species and ranges in size from ~29 kb in mouse to ~40 kb in chimpanzee.[77] As shown in Figure 2, this gene spans 18 exons with 17 intervening introns, with the matriptase-2 protein domain boundaries corresponding with intron/exon junctions of the encoding gene across all species. The first CUB domain is encoded by two exons (7 and 8), and the second CUB domain by three exons (9 to 11). The three LDLR domains of matriptase-2 are each encoded by separate exons (12, 13 and 14). Finally, the serine-protease domain, including the activation domain, is encoded by four exons (15 to 18). Accordingly, structural features are absolutely conserved across human, macaque, dog, cow, mouse and rat, with the human protein sharing 95.6%, 91.1%, 85.6%, 80.1% and 80.4% identity, respectively, to matriptase-2 from these species.[77] Consistently, by Western blot analysis of lysates from transiently transfected cells, both human and mouse matriptase-2 migrate close to the predicted molecular mass of ~90 kDa.[22,66] The matriptase-2 proteolytic domain has all the features common to members of the serine protease S1 family. These include the serine protease triad of ^{617}H, ^{668}D and ^{762}S residues (human numbering) required for catalytic activity, and an SWG motif predicted to be located at the top of the substrate S1 binding pocket positioning the scissile bond of the substrate in the correct orientation. Proteolytic activation of matriptase-2 is predicted to occur within a motif (^{576}RIVGG) at the junction of the pro- and catalytic domains which is charac-

teristic of serine proteases and conserved across species. Consistent with the presence of an aspartate residue 6 amino acids before the catalytic serine, which specifies preference for trypsin-like serine protease cleavage following arginine or lysine residues, the recombinant matriptase-2 protease domain cleaves following arginine but not alanine.[22]

In adult human and mouse tissues, the primary site of matriptase-2 mRNA expression is the liver, while the kidney was also a site of high mRNA transcription, with lower levels in uterus and much smaller amounts detected in many other tissues.[22,66] Further, matriptase-2 mRNA expression was demonstrated to be restricted to hepatocytes in the liver, predominately within glandular columnar epithelial cells in the uterus and ubiquitous throughout the kidney. In murine embryos, matriptase-2 mRNA expression peaked at day 13.5 *post coitus*. In addition to high expression in liver, matriptase-2 mRNA was strongly detected in olfactory epithelial cells of the nasal cavity and in pharyngo-tympanic tubes. These data suggest that a role may exist for this enzyme in embryogenesis and olfactory processes. Significantly, recent studies by our laboratory and others have attributed to liver matriptase-2 expression an essential regulatory role in systemic iron homeostasis.[7,8]

Systemic iron regulation by matriptase-2

Iron is an essential trace element in mammalian metabolism, whose physiological levels, due to its generation of bio-reactive superoxide anions and hydroxyl radicals, require tight regulation.[78] As the capacity for iron excretion is limited, systemic iron levels are primarily controlled through iron absorption by mature enterocytes of the duodenum. Further, balanced extracellular iron levels depend upon iron release from macrophages and hepatocyte stores. An integral player in regulating body iron supply in response to its requirements is the hepatic peptide hormone, hepcidin.[79,80] Hepcidin mediates the internalization and degradation of the iron export molecule ferroportin, located on the surface of intestinal enterocytes, macrophages and hepatocytes, thereby negatively regulating iron entry into the plasma.[81] Consistently, mice deficient in hepcidin[82] and humans with mutations of this gene develop severe iron overload disorders.[83] Conversely, mice with increased transgenic expression of hepcidin in the liver manifest severe iron deficiency anemia.[84] As such, control of hepcidin expression represents a critical checkpoint for maintaining iron balance. Recent studies performed in our laboratory as well as by Du and colleagues, have demonstrated that matriptase-2 functions as a negative regulator of hepcidin expression.[7,8] Mice deficient in the *Tmprss6* gene have a marked upregulation in hepcidin transcription and demonstrate an overt phenotype of alopecia and severe iron deficiency anemia.[7] Further, the *Tmprss6*$^{-/-}$ mice have reduced protein levels of ferroportin on the basolateral membrane of enterocytes of the duodenum, leading to the retention of iron within these cells. Supplementation of plasma iron levels through subcutaneous delivery of iron dextran effectively rescues the phenotype, reversing the hematologic deficiencies and restoring normal hair growth. Critically, the pathophysiological alterations in *Tmprss6*$^{-/-}$ mice are directly attributable to an absence of matriptase-2 proteolytic activity, as is clearly demonstrated by the presentation of identical phenotypic abnormalities in *mask* mice, a murine model that through chemical manipulation expresses a proteolytically inactive form of matriptase-2.[8]

The very recent work of Silvestri *et al.* has demonstrated that the negative regulation of hepcidin by matriptase-2 is mediated via its proteolysis of the membrane receptor hemojuvelin.[9] As shown in Figure 3, hemojuvelin is synthesized by hepatocytes as a membrane GPI linked protein that behaves as a co-receptor for BMP-2, -4 and -6.[85-87] BMP stimuli, transduced, via SMAD (Son of Mother Against Decapentaplegic) proteins, is the primary activator of hepcidin expression, as evidenced by the loss of hepcidin expression in mice with targeted liver deletion of SMAD4[88] and the strong

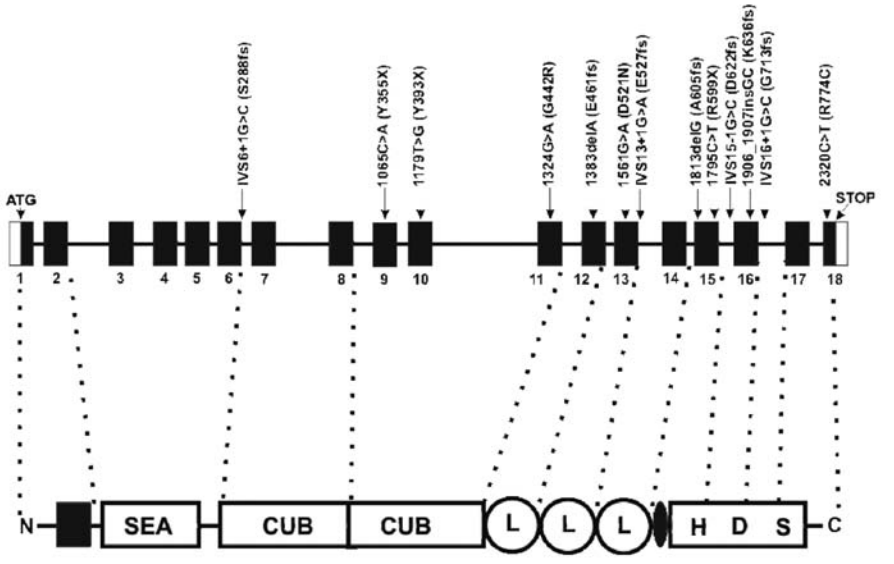

Figure 2. Matriptase-2 gene structure and locations of IRIDA mutations. The upper panel of the figure depicts the genomic organization of matriptase-2 with black and white boxes representing coding and non-coding regions respectively. All currently identified IRIDA patient mutations are marked with arrows on the matriptase-2 gene corresponding to their genomic locations. The encoded protein is shown in the lower panel. Dashed lines mark exon encoding boundaries for each of the matriptase-2 protein structural domains, including transmembrane (black box), one SEA, two CUB, three LDLa (L), activation (black oval) and proteolytic (boxed HDS).

stimulation of hepcidin *in vitro* by BMPs.[87,89,90] Further, hemojuvelin deficient mice display loss of hepcidin expression and present with iron overload.[91,92] A soluble form of hemojuvelin, produced by the liver and skeletal muscle, antagonizes BMP-2 and -4 stimulated hepcidin expressions.[93] Consistently, high-dose administrations of soluble hemojuvelin induce an increase in serum iron levels *in vivo* by suppressing hepcidin production.[86] The hemojuvelin/hepcidin regulatory pathway has been causally linked to the murine *Tmprss6*$^{-/-}$ and *mask* phenotypes through *in vitro* experiments that demonstrate matriptase-2 proteolytically processes membrane hemojuvelin, significantly reducing hepcidin transcription in response to BMP-2 stimulus.[9] Interestingly, matriptase-2 does not cleave the soluble form of hemojuvelin, suggesting that matriptase-2 functions to dampen BMP stimuli directly through proteolysis of membrane hemojuvelin, and indirectly by creating an imbalance in levels of BMP co-receptor and antagonist. Critically, validation of these *in vitro* demonstrations *in vivo*, are an essential foundation for further delineation of the mechanism(s) of matriptase-2 in systemic iron regulation.

The relevance of the physiological observations made in *Tmprss6*$^{-/-}$ and *mask* mice for correlations with human iron disorders were provided proof of principle by Finberg and colleagues through their description of human *matriptase-2* gene mutations in IRIDA patients.[94]

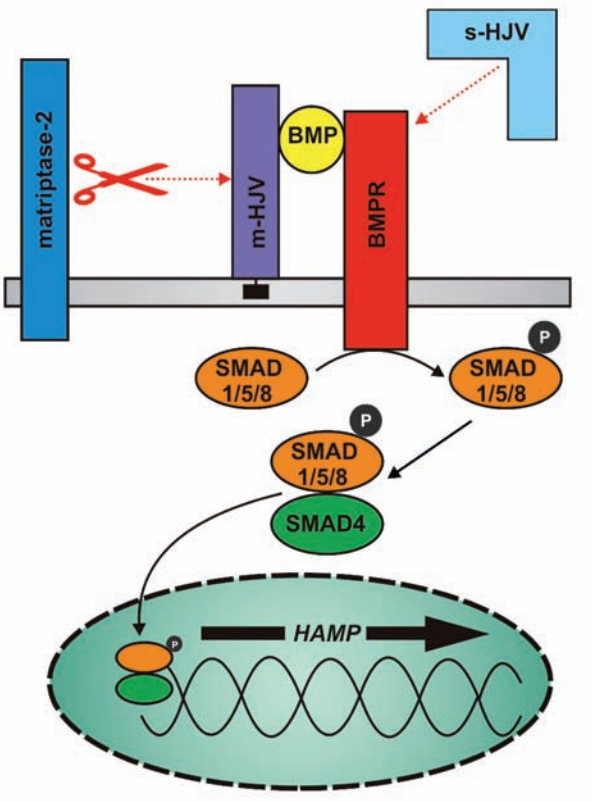

Figure 3. The role of matriptase-2 in the hepcidin regulatory pathway. Beginning extracellularly (top of panel), BMP-2,-4 or -6 bind to the co-receptor membrane hemojuvelin (m-HJV) and the BMP receptor (BMPR). This initiates phosphorylation of SMAD-1,-5 and -8 and formation of heteromeric complexes with the common mediator Smad4. Following nuclear translocation, the heteromeric SMAD complexes stimulate transcription of the *HAMP* gene. Negative regulation of the BMP-HJV-hepcidin pathway (designated by dashed red arrows) is mediated through the proteolytic processing of m-HJV by matriptase-2. Additional negative regulation of hepcidin transcription is provided by soluble hemojuvelin (s-HJV), which acts as an antagonist of the BMP pathway by competing with m-HJV for BMP ligands.

Matriptase-2 mutations in human iron disorders

Iron-limited anemias are categorized as those that are caused by chronic disease states or genetic mutations. The anemia of chronic disease is an acquired disorder seen in patients with a variety of inflammatory disorders, including infections, malignancies, and rheumatological disorders.[95] Currently, there is a limited understanding of genetically acquired iron-limited anemias. In accordance with its role in co-ordinating body iron levels, alterations in the genes encoding hepcidin[83] or its key regulators[96-98] induce iron overload syndromes such as hereditary hemochromatosis (HH). Consistently, HH disorders result from inadequate hepcidin production relative to body iron stores.[99] Conversely, elevated hepcidin levels have been described in iron deficiency anemia patients that are insensitive to oral iron therapy and display an incomplete hematologic recovery with parenteral iron administrations, a condition termed iron refractory iron deficiency anemia (IRIDA).[94,100] Heterozygous and homozygous biallelic human matriptase-2 mutations have been identified by 3 independent studies in 14 IRIDA patients from Northern European, African and Afro-American,[94] Mediterranean[100] and English ancestries.[101] Further, 2 additional IRIDA patients who harbored a single mutated allele have been described,[94] although as only exon/intron boundaries and coding regions were sequenced, deep intronic mutations of the second allele may exist.

As shown in Table 1, hematologic parameters of IRIDA patients mirror those of *Tmprss6*$^{-/-}$ and mask mice, including a congenital hypochromic, microcytic anemia, low corpuscular erythrocyte volume, low transferrin saturation and abnormal iron absorption. The mutations, as summarized in Table 1 and their genomic positions noted in Figure 2, include frame-shift, splice junction, missense and nonsense mutations, distal to exon 6. Predominately the identified mutations, including 1906_1907insGC, 1813delG, IVS13+1G>A, IVS15-1G>C, 1383delA, IVS6+1G>C, 1179T>G and 1795C>T, encode for matriptase-2 proteins which lack functional protease domains. Overexpression of the protease domain deficient *mask* human matriptase-2 in zebrafish results in reduced hemoglobinization in comparison to wild-type human matriptase-2, illustrating the *in vivo* impact a deficiency in matriptase-2 proteolytic activity renders.[9] Further, it is hypothesized that overexpression of *mask* human matriptase-2 elicits a dominant negative effect by binding, potentially through stem domain interactions, and sequestering hemojuvelin from endogenous zebrafish matriptase-2. Collaborating with matriptase-2 stem domain importance for *in vivo* function, heterozygous missense mutations in the CUB (1324G>A) and LDLa (1561>A) domains have been identified in IRIDA patients.[94]

Table 1. Hematologic parameters of *Tmprss6*$^{-/-}$ mice, "mask" mice and IRIDA patients.

	Tmprss6$^{-/-}$ mice (Folgueras *et al.*)[7]	*mask* mice (Du *et al.*)[8]	IRIDA patients (Finberg *et al.*)[94]	IRIDA patient (Melis *et al.*)[100]	IRIDA patient (Guillem *et al.*)[101]
Matriptase-2 allele and encoded protein mutations	Null/null	IVS14-2A>G/IVS14-2A>G (C566fs)/(C566fs)	1906_1907ins GC/1906_1907ins GC (K636fs)/(K636fs) IVS15-1G>C/IVS15-1G>C (D622fs)/D622fs) 1813delG/IVS13+1G>A (A605fs)/(E527fs) 1324G>A/1561G>A (G442R)/(D521N) 1065C>A/1383delA (Y355X)/(E461fs) IVS16+1G>C/not identified (G713fs)/- 2320C>T/not identified (R774C)/-	IVS6+1G>C/IVS6+1G>C (S288fs)/(S288fs)	1179T>G/1795C>T (Y393X)/(R599X)
Hemoglobin	Low	Low	Low	Low	Low
Hepcidin levels	High (liver mRNA)	High (liver mRNA)	High (urine protein)	High (urine/serum protein)	High (serum protein)
Iron absorption	Impaired	Impaired	Impaired	Impaired	Impaired
Iron stores	ND	high in spleen	ND	normal in liver	ND
MCV	Low	Low	Low	Low	Low
Reticulocytes	ND	ND	Low	ND	Low
Serum iron	Low	Low	ND	Low	Low
Transferrin saturation	Low	ND	Low	Low	Low

For IRIDA patients, "low" refers to values below the standard reference range. "Low" in Tmprss6$^{-/-}$ *and "mask" mice refers to values significantly lower than wild-type counterparts. Impaired iron absorption was determined by dietary iron uptake ("mask" mice), duodenal enterocyte iron retention (Tmprss6*$^{-/-}$*) and lack of response to oral iron treatment (IRIDA patients). MCV, mean cell volume; ND, not determined.*

With the recently identified suppression of hepcidin expression through proteolysis of membrane hemojuvelin by matriptase-2, it would be of great interest in future studies to confirm the *in vivo* status of hemojuvelin in IRIDA patients in comparison to healthy controls. Collectively these data suggest that restoration of matriptase-2 function and subsequent reduction of hepcidin levels represents a clinical opportunity in treating IRIDA. As such, liver-directed gene therapy through *ex vivo* hepatocyte manipulation and transplantation may offer one such avenue to supplement the livers of IRIDA patients with functional matriptase-2.[102] Conversely, hepcidin stimulation through matriptase-2 inhibition presents an intriguing clinical opportunity to counteract hepcidin deficiencies seen in patients with HH disorders.[99]

Conclusions and future perspectives

Our knowledge of the physiological roles of the TTSP family continues to evolve with the generation of loss-of-function animal models. Matriptase-2 becomes the most recent member of this family attributed with fulfilling a critical physiological role. The identity of matriptase-2 as a key element of an elaborate iron homeostatic network fosters a number of exciting avenues for future work in this area. A central element of these future challenges is to understand how matriptase-2 maintains hepcidin levels within the narrow limits required for normal physiological function. A critical aspect of these challenges is defining how body iron levels exert transcriptional control over the *HAMP* gene. Exposure of primary hepatocytes *in vitro* to holotransferrin induces hepcidin transcription through a hemojuvelin/BMP-dependent pathway.[90] As BMP-6 is currently the only component of the hemojuvelin/BMP signaling pathway that has been demonstrated to be iron regulated,[103] matriptase-2 may represent an additional element of this pathway whose expression is regulated in accordance with body iron stores.

The domain composition of matriptase-2 may potentially accord further mechanisms for calibrating hepcidin levels. Foremost amongst these are modulations of the protease domain. Inhibitor interactions, the *in vivo* relevance of which is exemplified by the interactions of the highly similar family member matriptase and its cognate inhibitor HAI-1, would have significant *in vivo* ramifications. HAI-1 both abolishes matriptase activity through active site inhibition, and paradoxically, controls zymogen matriptase activation, fundamentally governing the proteolytic activities that are essential for embryogenesis and epidermal barrier formation.[104] Consequently, modulation of the *in vivo* activities of matriptase-2 through either active site inhibition or zymogen conversion would afford exquisite control over hepcidin suppression. Interestingly, *in vitro* demonstrations of cell surface shedding of the matriptase-2 protease domain suggests that a circulating form of the enzyme may exist, potentially with a differing proteolytic agenda to the peri-cellular activities of the membrane precursor.[9] Further, the cytoplasmic tail of matriptase-2 offers intriguing possibilities for hepcidin control. Overexpression *in vitro* of a matriptase-2 mutant in

which the extracellular portion was substituted with GFP exhibited suppression of *HAMP* promoter activity, implying direct signal transduction involvement of matriptase-2 in hepcidin suppression.[8] However, none of the intracellular consensus phosphorylation sites are conserved between species, suggesting that potential phosphorylation of the matriptase-2 N-terminus may be species dependent.

Hopefully, clarification of these fundamental biochemical questions will contribute to a better understanding of the functional relevance of matriptase-2 in regulating iron homeostasis and to translate this information into clinical answers for patients with IRIDA or other deficiencies in iron metabolism.

Authorship and Disclosures

AJR, JDH and CLO wrote the paper; AJR and JDH formatted figures and tables; ARF and GVC analyzed literature and revised the manuscript.

All authors equally contributed to this paper. The authors declare they have no conflicts of interest. The authors declare no competing financial interests.

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