



# Iron and hepcidin: a story of recycling and balance

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To avoid iron deficiency and overload, iron availability is tightly regulated at both the cellular and systemic levels. The liver peptide hepcidin controls iron flux to plasma from enterocytes and macrophages through degradation of the cellular iron exporter ferroportin. The hepcidin-ferroportin axis is essential to maintaining iron homeostasis. Genetic inactivation of proteins of the hepcidin-activating pathway causes iron overload of varying severity in human and mice. Hepcidin insufficiency and increased iron absorption are also characteristic of anemia due to ineffective erythropoiesis in which, despite high total body iron, hepcidin is suppressed by the high erythropoietic activity, worsening both iron overload and anemia in a vicious cycle. Hepcidin excess resulting from genetic inactivation of a hepcidin inhibitor, the transmembrane protease serine 6 (TMPRSS6) leads to a form of iron deficiency refractory to oral iron. Increased hepcidin explains the iron sequestration and iron-restricted erythropoiesis of anemia associated with chronic inflammatory diseases. In mice, deletion of *TMPRSS6* in vivo has profound effects on the iron phenotype of hemochromatosis and beta-thalassemia. Hepcidin manipulation to restrict iron is a successful strategy to improve erythropoiesis in thalassemia, as shown clearly in preclinical studies targeting *TMPRSS6*; attempts to control anemia of chronic diseases by antagonizing the hepcidin effect are ongoing. Finally, the metabolic pathways identified from iron disorders are now being explored in other human pathologic conditions, including cancer.

## Introduction

Iron is essential for multiple cell functions, but is also potentially deleterious because of its ability to generate free oxygen radicals. Due to the absence of an active excretory mechanism, iron balance in mammals is maintained by limiting its intestinal uptake and by continuously recycling and reusing cellular iron. Multiple safety mechanisms, such as binding to chaperone proteins, storage in ferritin, and export through ferroportin (FPN), protect cells from free iron toxicity. The mechanisms of cellular iron handling are summarized in Figure 1. Iron is used in mitochondria for heme synthesis and iron sulfur cluster biogenesis. There is increasing interest in the latter pathway because iron sulfur clusters are prosthetic groups for key enzymes of DNA duplication, repair, and epigenetics. Iron-regulatory proteins (IRPs) and hepcidin exert iron homeostatic control at the cell and systemic levels, respectively.<sup>1</sup> Disruption of iron control mechanisms leads to genetic iron disorders and may also contribute to the pathophysiology of common pathologic conditions including inflammation, neurodegeneration, metabolic disorders, and cancer.

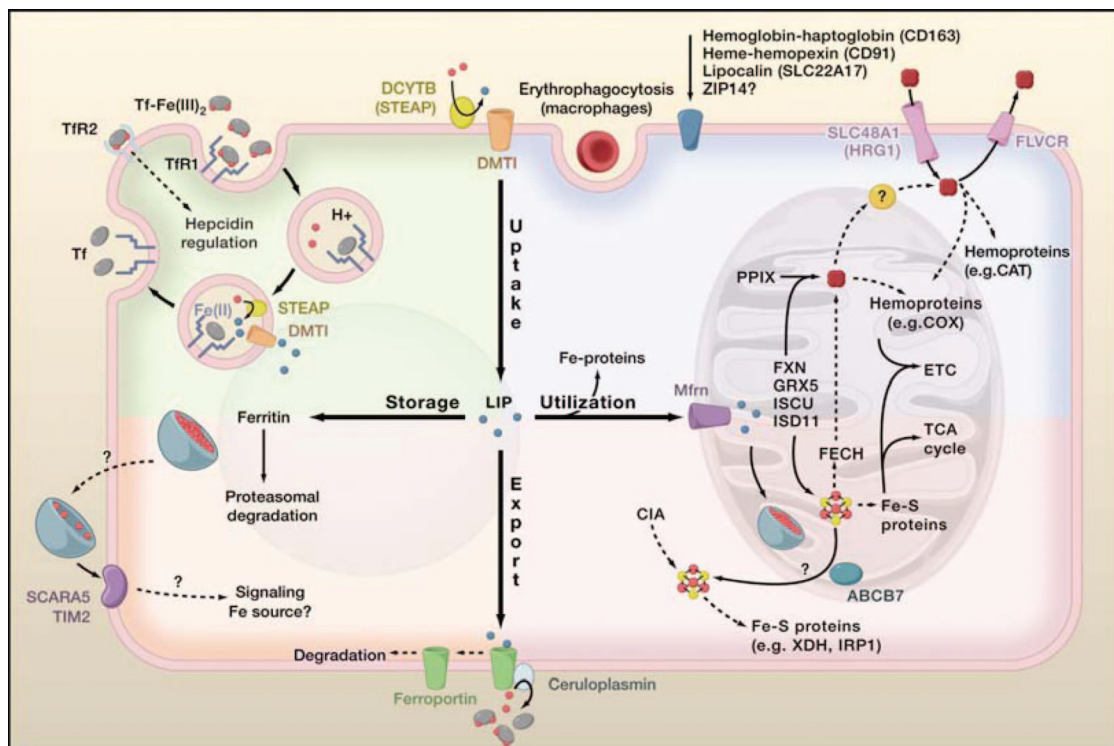
At the cellular level, IRP1 and IRP2 orchestrate the coordinated expression of iron importers (transferrin receptor 1 [TFR1] and divalent metal transporter 1 [DMT1]) and of storage (ferritin light and heavy chains) and export (FPN) proteins. IRPs regulate their targets posttranscriptionally by binding to special stem loop elements in the untranslated regions of mRNA-encoding proteins involved in iron metabolism; binding activity is high in iron deficiency and hypoxia and is suppressed by iron and oxygen (for review, see Hentze et al<sup>1</sup>). Recently, differential target specificity of the 2 IRPs has been identified, with IRP1 specifically controlling the hypoxia mediator HIF2- $\alpha$ <sup>2</sup> and IRP2 controlling ferritin.<sup>3</sup> Control of HIF2- $\alpha$  by IRP is one of the multiple links between iron and hypoxia. Undoubtedly, conditional deletion of either IRP in animal models will clarify other tissue- and IRP-specific roles.

At the systemic level, the liver peptide hepcidin regulates iron homeostasis by binding and degrading the sole cellular iron exporter FPN, which is highly expressed at the basolateral surface of duodenal enterocytes and on the cell membrane of macrophages. In this way, hepcidin restricts the amount of iron delivered to its plasma carrier transferrin.<sup>4</sup> The concentration of both circulating and tissue iron provides distinct signals that modulate hepcidin. The result is low hepcidin and active iron delivery to plasma in iron deficiency and high hepcidin with reduced iron flux to plasma in iron overload (Figure 2).

How IRP-based and systemic regulatory pathways interconnect and work together in general iron homeostasis is only partially understood and is a subject of intensive investigation.

## Hepcidin up-regulation

Hepcidin transcription in hepatocytes is dependent on the bone morphogenic protein (BMP)-SMAD signaling cascade (Figure 3A).<sup>1</sup> BMP6 is the iron-related BMP receptor (BMPR) ligand in vivo, as shown by *Bmp6*<sup>-/-</sup> mice, which have severe iron overload and very low hepcidin.<sup>5</sup> In the liver, BMP6 is mainly expressed in nonparenchymal cells such as sinusoidal endothelial and Kupffer cells.<sup>6</sup> Binding of the ligand to BMPR complex on the hepatocyte surface triggers phosphorylation of SMAD proteins, which translocate to the nucleus to activate target genes including hepcidin (Figure 3A). In mice, liver-specific disruption of the BMPR *ALK2* and *ALK-3* or of *SMAD4* molecule results in iron overload with low hepcidin.<sup>7</sup> Hemojuvelin (HJV), a protein mutated in juvenile hemochromatosis type A (Table 1), is the essential BMP coreceptor in this pathway. In humans, its inactivation causes severe, early onset iron overload indistinguishable from hemochromatosis caused by inactivation of the hepcidin gene itself.<sup>8</sup> Hemochromatosis type 1, 2 and 3 (Table 1) and their corresponding murine models show defective BPM signaling that results in hepcidin insufficiency. Whereas the function of membrane-HJV



**Figure 1. Cellular iron handling.** The 4 general mechanisms of cellular iron handling are shown. Iron is taken up through the endosomal cycle of TFR1 by all cells, but also by DMT1 (enterocytes) or RBC breakdown (macrophages). Within the cell, the labile iron pool (LIP) is used for metabolic needs. Most iron is transported to mitochondria to be used for iron sulfur cluster and heme biogenesis. In the cytosol, excess iron is stored in ferritin, a molecule that is also secreted into the circulation. Excess iron can also be exported through FPN. Used with permission from Hentze et al.<sup>1</sup>

in hepcidin activation is clearly defined, that of other hemochromatosis proteins (TFR2 and HFE) remains uncertain. To add further complexity, in mice, hepcidin, *Hfe*, and *Hjv* are modulated by microRNA miR-122.<sup>9</sup>

In inflammation, hepcidin is activated by IL-6, IL-1-beta, and other cytokines as well as by lipopolysaccharide through the JAK2/STAT3 signaling pathway (Figure 3B). The integrity of the BMP pathway is essential for a full hepcidin response in inflammation<sup>1</sup> and the cross-talk between the 2 pathways is the subject of intensive investigation.

### Hepcidin down-regulation

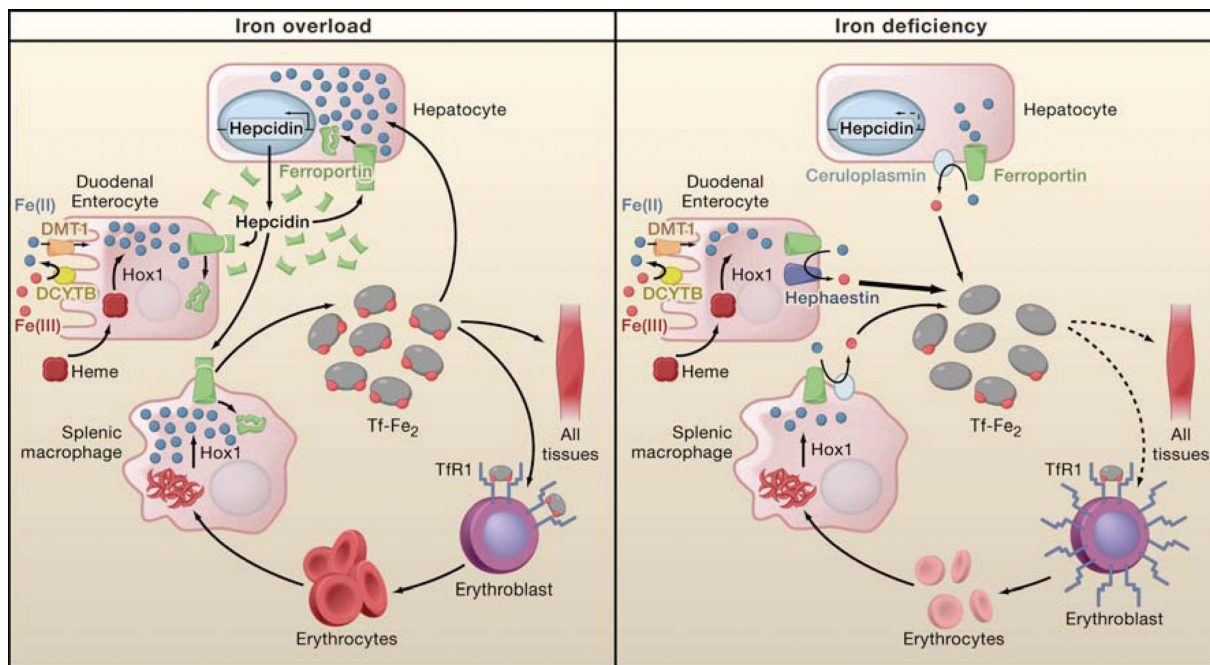
Erythropoiesis consumes most of the recycled and absorbed iron (~25 mg/daily). Therefore, it is not surprising that hepcidin expression is suppressed in all conditions characterized by increased erythroid demand for iron and elevated circulating erythropoietin levels, such as iron deficiency, hypoxia, and erythropoietic expansion. A key hepcidin inhibitor was discovered by positional cloning of the gene responsible for the phenotype of the *Mask* mice, which have microcytic anemia, high hepcidin, and are unable to absorb oral iron.<sup>10</sup> Matriptase-2, encoded by *TMPRSS6*, is a liver-expressed type II transmembrane serine protease that is inactivated in *Mask* mice due to truncation of the protein and loss of the catalytic domain. *TMPRSS6* interacts with and cleaves the BMP coreceptor HJV, switching off BMP signaling and thereby decreasing hepcidin transcription (Figure 3A).<sup>11</sup> It is unknown whether, in vivo, *TMPRSS6* cleaves other substrates. In iron deficiency, the function of *TMPRSS6* is essential to suppress hepcidin and to allow iron absorption. In vitro, the expression of *TMPRSS6* is up-regulated by hypoxia and iron deficiency and its proteolytic activity is inhibited

by hepatocyte growth factor activator inhibitor type-2 (HAI-2), an inhibitor of the homologous protease matriptase-1.<sup>12</sup> The regulation of *TMPRSS6* in vivo is largely unknown.

Several other candidate hepcidin inhibitors have been proposed. Any direct effect of the hypoxia mediator HIF-1-alpha on the hepcidin promoter remains uncertain. Growth differentiation factor 15 (GDF15) and twisted gastrulation protein homolog 1 (TWGS1) are released by erythroblasts and have been proposed as hepcidin suppressors, especially in thalassemia, but their physiologic or pathologic role remains uncertain.<sup>1</sup> The longstanding known soluble (s)TFR1 and the recently identified sTFR2 (C.C., unpublished data, April 2013) could in theory contribute to hepcidin suppression, but experimental proof is lacking. In vitro, soluble HJV produced by furin cleavage<sup>13</sup> competes as a decoy molecule with membrane-HJV for BMP6 binding, but its in vivo function is unclear. HJV is highly expressed in muscle, where it might have an iron-unrelated function, because muscle-specific *Hjv*-knockout mice have normal iron homeostasis.<sup>14</sup> It is conceivable that multiple inhibitors triggered by different signals from iron-deficient, expanded, or abnormal erythropoiesis all converge on the same final pathway.

### Lessons from human iron disorders

Genetic disruption of the finely tuned regulation of the hepcidin-FPN axis causes either iron overload or iron deficiency (Table 1). Hemochromatosis, a heterogeneous genetic disorder leading to iron overload and ultimately to organ failure, provided important clues to understanding hepcidin up-regulation, and autosomal recessive iron-refractory iron-deficiency anemia (IRIDA) provided clues to the mechanisms of hepcidin suppression.



**Figure 2. Systemic iron regulation according to iron status.** Shown is the differential regulation of liver hepcidin in conditions of iron overload (left) and iron deficiency (right). DMT1 in duodenal enterocytes takes up iron from the lumen after DCYTB reduces ferric to ferrous iron. At the basolateral membrane, iron is exported by FPN and oxidized by hephaestin. Iron-loaded transferrin (Tf-Fe<sub>2</sub>) delivers iron to cells by binding TFR1. After phagocytosis of RBCs by macrophages, heme released from hemoglobin is processed by heme oxygenase-1 (HOX1) to generate iron, which is then delivered to plasma through FPN. In iron overload (left), the high concentration of hepcidin binds and degrades FPN, blocking iron export. BMP6, stimulated by intracellular iron, increases hepcidin transcription in the liver. In iron deficiency (right), iron is released by FPN into the circulation. Production of hepcidin is low because of low BMP6 expression and because of inhibitory signals generated by iron-deficient erythropoiesis. Used with permission from Hentze et al.<sup>1</sup>

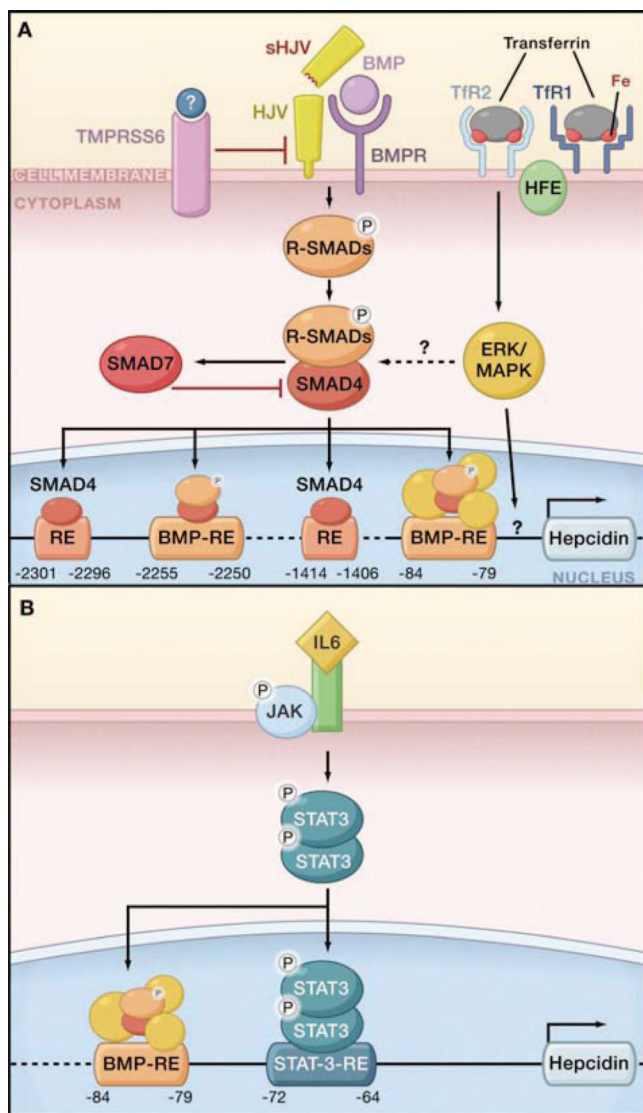
Hemochromatosis patients are natural human mutants of iron homeostasis. In the recessive disease, mutations affect genes involved in hepcidin activation (*HFE*, *HJV*, *HAMP*, *TFR2*); in the dominant type, they affect the hepcidin receptor FPN (Table 1; for review, see Pietrangelo<sup>15</sup>). In hemochromatosis, the severity of iron overload correlates with the degree of hepcidin deficiency, suggesting a hierarchy of the corresponding proteins in the regulatory pathway. *HAMP* (hepcidin) and *HJV*, the genes of the most severe juvenile form of hemochromatosis, have a central role in hepcidin regulation, whereas *HFE* and *TFR2* have ancillary roles. In the current model, *HFE* and *TFR2* function as a complex to activate hepcidin<sup>1</sup> (Figure 3A); however, distinct disorders result from their individual inactivation. *HFE* disease has adult onset, low penetrance, and male predominant expression, suggesting a modest biological effect of the protein. Although the number of cases reported is limited, *TFR2* hemochromatosis affects both genders and has early onset, but its clinical course is not as severe as the juvenile form. These clinical observations suggest 2 distinct, perhaps age-dependent, mechanisms of hepcidin regulation. In addition, the hepcidin response after an oral iron challenge that increases only plasma and not tissue iron is blunted in *HFE* but absent in *TFR2* patients,<sup>16</sup> and the same response is observed in the corresponding animal models.<sup>17</sup> The evidence that normal *HFE* and *TFR2* do not compensate for deficient *HJV* suggests that all of these hepcidin-regulatory proteins converge on the same pathway. Indeed, *HFE*, *TFR2*, and *HJV* in vitro interact to form a cell surface complex.<sup>18</sup> The simplest explanation is that *TFR2*, which binds iron-loaded transferrin, controls hepcidin according to plasma iron levels to avoid iron overload when iron demands are high, as in young individuals. *HFE* might control hepcidin according to tissue

iron concentration to avoid iron accumulation in adults. An additive effect of *HFE* and *TFR2* is evident in a patient who presented with mutations in both genes and had severe disease.<sup>15</sup>

On the opposite side, the only known genetic disorder with high hepcidin is the recessive *IRIDA*<sup>19</sup> due to *TMPRSS6* inactivation, pointing to the unique role of this protease in hepcidin suppression. Inhibition of hepcidin expression in iron deficiency serves to increase the iron supply to plasma. *IRIDA* patients have moderate anemia, severe microcytosis and hypochromia, very low transferrin saturation, and inappropriately normal/high hepcidin levels.<sup>19</sup> Anemia is more severe in children than in adults,<sup>20</sup> supporting the concept that *TMPRSS6* mediates the physiologic response to increased iron demand.

All of these clinical observations point to a single iron-responsive hepcidin regulatory pathway with hepcidin production reflecting the balance between positive (*BMP6*) and negative (*TMPRSS6*) hepcidin regulators (Figure 4). Interestingly, hepcidin up-regulation by increased plasma iron does not require *BMP6* to increase. One possible mechanism causing a rapid increase in hepcidin when *BMP6* is low is blocking *TMPRSS6* activity. Hemochromatosis resulting from mutations of *FPN* is dominant (Table 1) and the homozygous state has not been reported, likely because homozygous inactivation of *FPN* would be incompatible with life; this again points to the critical importance of cellular iron export and macrophage iron recycling. Disorders of *FPN* are heterogeneous: mutations that decrease its surface expression or the ability to export iron result in relatively benign iron accumulation in macrophages.<sup>15</sup> In contrast, mutations at the hepcidin-binding site of *FPN* cause true





**Figure 3. Regulation of hepcidin by iron and inflammation.** (A) Hepcidin regulation by iron. Binding of BMP6 to BMP receptor complex on the hepatocyte surface, in the presence of the coreceptor HJV, activates the receptor kinase to phosphorylate SMAD1, SMAD5, and SMAD8 proteins. Phosphorylated SMADs, together with SMAD4, translocate into the nucleus to induce transcription of hepcidin and of other target genes. HFE displaced from TFR1 by high circulating iron binds to TFR2 to activate hepcidin through uncertain mechanisms. TMPRSS6 cleaves HJV from the cell membrane to dampen BMP receptor signaling. Relevant sequence motifs of the hepcidin promoter are shown. (B) IL-6 and other cytokines activate the JAK2 and STAT signaling pathway to activate hepcidin via a STAT-binding motif in the hepcidin promoter. Modified and used with permission from Hentze et al.<sup>1</sup>

hemochromatosis with parenchymal iron overload because FPN is not degraded by hepcidin (hepcidin resistance). The similarity of the phenotype caused by hepcidin deficiency and by FPN mutations that cause resistance to hepcidin attests to the essential role of the hepcidin-FPN interaction in iron homeostasis.<sup>15</sup>

Hepcidin insufficiency causes the development of secondary iron overload also in “iron-loading anemias,” which include the non-transfusion-dependent thalassemia syndromes such as thalassemia

intermedia<sup>21</sup> and congenital dyserythropoietic and inherited nonsyndromic sideroblastic anemias (Table 1). Beta-thalassemia intermedia, which has a clinical course of severity intermediate between transfusion-dependent patients and asymptomatic carriers, is the prototype of conditions characterized by ineffective erythropoiesis and high iron stores. Despite often severe iron overload, hepcidin is suppressed by the expanded erythropoiesis. The observation of increased iron absorption irrespective of high iron stores in iron-loading anemias antedated the discovery of hepcidin.

We learned from patients with inflammatory disorders that hepcidin production is up-regulated by cytokines. Hepcidin is an acute phase protein and an essential mediator of the complex anemia of inflammation or anemia of chronic diseases, a multifactorial form of anemia present in numerous disorders<sup>22</sup> in which the blockage of iron absorption and recycling plays a major role. In addition to the systemic effects of increased hepcidin production by the liver, inflammatory macrophages also express hepcidin and may induce iron retention by an autocrine mechanism. The blockage of macrophage iron recycling and the resulting hypoferrremia is considered a protective mechanism against extracellular pathogens, likely reflecting the true “antimicrobial” function of hepcidin. Recent studies indicate that serum ferritin is predominantly secreted by macrophages; if so, hepcidin-induced iron sequestration in macrophages and the resulting stimulation of ferritin synthesis would explain the high serum ferritin observed in inflammation and also the high correlation between serum hepcidin and ferritin levels reported not only in healthy subjects<sup>23</sup> but also in inflammation.

### Lessons from animal models of *TMPRSS6* inactivation

*Tmprss6*<sup>-/-</sup> mice show the same phenotype of iron deficiency with high hepcidin described in *Mask* mice. Crossing *Tmprss6*<sup>-/-</sup> mice with iron-loaded mice provided important insights into the hierarchy of the hepcidin pathway proteins (Figure 5). *Tmprss6*-*Bmp6* double knockout mice are as severely iron loaded as *Bmp6*<sup>-/-</sup> mice, implying that the function of *Tmprss6* requires an active Bmp-Smad pathway.<sup>24</sup> *Tmprss6*-*Hjv* double mutant mice are as iron loaded as *Hjv*<sup>-/-</sup> mice, a finding consistent with *Hjv* being downstream of *Tmprss6* and likely its substrate.<sup>25</sup> On the contrary, the genetic loss of *Tmprss6* in *Hfe*<sup>-/-</sup> mice reverts the body iron status to an IRIDA-like phenotype,<sup>26</sup> indicating that *Hfe* acts genetically upstream of *Tmprss6*, promoting Bmp/Smad signaling and increasing hepcidin.<sup>26</sup> In addition, *Tmprss6*-*TFR2*-null mice have iron deficiency, with increased RBC and reticulocyte count and even more severe microcytosis than *Tmprss6*<sup>-/-</sup> mice<sup>27</sup> (C.C., unpublished data, April 2013). Hepcidin is increased, although it does not attain the levels found in *Tmprss6*<sup>-/-</sup> mice, likely because the expanded erythropoiesis in the double mutants drives partial hepcidin suppression. Interestingly, TFR2 is a component of the erythropoietin receptor complex and is necessary for its efficient transport to the erythroblast surface.<sup>28</sup> The development of a chimeric mouse with *TFR2*-deficient BM is in progress in our laboratory and hopefully will help to clarify the function of TFR2 in erythropoiesis.

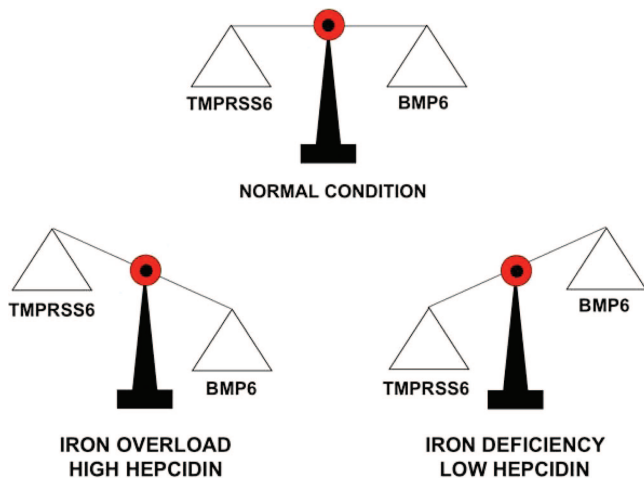
Unexpected results were obtained when crossing *Tmprss6*<sup>-/-</sup> with *Hbb*<sup>th3/+</sup> mice, which recapitulate features of thalassemia intermedia patients, such as microcytic anemia, ineffective erythropoiesis, splenomegaly, low hepcidin levels, and liver iron overload. As expected, the ablation of *Tmprss6* increased hepcidin expression and reduced liver iron concentration, but, surprisingly, it also decreased ineffective erythropoiesis and spleen size, improving

**Table 1. Classification of hepcidin disorders**

	Gene	Phenotype	Mechanism	Current therapy	Potential treatment
Genetic disorders					
I. Low hepcidin: iron overload					
Hemochromatosis type 1	<i>HFE</i>	Classic type	Reduced hepcidin activation	Phlebotomy	Hepcidin agonists
Hemochromatosis type 2A	<i>HJV</i>	Juvenile type	Ablation of BMP co-receptor	Phlebotomy	Hepcidin agonists
Hemochromatosis type 2B	<i>HAMP</i>	Juvenile type	Ablation of hepcidin	Phlebotomy	Hepcidin agonists
Hemochromatosis type 3	<i>TFR2</i>	Early onset	Reduced hepcidin activation	Phlebotomy	Hepcidin agonists
Hemochromatosis type 4A	<i>FPN</i>	Macrophage type	Decreased iron export	Phlebotomy	Unknown
Hemochromatosis type 4B	<i>FPN</i>	Classic type	Hepcidin-resistance	Phlebotomy	Unknown
II. High hepcidin: iron deficiency					
IRIDA	<i>TMPRSS6</i>	Iron deficiency, iron refractoriness	Lack of hepcidin inhibition	Parenteral iron	Hepcidin antagonists
Other disorders					
I. Low hepcidin: iron overload					
Beta-thalassemia intermedia		Anemia, iron overload	Hepcidin suppression	Iron chelation	Hepcidin agonists
Congenital dyserythropoietic anemias		Anemia, iron overload	Hepcidin suppression	Iron chelation	Hepcidin agonists
Sideroblastic anemia		Anemia, iron overload	Hepcidin suppression	Iron chelation	Hepcidin agonists
II. High hepcidin: iron deficiency					
Inflammation/cancer		ACD	Cytokine effects Hepcidin overexpression	No specific treatment EPO (selected cases)	Hepcidin antagonists
Chronic renal failure		Severe anemia ACD	EPO insufficiency Reduced hepcidin clearance	EPO + iron	Hepcidin antagonists
Hepcidin-producing adenoma		Iron deficiency	Autonomous production	Adenoma surgical removal	Unknown

ACD indicates anemia of chronic diseases; and EPO, erythropoietin.

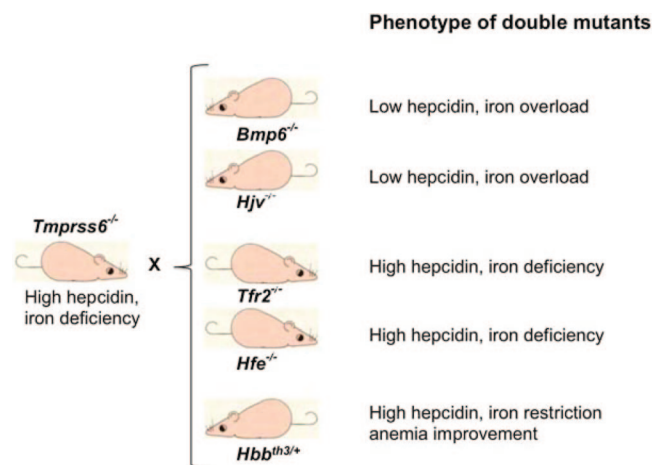
anemia and erythrocyte survival.<sup>29</sup> These results provided the proof of principle for novel thalassemia therapeutic strategies based upon *Tmprss6* manipulation. In addition, the characterization of this double mutant mouse showed that full hepcidin suppression by ineffective erythropoiesis requires a functional *Tmprss6*, in agreement with the observation that *Tmprss6*<sup>-/-</sup> mice are resistant to the effect of exogenous erythropoietin.<sup>29,27</sup> Only hypoxia seems to fully suppress hepcidin in *Tmprss6*<sup>-/-</sup> animals,<sup>27</sup> suggesting an effect downstream of *Tmprss6* and consistent with the concept of multiple inhibitors of the pathway.



**Figure 4. Role of BMP6 and TMPRSS6 in hepcidin production.** A model for adequate hepcidin regulation is based on the balance between BMP6 and TMPRSS6. In normal conditions (top middle), hepcidin production and iron levels are adequate because of the balanced activity of TMPRSS6 and BMP6. In iron deficiency (bottom left), TMPRSS6 activity is increased whereas BMP6 is low because of low tissue iron: this combination results in low hepcidin expression. In iron overload (bottom right), TMPRSS6 activity must be repressed (although the mechanisms are unknown), whereas BMP6 expression is enhanced by increased tissue iron.

**Lessons from genome-wide association studies for iron and erythrocyte parameters**

Several genome-wide association studies have shown that common *TMPRSS6* genetic variants are associated with erythrocyte traits and iron parameters, highlighting a role for *TMPRSS6* in the control of erythropoiesis in healthy individuals.<sup>30</sup> A common associated single nucleotide polymorphism (rs855791) causes a nonsynonymous alanine to valine change in the catalytic domain of the



**Figure 5. Phenotypes of crosses between *Tmprss6* knockout mouse and other models of iron disorders.** *Tmprss6*<sup>-/-</sup> mice with an iron-deficient phenotype (left) have been crossed with different models of iron overload (middle). The phenotype of the double knockout mice is shown. Double *Tmprss6*-*Bmp6* and *Tmprss6*-*Hju* knockout (top) develop iron overload. Crossing *Tmprss6*<sup>-/-</sup> with both *Hfe*<sup>-/-</sup> and *Tfr2*<sup>-/-</sup> (middle) leads to iron-deficient double knockout mice. Loss of *Tmprss6* activity in beta-thalassemia mice (bottom) reduces iron overload and improves anemia (see “Lessons from animal models of *TMPRSS6* inactivation” for details).

**Table 2. Hecpudin agonists and antagonists of potential therapeutic utility**

Agonists	Mechanism	Preclinical studies	Reference
Hecpudin mimetics			
Mini-hecpudin peptides	FPN binding and degradation	Wild-type mice <i>Hamp</i> <sup>-/-</sup> mice	Preza et al <sup>41</sup> Ramos et al <sup>32</sup>
TMPRSS6 inhibitors			
siRNA	Reduction of TMPRSS6 activity	<i>Hfe</i> <sup>-/-</sup> , <i>Hbb</i> <sup>th3/+</sup> mice	Schmidt et al <sup>33</sup>
ASO	Reduction of TMPRSS6 activity	<i>Hfe</i> <sup>-/-</sup> , <i>Hbb</i> <sup>th3/+</sup> mice	Guo et al <sup>34</sup>
Antagonists			
Antibodies			
Anti-IL-6 receptor Ab	Reduction of IL-6 activation	Arthritic monkey	Hashizume et al <sup>42</sup>
Anti-hecpudin Ab	Hecpudin neutralization	<i>Brucella abortus</i> mice model	Sasu et al <sup>43</sup>
Interference with BMP signaling			
Dorsomorphin derivatives	BMP signaling inhibition	IL-6- or turpentine-treated mice	Steinbicker et al <sup>44</sup>
Dorsomorphin derivatives	BMP signaling inhibition	Rat models of inflammation	Theurl et al <sup>45</sup>
Soluble HJV	BMP6 inhibition	Wild-type mice	Babitt et al <sup>46</sup>
Non-anticoagulant heparins	Interference with BMP signaling	LPS-treated mice	Poli et al <sup>47</sup>
Spiegelmer NOX-H94	Hecpudin binding modified oligonucleotides	Cynomolgus monkey	Schwoebel et al <sup>48</sup>

ASO indicates allele-specific oligonucleotide; and LPS, lipopolysaccharide.

protease (A736V), leading to the hypothesis that the hematological effects are mediated by variations of hecpudin levels secondary to the different inhibitory activity of TMPRSS6 polymorphic alleles. The rs855791 allele (736V), associated with low MCV and MCH, induced the hecpudin promoter more efficiently in an in vitro assay and was associated with higher serum hecpudin and lower iron levels than the 736A allele in a large series of healthy individuals.<sup>31</sup> Variants of iron genes, such as *TMPRSS6* and *HFE*, are associated with serum hecpudin, iron parameters and erythrocyte traits, underlining the influence of hecpudin and iron levels on normal erythropoiesis.<sup>31</sup> These results further reinforce the concept that TMPRSS6 modulates the BMP-SMAD pathway in physiological conditions and that hecpudin production results from the balance between opposing forces (Figure 4). This also implies that the role of TMPRSS6 is especially relevant when BMP6 is low. As a corollary, in the latter condition, the only possibility for rapidly increasing hecpudin expression is to suppress TMPRSS6 activity. From these data, TMPRSS6 appears to be an important target for the manipulation of hecpudin levels in different pathological conditions.

### TMPRSS6 as a therapeutic target in disorders of low hecpudin

Because the hecpudin pathway is deranged in several disorders, its manipulation is an attractive novel therapeutic strategy. Hecpudin agonists might replace hecpudin when insufficient, restoring the correct balance and controlling iron overload in disorders with low hecpudin (Table 1). Small synthetic peptides (minihecpidins) may decrease serum iron in healthy mice and prevent iron overload and promote at least partial iron redistribution in hecpudin-deficient mice (Table 2).<sup>32</sup> The pharmacologic inhibition of TMPRSS6 is emerging as an alternative strategy to increase hecpudin levels. This strategy has been exploited in preclinical studies using *Tmprss6* small interfering RNA formulated in lipid nanoparticles with high liver affinity<sup>33</sup> or by *Tmprss6* allele-specific oligonucleotides<sup>34</sup> (Table 2). *Tmprss6* silencing successfully increased hecpudin levels and reduced serum and liver iron concentration in both hemochromatosis *Hfe*<sup>-/-</sup> and thalassemia *Hbb*<sup>th3/+</sup> murine models. In thalassemic mice, the effect overlaps that observed in the *Tmprss6*<sup>-/-</sup> *Hbb*<sup>th3/+</sup> double mutant,<sup>29</sup> with improvement of RBC maturation and survival and partial correction of anemia. The positive effect was shown to be thalassemia specific because *Hfe*<sup>-/-</sup> mice showed mild anemia as a side effect of *Tmprss6* inhibition.

On the opposite side, excessive hecpudin production can be antagonized at different levels. Antagonists are under development with the aim of reducing excessive hecpudin in anemia of chronic diseases and are being tested in animal models of inflammation (Table 2). However, it remains to be proven that reduction of hecpudin alone can improve anemia in inflammatory disorders because of the multifactorial nature of anemia of inflammation.

### Iron as a modifier of erythropoiesis

Why does enhancing hecpudin production improve anemia in beta-thalassemia? The analysis of the cross-talk between iron homeostasis and erythropoiesis in *Hbb*<sup>th3/+</sup> mice led to the conclusion that limiting iron may be beneficial in transfusion-independent beta-thalassemia. Beta-thalassemia erythroblasts are unable to produce adequate amounts of beta globin chains but synthesize normal amounts of alpha chains and heme. Excess unpaired alpha chains causes severe oxidative damage through hemichrome formation that induces apoptosis and death of the erythroid precursors, leading to “a high proliferation low differentiation state.”<sup>21</sup> Limiting iron might reduce the proliferation of immature cells and increase RBC maturation. Positive effects were reported first in *Hbb*<sup>h1/th1</sup> mice, a model of mild thalassemia intermedia, treated by infusions of exogenous transferrin<sup>35</sup>; then in *Hbb*<sup>th3/+</sup> mice engineered to overexpress moderate amounts of hecpudin<sup>36</sup>; and finally by *Tmprss6* pharmacological ablation.<sup>33,34</sup> In all cases, the RBC count was increased, although MCV and MCH were further reduced. This suggests that in all of these situations, less iron was delivered to a higher number of erythroid precursors than in control thalassemia animals. Because heme is a strong regulator of protein translation in erythroblasts, it is highly likely (but not yet proven) that decreased heme synthesis due to low iron impairs alpha globin translation, reducing globin chain imbalance, oxidative damage, and ineffective erythropoiesis.<sup>37</sup>

Anemia may also improve after iron removal by chelators in sideroblastic anemia, which is characterized by mitochondrial iron accumulation in ringed sideroblasts and excessive reactive oxygen species formation, and in some myelodysplastic syndromes.<sup>38</sup> Limiting iron excess improves erythropoiesis in a proportion of these patients, reinforcing the concept that iron overload has a negative effect on erythropoiesis and that iron is a modifier of erythroid maturation.



## Potential role of hepcidin in common nonhematologic disorders

Derangement of iron metabolism at the systemic and/or cellular level may accompany common pathological processes such as infections, inflammatory and metabolic disorders, neurodegeneration, and cancer. Hepcidin production by inflammatory macrophages to control local iron availability is a phenomenon that might be relevant in disorders characterized by low-grade inflammation, such as obesity, diabetes, and metabolic syndrome.

The hepcidin-FPN axis has been proposed to mediate the acute and chronic changes in iron distribution that contribute to host defense in major infections.<sup>39</sup> The relevance of iron for cell proliferation and its propensity to generate reactive oxygen species explain the increasing interest for the study of iron metabolism in cancer, considering that hypoxia and inflammation create a microenvironment that favors iron supply to neoplastic cells. The hepcidin-FPN axis plays an important role in breast cancer, in which cell retention of iron due to low FPN expression or the high hepcidin/high FPN combination is a prognostic signature of cancer itself,<sup>40</sup> an issue worth investigating in hematologic malignancies as well.

The lessons learned from rare genetic iron disorders are beginning to shed light on important pathogenic or protective mechanisms in major human diseases.

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