Supplementary data
Hepcidin at the nexus of various regulatory pathways

Momentarily four putative upstream regulatory pathways that control liver hepcidin production have been described: i) iron store-related regulation, ii) erythropoietic activity driven regulation, iii) inflammation related regulation), and iv) a mandatory signaling pathway. All are found to interact with liver cells to initiate the production of sufficient hepcidin for a proper maintenance of iron homeostasis. In Figure 3 we depict a model of pathways involved in hepcidin regulation that builds upon recently acquired insights, in general derived from mice studies and in vitro cell culture work. The model is focused on three relevant sites involved in hepcidin regulation: kidney, bone marrow and liver cells. Notably, the iron efflux regulation in macrophages by hepcidin, just as skeletal muscles which express high levels of hemojuvelin are kept out of this picture.

i) Iron Store-related Regulation

Information on the amount of iron in depot is communicated by a “store regulator” ¹. How this stores regulator acts upon the hepcidin producing liver cells is unclear, although in vitro work on interactions between transferrin and the membrane proteins HFE, and transferrin receptor (TfR) 1 and 2 has lead to a hypothetical model in which circulating iron bound to transferrin, affects the formation of a complex of TfR2, and HFE on the surface of liver cells. This regulation mechanism is clarified in figure 3 and the corresponding legend. This complex is capable of increasing the hepcidin production by a thus far unknown intracellular signaling pathway²,³.
ii) Erythropoietic activity-driven regulation

An erythropoietic activity derived regulator is proposed to act as the communicator between the erythron and the liver. In case of hypoxia or anemia, low oxygen pressure (pO\textsubscript{2}) levels induce hypoxia inducible factor (HIF)-1α stabilization in kidney cells, which results in erythropoietin (EPO) production of the kidney. EPO increases the erythropoietic activity and thus the need for iron of the bone marrow, resulting in a fast iron mobilization from the stores. This results in an increased duodenal iron absorption by diminishing the circulating hepcidin concentration regardless the status of the iron stores. This suggests that the erythropoietic activity derived regulation interacts with the store regulator by means of a humoral factor which controls the induction of hepcidin. Different candidates for this role have been proposed like soluble transferrin receptor (sTfR)\textsuperscript{4} and recently Growth Differentiation Factor (GDF)-15\textsuperscript{5}.

iii) Inflammation-related regulation

A third upstream regulator of hepcidin is controlled by infection and inflammation. This “inflammatory regulator” pathway has lately been shown to be predominantly induced by interleukin (IL)-6 followed by Janus kinase (JAK)/Signal transducer and activator of transcription (STAT)-3 signalling\textsuperscript{6-8}. It is suggested that this pathway might act more independently from the other pathways\textsuperscript{9-14} although results lack consensus on this matter\textsuperscript{15}. Interactions between inflammation and HJV/BMP regulation through STAT-3 and SMADs as a result of TGF-β cell signalling\textsuperscript{16-18}, illustrate the complexity of the signalling cascades involved in hepcidin regulation\textsuperscript{19,20}.
iv) Mandatory signalling pathway

A recent report hypothesized that the functional effect of both the store regulator and erythroid regulator fully depends on the activity of an additional pathway that is controlled by the glycosylphosphatidylinositol (GPI)-linked cell associated hemojuvelin (HJV). HJV has been suggested to maintain a mandatory regulation pathway by Bone Morphogenetic Protein (BMP)/SMAD signalling in which SMAD4 seems to be essential. Disruption of this pathway by HJV mutations cripples the functionality of both store and erythropoietic activity related regulation, hereby claiming a critical role in hepcidin production. Next to the membrane-linked HJV, the presence of a soluble form was reported to be detectable in human serum. This soluble HJV (sHJV) is suggested to be a cleavage product of the membrane-anchored protein, and in some way under control of circulating iron. In addition, in vitro experiments have shown that recombinant soluble hemojuvelin is capable of suppressing hepcidin mRNA expression. Together these data suggest an iron controlled binding competition between membrane-bound and sHJV that result in the control of hepcidin production. However, many details of this mandatory hepcidin signaling pathway and its nexus with other regulatory pathways are still unknown.

Transcriptional regulation

Several transcription factors are reported as important for the Hepcidin promoter function such as C/EBPα, hepatic nuclear factor (HNF4α), upstream stimulatory factor (USF) and p53 and probably cooperate to allow opening of the chromatin at the hepcidin locus and initiation of transcription.

Simultaneously, some of these factors are also mentioned in association with metabolic syndrome, alcohol metabolism-mediated oxidative stress and hypoxia. Involvement of the von Hippel-Lindau (VHL)/HIF-axis is recently reported as possible regulation pathway
related to erythropoietic activity. So far, nothing is known of the signaling pathway responding to the HFE-TfR2 interactions and its interference with ubiquitous or hepatic-specific transcription factors indicating that our understanding of this last step in hepcidin gene regulation is far from complete. Taken together, what once was considered as a regulation system with only a few roads now appears to be part of a complex regulatory network in which hepcidin is in fact a protein that has numerous irons in the fire.

References


Supplemental Table
Kemna et al. Hepcidin: from discovery to differential diagnosis.

Hepcidin values in the various pathological conditions of mice and man.

A. Pathological conditions

<table>
<thead>
<tr>
<th>Pathological conditions</th>
<th>Hepcidin#</th>
<th>Human</th>
<th>Animal</th>
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<tr>
<td>Elevated iron stores/ iron overload</td>
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<td>Liver disease‡</td>
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B. Hereditary Hemochromatosis

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† After phlebotomy or in iron loading anemia’s; ‡ Depending on status of inflammation, iron loading or fibrosis stage. § OMIM, Online Mendelian Inheritance in Man. # ↓↓, strongly decreased; ↓, mildly decreased; N, normal; †, mildly increased; ↑↑, strongly increased. n.d., not detectable.
U, Urine; S, Serum
References


Wallace DF, Summerville L, Subramaniam VN. Targeted disruption of the hepatic transferrin receptor 2 gene in mice leads to iron overload. Gastroenterology 2007;132:301-10.

