Liver and Serum Iron: Discrete Regulators of Hepatic Hepcidin Expression

To prevent pathological excesses or deficiencies, body iron balance must be tightly controlled due to the lack of a highly evolved mechanism for iron excretion. This is achieved through the liver peptide hepcidin, which efficiently regulates the processes of duodenal iron absorption, macrophage iron release and tissue iron storage, primarily in the liver. Hepcidin is released into the circulation and targets ferroportin, the iron exporter expressed on the surface of duodenal enterocytes, macrophages, and hepatocytes. The binding of hepcidin to ferroportin induces its internalization and degradation, thereby restricting iron entry from the absorptive enterocytes as well as iron release from macrophages and liver iron stores. Hence, appropriate hepcidin expression is paramount for accurate regulation of iron distribution. Indeed, impaired regulation of hepcidin synthesis caused by mutations in key upstream genes in hepcidin regulation—the classical hemochromatosis gene (HFE), transferrin receptor 2 (TFR2), hemojuvelin (HJV), or the hepcidin gene itself (HAMP)—underlies the pathogenesis of the iron overload disorder hereditary hemochromatosis (HH).

HJV is a member of the repulsive guidance molecule family and a coreceptor for bone morphogenetic proteins (BMPs), implicating a role for BMP signal transduction in the transcriptional regulation of hepcidin in the liver. The signaling pathway is initiated when BMP binds to its receptors, a complex of BMP receptor (BMPR) types I and II, inducing the phosphorylation of BMP-R-I by BMPR-II. This, in turn, activates phosphorylation of the intracellular small mothers against decapentaplegic homologue (SMAD) proteins SMAD1, SMAD5, and SMAD8, which then bind SMAD4, and the complex is translocated to the nucleus, promoting transcription of hepcidin. HJV has been shown to interact directly with BMP6, and their interaction facilitates activation of the BMPR complex and enhances BMP-SMAD signaling to modulate hepcidin expression. Although several BMPs including BMP2, 4, 5, 6, 7, and 9 can stimulate hepcidin expression, BMP6 is physiologically the most relevant. BMP6 is regulated by liver iron levels, increasing with iron loading and decreasing with iron depletion, inducing an up-regulation and down-regulation of Smad1/5/8 phosphorylation and HAMP expression.

Studies in Bmp6 null mice have demonstrated that the absence of Bmp6 induces severe iron overload and hepcidin deficiency, highlighting the noncompensatory roles of other functional Bmps. The lack of Bmp6 resulted in inhibition of Smad1/5/8 phosphorylation and their translocation to the nucleus. In contrast, administration of exogenous Bmp6 to mice increased hepatic Hamp expression and reduced both serum iron and transferrin saturation (TS). Liver-specific Smad4 null mice also developed iron overload and impaired BMP signaling, suppressing hepcidin production. Taken together, these observations strongly support BMP6 as the key endogenous regulator of hepcidin synthesis and iron metabolism in vivo. Recently, it was shown that inhibitory Smad7 tem- pers HAMP expression by blocking the interaction of Smad1/5/8 with Smad4.

TFR2 and HFE are thought to act as iron-sensing molecules to receive signals from circulating holotransferrin to modulate hepatic HAMP expression. TFR2 is a strong candidate as a sensor of serum TS, because it binds holotransferrin and undergoes posttranslational stabilization. As TS increases, HFE dissociates from TFR1 and binds to TFR2 to possibly convey the necessary signal downstream to stimulate hepcidin synthesis. Some studies support the premise that TFR2 and HFE interact with the BMP6–SMAD pathway, because this signaling pathway is impaired in Tfr2 and/or Hfe null mice as well as in subjects with HFE-associated HH, whereas others report no interaction. TFR2 and HFE may also signal independently of each other, because disruption of both Tfr2 and Hfe in mice causes a more severe iron overload phenotype. TFR2 and HFE, however, are likely

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**Abbreviations:** BMP, bone morphogenetic protein; BMPR, BMP receptor; ERK1/2, extracellular signal-regulated kinase 1 and 2; HAMP, hepcidin gene; HFE, hemochromatosis gene; HH, hereditary hemochromatosis; HJV, hemojuvelin; LIC, liver iron concentration; MAPK, mitogen-activated protein kinase; SMAD, small mothers against decapentaplegic homologue; TFR1 and binds to TFR2 to possibly convey the necessary signal downstream to stimulate hepcidin synthesis. Some studies support the premise that TFR2 and HFE interact with the BMP6–SMAD pathway, because this signaling pathway is impaired in Tfr2 and/or Hfe null mice as well as in subjects with HFE-associated HH, whereas others report no interaction. TFR2 and HFE may also signal independently of each other, because disruption of both Tfr2 and Hfe in mice causes a more severe iron overload phenotype. TFR2 and HFE, however, are likely
to modulate SMAD signaling downstream of BMP6 due to their redundancies in BMP6 transcription. Holotransferrin, through TFR2 and HFE signaling, may also regulate hepcidin by activating the extracellular signal-regulated kinases 1 and 2 and mitogen-activated protein kinases (ERK1/2–MAPK) pathway and interact with the BMP–SMAD pathway. The interaction between BMP–SMAD and ERK–MAPK pathways is not fully understood.

The current study by Corradini et al. adds to an actively expanding body of work to unravel the complexities of hepcidin regulation by iron. Iron-dependent regulation of hepcidin appears to involve both liver iron and circulating iron levels. The modulation of hepcidin expression by liver iron is likely to be mediated through the BMP6–SMAD signaling pathway, whereas regulation by serum TS is mediated by TFR2 and HFE signaling, although the latter mechanism remains poorly defined. Corradini et al. show that, in a setting where there was a sudden surge in circulating iron levels with unaltered liver iron concentration (LIC), hepcidin responded according to the changes in serum TS. Mice administered 2 mg/kg iron (through oral gavage) had increased serum iron and TS levels after 1 hour of iron dosing, which returned to baseline levels by 8-24 hours, whereas LIC was unchanged over 24 hours. Hamp expression was increased after 4 hours, returning to baseline levels by 24 hours, and similar changes were observed for pSmad1/5/8 protein expression. Despite these changes, hepatic Bmp6 messenger RNA expression remained unaltered. This prompted the authors to suggest that TS regulates
hepcidin independently of LIC through Smad1/5/8 signaling downstream of Bmp6 by a mechanism that does not appear to involve HJV. In contrast, mice fed a 2% iron diet for up to 3 weeks exhibited increased serum iron, TS, and Hamp expression after 1 day, which plateaued thereafter, whereas LIC and Bmp6 expression continued to increase over the 3 weeks of iron feeding. LIC correlated positively with Hamp and Bmp6 expression, whereas pSmad1/5/8 protein expression, which was increased at all time points, paralleled the increases in LIC and Bmp6 expression, consistent with previous studies.6,7 In this setting (increasing LIC with high but stable serum iron levels), transcription of hepcidin was initiated through LIC, which promoted Smad1/5/8 signaling through induction of Bmp6 expression. Similarities were observed with regard to hepcidin expression induced by the differing TS- and LIC-induced pathways: TS was an independent predictor of Hamp expression, inhibitory Smad7 expression paralleled changes in pSmad1/5/8 expression, and neither ERK nor interleukin-6 signaling was activated. These data suggested that Smad7 may be involved in a negative feedback regulation of hepcidin and iron-dependent regulation of hepcidin did not involve ERK–MAPK and interleukin-6–STAT3 signaling. Moreover, TS was an important signal for hepcidin regulation in vivo, because it activated Hamp expression both in the absence and presence of increased LIC. It is curious that Corradini et al. did not observe an activation of ERK signaling by TS in their iron loading models as reported in other studies.19,20 The importance of ERK activation in in vivo regulation of iron metabolism, however, is currently not known.

Corradini et al. provide evidence for differential regulation of hepcidin by serum TS and liver iron.21 This is consistent with studies that have shown hepcidin regulation by exogenous holotransferrin7 as well as increased LIC.6,7 Liver iron signals predominantly through the BMP6–SMAD pathway to regulate hepcidin synthesis, as seen in iron overload conditions where high LIC induces BMP6 expression6,7 and triggers downstream activation of the SMAD signaling cascade to stimulate hepcidin transcription. Thus, BMP6 acts as a signal transducer of liver iron stores. It is unclear whether the transcribed BMP6 then proceeds to further enhance BMP6–SMAD signaling through positive feedback regulation. Whereas TS also activates SMAD signaling, this occurs in the absence of hepatic BMP6 messenger RNA induction, suggesting that the regulation is independent of BMP6. TFR2 and HFE are, however, required in hepcidin induction by TS, because subjects with TFR2- and HFE-associated HH have an impaired hepcidin response to oral iron challenge.22 The nature of TFR2 and/or HFE involvement in the modulation of hepcidin expression by this or other pathways has not been ascertained. A current model of hepcidin regulation is depicted in Fig. 1.

Our understanding of the role of iron in health and disease has progressed tremendously over the last decade since the identification of the iron regulatory peptide hepcidin. Although this area of research has forged ahead, many unanswered questions remain. Further studies are required to fully elucidate how extracellular and intracellular iron signals independently and yet coordinately modulate hepcidin expression to maintain iron homeostasis. The precise functions of HH-related proteins and other iron regulatory proteins in the governance of hepcidin synthesis have not yet been completely decoded. Whether there is cross-talk between known signaling pathways of iron regulation or between regulatory pathways of iron and inflammation or a role for other liver cells as well as hepatocytes in hepcidin regulation remains to be confirmed.

References


