Journal of Nutrition & Food Sciences

Rodova et al., J Nutr Food Sci 2016, 6:4
DOI: 10.4172/2155-9600.1000521

Review Article Open Access

Hepcidin Regulation by Bone Morphogenetic Protein Signaling and Iron Homeostasis

Marianna Rodova[1], **Seunghwan Kim**[1], **M. Abdul Mottaleb**[2] **and M. Rafiq Islam**[1*]

[1]*Biochemistry Laboratory, Northwest Missouri State University, Maryville, Missouri, USA*

[2]*Center for Innovation and Entrepreneurship, Northwest Missouri State University, Maryville, Missouri, USA*

[*]**Corresponding author:** Rafiq Islam, Biochemistry Laboratory, Northwest Missouri State University, Maryville, USA, Tel: 660-562-3118; E-mail: islamr@nwmissouri.edu

Received date: May 5, 2016; **Accepted date:** June 10, 2016; **Published date:** June 16, 2016

Keywords: Hepcidin; Protein signaling; Homeostasis; Bone morphogenetic protein; Hepcidin regulation

Abbreviations:

BMP: Bone Morphogenetic Protein; BMPRIIA: BMP Receptor Type IIA; BMPRI: BMP Receptor Type I; ALK 2/3: ALK, Activin-Like Kinase 2/3; Endofin: Endosome-associated FYVE-domain; SMAD1/5/8: Sma and Mothers Against Decapentaplegic Homolog 1/5/8; SMAD4: SMAD Homolog 4; ATOH8: Atonal Homolog 8; BMPRE: BMP Response Elements; HFE: Hemochromatosis Protein; HJV: Hemojuvelin; IL6: Interleukin 6; PARP-1: Poly ADP Ribose Polymerase 1; TMPRSS6: Transmembrane Protease Serine 6; Tf: Transferrin; TfR2: Transferrin Receptor 2

Introduction

Iron is an important mineral for most life on Earth, including humans. It is required as cofactor for a number of proteins including cytochromes and plays a major role in oxygen transport by erythrocyte hemoglobin and oxygen storage by muscle myoglobin. Just as iron deficiency is detrimental, iron overload is also deleterious as there is no specific pathway to excrete iron. Therefore, iron is strictly regulated both at the cellular and systemic levels. Its absorption by intestinal enterocytes and concentration in serum is strictly regulated by liver produced hormone, hepcidin in association with iron exporter, ferroportin. Hepcidin expression in response to body iron level is primarily regulated via Bone Morphogenetic Protein/Sma and Mothers against Decapentaplegic (BMP/SMAD) pathway, although other signaling pathways may exist [1]. This mini review summarizes present day knowledge of the key players involved in iron absorption, transport, storage, and homeostasis. It also synopsizes regulation of hepcidin by known major pathways.

Absorption, Transport and Storage

As a vital mineral, iron concentration is strictly maintained in circulation within 10-30 microM to ensure adequate supply for biosynthesis of hemoglobin (erythropoiesis), myoglobin and cytochromes, and for other essential metabolic functions. However, its concentration is kept at low to prevent generation of free radicals [2] in the presence of intracellular H_2O_2 that can cause damages to cellular components. It is virtually insoluble under physiological condition, so animals have developed specialized molecules for its acquisition, transport, and storage in a soluble and nontoxic form. Another set of molecules evolved for its intracellular and systemic regulation.

Under steady state, very little iron is absorbed or lost. Most of the iron requirement is met by recycling from erythrocyte turn-over. Senescent erythrocytes are catabolized by macrophages in spleen, liver and reticuloendothelial system, and the released iron is used by bone marrow reticulocytes for synthesis of hemoglobin. There is no specific excretory pathway for iron, although small amount (1-2 mg) is lost from the body through a variety of ways including urination, defecation, sweating, and exfoliating old/matured cells [3]. Thus, vast majority of body iron is recycled into hemoglobin of circulating erythrocytes (2500 mg in human) and bone marrow erythroid precursors (300 mg), and the excess is stored in the liver (1000 mg) and splenic macrophages (600 mg). Hepatocytes serve as the important reservoir that stores or releases iron to maintain homeostasis. Under normal condition, a small amount of iron is found in circulating plasma.

Dietary ferric (Fe^{3+}) iron is absorbed by mature enterocytes-cells of intestinal epithelia, following reduction to ferrous (Fe^{2+}) in the intestinal lumen by ferric reductases. Two main pathways to absorb iron from the lumen are associated with HCP1 (heme carrier protein 1) for organic heme iron and DMT1 (divalent metal transporter 1) for inorganic non-heme iron. They are expressed on the enterocyte apical membrane and transfer Fe^{2+} ions into its cytoplasm [4].

Absorbed dietary iron in enterocytes (and stored iron in hepatocytes and macrophages) is secreted into plasma via iron exporter ferroportin [5], after being re-oxidized to Fe^{3+} by ferroxidases, ceruloplasmin and hephaestin [6]. Once in plasma, Fe^{3+} binds tightly, but reversibly transferrin Tf, a plasma carrier protein highly expressed and actively secreted by hepatocytes. Cells acquire iron from circulating Fe^{3+} bound Tf via transferrin receptor 1 (TfR1) localized on the cell membrane through endocytosis [7]. Ferric iron is released from endosomes carrying Fe^{3+}-Tf-TfR1 ternary complex and undergoes reduction to Fe^{2+} by reductases.

Excess iron in hepatocytes is stored in complex form with cytoplasmic iron-storage protein ferritin. Within its ferooxidase site, Fe^{2+} is oxidized with dioxygen [8,9] to ferric Fe^{+3} ion, and stored in solid, nanoparticular, and mineral form [10]. This limits radical production via Fenton reaction between ferrous iron and hydrogen peroxide. When needed, ferritin is degraded by lysosomes [11] and the stored Fe^{+3} is reduced for its exit [12] and transit to the exporter ferroportin. It is not known how this reduction and transit to ferroportin is carried out, but a ferrireductase may be involved. Copper-containing ferroxidases ceruloplasmin, hephaestin, and possibly zyklopen [13] use molecular oxygen to re-oxidize ferroportin bound Fe^{+2} to Fe^{+3} before being taken up by Tf. Thus, both ferritin and Tf keep iron in unreactive form and prevent formation of oxygen radicals and cellular damage [14].

Intracellular Regulation

Dietary iron is absorbed by enterocytes to replace iron losses from the body. Cellular iron uptake, storage and export have to be coordinately regulated in the body. Cells monitor iron concentration through a feedback control mechanism involving cytosolic iron-regulatory proteins 1 and 2 (IRP1/2). This regulation is exerted via posttranscriptional mechanism utilizing Iron Responsive Elements (IREs) located in the untranslated region (UTR) of mRNAs [15] of proteins involved in iron metabolism. IRE is present in the 5' UTR of ferritin and ferroportin mRNA, and 3' UTR of TfR1 and DMT1 mRNAs. With high iron concentration, IRPs lose affinity for IREs resulting in increased translation of 5′ IRE-containing mRNAs and degradation of the 3′ IRE-containing mRNAs [15]. Thus, precisely regulated control by IRPs allows cells to acquire enough iron for their metabolism without reaching toxic concentration.

Hepcidin is the "Master Regulator" of Iron Homeostasis

In addition to intracellular control, iron homeostasis is maintained at the systemic level by controlling absorption through intestinal enterocytes and export from body stores. Key to this maintenance is a 25-amino-acid peptide hormone, hepcidin (*HAMP* gene product) produced predominantly by hepatocytes and secreted into circulation [16]. Secreted hepcidin in serum is transported in complex with alpha 2-macroglobulin [17]. Hepcidin negatively regulates absorption of dietary iron and release from stored sources into circulation [18] through binding to the exporter ferroportin and leading to its degradation. Thus, high serum hepcidin level decreases iron absorption by enterocytes as well as iron export from hepatocytes and macrophages leading to reduced iron entry into circulation and intracellular retention [19,20]. Sustained elevation of hepcidin in circulation, as occurs in many inflammations, leads to anemia due to reduced availability of iron for erythropoiesis, a condition known as anemia of inflammation. On the other hand, below normal hepcidin concentration accounts for increased dietary iron absorption and tissue iron overload, leading to iron overload disease, hemochromatosis. This disease is associated with disrupted control of dietary iron absorption and progressive iron overload in tissues leading to multiple organ damage and failure in humans. The most frequent type is hereditary hemochromatosis (HH), developed by chronic hyper-absorption of dietary iron in individuals with mutations in the HFE gene [21]. HFE encodes an atypical major histocompatibility complex (MHC) class I protein, however, unlike other members of MHC class I, it does not possess peptide antigen presentation. The majority of HH patients carry a C282Y mutation that abolishes a disulfide bridge in the HFE protein, preventing its association with beta -2-microglobulin for proper processing, transport to the membrane and cell surface expression [22]. Unprocessed C282Y mutant protein is degraded by proteasome in the cells [23]. Mutations in a number of other genes such as TfR2, hemojuvelin (HJV), and bone morphogenetic protein 6 (BMP6) lead to low hepcidin level and develop HH. In fact, *Hamp1* regulation is more severely impaired in $Bmp6^{-/-}$ and $Hjv^{-/-}$ mice than in $Hfe^{-/-}$ and $Tfr2^{-/-}$mice.

Regulation of Hepcidin

Current knowledge of hepcidin regulation appears to occur at the transcriptional level. Two major stimuli that regulate hepcidin promoter activity are body iron status and inflammation: hepcidin transcription in liver is induced by serum iron level or inflammation, and is inhibited by iron deficiency, increased erythropoietic drive or hypoxia [24,25].

Iron-mediated regulation

BMP/SMAD pathway: Iron induced hepcidin activation is mainly regulated through BMP (bone morphogenetic protein) signaling pathway [1]. BMP6 binds as a ligand to BMP receptors (BMPR) type I and II, promoting phosphorylation of downstream BMP mediators such as SMAD1, SMAD5 and SMAD8 (SMAD1/5/8=Sma and mothers against decapentaplegic homolog 1, 5 or 8). Among BMPR type I, only ALK2 and ALK3 are expressed in human [26] and murine [27] hepatocytes. As for BMPR type II, hepatocytes express only ActRIIA [26]. Transgenic mice with liver specific deletion of Alk2 or Alk3 [27] showed impaired iron induced expression of hepcidin and iron overload.

The phosphorylated SMAD1/5/8 associates with cytoplasmic SMAD4 and translocates to the nucleus, where the complex activates hepcidin transcription, in addition to other target genes, upon binding to BMP responsive elements (BMP-RE1 and BMP-RE2) at proximal and distal sites of *HAMP* promoter [28]. Mice with liver-specific ablation of Smad4 have low plasma hepcidin level, and hepatocytes with knocked down SMAD4 do not respond to iron overload and BMP signaling [29], supporting an important role of SMAD4 in iron-mediated hepcidin expression.

BMP signaling pathway is the critical iron-mediated regulator of hepcidin as BMP6, unlike other members of BMP protein family, is responsive to iron concentrations. BMP6 is produced in non-parenchymal cells of the liver and activated by iron; its expression is proportional to hepatic iron level [30]. Transgenic mice with ablated BMP6 lose the ability to activate hepcidin in response to iron overload, which can be rescued only by BMP6 [31,32]. In addition, neutralizing BMP6 antibody can decrease hepcidin expression and trigger an increase in serum iron concentrations in mice [32]. These data suggest a unique, perhaps central, role of BMP6 in hepcidin transcriptional regulation in response to iron level.

BMP signaling in liver requires BMP co-receptor hemojuvelin (HJV), since it forms complex with BMP receptors to enhance downstream signaling. Mutations in HJV cause decreased BMP signaling, resulting in decreased hepcidin expression, followed by severe iron overload [33] and HH. Consistent with this, cleavage of HJV by liver expressed, membrane serine-threonine protease, *matriptase-2* encoded by *TMPRSS6* results in suppressed signaling through the BMP pathway, and consequently, decreased hepcidin expression [34]. $Tmprss6^{-/-}$ mice have increased *Hamp1* expression, decreased ferroportin level and severe iron-deficient anemia. Mutations in *TMPRSS6* produce iron-refractory iron-deficiency anemia in humans [35]. Evidences suggest that *TMPRSS6* expression is positively correlated by iron level through BMP6 that requires intact BMP/SMAD pathway and BMP6 mediated induction of the transcription factor, Id1 [36]. In contrast, a recent result showed that low iron level increased *TMPRSS6* expression in hepatocyte cell culture [37]. In any case, *matriptase-2 serves as* a negative regulator of hepcidin activation by the BMP pathway.

Besides *matriptase-2*, other important modulators of the BMP/SMAD pathway include endofin, ATOH8 and SMAD7. Endosome-associated FYVE-domain protein, endofin anchors SMAD1/5/8 proteins to BMP receptors and enhances SMADs phosphorylation, and

thus, activates BMP signaling [38]. Using Endofin specific siRNA in hepatic cultured cells, Goh et al. [39] showed that endofin is required for BMP6 induced hepcidin activation as it facilitates SMAD1/5/8 phosphorylation.

Transcription factor ATOH8 also activates hepcidin expression by enhancing SMAD1/5/8 phosphorylation, although this activation in HEK 293 cells is also suggested by direct binding to E-boxes in hepcidin proximal promoter [40]. Atoh8 contributes to hepcidin sensitivity to iron as it is correlated with plasma iron and erythroid activity in mice [40,41]

Activation of inhibitory SMAD7, which regulates BMP pathway by negative feedback loop, decreases hepcidin transcription [42]. Evidences suggest SMAD7 directly suppresses hepcidin expression as specific siRNA resulted in increased HAMP transcription. In murine hepatocytes, overexpression of SMAD7 suppresses *Hamp1* expression and prevents *Hamp1* induction by BMP6.

Pathways involving HFE: Although evidences described above clearly place MBP6 at the center of sensing iron level and BMP/SMAD as the central pathway of hepcidin regulation in response to iron, alternative model of hepcidin regulation emerged through sensing iron level via the interaction of Fe^{3+}-Tf with its receptors. Studies in $Hfe^{-/-}$ mice showed that even with iron overload and corresponding increase in BMP6 mRNA, no increase in phosphorylation level of SMAD1/5/8 or *Hamp1* transcription was observed. Consistently, despite increase in BMP6 mRNA, phosphorylation of SMAD1/5/8 and expression of *HAMP* are inappropriately low in individuals with HH carrying C282Y mutations. No change in SMAD4 mRNA level is noticed. Interestingly, inhibitory Smads, SMAD7 and SMAD6, are upregulated in these patients. All these suggest existence of additional pathways for *HAMP* regulation.

The first pathway involving HFE, reported by Bennet et al. [43], demonstrated that HFE binds both transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2) [44] on the cell membrane. However, the choice between TfR1 and TfR2 is determined by concentration of Fe^{3+}-Tf: increased concentration disrupts HFE-TfR1 interaction and shifts HFE binding towards TfR2. HFE-TfR2 complex, in turn, activates hepcidin transcription [45,46]. Mutated HFE fails to form this complex with TfR2 and suppresses hepcidin level. The mechanism of this regulation is still unclear, although both HFE and TfR2 probably contribute to hepcidin up regulation through BMP/SMAD signaling pathway in response to increased iron [31,47]. The role of hepatic TfR2 as a regulator of hepcidin transcription has been confirmed in transgenic mice studies. Low hepcidin level and iron overload in liver have been found in TfR2 null mice as well as in liver specific TfR2 knockout mice model [48,49]. Thus, in this pathway, HFE utilizes its interaction with transferrin receptors on the cell membrane to regulate hepcidin expression upon changes in iron bound transferrin concentration in serum.

The second pathway of hepcidin regulation by HFE has been recently reported by Wu et al. [50]. They demonstrated that HFE regulates hepcidin transcription via BMP/SMAD pathway. BMP6 phosphorylates BMPR type I Alk2 and/or Alk3 that are expressed in the liver and critical for hepcidin transcription [27]. Analysis of HH patients with mutated HFE and HH model mice showed impaired BMP signaling suggesting critical role of HFE in BMP/SMAD mediated hepcidin regulation [51]. Wu et al. [50] determined that Hfe binding to hepatic Alk3 reduces its degradation by proteasome and activates hepcidin expression, thus linking HFE to BMP/SMAD pathway. Consistent with this, Hfe deficient mice show reduced Alk3 protein level and, consequently, reduced hepcidin expression. This is the first evidence of hepcidin transcription activation by HFE via BMP signaling.

Our results in cultured cells showed that PARP1 (poly ADP ribose polymerase 1), a nuclear protein, strongly represses HFE expression via binding to a cruciform structure in the distal HFE promoter. PARP1 specific siRNA increases HFE mRNA and protein. Likewise, PARP1 degradation, induced by iron containing molecules such as hemin and $FeCl_3$, increases HFE expression by releasing the repression [52]. Other studies demonstrated that PARP1 disrupts SMAD complex assembled on DNA target sites by ADP-ribosylating (PARylating) Smad4 and attenuated SMAD mediated response [53]. PARylation of SMAD proteins by PARP1 is a key step in controlling the strength and duration of SMAD mediated transcription [53,54]. Thus, PARP1 negatively regulates transcription of SMAD target genes. Taken together, PARP1 can reduce hepcidin expression in two ways: via PARylation of SMAD4 assembled on BMP-RE1 and -RE-2 sites in the promoter in BMP/SMAD mediated pathway and/or via reducing HFE expression in pathways requiring HFE. Therefore, degradation of PARP1 in response to high iron can apparently release these repressions on hepcidin transcription. Likewise, PARylation activity inhibitors can increase hepcidin expression in BMP/SMAD pathway. It will be interesting to investigate if PARP1 inhibitors can alleviate iron overload symptoms in HH mice.

Inflammation mediated regulation

The second major signaling pathway known to regulate hepcidin transcription is the JAK (Janus kinase)–STAT3 (signal transducer and activator of transcription 3) pathway; the stimulus for this regulation comes mainly through inflammatory cytokines such as interleukin-6 (IL-6). IL-6, released upon inflammation, binds to its receptors on the cell membrane leading to activation of JAK which phosphorylates predominantly STAT3 protein in hepatocytes as well as in leukocytes. Phosphorylated STAT3 translocates to the nucleus and binds to a STAT-specific site in target gene promoters. It directly regulates hepcidin through a STAT binding site in proximal region of its promoter [55-57]. IL-6 also binds BMP receptor Alk3 and induces hepcidin expression in mice [58], suggesting a cross-talk exists between BMP and IL-6 regulation. Another mechanism of hepcidin up-regulation upon inflammation involves activin B, a member of TGF-beta protein superfamily that includes BMP6 [59]. Activin B binds to BMP receptor type I, ALK3 to activate hepcidin transcription in hepatocytes via BMP/SMAD pathway (Figure 1) [60-65].

Conclusion

Both iron deficiency and iron overload are deleterious. Thus, body iron levels are strictly maintained by regulating absorption of dietary iron and release of stored iron by the hepatic hormone, hepcidin, its impaired regulation is associated with all iron related disorders. Over the past two decades, an incredible amount of progress has been made in our understanding of iron homeostasis and hepcidin regulation. Hepcidin expression is tightly regulated at transcription level primarily via BMP/SMAD pathway. BMP6 produced in non-parenchymal cells of liver is the predominant ligand and main upstream positive regulator of hepcidin responding to iron level.

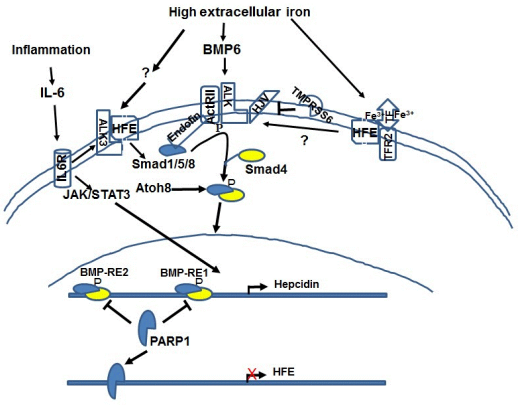

Figure 1: Overview of hepcidin transcription regulation in hepatocytes [In response to high body iron, in primary pathway BMP6 binds activated BMP receptor complex, consisting of BMPRIIA (Act RII) and BMPRI (ALK) and co-receptor HJV, which phosphorylates SMAD1/5/8 anchored by endofin protein. ATOH8 enhances this phosphorylation, while matriptase2 encoded by *TMPRSS6* attenuates this phosphorylation via proteolytic cleavage of HJV. The phospho-SMAD1/5/8 recruits the common SMAD4 to form a transcription complex, which upon transport to the nucleus, binds BMP response elements (BMP-RE1 and -RE2) on the hepcidin promoter and upregulates hepcidin transcription. In pathways involving HFE, complexes are formed between HFE with either holotransferrin bound transferrin receptor 2 ($2Fe^{3+}$-Tf-TfR2) or ALK and transmit signal via BMP or other pathway-the mechanisms are not fully characterized. Inflammation induced pathway through IL6 and JAK/STAT3 is also shown. Evidence shows that PARP1 represses HFE directly via binding to HFE promoter, while HAMP indirectly via dissociation of SMAD4-SMAD1/5/8 complex through PARylation of SMAD4. Iron induced breakdown of PARP1 releases the repression on HFE promoter. Both iron and PARP1 activity inhibitors thus can remove PARP1 inhibitory activity on HAMP promoter].

However, several key questions still remain. For example, it is still not clear how BMP6 sense iron in the non-parenchymal cells and what other molecules are involved. What are the DNA binding and transcription partners that drive transcription of *HAMP*? How iron is sensed in HFE-ALK3 pathway? Since inhibitory SMAD7 is up-regulated in HFE deficient mice and individuals, is it the role of HFE to keep in check the inhibitory pathway? Whether PARP1 plays a significant r o le in *HAMP* regulation and its inhibitors can alleviate iron overload symptoms in HH mice. The answers to these questions will provide further insight into hepcidin mediated iron homeostasis.

References

1. Parrow NL, Fleming RE (2014) Bone morphogenetic proteins as regulators of iron metabolism. Annu Rev Nutr 34: 77-94.
2. Papanikolaou G, Pantopoulos K (2005) Iron metabolism and toxicity. Toxicol Appl Pharmacol 202: 199-211.
3. Ganz T (2013) Systemic iron homeostasis. Physiol Rev 93: 1721-1741.
4. Przybyszewska J, Zekanowska E (2014) The role of hepcidin, ferroportin, HCP1, and DMT1 protein in iron absorption in the human digestive tract. Prz Gastroenterol 9: 208-213.
5. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, et al. (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403: 776-781.
6. Vashchenko G, MacGillivray RT (2013) Multi-copper oxidases and human iron metabolism. Nutrients 5: 2289-2313.
7. Aisen P (2004) Transferrin receptor 1. Int J Biochem Cell Biol 36: 2137-2143.
8. Levi S, Luzzago A, Cesareni G, Cozzi A, Franceschinelli F, et al. (1988) Mechanism of ferritin iron uptake: activity of the H-chain and deletion mapping of the ferro-oxidase site. A study of iron uptake and ferro-oxidase activity of human liver, recombinant H-chain ferritins, and of two H-chain deletion mutants. J Biol Chem 263: 18086-18092.
9. Lawson DM, Artymiuk PJ, Yewdall SJ, Smith JM, Livingstone JC, et al. (1991) Solving the structure of human H ferritin by genetically engineering intermolecular crystal contacts. Nature 349: 541-544.
10. Chasteen ND, Harrison PM (1999) Mineralization in ferritin: an efficient means of iron storage. J Struct Biol 126: 182-194.
11. Linder MC (2013) Mobilization of stored iron in mammals: a review. Nutrients 5: 4022-4050.
12. Theil EC (2013) Ferritin: the protein nanocage and iron biomineral in health and in disease. Inorg Chem 52: 12223-12233.
13. Kosman DJ (2010) Redox cycling in iron uptake, efflux, and trafficking. J Biol Chem 285: 26729-26735
14. Gkouvatsos K, Papanikolaou G, Pantopoulos K (2012) Regulation of iron transport and the role of transferrin. Biochim Biophys Acta 1820: 188-202.
15. Nicole Wilkinson and Kostas Pantopoulos (2014) The IRP/IRE system in vivo: insights from mouse models. Front Pharmacol 5: 176.
16. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, et al. (2000) LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 480: 147-150.
17. Peslova G, Petrak J, Kuzelova K, Hrdy I, Halada P, et al. (2009) Hepcidin, the hormone of iron metabolism, is bound specifically to alpha-2-macroglobulin in blood. Blood 113: 6225-6236.
18. Nemeth E, Ganz T (2006) Regulation of iron metabolism by hepcidin. Annu Rev Nutr 26: 323-342.
19. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, et al. (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306: 2090-2093.
20. De Domenico I, Ward DM, Langelier C, Vaughn MB, Nemeth E, et al. (2007) The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell 18: 2569-2578.
21. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, et al. (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13: 399-408.
22. Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, et al. (1997) Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci U S A 94: 12384-12389.
23. de Almeida SF, Picarote G, Fleming JV, Carmo-Fonseca M, Azevedo JE, et al. (2007) Chemical chaperones reduce endoplasmic reticulum stress and prevent mutant HFE aggregate formation. J Biol Chem 282: 27905-27912.
24. Ganz T, Nemeth E (2011) The hepcidin-ferroportin system as a therapeutic target in anemias and iron overload disorders. Hematology Am Soc Hematol Educ Program 2011: 538-542.
25. Flanagan JM, Truksa J, Peng H, Lee P, Beutler E (2007) In vivo imaging of hepcidin promoter stimulation by iron and inflammation. Blood Cells Mol Dis 38: 253-257.
26. Xia Y, Babitt JL, Sidis Y, Chung RT, Lin HY (2008) Hemojuvelin regulates hepcidin expression via a selective subset of BMP ligands and receptors independently of neogenin. Blood 111: 5195-5204.

27. Steinbicker AU1, Bartnikas TB, Lohmeyer LK, Leyton P, Mayeur C, et al. (2011) Perturbation of hepcidin expression by BMP type I receptor deletion induces iron overload in mice. Blood 118: 4224-4230.
28. Casanovas G, Mleczko-Sanecka K, Altamura S, Hentze MW, Muckenthaler MU (2009) Bone morphogenetic protein (BMP)-responsive elements located in the proximal and distal hepcidin promoter are critical for its response to HJV/BMP/SMAD. J Mol Med (Berl) 87: 471-480.
29. Wang RH, Li C, Xu X, Zheng Y, Xiao C, et al. (2005) A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. Cell Metab 2: 399-409.
30. Ramos E, Kautz L, Rodriguez R, Hansen M, Gabayan V, et al. (2011) Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. Hepatology 53: 1333-1341.
31. Meynard D, Kautz L, Darnaud V, Canonne-Hergaux F, Coppin H, et al. (2009) Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat Genet 41: 478-481.
32. Andriopoulos B Jr, Corradini E, Xia Y, Faasse SA, Chen S, et al. (2009) BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nat Genet 41: 482-487.
33. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, et al. (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet 38: 531-539.
34. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, et al. (2008) The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab 8: 502-511.
35. Finberg KE (2009) Iron-refractory iron deficiency anemia. Semin Hematol 46: 378-386.
36. Meynard D, Vaja V, Sun CC, Corradini E, Chen S, et al. (2011) Regulation of TMPRSS6 by BMP6 and iron in human cells and mice. Blood 118: 747-756.
37. Zhao N, Nizzi CP, Anderson SA, Wang J, Ueno A, et al. (2015) Low intracellular iron increases the stability of matriptase-2. J Biol Chem 290: 4432-4446.
38. Shi W, Chang C, Nie S, Xie S, Wan M, et al. (2007) Endofin acts as a Smad anchor for receptor activation in BMP signaling. J Cell Sci 120: 1216-1224.
39. Goh JB, Wallace DF, Hong W, Subramaniam VN (2015) Endofin, a novel BMP-SMAD regulator of the iron-regulatory hormone, hepcidin. Sci Rep 5: 13986.
40. Patel N, Varghese J, Masaratana P, Latunde-Dada GO, Jacob M, et al. (2014) The transcription factor ATOH8 is regulated by erythropoietic activity and regulates HAMP transcription and cellular pSMAD1,5,8 levels. Br J Haematol 164: 586-596.
41. Kautz L, Meynard D, Monnier A, Darnaud V, Bouvet R, et al. (2008) Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. Blood 112: 1503-1509.
42. Mleczko-Sanecka K, Casanovas G, Ragab A, Breitkopf K, Müller A, et al. (2010) SMAD7 controls iron metabolism as a potent inhibitor of hepcidin expression. Blood 115: 2657-2665.
43. Bennett MJ, Lebrón JA, Bjorkman PJ (2000) Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. Nature 403: 46-53.
44. Goswami T, Andrews NC (2006) Hereditary hemochromatosis protein, HFE, interaction with transferrin receptor 2 suggests a molecular mechanism for mammalian iron sensing. J Biol Chem 281: 28494-28498.
45. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, et al. (2009) Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab 9: 217-227.
46. D'Alessio F, Hentze MW, Muckenthaler MU (2012) The hemochromatosis proteins HFE, TfR2, and HJV form a membrane-associated protein complex for hepcidin regulation. J Hepatol 57: 1052-1060.
47. Corradini E, Garuti C, Montosi G, Ventura P, Andriopoulos B Jr, et al. (2009) Bone morphogenetic protein signaling is impaired in an HFE knockout mouse model of hemochromatosis. Gastroenterology 137: 1489-1497.
48. Wallace DF, Summerville L, Crampton EM, Subramaniam VN (2008) Defective trafficking and localization of mutated transferrin receptor 2: implications for type 3 hereditary hemochromatosis. Am J Physiol Cell Physiol 294: C383-C390.
49. Roetto A, Di Cunto F, Pellegrino RM, Hirsch E, Azzolino O, et al. (2010) Comparison of 3 Tfr2-deficient murine models suggests distinct functions for Tfr2-alpha and Tfr2-beta isoforms in different tissues. Blood 115: 3382-3389.
50. Wu XG, Wang Y, Wu Q, Cheng WH, Liu W, et al. (2014) HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. Blood 124: 1335-1343.
51. Ryan JD, Ryan E, Fabre A, Lawless MW, Crowe J (2010) Defective bone morphogenic protein signaling underlies hepcidin deficiency in HFE hereditary hemochromatosis. Hepatology 52: 1266-1273.
52. Pelham C1, Jimenez T, Rodova M, Rudolph A, Chipps E, et al. (2013) Regulation of HFE expression by poly(ADP-ribose) polymerase-1 (PARP1) through an inverted repeat DNA sequence in the distal promoter. Biochim Biophys Acta 1829: 1257-1265.
53. Lönn P, van der Heide LP, Dahl M, Hellman U, Heldin CH, et al. (2010) PARP-1 attenuates Smad-mediated transcription. Mol Cell 40: 521-532.
54. Dahl M, Maturi V, Lönn P, Papoutsoglou P, Zieba A, et al. (2014) Fine-Tuning of Smad Protein Function by Poly(ADP-Ribose) Polymerases and Poly(ADP-Ribose) Glycohydrolase during Transforming Growth Factor β Signaling PLoS One 9: e103651.
55. Wrighting DM, Andrews NC (2006) Interleukin-6 induces hepcidin expression through STAT3. Blood 108: 3204-3209.
56. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, et al. (2007) STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood 109: 353-358.
57. Pietrangelo A, Dierssen U, Valli L, Garuti C, Rump A, et al. (2007) STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. Gastroenterology 132: 294-300.
58. Mayeur C, Lohmeyer LK, Leyton P, Kao SM, Pappas AE, et al. (2014) The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6. Blood 123: 2261-2268.
59. Besson-Fournier C, Latour C, Kautz L, Bertrand J, Ganz T, et al. (2012) Induction of activin B by inflammatory stimuli up-regulates expression of the iron-regulatory peptide hepcidin through Smad1/5/8 signaling. Blood 120: 431-439.
60. Lebrón JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, et al. (1998) Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 93: 111-123.
61. Lee P, Peng H, Gelbart T, Wang L, Beutler E (2005) Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc Natl Acad Sci U S A 102: 1906-1910.
62. Meynard D, Babitt JL, Lin HY (2014) The liver: conductor of systemic iron balance. Blood 123: 168-176.
63. Nicolas G, Viatte L, Bennoun M, Beaumont C, Kahn A, et al. (2002) Hepcidin, a new iron regulatory peptide. Blood Cells Mol Dis 29: 327-335.
64. Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, et al. (2001) A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 276: 7811-7819.
65. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, et al. (2002) Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood 100: 3776-3781.