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Hepcidin

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Dysregulation of metabolism and utilization of iron can lead to the development and maintenance of anemia of CKD. Anemia is prevalent among patients with CKD. The markers of iron sufficiency or availability of iron are far from perfect which results in inaccurate diagnosis and treatment of anemia with poor outcomes. Hepcidin, a 25 amino acid peptide produced by the hepatocytes, has emerged as the key regulator of uptake and release of iron in the tissues to maintain a steady supply of iron to erythron and other tissues while avoiding higher levels of iron that could be detrimental to the organs. Hepcidin itself is regulated by the supply of iron, the need for erythropoiesis, and the state of inflammation. Alterations in hepcidin levels are associated with restricted erythropoiesis, anemia, and iron overload. Discovery of hepcidin and elucidation of its mechanism of action and consequences of its upregulation and suppression have unraveled important insight into many hematologic disorders including anemia of CKD. This knowledge has also unlocked unique opportunities to modulate hepcidin via agonists and antagonists of hepcidin and its feedback pathways to treat clinical conditions. Many such agents are being developed and have potential therapeutic utility in future.

Key Words: Anemia, Hepcidin, Ferroportin, BMP, Iron

An adequate amount of iron is essential for many molecular processes including energy production as well as heme formation to facilitate oxygen delivery to the tissues. An excessive amount of iron, however, can be detrimental through the formation of reactive oxygen species with resultant oxidative stress, damage to DNA, and potentiation of lipid peroxidation and ferroptosis of cells. Even in the presence of adequate iron stores, a maldistribution of iron can result in anemia as in inflammation and chronic disease such as CKD. As iron losses are relatively constant and not regulated, the absorption and release of iron from cells is regulated through a multitude of proteins to carefully maintain intracellular and systemic levels of iron that are both adequate and safe.

HEPCIDIN PRODUCTION, ACTION, AND REGULATION

Hepcidin was discovered as the liver-expressed antimicrobial peptide in 2000.[1,2] Hepcidin is produced primarily by the hepatocytes which are strategically located in the vicinity of portal veins (carrying dietary iron) as well as the Kupffer cells (sensing microbes and recycling erythrocytes).[3] Hepcidin is also produced by macrophages and adipocytes in a small quantity.[4,5]

Hepcidin is encoded by the hepcidin antimicrobial peptide (HAMP) gene. It is initially synthesized as an 84 amino acid pre-pro-hepcidin. It is then processed to 60 amino acid pro-hepcidin and ultimately sliced to a mature C-terminal 25 amino acid active peptide.[6] Hepcidin is a tightly folded peptide hormone that forms a simple hairpin structure stabilized by 4 disulfide bonds (Fig 1).[7] Mutations of the HAMP gene are associated with severe iron overload and hemochromatosis. Hepcidin expression is increased in iron overload and inflammation and is diminished in states of iron deficiency and hypoxia.[8]

Hepcidin transcription is regulated by bone morphogenic protein (BMP) and its coreceptor hemojuvelin (HJV) in the liver. A number of BMPs, specifically BMP-6, can induce HAMP expression and upregulate hepatocyte hepcidin expression, a process enhanced by HJV and blunted in hemojuvelin knockout (*Hfe2*$^{-/-}$) hepatocytes.[9] BMPs bind to type I and type II receptors leading to signal transduction. BMP and transforming growth factor-beta induce hepcidin expression via "small" worm phenotype and Mothers Against Decapentaplegic (SMAD) signaling.[10] HJV is a member of the repulsive guidance molecule family and acts as a BMP coreceptor. *HJV gene* (also known as HFE2) mutations can result in hepcidin deficiency and juvenile hemochromatosis.[11] A number of other pathways and compounds partake in hepcidin regulation in a complex manner.[3]

Hepcidin gene expression is upregulated by inflammation and iron through the Janus kinase/signal transducers and activators of transcription (JAK/STAT) and BMP/SMAD pathways, respectively.[6] The BMP-6 signal acts through its receptor (BMPR), and is modulated by HJV. The *TMPRSS6* (transmembrane serine protease-2) gene encodes matriptase-2 (MT2) which cleaves membrane-bound HJV. BMPs can also signal through SMAD-independent pathways, notably via mitogen-activated protein (MAP) kinases. Dorsomorphin inhibits BMP signaling through the SMAD pathway. SMAD complexes bind to BMP-responsive elements. Tumor necrosis factor (TNF), pathogens, and interleukin-6 (IL-6) stimulate hepcidin synthesis via signal transducer and activator of transcription 3 (STAT-3) activation. The P38 MAP-kinase and extracellular signal-regulated kinases (ERK) 1-2 pathways are activated in response to iron signals. Diferric transferrin (Tf) binds to TF-receptor 1 (TfR-1) on the cell surface and the complex undergoes

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endocytosis. HFE is a protein that competes with Tf for binding to TfR-1. Hepcidin expression in macrophages is regulated mainly through TLR4 receptors associated with adaptor proteins. Hepcidin mRNA expression in macrophages induced by lipopolysaccharide (LPS) or high mobility group protein B1 (HMGB1) depends on nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB).

Vitamin D is a potent negative regulator of hepcidin transcription in cultured monocytes and hepatocytes and also induces antibacterial proteins such as cathelicidin.[12] In an in vivo and in vitro study, seven healthy volunteers received a single oral dose of vitamin D (100,000 IU vitamin D_2) which increased serum levels of 25-hydroxyvitamin D from 27 ± 2 ng/mL before supplementation to 44 ± 3 ng/mL after supplementation ($P < .001$). This response was associated with a 34% decrease in circulating levels of hepcidin within 24 hours of vitamin D supplementation. The high levels of hepcidin in patients with CKD may possibly be due not only to reduced renal excretion of hepcidin but also to vitamin D deficiency, which is widely prevalent in this population.

Hepcidin 25 has both iron regulatory and antimicrobial activities, but hepcidin 22 and 20 have only antimicrobial activity. Hepcidin 25 binds to ferroportin, a transmembrane protein, and leads to its degradation and consequent inhibition of iron release from enterocytes, macrophages, and hepatocytes to regulate iron absorption and utilization. Hepcidin is cleared via its internalization and degradation with ferroportin as well as through its excretion by the kidneys. Although hepcidin controls iron transport, its production is regulated by the systemic availability of iron, erythropoietic requirement, as well as by the state of inflammation (Fig 2).[3]

Hepcidin production is impacted by the feedback loop of systemic availability of iron. Hepcidin is produced in the iron-rich environment (whether in plasma or in tissues) and its production is reduced or stopped when iron is deficient or in high demand. In the presence of active erythropoiesis, production of hepcidin is inhibited, at least partially via an erythroid factor (erythroferrone, ERFE) produced by the erythroblasts that suppresses the BMP/SMAD pathway in the liver. In vitro, ERFE decrease SMAD1, SMAD5, and SMAD8 phosphorylation and inhibits expression of BMP target genes, suppressing the induction of hepcidin by BMP5, BMP6, and BMP7 without affecting hepcidin induction by BMP2, BMP4, BMP9, or activin B.[13,14]

Hepcidin levels decrease in hypoxia, although the exact mechanism is unclear. Hypoxia inducible factor may suppress hepcidin transcription directly or through activation of furin to release soluble HJV which interferes with BMP signaling and leads to inhibition of hepcidin activation.[15,16] Hepcidin synthesis is increased during periods of infection or inflammation. The hepatocyte production of hepcidin in such states is regulated by IL-6 through the STAT-3 signaling pathway.

HEPCIDIN IN IRON REGULATION AND ERYTHROPOIESIS

It is important to understand iron metabolism in order to understand therapeutic modulation of hepcidin. Total body iron stores are approximately 4 g with daily iron losses of 1-2 mg, which must be replenished via the absorption of dietary iron. Erythropoiesis requires 20-25 mg of iron daily, most of it coming from recycling by erythrophagocytosis of senescent red cells. Daily requirement of dietary iron differs among individuals according to their age and gender, especially in young females due to a much larger iron loss during menstruation. Iron absorption and availability in the plasma are regulated by hepcidin.

Iron uptake is mediated by the H^+ coupled Fe^{2+} transporter known as divalent cation transporter 1/divalent metal ion transporter 1 (DMT1) at the brush border of the duodenal enterocytes.[17] This is facilitated by the membrane ferrireductase, duodenal cytochrome B. Iron enters via enterocytes, is stored in the hepatocyte cytoplasm and macrophages, and must exit the cell on demand. The exit of the reduced iron from the cell requires the presence of ferroportin, a transmembrane protein, which is the only way for iron to exit into the blood. Hepcidin-ferroportin axis is an integral mechanism regulating the export of iron from the cells. The iron is again oxidized by the membrane bound ferroxidase hephaestin and ceruloplasmin and is then taken up by Tf. Tf releases iron to the cells by binding primarily to the TfR-1 on the cell membrane which stimulates its endocytosis and transport to mitochondria for heme synthesis or to cytoplasm to be stored as ferritin. Lack of ferroportin would render intracytoplasmic iron to be trapped within the cell. Thus, ferroportin degradation by hepcidin leads to inhibition of the transport of iron out of the duodenal enterocytes and a blockade of iron release from the macrophages and hepatocytes resulting in iron sequestration and depletion of the blood pool of iron. Cells that have intracellular iron but lack ferroportin have to be recycled upon cell death by macrophages.

CLINICAL SUMMARY

- Hepcidin, produced by hepatocytes, is the key regulator of uptake and release of iron in tissues; hepcidin is regulated by iron supply, erythropoietic requirements, and inflammatory status.
- Changes in hepcidin concentrations are associated with iron-restricted erythropoiesis, anemia, and iron overload.
- Hepcidin levels exhibit biological variation and subject to alterations in renal excretion and inflammation, thereby rendering hepcidin an unsuitable biomarker of iron status or predictor of ESA-responsiveness.
- Modulation of the hepcidin-ferroportin axis by agonists/antagonists represents an attractive future target for novel preventive or therapeutic strategies for disorders of iron metabolism and erythropoiesis.

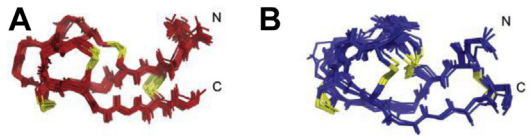

Figure 1. Two distinct conformations of hepcidin resolved at high and low temperature. *A*, high temperature nuclear magnetic resonance (NMR) ensembles; *B*, low temperature NMR ensembles. Adapted with permission from Jordan et al[7]; © 2009 by The American Society for Biochemistry and Molecular Biology, Inc.

Coordination and balance between iron uptake at the brush border and its exit from the basolateral membrane is tightly regulated. This involves a complex relationship between cellular iron accumulation, leading to activation of hepcidin with its effect on iron-binding proteins, and decreased formation of DMT1, degradation of hypoxia inducible factor, and activation of transubiquitination of DMT1.[17-19]

Iron overload induces the formation of BMPs by the hepatic sinusoidal endothelial cells. BMPs activate phosphorylation of Smad1/5/8 phosphorylation, which forms a transcriptional activator complex with Smad4 to stimulate hepcidin transcription.[20] HJV or HFE2, a membrane bound GPI-anchored protein, acts as a BMP coreceptor and promotes hepcidin transcription.[9] A soluble form of HJV (sHJV) blocks BMP-6 and inhibits hepcidin expression.[21] HJV is cleaved by matriptase 2 (MT2), and BMP receptor, HJV, and MT2 are stabilized by neogenin.[22]

Degradation of ferroportin by hepcidin requires direct binding of hepcidin to its receptor ferroportin.[23] The binding depends upon the presence of amino acid cysteine in position 326 on the extracellular loop of ferroportin. Absence of this amino acid in this position, as in the variant ferroportin, renders hepcidin unable to bind to ferroportin and can result in iron overload. The hepcidin-ferroportin complex then undergoes a conformational change leading to its endocytosis and lysosomal degradation. Studies of hepcidin structure also reveal that the first 9 N-terminal amino acids can internalize ferroportin—a conformational concept known as mini-hepcidin.[24]

HEPCIDIN IN HOST DEFENSE AND INFECTION AND ANEMIA OF INFLAMMATION

Growth and pathogenicity of many microbes require presence of iron.[25] Reduction in extracellular iron concentration has evolved over time as a host defense mechanism against infection. Protein-bound iron, as in ferritin, Tf, lactoferrin, or ovotransferrin, is not readily available for uptake by the microbes. Through ferroportin degradation, hepcidin decreases blood iron level to defend against infection.

Inflammation and infection result in increased production of IL-6 and TNF-alpha. IL-6 directly regulates hepcidin production through induction and subsequent promoter binding of STAT-3.[26] STAT-3 is not only necessary but also sufficient for the IL-6 responsiveness of the hepcidin promoter. STAT-3 signaling is influenced by BMP-dependent Smad activation.[10] An increase in hepcidin reduces iron concentrations in the blood which might

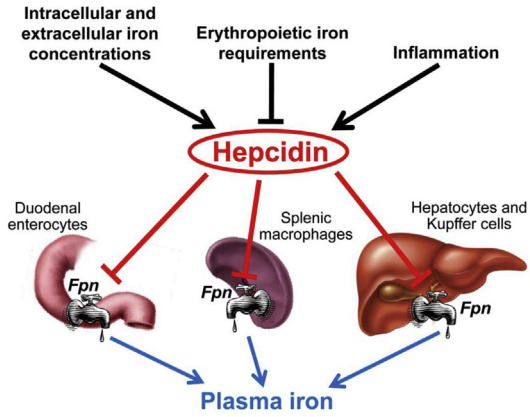

Figure 2. Hepcidin has a central role in maintenance of iron homeostasis. Hepcidin synthesis is regulated at the transcriptional level by multiple stimuli. Intracellular and extracellular iron concentrations increase hepcidin transcription, as does inflammation, whereas increased erythropoietic activity suppresses hepcidin production. In turn, hepcidin regulates plasma iron concentrations by controlling ferroportin concentrations on iron exporting cells including duodenal enterocytes, recycling macrophages of the spleen and liver, and hepatocytes. Adapted and reprinted from Ganz and Nemeth,[3] with permission from Elsevier.

be helpful in defense against iron-dependent microbes. However, this very action of hepcidin forms the basis of iron sequestration and anemia of inflammation. Inflammatory conditions may be associated with other hepcidin independent mechanisms such as decreased red cell survival and bone marrow suppression that contribute to anemia.

CLINICAL IMPLICATIONS

Dysregulation of hepcidin (*HAMP*) or related genes such as hemochromatosis (*HFE*), transferrin receptor 2 (*TFR2*), and *HJV* and conditions such as beta-thalassemia intermedia are known to result in states of dysregulated ferroportin. These conditions are often associated with increased iron absorption as well as export, resulting in excessive amounts of free systemic iron and excessive iron uptake by the tissues with consequent tissue damage. Insufficient production of hepcidin mediated by the mutations in hepcidin or *HJV* gene is associated with iron accumulation as in hereditary hemochromatosis. Beta-thalassemia, characterized by defective beta globin production and ineffective erythropoiesis, is also associated with hyperabsorption of dietary iron, which is linked to the presence of hepcidin suppressors such as ERFE. Hepcidin suppression is also mediated by the growth differentiation factor 15 (GDF15), a member of the transforming growth factor beta superfamily.[27] The twisted gastrulation protein homolog 1 TWSG1 also interferes with BMP 2 and 4-mediated hepcidin expression and may act with GDF15 to dysregulate iron homeostasis in thalassemia syndromes.[28] Therapy with hepcidin agonists may in future become a potential therapy for iron overload disorders.

Hepcidin circulates in blood in a protein-bound form, but with low affinity, leading to the presence of free hepcidin which is filtered by the kidney and degraded in

Table 1. Hepcidin Levels in Health and Disease

Serum hepcidin level	Low	High
Gender	Women	Men
Diurnal variation	Morning	Afternoon
Disease states	Iron deficiency, beta thalassemia intermedia, juvenile hereditary hemochromatosis	Secondary iron overload (such as iron transfusions), iron refractory iron deficiency anemia, adult hereditary hemochromatosis (though inappropriately low for the degree of iron overload), inflammation, infection, cancers such as multiple myeloma, and Hodgkin's disease

proximal tubule. Hepcidin levels in plasma are significantly elevated in patients on hemodialysis and can aggravate iron sequestration. Hepcidin levels decrease significantly after hemodialysis.[29] The improvement in anemia management in patients on prolonged dialysis may be partly related to hepcidin clearance.[30]

On the other end of the spectrum, hepcidin overexpression can result in iron restrictive anemia as in the case of hepcidin-expressing hepatic adenomas or in familial iron refractory iron deficiency anemia due to the mutations in MT2 which is an inhibitory regulator of hepcidin. Ferroportin deficiency due to missense mutations can also lead to impaired export of iron from cells and lead to iron restricted erythropoiesis. Anemia of some malignancies can also be related to hepcidin overproduction. Local levels of BMP are more important than the systemic level in these circumstances.

ANEMIA OF CKD AND HEPCIDIN AS A BIOMARKER

Anemia of CKD is characterized by inappropriately decreased production of erythropoietin by the kidneys where erythropoietin levels are indeed elevated but not commensurate with the severity of anemia. Anemia of CKD is truly multifactorial, resulting from a relative erythropoietin deficiency compounded by the deficiency, loss, sequestration, and poor utilization of iron with characteristics of anemia of inflammation. Interestingly, patients with anemia of CKD do respond to the supraphysiologic doses of erythropoiesis-stimulating agent (ESA) as well as high-dose intravenous iron even in the presence of adequate levels of iron.[31] High serum levels of hepcidin have been demonstrated in patients with kidney disease. In a study of CKD patients, bioactive serum hepcidin using a novel competitive ELISA was used to accurately measure hepcidin.[32] Hepcidin levels were significantly elevated compared with respective age controls as tested by analysis of variance. Additionally, each quartile of CKD patients had significantly different hepcidin levels. In this study, hepcidin levels were shown to be predicted by ferritin, C-reactive protein, and stage of kidney disease.

A variety of laboratory tests have been used to assess iron deficiency in patients with anemia. These include ferritin, Tf saturation, percent of hypochromic red cells, and soluble TfR. However, current methods of assessing adequacy of iron stores and iron availability to the erythron are grossly inadequate, especially in the presence of inflammation, and are unable to guide the clinician to formulate a strategy to treat anemia. Tf saturation, soluble TfR, and ferritin levels do not provide accurate insight. The association of hepcidin levels with the stage of CKD and ferritin and its inverse relationship with ESA therapy support the possibility that hepcidin could be a biomarker of iron status.[33] However, there is significant intrapatient variability in hepcidin level measurements. Additionally, hepcidin is dependent on kidney function for its excretion, and the levels increase with the presence of inflammation and are not necessarily different in patients sensitive or resistant to ESA.[34] Considering these factors along with nonharmonization of different hepcidin assays, the hepcidin levels cannot be considered a biomarker of iron status or a predictor of ESA response at this time.

HEPCIDIN ASSAYS

The availability, accuracy, interpretation, and clinical implications of hepcidin assays have been controversial. One study tested 11 different assays (5 mass spectrometry-based and 6 immunochemical-based) to quantify native individual plasma samples ($n = 32$) and native plasma pools ($n = 8$) to assess analytical performance and current and achievable equivalence.[35] Absolute hepcidin values and reproducibility (intrameasurement procedure coefficient of variation 2.9%–8.7%) differed substantially between measurement procedures, but all were linear and correlated well. The current equivalence (intermeasurement procedure coefficient of variation 28.6%) between the methods was mainly attributable to differences in calibration and thus could be improved by harmonization with a common calibrator.

Antibody-based immunoassays and mass spectrometric assays have been available to measure hepcidin, and measurements of bioactive serum hepcidin using a novel competitive ELISA are now available and have shown clinical correlation. Due to the decreased hepcidin clearance in the presence of kidney disease, serum measurements of hepcidin might be more accurate than urinary hepcidin measurements. It is important to remember that hepcidin has a biological variation (women have a lower level of hepcidin than men) and a diurnal variation (levels are lower in the morning and higher in the afternoon) (Table 1).

HEPCIDIN AS TARGET FOR THERAPY

Current treatment of anemia associated with dysregulated iron metabolism comprises of a spectrum of therapies.

These range from iron administration with and without ESA for iron deficiency to chelation and phlebotomy for iron overload. As hepcidin has a central role in iron regulation and a pathogenetic role in most clinical conditions associated with anemia, there is a potential to modulate hepcidin-ferroportin axis for treatment or prevention of such conditions. A number of such approaches are in development.[36]

Hepcidin Agonists

Hepcidin agonists may be able to prevent and treat iron overload. Natural hepcidin is expensive and not suitable for therapeutic use due to a short half-life and rapid renal clearance. A minimal structure with 7-9 N-terminal amino acids of hepcidin, including a single thiol cysteine, retains hepcidin activity, as shown by the induction of ferroportin degradation in reporter cells. This "mini-hepcidin" acts as a hepcidin-mimetic and has been further modified to improve its half-life and absorption. In mouse models, mini-hepcidin has been shown to control iron overload.[24,37] Such therapy may have potential use in hereditary hemochromatosis and beta-thalassemia.

Apart from the hepcidin mimetics, there are other stimulators of hepcidin production. Soy-derived isoflavone genistein is a small molecule activator of the Stat/Smad pathway that has been shown to enhance hepcidin expression in preclinical models.[38] MT2 is a negative regulator of hepcidin production and its inactivation can be useful in iron overload disorders. Antisense oligonucleotides and single stranded nucleic acids that lead to degradation of their target mRNA, have been used to increase hepcidin levels and decrease iron load in mouse models.[39,40] Another approach involves use pf lipid nanoparticles loaded with double-stranded small interfering RNAs (siRNAs) to inhibit the expression of its target genes through RNA interference. Small-molecule inhibitors of MT2 increase hepcidin production by inhibiting cleavage of HJV. In a mouse model this approach was used to deliver the MT2 siRNA to the liver to block MT2 RNA translation; similar to the antisense oligonucleotide approach, it was effective in β-thalassemia and iron overload.[41] These agents may also be able to help treating siderophilic infections.

BMP6 has also been used in animal models to increase hepcidin transcription and decrease serum iron.[42] However, such an approach is compromised by the off-target actions of BMP6.

Hepcidin Antagonists

Inhibition of hepcidin activity can form the basis of treatment of anemia characterized by iron restriction, such as anemia of inflammation and cancer. Hepcidin antagonism can be approached in multiple ways that include direct inhibition of hepcidin, inhibition of hepcidin ferroportin binding, inhibition of hepcidin-induced ubiquitination of ferroportin, and endocytosis of ferroportin. Suppression of inflammation can also indirectly suppress hepcidin levels.

Direct hepcidin inhibition can be achieved via antibodies to hepcidin, hepcidin-binding oligoribonucleotides, and inhibitors of hepcidin expression. Hepcidin neutralizing monoclonal antibodies have been shown to be effective in anemia of inflammation in a mouse model.[43] Hepcidin neutralization with antibodies in AI mice substantially improved iron-dependent red cell parameters. The use of anti-hepcidin antibody along with ESA therapy was able to correct anemia, although neither agent was effective alone in inducing reticulocytosis, demonstrating synergy between the 2 treatments. This finding suggests that by improving effective hemoglobinization of progenitors, hepcidin neutralization may have created permissive conditions to allow an effective reticulocyte response to erythropoietin. Hepcidin neutralization likely improves distribution of iron and better dietary absorption of iron, which might also reduce the requirements of parenteral iron. A drawback of this approach is the high rate of production of hepcidin, although even a short-term suppression might be able to facilitate erythropoiesis.

Gene silencing using RNA interference is a common technique to study gene function but can also be utilized for therapeutic purposes. siRNAs have been developed against the HAMP gene and other targets including HJV, TFR2, HFE, neogenin, BMP6, BMPRI, BMPRII, SMAD4, and IL-6R to further characterize the hepcidin signaling pathway.[44] Treatment with HAMP-targeting siRNA was able to blunt hepcidin induction, hypoferremia, and the onset of anemia in a turpentine-induced mouse model of inflammatory anemia. Silencing other members of the hepcidin pathway resulted in decreased HAMP expression and increased Tf saturation to varying degrees. A single 0.1 mg/kg dose of a TFR2-targeting siRNA resulted in nearly 80% silencing of both TFR2 and HAMP and 100% Tf saturation within 24 hours postadministration in mice. In addition, TFR2 targeting resulted in the resolution of anemia in rodent models of anemia of inflammation. This mode of genetic manipulation may represent a novel approach for the treatment of anemia of chronic disease and anemia of CKD.

Spiegelmer technology utilizes L-enantiomeric oligonucleotides that can inhibit specific targets, such as hepcidin. Spiegelmer NOX-H94 has been modified to NOX-H94002 and its PEGylated variant binds human hepcidin with high affinity to block its biological function. It has been shown to counteract anemia of inflammation in cynomolgus monkeys.[45] Antisense oligonucleotides to inhibit translation of hepcidin or HJV are also in development.

Hepcidin suppression by ERFE may be the basis of anemia associated with ineffective erythropoiesis, and neutralization of ERFE may be a potential therapy of iron overload in such disorders. By suppressing hepcidin, ERFE could be a potential treatment of iron-restrictive anemia characterized by increased hepcidin as in inflammation, CKD, and iron-refractory iron-deficiency anemia.

It is also possible to target ferroportin for amelioration of hepcidin-induced iron restriction. Antibody to ferroportin was effective in raising serum iron levels in a monkey model.[46] A thiol-reactive compound fursultiamine interferes with the binding of hepcidin to ferroportin.[47] Fursultiamine acts in vitro by blocking ferroportin residue Cys326-SH but has a short half-life in vivo. Targeting

ferroportin synthesis or its endocytosis is also being investigated. A shortcoming of hepcidin antagonists is a homeostatic feedback response with increased expression via other pathways.

Many other pharmacologic compounds have anti-hepcidin activity. Some of these include cardiac glycosides, thioxolone, and pyrithione zinc, in low doses. Some other commonly used compounds such as vitamin C, vitamin A, and statins have been implicated in hepcidin suppression.

MT2, a membrane-bound serine protease, inhibits hepcidin expression by depressing BMP/SMAD signaling. It is mutated in iron refractory iron deficiency anemia leading to high hepcidin levels. Small molecule ferroportin inhibitors and TMPRSS6 antisense nucleotides increase hepcidin levels and reduce serum and liver levels of iron in preclinical models of hemochromatosis and thalassemia.

Anticalins are derivatives of lipocalins which are involved in the transport of lipids and steroids into the cell. Anticalin PRS-080 binds human hepcidin with subnanomolar affinity and blocks the hepcidin-FPN receptor interaction. A single 3 mg/kg intravenous bolus of PRS-080 showed dose-dependent and hepcidin-specific iron mobilization in cynomolgus monkeys.[48]

STAT-3 pathway is involved in hepcidin regulation and small molecule STAT-3 inhibitors such as AG490 and the synthetic peptide inhibitor of STAT-3 (PpYLKTK) abolish hepcidin expression in mice. AG490 inhibits the phosphorylation of STAT-3 by JAK2, and the PpYLKTK disrupts phospho-STAT-3 dimerization, which is required for binding target genes.[49] Curcumin also inhibits STAT-3, can be anti-inflammatory, and may have potential iron chelating activity.[50]

Mediators of inflammation can also be targeted to reduce hepcidin synthesis. Administration of tocilizumab, a humanized antibody to IL-6 receptor, reversed anemia in cynomolgus monkeys by decreasing hepcidin production.[51] Administration of tocilizumab to these arthritic animals rapidly decreased CRP levels and improved iron-deficient anemia within 1 week. Tocilizumab induced rapid but transient reduction in serum hepcidin-25. TNF-alpha blockers etanercept, adalimumab, and infliximab have also been reported to ameliorate anemia in patients with inflammatory arthritis, such as rheumatoid arthritis and psoriasis, although this effect may be either indirect via the suppression of IL-6 production or through a direct effect on erythropoiesis.[52] Etanercept is a soluble TNF receptor fusion protein and maintains efficacy longer as compared to the anti-TNF-alpha antibodies due to the absence of antidrug antibodies.

BMP pathway has been targeted to reduce hepcidin production. Humanized anti-BMP monoclonal antibody was shown in healthy mice to block hepatic hepcidin production and increase serum iron.[53] Small molecule dorsomorphin derivative LDN-193189 or the protein soluble hemojuvelin-Fc inhibit BMP type I receptor Smad-mediated signaling required for effective hepcidin transcription. In a rat model of anemia of chronic inflammation, LDN-193189 blocks endogenous hepcidin production and results in mobilization of iron from the reticuloendothelial system, stimulates erythropoiesis, and corrects anemia.[54] Heparins bind to BMP but cannot be used due to anticoagulant properties. Low molecular weight heparin in the treatment of deep vein thrombosis has shown a robust reduction in hepcidin levels. Non-anticoagulant heparins have been developed that interfere with BMP/SMAD signaling.[55] Alcohol can also inhibit BMP receptor activation and signaling.[56] As mentioned above, the potential issues include inhibition of other BMP-dependent pathways and a specific antagonist with no off-target effects of BMP antagonism would be desirable.

Finally, recent developments in the area of hypoxia-inducible factor prolyl hydroxylase inhibitor have shown the capability to overcome hepcidin in the treatment of anemia of kidney disease. These are being discussed elsewhere in this issue.

FUTURE DIRECTIONS

Iron is an essential nutrient regulated via multiple pathways and hepcidin has emerged as a central regulator of iron homeostasis. Discovery of these pathways and understanding of the mechanisms of hepcidin action have created an opportunity to intervene in many disease states related to hepcidin under- or over-production. Development of specific molecules to target a specific step in the hepcidin-ferroportin axis regulation has the potential to develop individualized treatment of specific conditions. These developments are providing further understanding of the intricacies of hepcidin metabolism and therapeutic developments will need to be specific to avoid unintended consequences of hepcidin modulation.

REFERENCES

1. Krause A, Neitz S, Magert HJ, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett.* 2000;480(2–3):147-150.
2. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem.* 2001;276(11):7806-7810.
3. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta.* 2012;1823(9):1434-1443.
4. Liu XB, Nguyen NB, Marquess KD, Yang F, Haile DJ. Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. *Blood Cell Mol Dis.* 2005;35(1):47-56.
5. Bekri S, Gual P, Anty R, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology.* 2006;131(3):788-796.
6. Rochette R, Gudjoncik A, Guenancia C, Zeller M, Cottin Y, Vergely C. The iron-regulator hormone hepcidin: a possible therapeutic target? *Pharmacol Ther.* 2015;146(7):35-52.
7. Jordan JB, Poppe L, Haniu M, et al. Hepcidin revisited, disulfide connectivity, dynamics, and structure. *J Biol Chem.* 2009;284(36):24155-24167.
8. Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest.* 2002;110(7):1037-1044.
9. Babitt JL, Huang FW, Wrighting DM, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat Genet.* 2006;38(5):531-539.
10. Wang RH, Li C, Xu X, et al. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab.* 2005;2(6):399-409.

11. Ramey G, Deschemin JC, Vaulont S. Cross-talk between the mitogen activated protein kinase and bone morphogenetic protein/hemojuvelin pathways is required for the induction of hepcidin by holotransferrin in primary mouse hepatocytes. *Haematologica.* 2009;94(6):765-772.
12. Bacchetta J, Zaritsky JJ, Sea JL, et al. Suppression of iron-regulatory hepcidin by vitamin D. *J Am Soc Nephrol.* 2014;25(3):564-572.
13. Arezes J, Foy N, McHugh K, et al. Erythroferrone inhibits the induction of hepcidin by BMP6. *Blood.* 2018;132(14):1473-1477.
14. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. *Blood.* 2006;108(12):3730-3735.
15. Silvestri L, Pagani A, Camaschella C. Furin-mediated release of soluble hemojuvelin: a new link between hypoxia and iron homeostasis. *Blood.* 2008;111(2):924-931.
16. Palaneeswari MS, Ganesh M, Karthikeyan T, Devi AJ, Mythili SV. Hepcidin—minireview. *J Clin Diagn Res.* 2013;7(8):1767-1771.
17. Gunshin H, Allerson CR, Polycarpou-Schwarz M, et al. Iron-dependent regulation of the divalent metal ion transporter. *FEBS Lett.* 2001;509(2):309-316.
18. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. *Cell Metab.* 2009;9(2):152-164.
19. Brasse-Lagnel C, Karim Z, Letteron P, Bekri S, Bado A, Beaumont C. Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. *Gastroenterology.* 2011;140(4):1261-1271.
20. Kautz L, Meynard D, Monnier A, et al. Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. *Blood.* 2008;112(4):1503-1509.
21. Papanikolaou G, Samuels ME, Ludwig EH, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat Genet.* 2004;36(1):77-82.
22. Enns CA, Ahmed R, Zhang A-S. Neogenin interacts with matriptase-2 to facilitate hemojuvelin cleavage. *J Biol Chem.* 2012;287(42):35104-35117.
23. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science.* 2004;306(5704):2090-2093.
24. Preza GC, Ruchala P, Pinon R, et al. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. *J Clin Invest.* 2011;121(12):4880-4888.
25. Weinberg ED. Iron availability and infection. *Biochim Biophys Acta.* 2009;1790(7):600-605.
26. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood.* 2006;108(9):3204-3209.
27. Tanno T, Bhanu NV, Oneal PA, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Med.* 2007;13(9):1096-1101.
28. Tanno T, Porayette P, Sripichai O, et al. Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells. *Blood.* 2009;114(1):181-186.
29. Zaritsky J, Young B, Gales B, et al. Reduction of serum hepcidin by hemodialysis in pediatric and adult patients. *Clin J Am Soc Nephrol.* 2010;5(6):1010-1014.
30. Schwartz DI, Pierratos A, Richardson RM, Fenton SS, Chan CT. Impact of nocturnal home hemodialysis on anemia management in patients with end-stage renal disease. *Clin Nephrol.* 2005;63(3):202-208.
31. Coyne DW, Kapoian T, Suki W, et al, the DRIVE Study Group. Ferric gluconate is highly efficacious in anemic hemodialysis patients with high serum ferritin and low transferrin saturation: results of the Dialysis Patients' Response to IV Iron with Elevated Ferritin (DRIVE) Study. *J Am Soc Nephrol.* 2007;18(3):975-984.
32. Zaritsky J, Young B, Wang HJ, et al. Hepcidin—a potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol.* 2009;4(6):1051-1056.
33. Ashby DR, Gale DP, Busbridge M, et al. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int.* 2009;75(9):976-981.
34. Kato A, Tsuji T, Luo J, et al. Association of prohepcidin and hepcidin-25 with erythropoietin response and ferritin in hemodialysis patients. *Am J Nephrol.* 2008;28(1):115-121.
35. van der Vorm LN, Hendriks JCM, Laarakkers CM, et al. Toward worldwide hepcidin assay harmonization: identification of a commutable secondary reference material. *Clin Chem.* 2016;62(7):993-1001.
36. Fung E, Nemeth E. Manipulation of the hepcidin pathway for therapeutic purposes. *Haematologica.* 2013;98(11):1667-1676.
37. Casu C, Nemeth E, Rivella S. Hepcidin agonists as therapeutic tools. *Blood.* 2018;131(16):1790-1794.
38. Zhen AW, Nguyen NH, Gibert Y, et al. The small molecule, genistein, increases hepcidin expression in human hepatocytes. *Hepatology.* 2013;58(4):1315-1325.
39. Guo S, Casu C, Gardenghi S, et al. Reducing TMPRSS6 ameliorates hemochromatosis and beta-thalassemia in mice. *J Clin Invest.* 2013;123(4):1531-1541.
40. Casu C, Aghajan M, Oikonomidou PR, Guo S, Monia BP, Rivella S. Combination of TMPRSS6-ASO and the iron chelator deferiprone improves erythropoiesis and reduces iron overload in a mouse model of beta thalassemia intermedia. *Haematologica.* 2016;101(1):e8-e11.
41. Schmidt PJ, Toudjarska I, Sendamarai AK, et al. An RNAi therapeutic targeting TMPRSS6 decreases iron overload in $Hfe^{-/-}$ mice and ameliorates anemia and iron overload in murine β-thalassemia intermedia. *Blood.* 2013;121(7):1200-1208.
42. Corradini E, Schmidt PJ, Meynard D, et al. BMP6 treatment compensates for the molecular defect and ameliorates hemochromatosis in Hfe knockout mice. *Gastroenterology.* 2010;139(5):1721-1729.
43. Sasu BJ, Cooke KS, Arvedson TL, et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. *Blood.* 2010;115(17):3616-3624.
44. Akinc A, Chan-Daniels A, Sehagal A, et al. Targeting the hepcidin pathway with RNAi therapeutics for the treatment of anemia [abstract]. *Blood.* 2011;118(21):688.
45. Schwoebel F, van Eijk LT, Zboralski D, et al. The effects of the anti-hepcidin Spiegelmer NOX-H94 on inflammation-induced anemia in cynomolgus monkeys. *Blood.* 2013;121(12):2311-2315.
46. Leung D, Hill KA, De Rosa DC, et al. LY2928057, an antibody targeting ferroportin, is a potent inhibitor of hepcidin activity and increases iron mobilization in normal cynomolgus monkeys [abstract]. *Blood.* 2013;122:3433.
47. Fung E, Sugianto P, Hsu J, et al. High-throughput screening of small molecules identifies hepcidin antagonists. *Mol Pharmacol.* 2013;83(3):681-690.
48. Hohlbaum A, Gille H, Christian J, et al. Iron mobilization and pharmacodynamics marker measurements in non-human primates following administration of PRS-080, a novel and highly specific anti-hepcidin therapeutic [abstract]. *Am J Hematol.* 2013;5(88):E41.
49. Fatih N, Camberlein E, Island ML, et al. Natural and synthetic STAT3 inhibitors reduce hepcidin expression in differentiated mouse hepatocytes expressing the active phosphorylated STAT3 form. *J Mol Med (Berl).* 2010;88(5):477-486.
50. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol.* 2006;71(10):1397-1421.

51. Hashizume M, Uchiyama Y, Horai N, Tomosugi N, Mihara M. Tocilizumab, a humanized antiinterleukin-6 receptor antibody, improved anemia in monkey arthritis by suppressing IL-6-induced hepcidin production. *Rheumatol Int.* 2010;30(7):917-923.
52. Papadaki HA, Kritikos HD, Valatas V, et al. Anemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. *Blood.* 2002;100(2):474-482.
53. Andriopoulos B, Corradini E, Xia Y, et al. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat Genet.* 2009;41(4):482-487.
54. Theurl I, Schroll A, Sonnweber T, et al. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. *Blood.* 2011;118(18):4977-4984.
55. Poli M, Asperti M, Naggi A, et al. Glycol-split nonanticoagulant heparins are inhibitors of hepcidin expression in vitro and in vivo. *Blood.* 2014;123(10):1564-1573.
56. Gerjevic LN, Liu N, Lu S, Harrison-Findik DD. Alcohol activates TGF-beta but inhibits BMP receptor-mediated Smad signaling and Smad4 binding to hepcidin promoter in the liver [published online October 23, 2011]. *Int J Hepatol.* 2012:459278. https://doi.org/10.1155/2012/459278.