Modulation of Hepcidin as Therapy for Primary and Secondary Iron Overload Disorders

Preclinical Models and Approaches

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KEYWORDS

• Iron metabolism • Ineffective erythropoiesis • Hereditary hemochromatosis • β-Thalassemia • Hepcidin/minihepcidins • Lipid nanoparticle siRNA/antisense oligonucleotide

KEY POINTS

- Dysregulation of iron metabolism is a primary or secondary cause of morbidity and mortality in many diverse diseases, including hereditary hemochromatosis and β-thalassemia.
- Hepcidin is the central hormonal regulator of iron metabolism.
- Tmprss6 is a serine protease that regulates hepcidin expression by the hepatocyte through a mechanism that involves several of the hereditary hemochromatosis proteins.
- Modulation of hepcidin activity has demonstrated potential as a treatment modality to treat iron overload disorders in preclinical animal models.

INTRODUCTION TO IRON METABOLISM

Because iron is highly toxic when present in excess, mammals have evolved elaborate mechanisms for the regulation of iron acquisition, transport, storage, and utilization. A typical adult human is endowed with approximately 4 g of iron, almost two-thirds of which is distributed in hemoglobin in red blood cells (RBCs). Nearly 25 mg of iron is

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required to support erythropoiesis each day, but most of the iron required for erythropoiesis derives from recycling of iron from effete RBCs by macrophages of the reticuloendothelial system. Under normal conditions, only 1 to 2 mg of iron is absorbed each day from the diet; that is only to offset iron losses, which are not regulated, and limited to physiologic and nonphysiologic epithelial cell (eg, skin and intestine) or blood loss. Accordingly, total body iron is regulated entirely at the level of intestinal absorption, which can be modulated according to the body's needs.

The Hepcidin-Ferroportin Iron Regulatory Axis

Hepcidin is a peptide hormone produced predominately by the liver in response to iron stores.[1] As iron levels increase, so does hepcidin,[2,3] which, as a negative regulator of iron release from cells, binds to and causes the internalization and degradation of ferroportin (FPN1), the only known iron exporter.[4] FPN1 is expressed in abundance on macrophages and duodenal enterocytes, the cells that are directly responsible for iron recycling from senescent RBCs and for iron absorption from the intestine (**Fig. 1**). Thus, hepcidin production simultaneously leads to decreased intestinal iron absorption and sequestration of iron in macrophages, limiting its availability for erythropoiesis. Conversely, decreasing hepcidin expression permits more nonheme iron to be taken up from the diet and released from internal stores. A failure of this stores regulator of systemic iron metabolism[5] underlies the pathophysiology of most forms of hereditary hemochromatosis (HH) (see later discussion).

In addition to systemic iron deficiency, hepcidin expression is also suppressed by anemia and hypoxia.[6] Anemias characterized by ineffective erythropoiesis (bone marrow erythroid hyperplasia with premature, intramedullary death of maturing erythroblasts) seem to uniquely potently suppress hepcidin production *even in the presence of systemic iron overload*.[7] The factor or factors that communicate this signal from the bone marrow to the liver to suppress hepcidin have been termed the *erythroid regulator* of iron metabolism.[5] It is the apparent supremacy of the erythroid regulator compared with the stores regulator that underlies the pathogenesis of iron overload in *iron-loading anemias*, such as β-thalassemia intermedia, which are characterized by ineffective erythropoiesis. Importantly, in these anemias, as well as in HH, the regulatory dysfunction leading to iron overload is a *relative if not absolute deficiency in hepcidin for the degree of iron overload.* It is on this theoretical basis that upregulation of hepcidin has been envisioned as a means to treat iron overload in these apparently diverse diseases.

Iron-Responsive Hepcidin Expression by the Hepatocyte

It is now evident that the autosomal recessive forms of HH caused by mutations in *HFE*, *HJV*, or *TFR2* result from a disruption of the hepatocyte's ability to translate systemic iron stores and availability for erythropoiesis represented by the transferrin saturation (or concentration of diferric transferrin) into a signal that promotes hepcidin gene transcription.[8–10] In this way, they are thought to disrupt the stores regulator of systemic iron homeostasis. This pathway has been reviewed comprehensively elsewhere.[11–13] Only elements that are fundamental to the therapeutic innovations discussed later are highlighted here.

There is strong evidence that the bone morphogenetic protein (BMP)–sons of mothers against decapentaplegic (SMAD) signaling pathway plays a key role in the regulation of hepcidin and systemic iron metabolism (**Fig. 2**). Hemojuvelin (HJV), which is mutated in patients with a severe, juvenile onset from of HH,[9] is a BMP coreceptor protein[14] that facilitates signaling through the BMP type I receptors (BMPRIs) ALK2 and ALK3[15,16] in response to BMP6,[17,18] which is itself upregulated in the liver by iron. Activated BMP receptors phosphorylate SMADS1, 5, and 8, which in turn