Iron Imports.

VI. HFE and regulation of intestinal iron absorption

Robert E. Fleming1,2 and Robert S. Britton3

1Department of Pediatrics, 2Edward A. Doisy Department of Biochemistry and Molecular Biology, and 3Division of Gastroenterology and Hepatology, Saint Louis University

Liver Center, Saint Louis University School of Medicine, St. Louis, Missouri

Fleming, Robert E., and Robert S. Britton. Iron Imports. VI. HFE and regulation of intestinal iron absorption. Am J Physiol Gastrointest Liver Physiol 290: G590–G594, 2006; doi:10.1152/ajpgi.00486.2005.—The majority of clinical cases of iron overload is caused by mutations in the HFE gene. However, the role that HFE plays in the physiology of intestinal iron absorption remains enigmatic. Two major models have been proposed: 1) HFE exerts its effects on iron homeostasis indirectly, by modulating the expression of hepcidin; and 2) HFE exerts its effects directly, by changing the iron status (and therefore the iron absorptive activity) of intestinal enterocytes. The first model places the primary role of HFE in the liver (hepatocytes and/or Kupffer cells). The second model places the primary role in the duodenum (crypt cells or villus enterocytes). These models are not mutually exclusive, and it is possible that HFE influences the iron status in each of these cell populations, leading to cell type-specific downstream effects on intestinal iron absorption and body iron distribution.

BECAUSE THERE ARE NO MECHANISMS for the regulated excretion of iron, homeostasis depends on the regulated absorption of dietary iron. Multiple genes appear to be involved in the feedback regulation between sites of iron utilization (primarily marrow), storage (spleen and liver), and absorption (duodenum). Many of these genes have been identified through the genetic analyses of pedigrees of individuals with iron overload. By far the most common of these heritable forms of iron overload is an autosomal recessive disorder caused by mutation in the HFE gene, designated HFE-associated hereditary hemochromatosis (HH). In western European populations, nearly 10% of individuals carry the C282Y mutation in HFE. Whereas most individuals homozygous for HFE mutations remain asymptomatic, some develop severe iron overload with consequent liver damage, heart failure, diabetes, arthritis, and/or sexual dysfunction. Despite extensive characterization of the protein encoded by HFE, important questions remain regarding the mechanism by which HFE regulates intestinal iron absorption. This review will describe the current understanding of the biology of HFE and will present hypotheses and controversies concerning the role of HFE in iron homeostasis.

HFE GENE AND PROTEIN

HFE is a 343-amino acid cell surface protein with homology to major histocompatibility complex (MHC) class I molecules (12). The structure includes a large extracellular domain consisting of three loops (α1 to α3), a single transmembrane domain, and a short cytoplasmic tail. Similar to other MHC class I molecules, HFE physically associates with β2-microglobulin (β2M) via its α3 loop (14). The most common mutation in HFE-associated HH leads to the substitution of tyrosine for cysteine at position C282 (C282Y). The loss of this cysteine disrupts the disulfide bond needed for formation of the α3 loop and thus interferes with the ability of HFE to interact with β2M (14, 36). The mutant HFE protein is consequently retained in the endoplasmic reticulum and middle Golgi compartments (36) where it is subject to accelerated degradation.

Cell surface expression of mutant HFE is thus considerably reduced (27). The hemochromatosis phenotype of β2M-knockout mice evidences the importance of the association between β2M and HFE for normal iron homeostasis (9, 31). HFE mutations other than C282Y have been identified in a comparatively small number of patients with iron overload. The most common of these is H63D. Compared with HFE knockout mice or C282Y homozygous mice, mice homozygous for the mutation orthologous to H63D have mild increases in parameters of iron status (33).

The tissue distribution of HFE offers few clues to its role in iron metabolism, because HFE is broadly expressed (12). However, expression in the liver is high relative to other tissues. In the liver, HFE has been localized to hepatocytes (21, 39), Kupffer cells (3), sinusoidal lining cells, and bile duct epithelial cells. HFE is also expressed in intestinal enterocytes. In the duodenum, the intestinal region where nearly all dietary iron absorption occurs, expression is greatest in crypt cells (28). Here HFE is found in association with transferrin (Tf) receptor 1 (TfR1) in recycling endosomes (35). However, HFE has also been identified in small intestine villus enterocytes as well (38). Because of the established role of these cell types in iron metabolism, nearly all investigative attention has focused HFE on duodenal crypt cells, hepatocytes, and Kupffer cells.

HFE gene expression is only modestly influenced by changes in cellular iron status. Approximately twofold increases in hepatic HFE mRNA have been reported in response to iron loading in mice (23, 32). No change was observed in response to phlebotomy. Experimental iron loading or deficiency has been associated with minor changes in HFE expression in the small intestine (16, 23, 32). Cell culture experiments have shown inconsistent results on the effect of exogenous iron on the expression of HFE (13, 20). No iron responsive elements are found in the HFE mRNA, and no metal response elements have been identified in the promoter region of the HFE gene (25, 30).

HFE EFFECTS ON CELLULAR IRON STATUS

Although changes in iron status appear to have little effect on the expression of HFE, the expression of HFE has profound effects on cellular iron status. Indeed, the first potential link
between HFE and cellular iron metabolism occurred with the discovery that HFE forms a complex with TfR1. The association between HFE and TfR1 has been observed in duodenal crypt enterocytes (35), liver homogenates (unpublished data), and transfected cell lines (19). HFE undergoes endocytosis with TfR1, with dissociation of the complex on acidification of the endosome (and release of iron from the Tf-TfR1 complex) (22). Crystallographic studies have identified the amino acids participating in the interaction between TfR1 and HFE (4). Interestingly, the binding site for HFE and diferric Tf on TfR1 overlap (18), suggesting that HFE can compete with Fe-Tf for binding to TfR1. As such, HFE would be expected to decrease TfR1-mediated uptake of Tf-bound iron. Indeed, transfected HFE has been demonstrated to decrease Tf-mediated iron uptake and lead to a decrease in cellular iron in some cell lines (6, 7, 29). Surprisingly, however, transfected HFE was also found to decrease iron uptake in a cell line that does not express TfR1 (6). Moreover, whereas transfected HFE has been found to decrease intracellular iron in many cell lines (37), in others, HFE instead leads to an increase in cellular iron by effecting a decrease in cellular iron efflux (8, 10). Thus it appears that HFE effects changes in cellular iron in a direction that depends on the relative TfR1-dependent and -independent iron transport activity in a particular cell type. Finally, HFE can indirectly influence the iron status of certain cell populations in vivo by modulating the expression of hepcidin (see below).

The interaction of HFE with TfR1 raises not only the possibility that HFE might influence the function of TfR1, but the converse as well; i.e., that TfR1 might influence the function of HFE. By binding HFE, TfR1 might decrease the bioavailability of HFE to interact with an additional protein involved in iron metabolism. This additional protein could be an iron transporter, as HFE appears to have direct affects on cellular iron export. Alternatively, HFE might interact with a protein involved in a signal transduction pathway which regulates expression of hepcidin (see below; see also Ref. 15a). In each of these proposed models, the amount of bioavailable HFE might be influenced by both the level of TfR1 expression and competing Fe-Tf, each of which is in turn influenced by iron availability. Nonetheless, there is currently no empiric evidence to demonstrate that HFE can be functionally modulated by TfR1. Moreover, it remains possible that the interaction of HFE with TfR1 is incidental to the role HFE plays in iron homeostasis.

HFE IN THE LIVER: HEPCIDIN HYPOTHESIS

The central role of hepcidin in the regulation of duodenal iron absorption is well established (16a). Studies on patients with HFE-associated HH (5) and knockout mice (1) clearly demonstrate that loss of HFE is associated with decreased liver hepcidin expression. Moreover, crossing HFE knockout mice with mice overexpressing hepcidin leads to normalization their iron overload phenotype (26). These studies strongly suggest that HFE participates in the regulation of intestinal iron absorption by modulating the expression of hepcidin. The proposed model is as follows (Fig. 1): loss of HFE leads to decreased circulating hepcidin, which results in increased ferroportin-mediated iron efflux from reticuloendothelial cells and duodenal enterocytes. Over time, circulating iron concentrations increase and Tf becomes saturated. Nontransferrin-bound iron (NTBI) appears in the circulation and is taken into those tissues that have high capability for NTBI uptake (particularly liver). In this model, increased enterocyte iron export might lead to secondary increases in the expression of genes involved in intestinal iron uptake (e.g., Dcytb, DMT1), as observed in some studies on HH patients or HFE knockout mice. Thus the iron homeostasis abnormalities, which characterize HFE-associated HH (i.e., increased intestinal iron absorption, increased circulating iron, and sparing of RE iron loading), are explicable on the basis of inappropriate underexpression of hepcidin.

Interestingly, whereas HFE knockout mice have inappropriately low hepcidin expression, they maintain the ability to regulate hepcidin expression with changes in iron status. Thus loss of HFE appears to attenuate but not eliminate the signal between iron status and hepcidin expression. Possibly, other

Fig. 1. Hepcidin hypothesis. In this model, diferric transferrin (Tf) in the portal circulation serves as the ligand for hepatocyte Tf receptor (TfR; TfR2 in particular). This interaction transduces a signal leading to increased hepatocellular expression of hepcidin. Hepcidin is secreted into the blood and acts on target cells (reticuloendothelial macrophages and duodenal enterocytes) to decrease iron export and thereby increase iron stores in these cells. In duodenal enterocytes, this leads to a decrease in the amount of dietary iron absorbed into the circulation. Thus circulating levels of diferric Tf are normalized, and homeostasis is maintained. In HFE-related hereditary hemochromatosis (HH), loss of functional HFE protein leads to aberrant hepatocellular sensing of plasma iron, inappropriately low levels of hepcidin, decreased reticuloendothelial iron stores, and increased duodenal iron absorption. RBC, red blood cells; fp1, ferroportin 1; HJV, hemojuvelin; NTBI, non-Tf-bound iron.
gene products (Tf receptor 2 and hemojuvelin) serve to transduce this signal. Whether HFE acts by modulating the function of either of these two molecules or acts independently of them to influence hepcidin expression is unknown.

Because hepatocytes are the major site of hepcidin synthesis, and because they express other genes known to modulate hepcidin expression (e.g. Tf receptor 2, hemojuvelin), it is attractive to postulate that hepatocellular HFE likewise modulates hepcidin expression. However, isolated hepatocytes in culture fail to demonstrate a change in hepcidin expression with changes in media iron concentrations. This observation has given consideration to the possibility that HFE expressed in another cell type modulates the signal that regulates hepcidin expression in hepatocytes. A very recent study supports such a role for HFE in Kupffer cells. The transplantation of wild-type reticuloendothelial (RE) cells into HFE knockout mice was associated with an increase in liver hepcidin expression and at least partial abrogation of hepatic iron loading (24). However, the converse experiment (transplanting HFE knockout RE cells into wild-type mice) had little effect. Ultimately, determining the relative contributions of HFE in hepatocytes compared with Kupffer cells in modulating hepcidin expression may require cell-specific knockout or rescue experiments.

Regardless of the cell type in which HFE plays its regulatory role, it is clear that loss of HFE results in decreased hepcidin expression and that the decreased hepcidin expression can at least theoretically account for the iron homeostasis abnormalities seen in HFE-associated HH. Does this mean that the effects of HFE on iron metabolism are mediated entirely through hepcidin? Several observations suggest that this might not be the case. As mentioned earlier, transfection of HFE into cultured cells (including enterocyte cell lines) profoundly affects their iron status without exogenous hepcidin. Moreover, transgenic ubiquitous overexpression of HFE in the mouse causes an iron-deficiency phenotype despite a compensatory downregulation of liver hepcidin expression (unpublished data). These observations lend support to models in which at least some of the effects of HFE on iron metabolism are not mediated through hepcidin. The most substantiated of these models is the “crypt cell hypothesis” described in HFE IN THE DUODENUM: CRYPT CELL HYPOTHESIS.

HFE IN THE DUODENUM: CRYPT CELL HYPOTHESIS

It has long been proposed that the sensing of body iron status by duodenal crypt cells might effect a change in the iron absorptive activity of daughter enterocytes (2). As mentioned previously, HFE is expressed in duodenal crypt cells in physical association with TfR1. These observations led to the hypothesis that HFE influences the ability of crypt cells to sense body iron status by modulating their Tf-mediated uptake of iron. This model postulates that HFE in duodenal crypt cells facilitates the TfR1-mediated uptake of plasma iron and that mutant HFE lacks this facilitating effect. Functional loss of HFE would thereby be anticipated to result in a relatively iron-deficient state in these cells and daughter enterocytes. This would, in turn, lead to an increase in expression of iron-regulated genes participating in dietary iron absorption, including DMT1, Dcytb, and/or ferroportin (Fig. 2). This “crypt cell hypothesis” is supported by the observations that iron uptake from plasma Tf by the duodenum is impaired in HFE knockout mice (34) and that expression of the iron transport genes are increased in HFE knockout mice (11).

HFE expression has also been reported, albeit at low levels, in villus enterocytes, suggesting the possibility that HFE may play a role in modulating the uptake or release of dietary iron by these cells. However, given the profound influence of hepcidin on enterocyte iron release, any direct effect of HFE on iron uptake or release by intestinal enterocytes (crypt or villus) might be less important than the indirect effect of HFE on hepcidin expression (15).

In summary, HFE clearly interacts with TfR1, changes the iron status of cells in which it is expressed, and influences hepcidin expression. It is attractive to put these observations together and propose that HFE modulates hepcidin expression by changing the iron status of a TfR1-expressing liver cell population. However, the broad expression of HFE and cell-specific effects on iron status suggest that such a model may be too simplistic. One of the difficulties in testing the role of HFE in vivo is distinguishing primary effects of HFE on cellular iron status from secondary effects resulting from changes in

Fig. 2. Duodenal crypt cell programming hypothesis. Duodenal villus cells are the major site of iron absorption from the diet. Before uptake, dietary ionic iron requires reduction from the ferric to the ferrous state. This is accomplished by the ferric reductase Dcytb that is expressed on the luminal surface of villus cells. Ferrous iron is taken up by the apical transporter DMT1. Iron may be stored within the cell as ferritin and lost with the sloughed senescent enterocyte or transferred across the basolateral membrane to the plasma. This latter process occurs via the transporter ferroportin1 and requires oxidation of iron to the ferric state by the molecule hephaestin. Villus enterocytes differentiate from crypt cells during migration from the crypts to the apex of the villus. The crypt cells may sense plasma iron via the HFE-TfR1 complex on the basolateral surface and program the level of expression of the iron transport genes expressed on differentiation of these cells to villus absorptive enterocytes. In HFE-related HH, loss of functional HFE protein leads to decreased TfR1-mediated iron uptake by the crypt cells, a relatively iron-deficient phenotype of the enterocytes, and increased iron absorption by the villus enterocytes.
REFERENCES


