The liver, a major site of body iron stores, mediates key responses that preserve systemic iron homeostasis. In this issue of the JCI, Guo et al. demonstrate that administration of antisense oligonucleotides that reduce expression of Tmprss6, a hepatic protein that plays an essential role in maintaining iron balance, can attenuate disease severity in mouse models of human iron overload disorders. These data reveal the potential of novel Tmprss6-targeted therapies for the treatment of clinical conditions such as hereditary hemochromatosis and β-thalassemia.

Hepcidin and the regulation of systemic iron balance

The majority of iron required daily by the adult human body is used to meet the demands of hemoglobin synthesis. Most of this iron is obtained through the recycling of senescent erythrocytes by macrophages in the spleen, liver, and bone marrow, while a small amount is absorbed from the diet in the duodenum. Hepcidin, a small circulating peptide released by the liver, regulates iron balance by limiting both the absorption of iron from the diet and the release of iron from macrophage stores (1).

Hepcidin mediates these effects by triggering the internalization and degradation of ferroportin, a cellular iron exporter that is highly expressed at the basolateral membrane of enterocytes and the cell membrane of macrophages (Figure 1). In hepatocytes, hepcidin transcription is modulated by an intracellular signaling cascade that is activated by binding of BMP ligands to a cell-surface receptor complex (Figure 2). The liver, a major site of iron storage, increases production of the BMP family member BMP6 in response to rising local iron stores; this leads to increased signaling for hepcidin production, which in turn limits further dietary iron absorption. Appropriate regulation of intestinal iron absorption is critical, as there is no regulated mechanism for eliminating surplus iron from the body.

Hepcidin insufficiency in iron overload disorders

Inherited forms of iron overload (hemochromatosis) result from mutations in gene products that are required locally in the liver for hepcidin production. In these disorders, the resulting hepcidin insufficiency leads to gastrointestinal iron absorption that exceeds the body’s needs. The accumulation of excess iron promotes oxidative damage to tissues, which can ultimately lead to failure of organs such as the heart, liver, and endocrine glands. Hepcidin levels are inappropriately low relative to body iron stores in another class of clinical disorders associated with systemic iron loading: congenital anemias that are characterized by ineffective erythropoiesis (IE) (2). IE describes a defective form of erythroid maturation characterized by an increased proportion of erythroid precursors, which, due to excessive apoptosis, fail to produce the normal complement of mature erythrocytes. In β-thalassemia, the most common inherited form of IE, the primary genetic defect leads to reduced synthesis of the β-globin component of adult hemoglobin. The result is an excess
of unpaired α-globin chains; these form toxic aggregates that promote apoptosis of erythroid precursors. Patients with β-thalassemia major, the most severe form of the disease, develop iron overload from blood transfusions that are required to sustain life. Iron overload also develops in patients with β-thalassemia intermedia (TI), who by definition are not transfusion dependent, due to the hepcidin suppression associated with IE. In these patients, the hepcidin response increases iron availability for erythropoiesis but is also maladaptive, as it promotes the development of systemic iron overload. The molecular basis by which IE leads to hepcidin suppression is not yet understood.

Transgenic overexpression of hepcidin has been shown to limit iron loading in a mouse model of TI (3) and to prevent hepatic iron overload in a mouse model of HFE-associated hemochromatosis (HFE-HH), the most common inherited form of hemochromatosis resulting from mutation in the HFE gene (4). Additionally, chronic injection of synthetic hepcidin lowered plasma iron levels in the HFE-HH mouse model (5). However, the bioactive form of hepcidin shows rapid renal excretion, and disulfide bridging within the molecule makes chemical synthesis expensive. These limitations have spurred the development of small-peptide hepcidin mimetics (termed “minihepcidins”), which have shown early therapeutic promise in mouse models (6). In this issue of the JCI, Guo et al. demonstrate an alternative pharmacological strategy that addresses the hepcidin insufficiency in iron overload disorders by increasing endogenous hepcidin production (7).

**Tmprss6 as a therapeutic target**

In the liver, hepcidin synthesis through BMP signaling involves a number of extracellular, membrane-bound, and intracellular proteins (Figure 2). A key negative regulator of hepcidin synthesis by this pathway is TMPRSS6 (also known as matriptase-2), a transmembrane protein primarily expressed in the liver (8). TMPRSS6, which contains a serine protease domain, is thought to downregulate BMP signaling by cleaving a membrane-associated protein termed hemojuelin (HJV), which functions as a BMP coreceptor, from the cell surface (9). Accordingly, both humans and mice with TMPRSS6 mutations exhibit inappropriately elevated levels of hepcidin, leading to impaired dietary iron absorption, systemic iron deficiency, and iron deficiency anemia. The effects of Tmprss6 disruption are thus similar to those obtained by transgenic overexpression of hepcidin, and targeted genetic disruption of Tmprss6 can reduce iron loading in mouse models of HFE-HH (10) and TI (11).

To induce endogenous hepcidin expression, Guo et al. developed antisense oligonucleotides (ASOs) that target Tmprss6 mRNA (7). ASOs are single-stranded, chemically modified nucleic acid analogs, which following Watson-Crick base pairing, induce selective degradation of their target mRNA by the natural enzyme RNase H (12). Of over 150 ASOs designed to target murine Tmprss6, two were identified that suppressed Tmprss6 mRNA in a dose-dependent manner when introduced into isolated murine hepatocytes and when injected into healthy mice. Tmprss6-ASOs appeared well tolerated, and the treated mice did not show biochemical or histological evidence of hepatic inflammation or injury.

The authors then demonstrated efficacy of Tmprss6-ASOs in mouse models of human iron overload disorders. In a model of HFE-HH, biweekly Tmprss6-ASO injections for six weeks raised hepcidin expression and reduced iron concentrations in the serum and liver. Tmprss6-ASO treatment also led to sequestration of iron within splenic macrophages, reflecting the ability of hepcidin to limit ferroportin-mediated iron export, and to a moderate reduction in blood hemoglobin levels, likely due to the reduced availability of iron for erythropoiesis.

Tmprss6-ASOs were also effective in reducing serum and liver iron concentra-
recently, Schmidt et al. demonstrated that persistent for one week. Long-term treatment of mouse models of HFE-HH and TI with Tmprss6-siRNA modified iron homeostasis and erythropoiesis in a manner qualitatively similar to Tmprss6-ASOs.

Looking forward
In summary, the study of Guo et al. (7), together with that of Schmidt et al. (14), provides exciting proof of principle that Tmprss6-dependent hepcidin regulation can be exploited as a therapeutic strategy for iron overload disorders characterized by hepcidin insufficiency. Small molecules that selectively inhibit the Tmprss6 catalytic domain might be predicted to show similar therapeutic effects (15). Pharmacological approaches that effectively target human Tmprss6 and show acceptable safety profiles would increase management options for iron overload disorders. Currently, patients with HFE-HH with evidence of increased iron stores are treated by long-term phlebotomy (16), a treatment that while safe and effective, is inconvenient and may be limited by poor venous access. Patients with β-thalassemia, in whom phlebotomy is contraindicated due to preexisting anemia, are treated by pharmacological chelation (17). Treatment with deferoxamine, a subcutaneously administered chelator used extensively in β-thalassemia, may yield adverse effects such as infusion site reactions and audiologic, ophthalmologic, and bone toxicities. Side effects of deferasirox, a newer oral chelator, include gastrointestinal disturbances, changes in kidney function, and more rarely, hepatic and renal failure.

Whether Tmprss6-targeted therapies might be applied in isolation or in combination with other therapies remains uncertain. As hepcidin elevation causes a redistribution of iron to macrophages, Tmprss6-targeted treatments would require careful titration to prevent the development of iron-restricted anemia, particularly if used as an adjunct to phlebotomy in HFE-HH. Given that the availability of oral chelators has improved patient compliance and satisfaction with chelation therapy in β-thalassemia (17), the acceptance of Tmprss6-targeted therapies by this patient population might be influenced by the route of administration. As the majority of patients with β-thalassemia live in low- or middle-income countries, where access to chelation therapy still remains challenging (18), it would be important to consider how these novel therapies could be delivered to the areas of greatest clinical need.

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