Iron deficiency anemia - Section 11

Mechanisms, mishaps and manipulation of iron uptake

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Take home messages

- Disorders in systemic iron regulation or intestinal iron absorption can result in functional or absolute iron deficiency or iron overload.
- A generation of pharmaceuticals are now entering clinical trials aimed at manipulating systemic iron regulation to alleviate pathologic iron loading or depletion.

Introduction

Insights into mechanisms of iron absorption and systemic iron handling have improved understanding of the pathophysiology of a range of clinical conditions that cause iron deficiency (or iron withholding from the plasma, 'functional iron deficiency') or iron overload, and have led to rationally-designed novel therapeutics that may transform management of iron withholding or overload states, some of which are now entering clinical trials. Here, we review recent advances in iron-related physiology, pathology and pharmaceuticals.

Current state of the art
Mechanisms of iron uptake and distribution

To prevent iron deficiency and overload, iron utilization and losses must be balanced by iron uptake (Fig. 1). Systemic iron distribution is governed by the hepatic-derived hormone hepcidin. Hepcidin binds to the only known cellular iron exporter, ferroportin, to both occlude its channel[1] and cause its internalization and degradation, thus controlling entry of iron to the plasma from the duodenum (absorption) and reticuloendothelial system (recycling). Hepcidin is transcriptionally (epigenetically via

HDAC3)[2] regulated by BMP/SMAD signaling (iron availability via iron sensing in the liver, erythropoietic demand via erythroferrone[*3,*4] and acute serum iron deprivation[5]), and inflammation (IL6, JAK/STAT signaling) (Fig. 1).

Mishaps

Iron deficiency. Here we focus on clinical factors that impair iron uptake through either impaired luminal iron function or increased hepcidin expression.

Impaired luminal iron absorption causes iron deficiency. Defective acidification of the intestinal contents impairs solubility of ferric iron in the intestine, limiting absorption.[6] This poses a risk for patients who have undergone gastrectomy, and those who undergo gastric bypass for treatment of obesity.[7] *Helicobacter Pylori* infection may cause iron deficiency[8] by impairing the acidic gut environment and promoting gastrointestinal bleeding. Chronic gastric acid suppression (ie, proton pump inhibition or histamine receptor antagonism[9]) can increase risk of iron deficiency.

Intestinal (especially duodenal) dysfunction impairs iron absorption. Coeliac disease causes immune-mediated destruction of the intestinal absorptive surface resulting in diminished absorption of numerous nutrients including iron.[10] Iron deficiency may indicate occult coeliac disease and screening for this condition is now widely suggested for iron deficient patients. Other disorders of the intestinal functional surface (eg, environmental enteropathy in developing countries), or intestinal absorptive area (eg, resections due to inflammatory bowel disease) may promote iron deficiency.

Iron absorption and organ utilization is diminished in systemic conditions which physiologically or pathologically raise hepcidin (functional iron deficiency). Anemia of chronic disease occurs in inflammatory conditions (infections, cancer, autoimmune diseases) that increase hepcidin expression via IL6 (also IL22) mediated JAK-STAT signaling or directly abrogate ferroportin transcription via TLR signaling.[11] Among clinically-well but subclinically inflamed populations, elevated hepcidin is an important cause of iron deficiency: for example, in sub-Saharan Africa where subclinical plasmodium infection is common[12] and

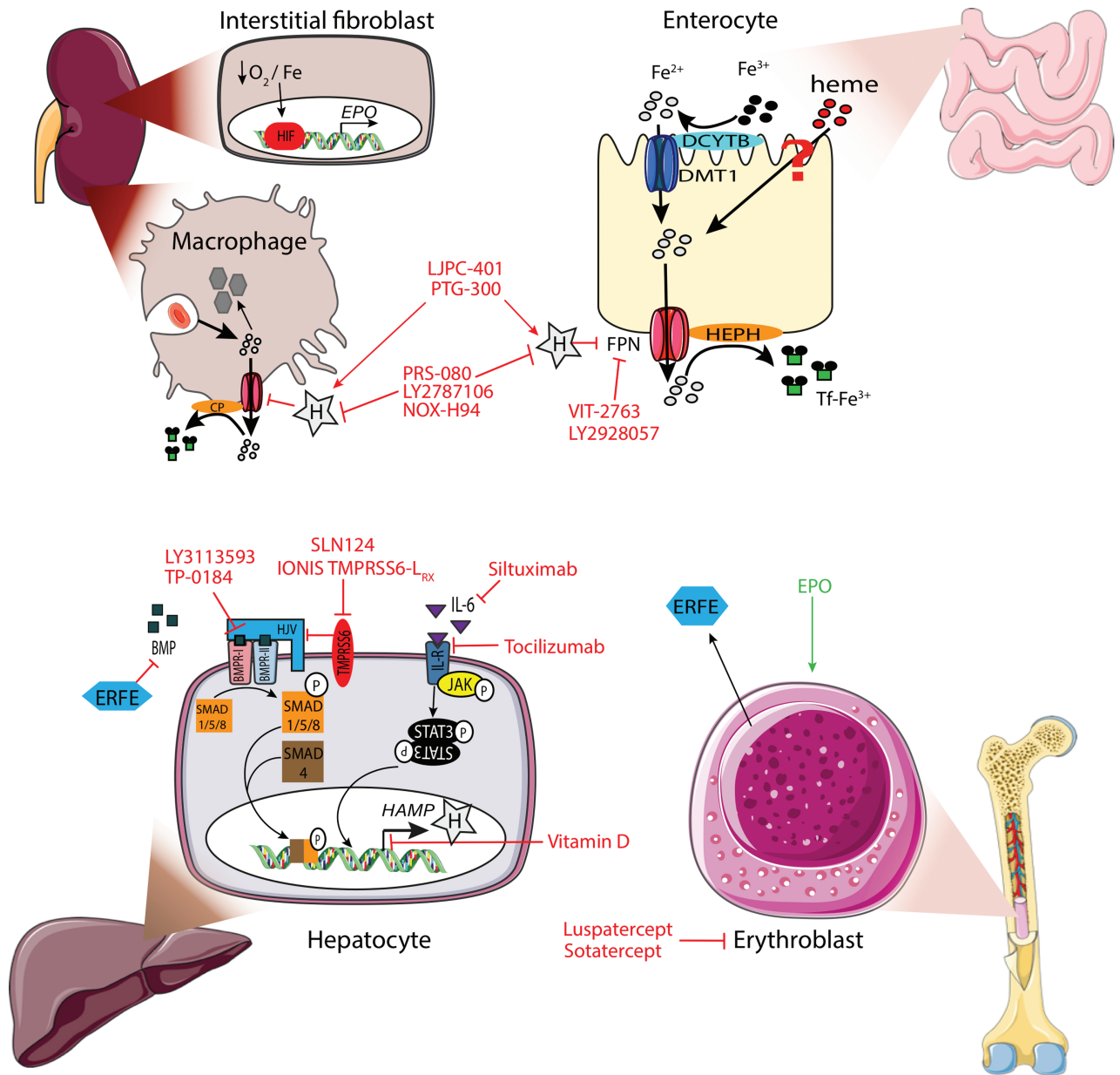**Figure 1. Systemic iron metabolism and therapeutics against the hepcidin-ferroportin axis.** Therapeutics that alter iron levels via the hepcidin-FPN axis are highlighted in red. Iron circulates in the plasma predominantly bound to transferrin (Tf; green squares). Iron reaches the plasma via absorption of dietary iron by duodenal enterocytes and recycling of iron from senescent erythrocytes by reticuloendothelial macrophages. The mechanism of dietary iron entry to enterocytes depends on the type of iron. Non-heme ferric iron (Fe^{3+}; black circles) is reduced to ferrous iron (Fe^{2+}; grey circles) by the ferrireductase DCYTB and transported across the apical membrane by DMT1. Heme iron (red circles) entry is probably via an undiscovered transporter. Once internalized, iron from both sources is exported to the bloodstream through the basolateral membrane via the only known iron exporter, ferroportin (FPN; red channel). The ferroxidase hephaestin (HEPH) co-localizes with FPN and oxidizes exported Fe^{2+} to Fe^{3+} allowing it to bind Tf. Reticuloendothelial macrophages phagocytose and degrade senescent erythrocytes, releasing heme into the phagolysosome, which is then exported into the cytoplasm and degraded. Iron can then be stored in the macrophages in ferritin (grey hexagons) or released via FPN where it will circulate bound to Tf. Systemic iron distribution is controlled by the hepatic derived hormone hepcidin (grey stars), which binds, occludes and leads to the degradation of FPN. Hepcidin expression is determined at the transcriptional level by BMP/SMAD and JAK/STAT (via IL-6/IL-22) signaling. The BMP/SMAD pathway is negatively regulated by TMPRSS6, which in low iron conditions cleaves hemojuvelin (HJV, a BMP co-receptor), inhibiting hepcidin expression. Immature red cells (erythroblasts), which accumulate during increased erythropoiesis, produce erythroferrone (ERFE; blue hexagons). ERFE negatively regulates hepcidin expression by dampening BMP/SMAD signaling. BMP = bone morphogenetic protein, BMPR = BMP receptor, CP = ceruloplasmin, DCYTB = duodenal cytochrome B, DMT1 = divalent metal transporter 1, EPO = erythropoietin, ERFE = erythroferrone, FPN = ferroportin, HAMP = hepcidin, HEPH = hephaestin, HIF = hypoxia inducible factor, HJV = hemojuvelin, IL-6 = interleukin 6, IL-R = interleukin receptor, JAK = Janus Kinase, STAT3 = signal transducer and activator of transcription 3, Tf = transferrin, TMPRSS6 = transmembrane serine protease 6.

Table 1

Therapeutic agents in clinical trial that influence plasma iron levels.

Therapeutic agent	Company	Administration route	Acts on	Effect on plasma iron	Clinical trials (Iron related)	Reference
PRS-080	Pieris Pharmaceuticals	Intravenous	Hepcidin	Increase	NCT03325621, NCT02754167	Moebius et al.[18]
NOX-H94	NOXXON Pharma AG	Intravenous	Hepcidin	Increase	NCT01691040	Schweobel et al.[19]
LY2787106	Eli Lilly and Company	Intravenous	Hepcidin	Increase	NCT01340976	Vadhan-Raj et al.[20]
LY2928057	Eli Lilly and Company	Intravenous	Ferroportin	Increase	NCT01991483	Barrington et al.[21]
LY3113593	Eli Lilly and Company	Intravenous	BMP6	Increase	NCT02144285, NCT02604160	
TP-0184	Tolero Pharmaceuticals	Oral	ALK2 (BMPR1) receptor	Increase	NCT03429218 (Phase-I, not iron related)	Peterson et al.[22,35]
Vitamin D (including Paricalcitol and Calcitriol)	Multiple	Oral	vitamin D receptor	Increase	Many including: NCT03145896, NCT0287621, NCT01768351	Bacchetta et al.[23]
Siltuximab	Multiple	Intravenous	IL-6	Increase	NCT01024036	Casper et al.[25]
Tocilizumab	Multiple	Intravenous	IL-6 receptor	Increase	NCT00951275, NCT01183598	Isaacs et al.[24]
LJPC-401	La Jolla Pharmaceutical Company	Subcutaneous	Ferroportin	Decrease	NCT03381833, NCT03395704	Lal et al.[27]
PTG-300	Protagonist Therapeutics	Subcutaneous	Ferroportin	Decrease	NCT0380220	Nicholls et al.[26]
IONIS-TMPRSS6-L$_{RX}$	Ionis Pharmaceuticals	Subcutaneous	TMPRSS6	Decrease	NCT03165864	Guo et al.[28]
SLN124	Silence Therapeutics	Subcutaneous	TMPRSS6	Decrease	CTA to be submitted in 2019	Altamura et al.[29]
VIT-2763	Vifor Pharma	Oral	Ferroportin	Decrease	Phase-I completed	http://www.viforpharma.com/~/media/Files/V/Vifor-Pharma/documents/en/media-releases/2019/pr-vifor-pharma-ferroportin-inhibitor-en.pdf
Sotatercept	Multiple	Subcutaneous	Activin receptor	Decrease	NCT01464164, NCT01712308	Komrokji et al. [32]; Cappellini et al.[33]
Luspatercept	Multiple	Subcutaneous	Activin receptor	Decrease	NCT03194542, NCT03342404	Platzbecker et al.[34]

in Western countries where prevalent obesity may elevated hepcidin elevation.[13] Iron deficiency may also occur in genetic mutations of *TMPRSS6* causing upregulation of hepcidin expression despite low iron stores ('Iron-Refractory Iron-Deficiency Anaemia, IRIDA').[*14]

Iron overload. Genetic mutations impairing hepcidin expression or function can result in excess iron uptake and iron overload. Mutations affecting the *HFE* gene are most common but of low penetrance, with rarer mutations in *HJV*, *TfR2* and *HAMP* producing a more severe phenotype. Mutations that quantitatively affect ferroportin or inhibit its degradation by hepcidin also result in iron overload (reviewed[15]).

Inherited (ie, haemoglobinopathies) and acquired (eg, myelodysplastic syndromes) genetic haematologic conditions can produce ineffective erythropoiesis. The expanded erythroid pool creates enormous iron demand and causes suppression of hepcidin likely via erythroblast production of erythroferrone.[16] Together with hypoxia-mediated upregulation of duodenal DMT1,[*17] this results in increased iron absorption and overload.

Future perspectives
Therapeutic manipulations in iron handling

At least 16 therapeutics entering clinical trials aim to increase or reduce plasma iron entry by manipulating the hepcidin-ferroportin axis (Table 1 and Fig. 1).

Downregulation of hepcidin. Several drugs prevent hepcidin function. PRS-080, an Anticalin protein linked to linear polyethylene-glycol, binds hepcidin and inhibits its function; it is being trailed in patients with chronic kidney disease.[18] Similarly, NOX-H94, a PEGylated anti-hepcidin L-RNA oligonucleotide inactivates hepcidin after binding, inhibited serum iron suppression in anemia of inflammation experimental models.[19] LY2787106 is a neutralizing hepcidin monoclonal antibody which has shown safety and efficacy in patients with cancer-related anemia.[20] LY2928057, a humanized FPN antibody, inhibits hepcidin function by protecting ferroportin from hepcidin-induced degradation and stabilizes FPN on the cell surface. It raised iron levels but not hemoglobin in a Phase I study in renal patients.[21]

Drugs that decrease hepcidin expression include: LY3113593, a humanized BMP6 monoclonal antibody, inhibits the canonical pathway of hepcidin transcription, and is being trialed in chronic kidney disease. TP-0184, an ALK2 receptor kinase inhibitor, likewise inhibits BMP/SMAD-driven hepcidin transcription[22] but appears currently to be being clinically tested for treatment of solid tumors. Vitamin D (as well as vitamin D analogs paricalcitol and calcitriol) may act via vitamin D receptor binding to vitamin D response elements in the hepcidin promoter.[23] Reducing hepcidin levels are potentially beneficial secondary benefits approved IL6 targeted treatments: anti-IL-6 (Siltuximab) and anti-IL-6 receptor (Tocilizumab) antibodies used to treat patients with rheumatoid arthritis,[24] cancer and Castleman's disease.[25]

Upregulation of hepcidin. Therapeutics that increase hepcidin levels or function aim to alleviate iron loading conditions. LJPC-401 is a synthetic full-length hepcidin which has entered Phase 2 clinical trials for β-thalassemia and haemochromatosis, while PTG-300 is a hepcidin mimetic which has likewise entered clinical trials for β-thalassemia. Both drugs induce reductions in serum iron in human Phase I studies,[26] and in both cases non-limiting local injection site reactions appeared to be the chief adverse effect.[27]

Inhibition of hepatic TMPRSS6 upregulates hepcidin expression At least 2 molecules with therapeutic potential that silence TMPRSS6 mRNA translation have been developed. IONIS-TMPRSS6-L$_{RX}$ is a ligand conjugated TMPRSS6 silencing molecule that upregulates hepcidin in preclinical models and has entered trials for thalassaemia.[*28] Likewise, SLN124 is a conjugated GalNac siRNA targeting TMPRSS6 which has shown pre-clinical activity in thalassemia models with clinical trials planned in the near future.[29] Beyond agents acting on hepcidin, VIT-2763 is a small molecule inhibitor of FPN and therefore replicates hepcidin function, and completed a Phase-I clinical trial in October 2018. Activin receptor ligand traps (Sotatercept, Luspatercept) have preclinical[30,31] and clinical[32–34] activity in diseases of ineffective erythropoiesis which indirectly counteracts hepcidin suppression.

Conclusions

New understanding of the molecular pathways governing iron homeostasis has led to a greater appreciation of the pathophysiology of iron related disorders and a pipeline of rationally designed novel therapeutics against the hepcidin-FPN axis.

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