REVIEW

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New insights into the links between hypoxia and iron homeostasis

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Purpose of review
This review outlines recent discoveries on the crosstalk between oxygen metabolism and iron homeostasis, focusing on the role of HIF-2 (hypoxia inducible factor-2) in the regulation of iron metabolism under physiopathological conditions.

Recent findings
The importance of the hepcidin/ferroportin axis in the modulation of intestinal HIF-2 to regulate iron absorption has been recently highlighted. Latest advances also reveal a direct titration of the bone morphogenetic proteins by the erythroferrone contributing to liver hepcidin suppression to increase iron availability. Iron is recycled thanks to erythrophagocytosis of senescent erythrocytes by macrophages. Hemolysis is frequent in sickle cell anemia, leading to increased erythrophagocytosis responsible of the macrophage polarization shift. New findings assessed the effects of hemolysis on macrophage polarization in the tumor microenvironment.

Summary
Hypoxia signaling links erythropoiesis with iron homeostasis. The use of HIF stabilizing or inhibiting drugs are promising therapeutic approaches in iron-associated diseases.

Keywords
erythropoiesis, hepcidin, hypoxia inducible factor, iron metabolism

INTRODUCTION

Iron is essential for all living organisms to support crucial biological processes, from DNA replication to oxygen transportation and ATP production. Iron is a key nutrient but has to be tightly regulated to avoid its remarkably high reactivity that could induce severe damages due to ROS production via the Haber Weiss/Fenton reaction. Iron is mainly used for the hemoglobin synthesis to support efficient oxygen transportation. Therefore, coordinated regulation between the physiology of hypoxic response and the control of iron availability need to take place. HIF (hypoxia inducible factors) transcription factors are central mediators of cellular adaptation to critically low oxygen levels (=hypoxia). Since the discovery of HIF-1 in 1992, a new research field has emerged leading to an important infatuation for these new basic loop helix transcription factors and the discovery of other HIF isoforms in 1997 and 2001. Many functions have been attributed to these factors [1], but we will focus in this review on the recent findings on the role of HIF-2 in iron-related physiologic and pathological conditions.

IRON HOMEOSTASIS

In humans, total iron content in the organism ranges between 3 and 5 g. Most of it is contained in red blood cells (RBCs), bound to the heme prosthetic group in hemoglobin, and in erythroid progenitor cells in the bone marrow. Senescent red blood cells are phagocytosed by the macrophages of the reticuloendothelial system, especially in the spleen, in a process called erythrophagocytosis [2,3].

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KEY POINTS

- The hepcidin/FPN axis regulates intestinal HIF-2 to modulate iron absorption.
- Direct titration of the bone morphogenetic proteins participates to ERFE-mediated hepcidin suppression.
- Recent implications of erythrophagocytosis in tumor microenvironment highlight a macrophage phenotype switch in hemolytic tumor area.
- The use of HIF-2 antagonists is a promising therapeutic approach in iron overload diseases.

The iron contained in RBCs is recycled by macrophages at a rate of 25–30 mg/day. There is no active excretory mechanism for the metal; however, small iron quantities (1–2 mg/day) are lost by desquamation or bleeding, and are replaced by dietary iron absorption, in the duodenum, the proximal part of the small intestine. Over the last two decades, major advances have been made in the iron homeostasis research topic. Hepcidin was identified as the key regulator of systemic iron homeostasis in 2001 [4]. This 25 amino acid peptide, highly conserved between species, has been shown to be mainly secreted by the liver in response to iron overload or inflammation [5,6]. It circulates in the blood flow and decreases plasma iron levels by blocking iron absorption in the duodenum and iron release from macrophages, thus targeting the two entrance gates for iron in the circulation. Molecularly, it binds the only known iron exporter ferroportin (FPN) inducing its internalization and consecutive degradation by the proteasome thanks to specific sites of ubiquitinylation [7,8].

Hepcidin deficiency is associated with hereditary hemochromatosis, a highly prevalent human genetic disease characterized by excessive iron accumulation. Iron accumulation in the organs leads to liver cirrhosis, diabetes, heart failure and increase risks of hepatocellular carcinoma. There are multiple origins for this abnormally low hepcidin level, from highly frequent mutations in the *Hfe* gene [9], to very rare cases of mutations in the hepcidin gene itself [10].

Although hepcidin is mainly produced by the liver, an increasing number of studies showed that hepcidin is also expressed by other tissues [11–14]. The generation of a liver-specific hepcidin knockout mouse model recapitulated the iron phenotype of the total hepcidin knockout mice, demonstrating that hepatic hepcidin is sufficient to ensure systemic iron homeostasis in physiological conditions [15] and suggesting that production of hepcidin by extrahepatic tissues may have local roles. Indeed, an essential role of heart hepcidin in cardiac iron homeostasis has recently been highlighted [16].

HIF-2: A KEY REGULATOR OF IRON HOMEOSTASIS IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

The richly perfused gastrointestinal mucosa is juxtaposed with the anaerobic lumen of the gut. As a consequence, intestinal epithelial cells experience a uniquely steep oxygen gradient. Adaptive transcriptional responses to oxygen deprivation are mediated by the hypoxia inducible factors (HIFs). HIFs are alpha/beta heterodimeric transcription factors playing key roles in adaptation to hypoxia. The beta monomer (HIF-1β, also known as ARNT) is constitutively expressed and the alpha monomers (HIF-1α, HIF-2α or HIF-3α) are regulated at the posttranslational level. Under normoxia, the prolyl-hydroxylase domain enzymes (PHDs) hydroxylate the α-subunit on two prolines. The proline-hydroxylated residues favor the interaction with the tumor suppressor protein von Hippel-Lindau (vHL) resulting in the degradation of the HIF-α subunit via the proteasome pathway. Conversely, under hypoxia or iron depletion, hydroxylation is inhibited increasing the stabilization of the alpha subunit and the heterodimerization with the beta subunit. The functional heterodimer translocates into the nucleus to regulate the transcription of HIF target genes by binding on specific sequences called hypoxia-responsive elements (HREs).

HIF-1 has been the most extensively subunit studied so far. An essential involvement of HIF-1 has been demonstrated in angiogenesis, glycolytic metabolism, apoptosis, cellular stress among other major biological processes [1]. HIF-1 has also been shown to regulate transferrin receptor 1 (TfR1) and heme oxygenase 1 (HO-1) expression [17,18], but is not essential for splenic macrophages erythrophagocytosis [19].

HIF-2 has a key role in adult erythropoiesis, by regulating the erythropoietin hormone (EPO) [20,21] but also by increasing iron mobilization via two essential mechanisms: in the enterocyte, HIF-2 regulates iron absorption via direct transcriptional activation of the divalent metal transporter 1 (DMT1), the ferric reductase DcytB and the iron exporter FPN [20,21]. In the liver, specific HIF-2 activation represses hepcidin production through an EPO-mediated stimulation of erythropoiesis [21,22].

HIF-2 is not only crucial to maintain normal absorption rates at basal level but also in different pathological settings. HIF-2 is essential to upregulate iron absorption genes in conditions of iron deficiency [23] or increased erythropoiesis [24] but

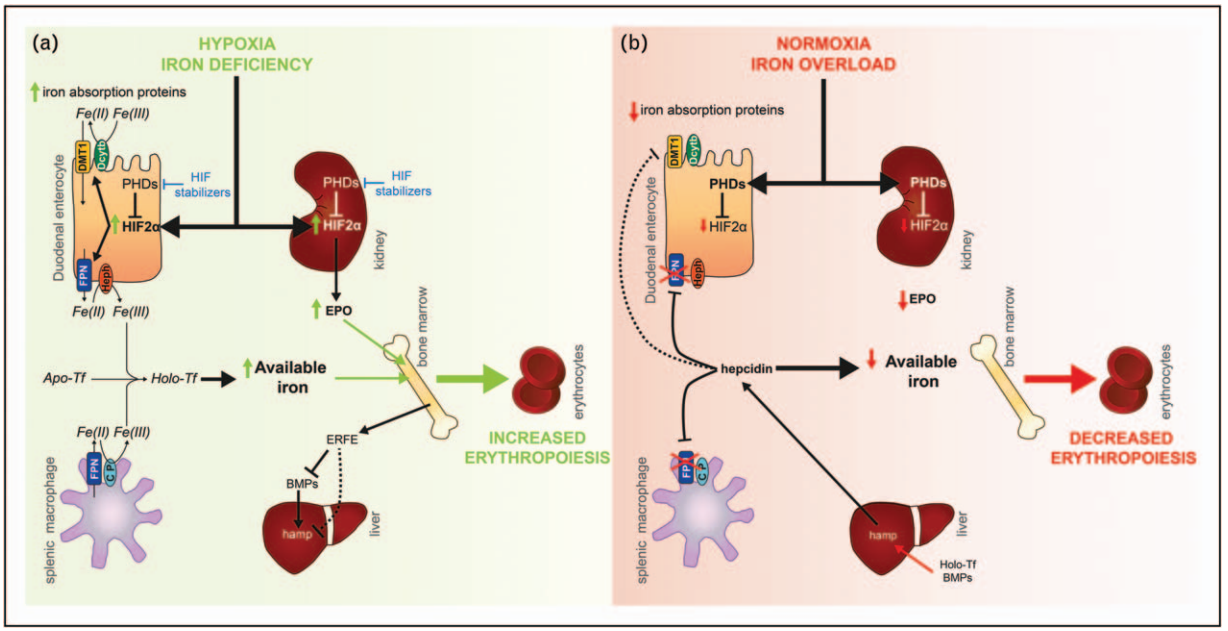

FIGURE 1. The links between hypoxia and iron metabolism. (a) Systemic regulation of iron metabolism under normoxia and/or iron deficiency. (b) Systemic regulation of iron metabolism under hypoxia and/or iron overload. Apo-Tf, Apo-transferrin; BMPs, bone morphogenetic proteins; CP, cerruloplasmin; EPO, erythropoietin; ERFE, erythroferrone; FPN, ferroportin; Heph, hephaestin; HIF, hypoxia inducible factor; Holo-Tf, Holo-transferrin; Ocytb, duodenal cytochrome b; OMT1, divalent metal transporter 1; PHOs, prolyl hydroxylase domain enzymes.

also contributes to iron overload in hemochromatosis [25] and sickle cell disease mouse models [24].

Schwartz *et al*. recently elucidated more precisely the mechanisms by which HIF-2 mediates regulation of iron absorption, thanks to inducible mouse models. They investigated the importance of the well described hepcidin/FPN axis to stabilize HIF-2 specifically in the intestine. In a context of an inducible knockout mouse model for hepcidin in the liver, duodenal FPN was stabilized and serum iron increased (leading to iron overload in case of sustained hepcidin deficiency mimicking the hereditary hemochromatosis [4]). FPN stabilization leads to a decrease in intracellular iron in the duodenal enterocytes. Under low cellular iron concentrations, the HIF-2α subunit is no longer hydroxylated by the PHDs, and is therefore stabilized, inducing the consecutive transcription of the iron absorption genes (DMT1, DcytB, FPN) (Fig. 1). Both iron overload and anemia models support the key role of the hepcidin/FPN axis in intestinal HIF-2 regulation [26]. Using inducible intestine-specific FPN and DMT1 knockout model, Schwartz *et al*. confirmed that enterocyte iron flux was the major mechanism by which the hepcidin/FPN axis regulates HIF-2α and demonstrated *in vitro* that the FPN-mediated efflux of iron triggers the stabilization of HIF-2α in a cell-autonomous manner. Interestingly, the use of a HIF-2 antagonist, recently developed [27] decreases systemic iron accumulation in hepcidin-deficient mice, confirming previous studies using mice lacking HIF-2 in the intestinal epithelium [25]. Noteworthy, in addition to the PHD-mediated posttranslational regulation, HIF2-α is also subjected to IRP-mediated translational regulation due to the presence of an IRE in its 5′UTR [28]. It provides a means by which HIF-2α activity can be attenuated and may limit the level of activation. Recently, an inhibitor of HIF-2 translation has been shown to reduce erythrocytosis/polycythemia in a mouse model of Chuvash polycythemia (Vhl^{R200W}) [29▪] Altogether, these recent studies confirm that HIF-2α is a potential pharmacological target downstream of the hepcidin/FPN axis in patients with iron overload

MAINTENANCE OF THE IRON BALANCE TO SUPPORT FUNCTIONAL ERYTHROPOIESIS

As mentioned previously, iron is mainly used to produce functional hemoglobin during erythropoiesis. The major actor for erythropoiesis activation is EPO, produced by the liver, during the embryonic stage and by the renal interstitial tissue after birth. Anemia results from insufficient functional red blood cells (low levels of hemoglobin or inefficient hemoglobin) in the blood flow causing a decrease in oxygen levels in tissues.

Hypoxia is a major inducer of EPO synthesis in the kidney, resulting in the synthesis of erythroferrone (ERFE) by the erythroblasts. ERFE mediates liver hepcidin suppression to mobilize iron to support erythropoiesis [30]. It also contributes to iron overload in a mouse model of β-thalassemia by decreasing liver hepcidin [31]. The mechanism involved in ERFE mediated hepcidin-suppression is still unknown. However, a recent work suggested a direct interaction of ERFE with BMP5, BMP6 and BMP7 that could participate to hepcidin suppression by inhibiting the hepatic BMP/SMAD signaling, critical for hepcidin control [32▪]. Interestingly, EPO has also been suggested to act directly on enterocytes through its receptor EPO-R to increase iron absorption [33].

In the case of chronic kidney disease (CKD) patients, the lack of EPO production by the renal interstitial tissue results in insufficient erythropoiesis, and subsequent anemia and hyperferremia. Anemia is a frequent complication contributing to increased morbidity and mortality in these patients. In physiologic state, response to anemia and renal hypoxia induces HIF-2 stabilization in the renal EPO-producing cells, resulting in increased EPO production. After submitting mice to an iron-rich diet or Iron-Dextran injections, HIF-2 stabilization is reduced and EPO induction lost [34], suggesting a vicious circle with an increased iron deposition in patients with EPO deficiency decreasing consecutively the EPO production.

IRON MODULATES MACROPHAGE POLARIZATION IN TUMOR

Macrophages play a critical role in the recycling of iron from RBCs. The consequences of macrophage exposure to hemolytic RBCs are usually studied in the context of hemolytic diseases, such as sickle cell disease, where pro-inflammatory 'M1 macrophages' reprogrammed by heme and iron aggravates tissue damage [35]. In a recent publication, Muckenthaler *et al*. investigated the impact of hemolysis in the tumor microenvironment on the reprogramming of tumor-associated macrophages. During the tumorigenesis process, bleedings appear in some area of the tumor microenvironment, with an increase in hemolytic RBCs number. The authors describe that the hemolytic area is associated with a subpopulation of macrophages expressing M1-like markers and heme scavenger genes to take heme iron from hemolytic RBCs. The presence of these iron-loaded pro-inflammatory tumor-associated macrophages (named iTAMs) correlates with reduced tumor size in patients with nonsmall cell lung cancer. Thanks to in-vitro experiments, they showed that aged/hemolytic RBCs switch M2-like macrophages into pro-inflammatory M1-like macrophages, able to kill Lewis lung carcinoma cells, thanks to increased ROS production. To test the therapeutic relevance of their findings, the authors used iron oxide nanoparticles inducing an iron increase exclusively in phagocytic cells. Co-injection of these nanoparticles with Lewis lung carcinoma cells in mice reduced tumor growth. These results demonstrate a new important role of iron and senescent/aged RBCs in tumorigenesis, and suggest implication of iron in antitumor response, at least for macrophages in this cancer [36▪].

THERAPEUTIC APPROACHES TO MODULATE HYPOXIA INDUCIBLE FACTOR

The increase in CKD cases underlines the current necessity of novel therapeutic approaches to treat anemia. Since the discovery of HIF, the concept of stabilizing HIF to support EPO production in CKD anemia has been suggested for many years. It is now conceivable thanks to a new class of drugs, the PHD inhibitors or HIF stabilizers, which prevents the HIF-alpha subunit from proteasome-mediated degradation. These small molecules are currently in phase III clinical trials. These drugs by stabilizing not only HIF-1 but also HIF-2 would coordinate erythropoiesis with iron metabolism. It could ameliorate the quality life of CKD patients, by avoiding multiple transfusions, iron supplementation and by reducing the doses of erythropoiesis stimulating agents administered [37]. However, there are several concerns related to putative side effects of HIF activation as HIF-1 and HIF-2 regulate a broad spectrum of cellular functions. Recent findings highlighted an increase of fibroblast growth factor 23 (FGF23) after HIF stabilizers treatment [38]. High levels of circulating FGF23 are associated with cardiac dysfunction and bone demineralization among others [39,40]. Further investigations will be needed to assess the benefit-risk of both treatments.

Recent works from the last 5 years converge to the critical role for HIF-2α in iron hyperabsorption in primary and secondary hemochromatosis [24,25,29▪]. A selective inhibitor of HIF-2α, PT2385, has recently been developed [27] and has been validated for the treatment of patients with clear-cell renal cell carcinoma [41▪]. Of note, by targeting HIF-2, PT2385 has also recently been shown to prevent obesity, type 2 diabetes and hepatic steatosis [42▪▪]. Therefore, the use of HIF-2 antagonists, such as PT2385, will be a promising therapeutic approach in the treatment of iron overload diseases characterized by dysfunction of the hepcidin/FPN axis.

CONCLUSION

Defects in oxygen supply are present in lots of pathologies indicating a need to better understand the mechanisms increasing iron import to support optimal erythropoiesis to maintain tissue normoxia. Since the discovery of liver hepcidin as the key iron regulator, new interest has emerged to identify other hepcidin producing tissues and understand their role. Recent publications investigated the crucial role of the hepcidin/FPN axis to regulate iron absorption in the intestine thanks to HIF-2. As this factor tightly regulates EPO production by the kidney, new therapeutic stabilizing HIF emerged to restore both iron import and sufficient erythropoiesis in anemia-associated diseases. Further investigations are needed to evaluate the side effects of HIF stabilizers and to assess the benefit-risk in comparison to current erythropoiesis stimulating agent treatments. As HIF factors are involved in many key biological processes, the next step will be to identify specific stabilizers for each HIF isoform. At the other spectrum of iron-related diseases, the use of specific HIF-2 antagonists will be a novel and promising therapeutic approach in the treatment of iron overload diseases.

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Conflicts of interest

There are no conflicts of interest.

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