Iron & Anemias - Section 11

Basis of Iron Regulation for Erythropoiesis

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Take-home messages:
- Iron regulates erythropoiesis at the level of early erythroid progenitors, heme and globin synthesis, and EPO-EPOR signaling.
- Hepcidin, a liver peptide hormone that regulates iron homeostasis, indirectly control erythropoiesis efficiency.
- The erythroid regulator erythroferrone increases iron availability for hemoglobin synthesis by inhibiting hepcidin.

Introduction
Iron is essential for many biological processes as oxygen delivery, mitochondrial respiration, nucleic acid replication and repair, metabolic reactions and host defense. However, due to its ability to accept/release electrons, excess iron can damage cell components. For this reason several mechanisms exist to avoid iron overload and deficiency (Fig. 1).

Iron, absorbed by duodenal enterocytes, reaches the bloodstream through ferroportin (FPN1), the sole iron exporter in mammals. In the circulation iron is bound to transferrin (TF) as holo-TF to allow safe delivery of the metal throughout the body. Excess circulating iron is stored in hepatocytes and spleen macrophages, and exported back to the circulation through FPN1, when serum iron concentration decreases.

Within cells iron is up-taken through TFR1 interaction with holo-TF in a regulated way and released to the cytosol by endocytosis. In mitochondria it is utilized for heme and Fe-S cluster synthesis and for the activity of enzymes of the respiratory chain, or safely stored into ferritin.

Since an active mechanism to excrete iron is not in place, its concentration is tightly regulated through hepcidin, a peptide hormone produced mainly by hepatocytes, which negatively regulates body iron by binding and blocking FPN1. Hepatocyte hepcidin expression is under the control of the BMP-SMAD pathway, whose activation requires the coordinated function of BMP ligands (BMP2 and BMP6), BMP receptors and regulatory proteins as HFE, the second transferrin receptor (TFR2) and the BMP-coreceptor hemojuvelin (HJV). When body iron increases, hepcidin is upregulated by BMP6 and cell surface stabilization of HJV and TFR2. Increased hepcidin inhibits FPN1 and reduces circulating iron. In agreement, defective hepcidin causes iron overload in Hereditary Hemochromatosis, characterized by impaired liver BMP-SMAD signaling. In ineffective erythropoiesis, as in β-thalassemia, hepcidin inhibition is due to expanded erythropoiesis, to allow increased supply of the metal to erythroid precursors that need large amounts of iron for Hb synthesis.

Current state-of-the-art

Since erythropoiesis requires around 25 mg of iron daily, any perturbation of body iron concentration influences the erythropoietic activity and vice-versa, being these two processes highly interconnected. Iron deficiency can evolve in anemia if untreated. The resulting anemia can be classified as iron deficiency anemia due to reduced dietary intake, decreased absorption or blood loss, or iron restricted anemia due to reduced iron availability for erythropoiesis, as in anemia of inflammation or the rare genetic disease Iron Refractory Iron Deficiency Anemia (IRIDA), both characterized by excessive hepcidin synthesis. The reduced iron supply affects erythrocytes production and Hb synthesis at multiple levels. Erythroid cells have several mechanisms to regulate their activity according to iron. In in vitro studies, iron deprivation blocks early erythroid progenitors differentiation due to inactivation of mitochondrial aconitase, a process that can be rescued by iron or isocitrate supplementation. Hb synthesis requires heme and globin. Heme biosynthesis takes place both in mitochondria and cytosol. The first enzymatic step to form the γ-aminolevulinic acid (ALA) is catalyzed by the rate-limiting enzyme ALA synthase 2 (ALAS2) exclusively expressed in erythroid cells. ALAS2 is regulated by iron at post-transcriptional level: it has an iron-responsive element in its 5’ untranslated region (UTR) that interacts with iron-regulatory proteins in iron deficiency to block translation, thus linking heme synthesis to iron availability. The final step in heme biosynthesis is the incorporation of iron into protoporphyrin IX, a process catalyzed by the iron-dependent rate-limiting enzyme ferrochelatase whose activity requires an iron-sulfur cluster. Iron indirectly controls α- and β-globin synthesis in erythroblasts, since the activity of heme-regulated elf2a kinase (HRI), the enzyme that coordinates both globin chain synthesis, is regulated by heme...
concentration. On the cell surface, the iron sensor TFR2, a partner of erythropoietin receptor (EPOR) in erythroid cells and a positive regulator of hepcidin in hepatocytes, has a crucial role in coordinating the erythroid response with transferrin iron. TFR2 is highly homologous to TFR1 and binds holo-TF, although with a lower affinity than TFR1. It is stabilized on cell surface by holo-TF and destabilized by its decreases. Binding EPOR, TFR2 attenuates the EPO-EPOR signaling. Decreased cell surface TFR2 in iron deficiency or in genetic models of Tfr2 deletion in bone marrow derived cells, favors the EPO-EPOR signaling causing erythrocytosis. In hepatocytes, decreased plasma membrane TFR2 attenuates the BMP-SMAD pathway and hepcidin expression to facilitate iron delivery to the circulation through FPN1 stabilization.

Given the huge iron need of erythropoiesis, this process also regulates iron homeostasis. The effect is quite impressive in ineffective erythropoiesis, as in β-thalassemia, a genetic disease in which defective β-globin synthesis leads to anemia and massive iron overload. The recent discovery of erythroferrone (ERFE) defined the nature of the long sought “erythroid regulator”. ERFE is a member of the tumor necrosis factor (TNF)-α family, produced by several tissues but whose expression in response to EPO is increased only in erythroid precursors. ERFE functions in an endocrine manner and inhibits hepcidin expression in hepatocytes attenuating the liver BMP-SMAD pathway by sequestering BMP ligands, mainly BMP6. Although Erfe-ko mice are not anemic, suggesting no major role of this protein in steady state, its inactivation worsens recovery from anemia in inflammation and malarial infection, and improves iron overload in beta thalassemia mice.

Future perspectives

Research achievements have translational implications for therapy of iron loading disorders. Reduction of iron supply is a promising approach for disorders as hemochromatosis or β-thalassemia since iron restriction decreases globin chains unbalance and improves erythrocyte survival, ameliorating anemia and iron burden. This can be achieved by several approaches:

1. by hepcidin agonists, as minihepcidins, inhibitors of hepcidin repressors as anti-TMPRSS6 molecules, compounds blocking ferroportin activity or ERFE blocking antibodies.

2. by transferrin infusions that reduces anemia and ineffective erythropoiesis and thus hepcidin inhibition. A similar effect is observed decreasing Tfr1 expression and reducing erythroid the iron up-take.

3. by selective bone marrow Tfr2 inactivation that, enhancing the EPO sensitivity of erythroblasts, improves anemia in a β-thalassemia model.

On the contrary blocking excess hepcidin in IRIDA and anemia of inflammation can favor iron delivery to bone marrow. Some of these successful preclinical studies offer new and original therapeutic opportunities for diseases due to inappropriate hepcidin synthesis.

References


This is a comprehensive review of the last achievements in iron metabolism.


This review describes the mechanisms of iron deficiency anemia and the current therapeutic approaches.


This paper describes for the first time the role of TFR2 in the regulation of erythropoiesis in vivo.


This paper demonstrates that erythroferrone is the long sought “erythroid regulator”.


This review describes the mechanisms of anemia of inflammation and the current therapeutic approaches.