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Erythropoietic regulators of iron metabolism

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Abstract

Erythropoiesis is the predominant consumer of iron in humans and other vertebrates. By decreasing the transcription of the gene encoding the iron-regulatory hormone hepcidin, erythropoietic activity stimulates iron absorption, as well as the release of iron from recycling macrophages and from stores in hepatocytes. The main erythroid regulator of hepcidin is erythroferrone (ERFE), synthesized and secreted by erythroblasts in the marrow and extramedullary sites. The production of ERFE is induced by erythropoietin (EPO) and is also proportional to the total number of responsive erythroblasts. ERFE acts on hepatocytes to suppress the production of hepcidin, through an as yet unknown mechanism that involves the bone morphogenetic protein pathway. By suppressing hepcidin, ERFE facilitates iron delivery during stress erythropoiesis but also contributes to iron overload in anemias with ineffective erythropoiesis. Although most of these mechanisms have been defined in mouse models, studies to date indicate that the pathophysiology of ERFE is similar in humans. ERFE antagonists and mimics may prove useful for the prevention and treatment of iron disorders.

Graphical Abstract

Keywords

Iron; anemia; erythropoiesis; hepcidin; erythroferrone

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1. Interdependence of erythropoiesis and iron homeostasis

Erythropoiesis dominates the iron economy in humans and other vertebrates—

Although jawed vertebrates (gnathostomes) are not the only animals with iron-rich hemoglobin in blood [1], vertebrate blood contains a high proportion of erythrocytes, a cell type with a relatively short lifespan compared to other cells. Thus the close coupling between erythropoiesis and iron metabolism in vertebrates is necessitated by the high content of iron in erythrocytes and by their high turnover [2]. Human erythrocytes contain about 1 mg of iron per ml of packed cells. An adult with 5 liters of blood and a hematocrit of 45% will have about 2.3 g of iron in erythrocytes. Erythrocytes normally last about 120 days before they are ingested by macrophages and their iron is recycled and delivered to the marrow to make new erythrocytes. About 20 ml of packed erythrocytes are produced each day and 20 mg of iron are flowing through blood plasma every day to serve erythropoiesis. The needs of nonerythroid tissues for iron are relatively small in comparison (about 10% of total iron flow). If plasma contains 2 mg of iron bound to transferrin, the compartment must turn over about 10-times a day to deliver sufficient iron for erythropoiesis.

Evolutionary constraints—

During human and other animal evolution, injury and infection were two major obstacles to survival and reproductive success, and these two threats constrained iron metabolism and its regulation in opposite ways. Injury was usually accompanied by blood loss, and recovery from blood loss required mobilization of iron from internal stores and increased absorption of iron from the diet. However, injury also predisposed to infection, which may be enhanced by excess iron promoting microbial growth. The transit compartment—plasma transferrin—can only hold about 5 mg of iron, i.e. enough iron to make 5 ml of erythrocytes. Flooding of this compartment with iron would saturate transferrin and generate non-transferrin bound iron (NTBI) species that enhance the severity of infection with gram-negative organisms [3], for which many injured individuals are already at increased risk. Excess of iron, especially in its chemically reactive forms, could also promote tissue injury [4]. These constraints may have favored relatively tight control of iron concentrations and the association of iron with proteins that act as high affinity chaperones.

Recovery from blood loss—

Rapid recovery from anemia likely provided a survival advantage because the physical and mental deficits of anemic and iron-deficient individuals may put them at further risk in a hostile environment. Restoration of erythrocytes and oxygen carrying capacity required accelerated erythropoiesis. In humans, maximum erythropoietic rates are estimated at 3-times baseline after acute hemorrhage, and up to 8-times baseline during repeated hemorrhage or chronic hemolysis [5]. These high rates of erythropoiesis obligate proportional increases in iron delivery to the marrow. Inadequate iron influx into plasma is deleterious to recovery from anemia because erythropoiesis is exquisitely sensitive to iron availability [6]; even baseline erythropoiesis is inhibited when the concentrations of iron-saturated transferrin are below the normal range.

Iron homeostasis—

Organismal iron homeostasis functions to deliver iron to cells and tissues according to their requirements without excessive accumulation of iron that could promote infections or result in tissue injury. Based on clinical observations and studies in humans and experimental animals, baseline iron losses were thought to be very minor and essentially unregulated, focusing the analysis on dietary iron absorption, internal recycling and storage of iron, and its distribution to tissues. Under steady state conditions, iron absorption, taking place in the proximal duodenum, balances out the relatively small daily losses, about 1–2 mg/day, incurred by desquamation of epithelial surfaces and microscopic bleeding. However, in iron deficiency, absorption can increase more than ten-fold. Two systems were postulated to regulate iron absorption and release from internal stores [7]. The first, the “stores” regulator, acted to increase iron absorption during iron deficiency, and decrease iron absorption in iron overload, maintaining about 1 g of iron in stores of the average adult male but much less in adult women. The second, the “erythroid” regulator, protected erythropoiesis from iron limitation by increasing iron absorption and release from stores when erythropoiesis accelerated and required more iron. Hereditary hemochromatosis was understood to be a disorder of the “stores” regulator causing hyperabsorption of dietary iron and eventual iron overload with consequent tissue injury. On the other hand, the iron overload in untransfused β -thalassemia was caused by dysfunction of the “erythroid” regulator responding to hyperproliferation of erythroid precursors and their demand for iron. Although the basic physiology of how iron metabolism and erythropoiesis are tightly coupled was largely worked out in the second half of the 20th century, the molecular circuitry that mediates the coupling is still not completely understood.

2. Molecular mechanism of iron homeostasis

The hepcidin-ferroportin axis—

The existence of an iron-homeostatic system was long anticipated based on the stability of iron concentrations in blood plasma (generally between 10–30 μ M) despite fluctuations in erythrocyte production and dietary iron supply, and the long term stability of total body iron content even in subjects who consumed excessive dietary iron. As we now understand it, the principal ironhomeostatic axis consists of the peptide hormone hepcidin negatively regulating the activity of the sole known cellular iron exporter, ferroportin [8]. Although ferroportin is found in nearly all plants and animals, hepcidin and its binding site on ferroportin make their evolutionary debut in vertebrates (fish) where blood circulation with high erythrocyte density and high hemoglobin content also first appeared [9]. Ferroportin mediates the major flows of iron into blood plasma, including from duodenal enterocytes absorbing dietary iron, from macrophages that recycle iron from senescent erythrocytes, and from hepatocytes that store iron. Hepcidin binds to ferroportin and causes its endocytosis and degradation [8], and, at higher concentrations, directly inhibits the iron transport function of ferroportin [10]. Injection of hepcidin into mice will therefore rapidly lower plasma iron concentrations [11], and chronic treatment with hepcidin agonists prevents the absorption of dietary iron and its accumulation in the liver [12]. The long term iron balance is assured by feedback stimulation of hepatic hepcidin production by stored iron, while short-term control of plasma iron concentration is maintained by the feedback stimulation of

hepcidin production by transferrin-bound iron. Interestingly, both of these feedback mechanisms are mediated by transcriptional control of hepcidin by the bone morphogenetic protein receptor pathway. Holotransferrin concentrations apparently modulate the sensitivity of the receptor [13–15] to its ligands (mainly BMP2 and BMP6) while the effective amount of these ligands appears to be driven by iron stores in the liver [13, 16–18].

3. Erythroid regulators of iron homeostasis

Evidence for the existence of one or more erythroid regulators of iron homeostasis—

In principle, erythroid regulators of iron homeostasis could function as mediators of appropriate responses to hemorrhage, anemia, hypoxia or erythropoietin administration (physiologic erythroid regulators) or as pathological products of severely dysregulated erythropoiesis, as is seen in β -thalassemia or other disorders with ineffective erythropoiesis (pathological erythroid regulators). Regarding physiologic regulators, the connection between erythropoiesis and iron absorption was noted more than 50 years ago, based on studies of duodenal absorption of radioactive iron in hypoxic mice, as well as in hypertransfused mice treated with erythropoietin [19], experimental designs that endeavored to isolate the effects of erythroid activity from the effects of iron deficiency on iron absorption. Iron absorption increased with either erythropoietin treatment or with hypoxia and detailed studies led to the recognition that either increased erythropoietic activity or intestinal hypoxia were capable of increasing iron absorption in the duodenum. The effect of erythropoietin on iron absorption was thought to be indirect, requiring the presence and activity of erythropoietic organs, marrow and spleen. After the discovery of hepcidin and its role in iron regulation, it became clear that at least some of the effects of the erythroid regulators were mediated through hepcidin, as anemia and hypoxia were noted to suppress hepcidin in mice [20]. The suppression of hepcidin could also be elicited by the administration of erythropoietin, and this effect was shown to be indirect, requiring intact erythropoietic organs [21–23]. In healthy human subjects, the administration of erythropoietin caused a hepcidin decrease within less than a day, even before any changes in iron parameters could be detected [24]. Currently, only erythroferrone (ERFE, Fam132b, CTRP15) fits the description of a physiologic erythroid regulator. Regarding potential pathological regulators, serum hepcidin was shown to be decreased in β -thalassemia and in other anemias with ineffective erythropoiesis, especially when the expected increase in hepcidin from iron overload was accounted for [25]. Hepcidin was especially low in β -thalassemia intermedia, where the physiology was not confounded by the suppression of erythropoiesis by transfusions and by transfusional iron overload [26], and in samples from patients with β -thalassemia major before their regular transfusion [27]. In β -thalassemia and congenital dyserythropoietic anemia two proteins, GDF15 and ERFE, show characteristics of a pathological erythroid regulator and suppressor of hepcidin.

GDF15—

Transcriptional profiling of TGF β family members in a model of *in vitro* human erythropoiesis identified GDF15 as a transcript that greatly increased with erythroblast maturation [28]. Another molecule identified in the same context was TWSG1 (twisted gastrulation) but the evidence for its role as an erythroid regulator of hepcidin is much

weaker, and so TWSG1 will not be discussed further here. GDF15 protein mildly stimulated hepcidin production in human but not murine hepatocytes at concentrations of 0–1 ng/ml, similar to those seen in healthy controls (0.5 ± 0.05 ng/ml) but potently suppressed hepcidin at higher concentrations, similar to those present in β -thalassemia patients' sera (66 ± 10 ng/ml), and in patients with congenital dyserythropoietic anemias type I (10 ± 3 ng/ml)[29] and type II (1.8 ± 1.7 ng/ml)[30]. In pyruvate kinase deficiency, an iron-loading anemia with ineffective erythropoiesis, serum hepcidin was also strongly suppressed (more than 10-fold despite iron overload) but GDF15 was only modestly increased, making it unlikely that GDF15 caused hepcidin suppression in this disease. Mouse models exploring the role of GDF15 have not supported the universal role of GDF15 as an erythroid regulator. Mice lacking GDF15 manifested the same expected suppression of hepcidin after phlebotomy as WT mice, showing that GDF15 is not a physiologic erythroid regulator in mice [31]. Unlike in human β -thalassemia, GDF15 expression was not increased in the marrow or spleen of Hbb^{th3/+} mice, a well-established mouse model of β -thalassemia intermedia [31], indicating that GDF15 is regulated differently in mice than in humans. In another study of the same mouse model, a small increase in GDF15 expression in the marrow and perhaps the spleen was seen but appeared to be a consequence of increased erythroid cellularity in these organs rather than increased GDF15 expression in individual erythroid precursors [32]. It therefore appears that the regulation and activity of GDF15 in mice and humans are so different that the mouse model cannot be used to test its pathological role in humans. Studies of GDF15 in healthy human volunteers indicate that GDF15 is not significantly changed when hepcidin is suppressed 24h after EPO treatment of human volunteers [33–35], ruling out a role in EPO-induced hepcidin suppression. In summary, GDF15 is not a physiologic erythroid regulator of hepcidin but it remains possible that very high concentrations of GDF15 contribute to erythroid suppression of hepcidin in some anemias with ineffective erythropoiesis.

Erythroferrone (ERFE)—

ERFE was implicated as a physiologic erythroid regulator of hepcidin in a systematic screen for the marrow transcripts that: 1) increased in mice subjected to large blood loss (0.5 ml) or treatment with a single dose of exogenous human EPO (200 u), and 2) encoded a secreted protein. In this model, hepatic hepcidin mRNA and serum hepcidin concentrations decrease to about 10% of baseline by 15 h after phlebotomy or EPO injection. The screen identified ERFE mRNA as a transcript that increased more than 30-fold by 9 h after either treatment and encoded a member of the TNF α -C1q superfamily. Although the mRNA was detectable in multiple tissues, the highest expression was seen in EPO-stimulated marrow, and specifically in erythroblasts. In isolated marrow cells, ERFE mRNA was inducible by EPO treatment more than 30-fold but the increase was completely suppressed by chemical inhibitors of Stat5, indicative of the involvement of the Jak2-Stat5 pathway in the regulation of ERFE expression by EPO.

Structural features of ERFE—

Mouse and human ERFE are encoded by very similar ~10 kb genes divided into 8 exons and located on mouse chromosome 1 or human chromosome 2. The mouse and human proteins are predicted to be secreted, and contain a 24 (mouse) or 28 (human) amino acid signal sequence followed by a 316 (mouse) or 326 (human) amino acid mature protein, with 4

(mouse) or 3 (human) predicted glycosylation sites [36]. Predicted protein masses (without glycosylation) are 34 kD (mouse) or 35 kD (human). Recombinant mouse Erfe (FLAG-tagged) expressed from cDNA in HEK293 cells is approximately 40 kD [37] and very similar rat erythroferrone isolated from splenic microsomes [38] runs on SDS-PAGE as two bands, 40 and 42 kD, which decrease to 37 kD after deglycosylation, indicating two glycosylation variants. The C-terminal domain of erythroferrone (~156 amino acids) is predicted to fold into a TNF α /C1q-like globular head and there is a weak proline-rich domain in the N-terminal third of the molecule, both of which may support multimerization, as has been documented for other members of the TNF α /C1q superfamily [39]. Multimeric ligands are expected to bind avidly to their cognate multimeric receptors.

4. Pathophysiological role of ERFE in iron metabolism

ERFE is a physiological erythroid regulator in mice—

Unstressed ERFE-deficient (ERFE KO) mice are phenotypically normal, except for a mild hypochromic anemia that develops around 6 weeks of age, during a period of very rapid growth with associated expansion of erythroid mass [37]. Unlike WT control mice, ERFE KO mice do not acutely (during the first 24 hours) suppress hepcidin after hemorrhage or EPO administration, although they will do so after multiple EPO injections (our unpublished data). At least some of this delayed suppression of hepcidin may be mediated by iron depletion of the plasma transferrin compartment causing decreased signaling through the BMP receptor pathway. ERFE KO mice take several days longer to recover from hemorrhage-induced anemia, presumably because the lack of early hepcidin suppression delays both the mobilization of iron from stores and the increase in absorption of dietary iron.

ERFE is a pathological suppressor of hepcidin in mouse models of anemias with ineffective erythropoiesis—

In the Hbb^{th3/+} mouse model of thalassemia intermedia (nontransfusion dependent), marrow and splenic ERFE mRNA concentrations were greatly increased (up to 32-fold) compared to WT mice [40]. In comparison, GDF15 and TWSG1 mRNAs were unchanged or at most slightly increased (less than 2-fold). Analysis of ERFE mRNA relative to the erythroid cell marker glycophorin A mRNA indicated that increased ERFE mRNA in the marrow was driven by increased expression in each erythroid precursor but that the splenic ERFE mRNA was in part driven by increased numbers of erythroid precursors in that organ. Serum ERFE concentrations were below the level of detection in WT mice but were increased in Hbb^{th3/+} mice to concentrations similar to those seen in WT mice after injection of EPO (200 u). Finally, erythrocyte transfusions (400 μ l packed erythrocytes) into Hbb^{th3/+} mice profoundly suppressed marrow and spleen ERFE mRNA by 48 hours after transfusion. After weaning, Hbb^{th3/+} mice have a profound (about 8-fold) suppression of hepcidin mRNA compared to WT mice but by adulthood they develop progressive iron overload and eventually their hepcidin mRNA concentration becomes similar to that of WT mice, but at 5-times higher liver iron concentrations than in WT mice. ERFE ablation in Hbb^{th3/+} mice completely reversed the hepcidin mRNA suppression but only partially prevented the development of iron overload and did not affect the anemia in the Hbb^{th3/+} mice. These results suggest that

increased iron absorption in $Hbb^{th3/+}$ mice is not entirely mediated by ERFE, and has ERFE-independent and hepcidin-independent components. One potential cause of increased duodenal iron absorption is the direct effect of anemia and associated hypoxia on duodenal iron transport, mediated by HIF2 α -induced increase in the synthesis of duodenal iron transporters DMT1 and ferroportin [41].

The role of ERFE in other anemias—

Endogenous EPO-driven ERFE increase and the resulting suppression of hepcidin help in mobilizing iron to facilitate recovery not only from anemia after blood loss [37] but also in other types of transient anemia, such as anemia of inflammation [42] and anemia after hemolysis [43]. In a mouse model of anemia of inflammation, induced by intraperitoneal injection of heat-killed *Brucella abortus*, mice develop a moderately severe anemia with a hemoglobin nadir about 14 days after the injection. As the anemia worsens, plasma EPO concentrations rise and hepcidin is suppressed, and when inflammation subsides, recovery from anemia is driven by high EPO together with iron mobilization enabled by low hepcidin concentrations. As shown using hepcidin-1 knockout mice, this anemia is strongly but not exclusively dependent on the inflammatory induction of hepcidin [44, 45]. During recovery, ERFE mRNA expression in the marrow and the spleen follows the same pattern as EPO concentrations in blood [42]. ERFE plays a limiting role in the recovery from anemia by suppressing hepcidin, as shown by ERFE KO mice which develop more severe anemia than WT mice, have higher plasma hepcidin concentration during recovery and have a less complete recovery from anemia by 28 days after the inflammatory stimulus. A very similar pattern of ERFE-driven hepcidin suppression during recovery was seen in a mouse model of transient hemolysis induced by Phenylhydrazine [43] but the contribution of this mechanism to hemoglobin restoration was not analyzed. In another study, increased ERFE mRNA concentrations were detected in the marrows and spleens in five murine models of anemia, including two models of β -thalassemia ($Hbb^{th3/+}$ and RBC14), the hemoglobin deficit mouse (hbd), dietary iron deficient mice and mice treated with phenylhydrazine to induce acute hemolysis. Iron deficiency anemia also greatly stimulated ERFE mRNA [43] expression in the marrow and the spleen, raising the possibility that ERFE may contribute to hepcidin suppression in iron deficiency anemia. It appears that EPO influences ERFE production by at least two mechanisms, i.e. by increasing the production of ERFE mRNA per erythroblast as well as increasing the number of erythroblasts that secrete ERFE.

Evidence for the role of ERFE as a physiological and pathological erythroid regulator in humans—

The protein sequence of human ERFE is highly similar to mouse ERFE (71% identity, 77% similarity). Like mouse ERFE, human ERFE is highly expressed in erythroblasts in the marrow or fetal liver and is induced further by EPO treatment [37]. The development of human ERFE immunoassay facilitated studies of human ERFE responses to physiological and pathological stimuli [46]. As was the case in phlebotomized mice, human blood donors responded to the loss of 2 units of packed erythrocytes (about 400–450 ml) by sustained increase in serum ERFE concentrations (lasting at least 2 weeks) and concurrent suppression of serum hepcidin concentrations, with a return to baseline in both hormones 16 weeks later. The administration of EPO to 4 human subjects caused a rapid rise in serum ERFE with a

peak 1–2 days later, and a corresponding decrease in serum hepcidin concentrations. Serum ERFE was profoundly increased in untransfused patients with β -thalassemia and also increased in patients with β -thalassemia before their regular transfusions but was only mildly increased in the week after β -thalassemia patients received transfusions. There was an inverse correlation between serum ERFE concentrations and serum hepcidin concentrations, so that patients with untransfused β -thalassemia had the highest levels of serum ERFE and the lowest hepcidin concentrations. These studies indicate that ERFE (patho)physiology in humans and in mice is similar, and that ERFE is a strong candidate for the principal erythroid regulator of iron and hepcidin in humans. The development and future testing of ERFE antagonists will provide a more stringent test of the role of ERFE in humans. As for other anemias, ERFE is increased in patients with anemia of chronic kidney disease, and also in end-stage kidney disease after treatment with exogenous EPO, suggesting that ERFE responds appropriately to endogenous and exogenous EPO, and is not the cause for EPO resistance in these anemias [47]. Increased serum ERFE was also reported in pediatric patients with moderate iron deficiency anemia, consistent with ERFE mRNA changes in a mouse model of this anemia [32]. It is not yet clear to what extent ERFE contributes to the profound suppression of hepcidin in this disorder. The proposed physiological and pathological roles of ERFE are summarized in Figure 1.

5. ERFE mechanism of action

Mechanism of ERFE effect on hepcidin—

Erythroferrone at subnanomolar concentrations suppresses hepcidin expression both in primary hepatocytes [37] and in the Hep3B hepatocytic cell line [48]. In the mouse model, this effect appears to be additive to the iron-regulatory signal. Although early studies raised the possibility that hepcidin suppression by ERFE is independent of the BMP pathway [37], more extensive subsequent analyses [48] have demonstrated that ERFE suppressed Smad1/5 phosphorylation in primary murine hepatocytes and in the Hep3B hepatocytic cell line, and that hepcidin suppression by EPO was dependent on intact Smad1/5 signaling in mice. When Smad1 and Smad5 were ablated in primary murine hepatocytes or in Hep3B cells, ERFE could no longer suppress hepcidin mRNA. These studies indicate that ERFE-induced hepcidin suppression is at least in part mediated by the BMP pathway.

Interaction of TMPRSS6 and ERFE—

TMPRSS6 (matriptase-2) is a transmembrane serine protease that acts as a negative regulator of the BMP pathway [49–51], likely through its ability to degrade several limiting components of the BMP receptor complex [52–54]. Loss of function mutations in *Tmprss6* increase plasma hepcidin concentrations, decreasing iron absorption and trapping iron in macrophages, thereby causing iron-refractory iron deficiency anemia (IRIDA) [50, 55]. Remarkably, patients with IRIDA are resistant to treatment with EPO suggesting that EPO cannot sufficiently suppress hepcidin in the context of loss of TMPRSS6 function [56]. Pointing to the same interaction, *Hbb*^{th3/+} mice with inactivated *Tmprss6* have high hepcidin, despite high serum EPO concentrations [57–59]. Nevertheless, *Tmprss6* KO mice express high levels of ERFE, consistent with their anemia and high EPO levels [56], raising the question whether *Tmprss6* is essential for the ability of ERFE to suppress hepcidin. One

potential explanation of these findings is that the effect of ERFE on hepcidin is linearly additive to that of the effect of the BMP pathway [37], so that when the BMP pathway is hyperactivated by the loss of inhibitory Tmprss6 activity the effect of ERFE becomes proportionally smaller. This explanation is supported by studies of the acute hepcidin-suppressive effect of phlebotomy in mice with various degrees of iron overload [37, 56], where the suppression of hepcidin proportionally decreases as iron overload becomes more severe. The other possibility, that Tmprss6 directly participates in the ERFE signaling pathway, is challenged by the normal suppression of hepcidin mRNA by recombinant ERFE treatment of hepatocytes from Tmprss6-deficient mice [60], and by the ability of EPO to suppress hepcidin mRNA in mice with pharmacologic inhibition of the BMP pathway [56].

6. Therapeutic applications

ERFE antagonists—

ERFE antagonists may be most useful for the prevention of iron overload in anemias with ineffective erythropoiesis, especially in patients who are not regularly transfused. Many patients with β -thalassemia have pathologically increased plasma concentrations of ERFE which are likely to contribute to the suppression of hepcidin, commonly seen especially in patients who are not regularly transfused or massively iron overloaded [25, 26, 46, 61]. Based on preclinical studies of ERFE ablation in mice [40], a therapeutic strategy targeting ERFE would increase hepcidin and help prevent iron overload. Even when present at pathologically excessive concentrations, ERFE is a subnanomolar hormone so neutralization by humanized monoclonal antibodies is likely to be feasible with relatively infrequent dosing. Other iron-loading anemias with a similar iron physiology include such genetic disorders as congenital dyserythropoietic anemias, some sideroblastic anemias and pyruvate kinase deficiency, as well as acquired conditions such as certain diagnostic subsets of myelodysplastic syndrome.

ERFE analogs and mimics—

Proteins, peptides or small molecules that mimic ERFE activity would be most useful in conditions where hepcidin concentrations are high, and the resulting iron restriction interferes with erythropoiesis. The most common such disorders include anemia of inflammation and anemia of chronic kidney disease.

7. Challenges and prospects

Studies to date leave some space for the identification and characterization of additional erythroid regulators, especially those associated with iron-loading anemias. We cannot assume in every case that homologous molecules must have the same function in human disease and its mouse model. Moreover, iron overload in mouse models appears to be much less cytotoxic, presenting additional challenges in extrapolating pathophysiological mechanisms to human disease. As for ERFE, the obvious missing piece of the puzzle is the ERFE receptor and its signaling pathway, where the availability of multiple high-throughput approaches should facilitate rapid progress. Other important tasks include the characterization of the role of multimeric ERFE species in its activity, standardization of

human and mouse ERFE assays, and exploration of the diagnostic utility of ERFE measurements in various anemias.

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Abbreviations:

BMP	bone morphogenetic protein
ERFE	erythroferrone
EPO	erythropoietin
GDF	growth/differentiation factor
MT2	matriptase 2 (same as TMPRSS6)
NTBI	non-transferrin bound iron
TGF	transforming growth factor
TMPRSS6	transmembrane serine protease 6
TWSG	twisted gastrulation

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Highlights

- Systemic iron homeostasis is controlled by the peptide hormone hepcidin
- Hepcidin binds to iron exporter ferroportin to control iron efflux into plasma
- Systemic iron homeostasis is closely coupled to erythropoiesis
- Erythroferrone is a glycoprotein secreted by erythropoietin-primed erythroblasts
- Erythroferrone signals erythroid demand for iron by suppressing hepcidin production

ERFE AS A PHYSIOLOGIC ERYTHROID REGULATOR **ERFE IN IRON-LOADING ANEMIAS** **Fig 1:**

Proposed roles of ERFE in matching iron supply to erythropoietic demand during stress erythropoiesis, such as after acute blood loss or acute hemolysis (left panel), contrasted with the pathological role of ERFE in iron-loading anemias with ineffective erythropoiesis, exemplified by β -thalassemia intermedia (right panel). During the physiological response (left panel), anemia causes increased EPO secretion by the kidneys. Driven by EPO, more erythroblasts enter the erythropoietic differentiation pipeline and EPO-stimulated ERFE secretion increases, lowering hepatic hepcidin production and thereby matching iron supply (from the spleen, liver, and duodenum) to increased erythropoiesis. In anemias with ineffective erythropoiesis (right panel), most erythroblasts do not successfully differentiate into mature erythrocytes, leading to anemia and increased EPO production by the kidneys. ERFE secretion is increased both because of EPO stimulation and because of the large numbers of dead-end but ERFE-secreting erythroblasts. Hepcidin is suppressed and iron is mobilized but erythrocyte production cannot increase and so the additional iron is not utilized. Excess iron saturates transferrin and generates NTBI, which is taken up in the liver and other vital organs, causing tissue injury and organ failure.