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Growth differentiation factor 15 in erythroid health and disease

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Abstract

Purpose of review—Growth differentiation factor 15 (GDF15) was identified as a hepcidin-suppression factor that is expressed at high levels in patients with ineffective erythropoiesis. This review addresses the regulation, expression and potential functions of GDF15 in the context of erythroid biology.

Recent findings—GDF15 expression during late erythroid differentiation was discovered as part of an erythroblast transcriptome project. Since GDF15 expression is associated with cellular stress or apoptosis, further investigation of the cytokine was focused upon its involvement in ineffective erythropoiesis. Remarkably high serum levels were detected in patients with thalassemia syndromes, congenital dyserythropoiesis and some acquired sideroblastic anemias. Similarly high-level GDF15 expression is not a feature of normal erythropoiesis, or erythroid recovery after bone marrow transplantation. Since GDF15 is a TGF-β superfamily member, it was investigated as an effector of ineffective erythropoiesis that suppresses hepcidin expression despite iron overloading.

Summary—In contrast to the low-levels of GDF15 expressed during normal erythropoiesis, ineffective erythropoiesis causes high-level expression of GDF15. In patients with thalassemia and related anemias, GDF15 expression may contribute to iron overloading or other features of the disease phenotype.

Keywords

GDF15; ineffective erythropoiesis; iron regulation

Introduction

Ineffective erythropoiesis describes the suboptimal production of mature erythrocytes from a proliferating pool of immature erythroblasts. In many patients, secondary hemochromatosis develops with iron loading in which stores are beyond the physiological levels needed to support erythropoiesis. Studies of iron homeostasis in patients with ineffective erythropoiesis led to the notion that erythroblast-expressed factors may cause excess iron loading through suppression of the hormone hepcidin. Recently, transcriptome studies of *ex vivo* human erythropoiesis identified growth differentiation factor 15 (GDF15) as a candidate molecule in this regard. In this review, erythroblast expression and the potential functions of GDF15 are discussed.

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The GDF15 gene and its expression

The human GDF15 locus was mapped by fluorescence in situ hybridization (FISH) to chromosome 19p12.1-13.1 [1]. As shown in Figure 1, the gene contains a single 1820 bp intron [1]. The GDF15 protein is encoded by two exons: the 309 bp Exon I contains a 71 bp 5' untranslated region (UTR) and 238 bp of coding region, and the 647 bp Exon II contains a 3' UTR. GDF15 is one of the major secreted proteins induced by the tumor suppressor protein p53 [2]. Two p53 binding sites are located within the −500 bp promoter with a site located in the 5' UTR [3,4]. Several studies suggest that GDF15 induction is associated with cell cycle arrest and apoptosis [5]. Hence, GDF15 may be an excellent *in vivo* biomarker of the p53 pathway activation [6]. However, p53 is not the only transcription factor regulating GDF15 expression. The GDF15 promoter contains motifs for several additional transcription factors [7]. Sp1 and COUP-TF1 transcriptional factors regulate the basal transcription of GDF15 through the GC box located within −133 bp of the GDF15 promoter [8]. The Egr-1 binding sites in the GDF15 promoter overlap with an Sp1 binding sites. GATA binding motifs are also encoded in the promoter region [3]. Thus, the transcriptional activity of GDF15 likely depends on the balance of transacting factors that may be regulated as part of an apoptotic or stress response as well as tissue differentiation. Since hypoxia or other cellular stresses increase p53, Sp1, and Egr-1 expression, increased serum levels of this cytokine may reflect cellular stress or death [9]. Additional transcription factors may be involved in the GDF15 response to hypoxia [10].

GDF15 is a member of transforming growth factor-β (TGF-β) superfamily that comprises more than 40 members. The TGF-β superfamily is involved in several processes including cell differentiation, development and apoptosis [11]. GDF15 is somewhat unique, in that it shares TGF-β homology according to its cysteine rich domain, but it otherwise shares less than 30% amino acid homology with other TGF-β family members. Among the superfamily, GDF15 is the most divergent member [12]. A single-nucleotide polymorphism at position 6 of the mature protein results in histidine to aspartic acid substitution (H6D, rs1058587) [13]. The H6D variant is associated with functional variation of the protein [14]. GDF15 is synthesized as a precursor protein that undergoes disulfide-linked dimerization like TGF-β. The precursor form mediates binding to theextracellular matrix, creating latent stromal stock of proGDF15. The precursor protein is cleaved at an RXXR furin-like cleavage site to form the mature C-terminal GDF15 peptide, which is subsequently secreted as a 25–30 kDa dimer [15,16]. Mature GDF15 is soluble and easily identified in blood, where it acts as an "extracellular" messenger [17]. Unfortunately, current knowledge regarding specific cellular membrane receptors and signaling cascades (Smad, MAPK, Akt) that transducer GDF15 signals remains superficial to date [18–20].

GDF15 expression in effective and ineffective erythropoiesis

Based upon the Human Genome Project, efforts were made over the last decade to better understand transcriptomes encoded in human erythroblasts. An erythroblast transcriptome project was initiated by first isolating human erythroblasts in real-time as they differentiate in *ex vivo* cultures [21]. mRNA from those cells was isolated and analyzed to provide a erythroid transcriptome map for further study. Due to the high-levels of erythroblast proliferation followed by incomplete maturation (without enucleation), the transcriptome generated using cultured cells was hypothesized to reflect ineffective erythropoiesis [22]. High-levels of GDF15 gene expression were noted in culture among the more mature erythroblasts, and the cytokine was selected for further study [23].

Along with studies in cultured erythroblasts, clinical studies of GDF15 expression *in vivo* were begun. A summary of clinical reports is shown in Figure 2. The levels of GDF15 in healthy

individuals are generally measured in 200–1,150 pg/ml range by commercial ELISA assays [23,24]. There are no significant differences of GDF15 concentrations among citrated plasma, EDTA-treated plasma, and serum. GDF15 is stable at room temperature in serum, plasma, and whole blood for at least 48 hours. GDF15 is resistant to at least 4 freeze thaw cycles. Anticoagulants, albumin, bilirubin, or hemoglobin do not significantly influence the measurement of GDF15 concentrations [25]. These features suggest GDF15 expression in the serum may eventually be utilized as a clinical marker of cell stress or apoptosis. Additional studies are needed to determine the sensitivity and specificity of GDF15 measurements in a broader range of disease settings.

Consistent with the high levels of GDF15 expressed *ex vivo* in the culture model described above, serum GDF15 levels are highly-elevated in patients with ineffective erythropoiesis or other hematopoietic disorders (Figure 2). The thalassemia syndromes (α-thalassemia and β-thalassemia) represent the most common causes of ineffective erythropoiesis. In the thalassemia syndromes, imbalances in the production of α- and β-globin chains result in increased apoptosis during erythroblast maturation [26]. Serum GDF15 levels in patients with thalassemia were dramatically elevated compared with healthy volunteers or patients with the thalassemia trait. The median in beta-thalassemia patients (48,000 pg/ml) was particularly high with a detected range of 5,000–250,000 pg/ml [23]. A second group of disorders associated with ineffective erythropoiesis and iron overloading is called dyserythropoietic anemias. Congenital dyserythropoietic anemia type I (CDAI) is caused by CDANI gene deficiency [27]. Like α- and β-thalassemia, very high serum levels of GDF15 are expressed in patients with CDAI [28]. In CDAI patients, serum GDF15 levels were correlated with the level of ineffective erythropoiesis, hepcidin-25, and ferritin. A third group of patients with ineffective erythropoiesis possess an erythroid defect that is characterized by accumulation of iron in mitochondria that “ring” the erythroblast nucleus during terminal maturation [29]. Intramedullary apoptosis is a feature of acquired anemia with ringed sideroblasts [30]. Significant elevations in GDF15 expression were also reported in this group (3,254 ± 1,400 pg/ml vs. 451 ± 87 pg/ml in healthy volunteers) [31]. Finally, significant ineffective erythropoiesis has been reported among some patients with pyruvate kinase deficiency, but the erythroid phenotype is variable [32,33]. GDF15 expression in patients with pyruvate kinase deficiency is elevated, but the magnitude of elevation is considerably lower than measured in patients with thalassemia [34].

Serum GDF15 levels from patients with other erythroid disorders have also been reported. Like thalassemia syndromes, sickle cell syndromes are characterized by increased levels of erythropoiesis, but the primary defect in sickle cell involves destruction of mature erythrocytes. In severe cases of sickle cell disease, some ineffective erythropoiesis may be found [35]. Interestingly, the elevation in the serum level of GDF15 is mild in sickle cell disease compared to thalassemia [23]. Studies of patients with effective erythropoiesis after bone marrow transplantation [36] or after injection of erythropoietin [37] failed to demonstrate major increases in serum GDF15. Indeed, preliminary studies at the National Institutes of Health suggest GDF15 expression is barely detectable in normal bone marrow, compared with the distinct staining in a subset of erythroid precursors in thalassemia bone marrow (Figure 3). Since GDF15 is not expressed specifically in erythroblasts, elevations of this cytokine in patients with cancers and inflammatory disease should not be attributed to erythroblast expression. This point may be particularly important when considering the high-levels of GDF15 reported in patients with anemia of chronic disease [38]. When combined, these data suggest GDF15 measurements may be helpful for predicting ineffective or apoptotic erythropoiesis.

Hepcidin, erythroblast iron loading, and GDF15

Dietary, tissue-stored, and erythrocyte-recycled iron molecules exit cells via a membrane channel named ferroportin. Ferroportin expression on the cell membrane is regulated by a protein named hepcidin [39–41]. Hepcidin is a small peptide that is produced in the liver that is most likely the major regulator of iron [42]. It acts by causing the endocytosis and degradation of ferroportin [43]. Based upon the importance of iron, multiple mechanisms exist for the regulation of hepcidin. Iron levels, inflammation, erythropoiesis and the combined effects of several proteins expressed on hepatocyte membranes are involved [44]. There exists a relatively broad range and diurnal variation in serum hepcidin levels among healthy volunteers. Hence, the interpretation of serum hepcidin levels in disease states may be complex. As an example, it is now recognized that simple iron deficiency causes serum hepcidin to be reduced to low or undetectable levels, and inflammatory disease causes increased serum levels [45]. However, the combination of iron deficiency with inflammation cause a balance of hepcidin-regulating mechanisms that results in serum levels within the normal range.

Hepcidin expression and iron overloading in patients with ineffective erythropoiesis may similarly be explained by a combination of regulatory mechanisms. Erythropoiesis in all forms requires high levels of iron for the production of hemoglobin. The robust demand for iron is supplied by transferrin-bound iron in the plasma [46]. Therefore, erythropoiesis causes the necessary suppression of hepcidin required to maintain adequate transport of iron from macrophage and other cells to support the large demand of iron for hemoglobin production. The mechanism by which normal erythropoiesis regulates the suppression of hepcidin is still not known. Based upon the relative lack of GDF15 regulation by EPO or during marrow recovery, GDF15 is not likely involved in the physiological suppression of hepcidin for normal erythropoiesis [36,37]. Importantly, ineffective erythropoiesis is unique among anemias in that it is associated with iron overloading of the host, which far exceeds the iron loading required for erythropoiesis. [47]. It is proposed here that ineffective erythropoiesis causes iron overloading by not permitting the appropriate rise in hepcidin expression to occur once the erythroid demand is met. The low or low-normal serum hepcidin levels in thalassemia support this hypothesis [48].

Since a pathological signal could explain the inappropriately normal serum hepcidin levels in iron-overloaded patients with ineffective erythropoiesis, studies were focused upon determining whether GDF15 modifies or is modified by iron homeostasis [23,49]. In cultured human hepatocytes, serum GDF15 in thalassemia patients, as well as recombinant GDF15 protein, suppressed hepcidin expression. However, significant suppression of hepcidin required high levels (>5,000 pg/ml) of GDF15, and the suppression was incomplete even after the addition of the highest dose of 100,000 pg/ml GDF15. In addition, serum of thalassemia patients suppressed hepcidin in the cultured cells even after GDF15 was immunoprecipitated [23]. As a result, it was concluded that GDF15 expression at the high levels achieved in the setting of ineffective erythropoiesis contribute to pathological iron overloading through a mechanism of incomplete hepcidin suppression. Others have suggested that in addition to the regulation of hepcidin, iron depletion or chelation in the host may regulate GDF15 expression [50].

A summary model of iron homeostasis in patients with ineffective erythropoiesis is shown in Figure 4. Based upon the vast expansion of medullary and extramedullary erythropoiesis, the erythroblast demand for iron is enormous. There is a clear increase in iron kinetics, tissue hypoxia, and erythropoietin in these patients that act together with other physiological mechanisms to suppress hepcidin and meet the erythroblast demand for iron. Once the host tissues load enough iron to meet the erythroblast demand, hepcidin expression should increase to levels sufficient for prevention of tissue iron overloading. Instead, hepcidin expression

remains within the normal range, partially due to over-expression of GDF15. Among patients who are transfused, the effects of iron loading are made worse by the host's inability to express sufficient hepcidin to maintain the sequestration of erythrocyte-recycled iron safely inside macrophages.

The curiosity of GDF15 function

GDF15 expression is not erythroid-specific. Human GDF15 is highly expressed in the placenta during pregnancy in the second and third trimester [51,52]. Increased serum GDF15 has also been described in several disease states including acute injury, inflammation, and cancer [53–55]. In contrast, murine GDF15 is not expressed at high levels in the placenta [56]. Instead, murine GDF15 is found in the CNS, its site of highest expression being the choroid plexus [57]. GDF15 mRNA is prominently upregulated in neurons [58]. Recently, Strelau *et al.* demonstrated that GDF15 is a genuine novel trophic factor for motor and sensory neurons using GDF15 deficient mice [59]. GDF15 shows remarkably low homology conservation between human and murine species. While mature forms of human and murine TGF-β1 and BMP-2 show 99% and 100% sequence similarity between the murine and human species, GDF15 shares only 66% amino acid sequence similarity [60,61]. Promoter homology between human and mouse is even less, with only 39% homology [8]. GDF15 null mice show few obvious abnormalities [56]. Overexpression of GDF15 in mice demonstrated a reduction in body weight [62]. GDF15 also suppresses intestinal and gastrointestinal tumorigenesis in the transgenic mice, suggesting that GDF15 may act as a tumor suppressor gene in those animals [19,63]. Despite these interesting findings, the interpretation for clinical application is blurred by major differences in homology and tissue expression between mice and men.

Beyond having a potential role in iron regulation, additional functions for human GDF15 have been proposed. Human GDF15-encoded protein has many alternative names [placental bone morphogenetic protein (PLAB), placental transforming growth factor-β (PTGF-β), macrophage inhibitory cytokine-1 (MIC-1), prostate derived factor (PDF), and nonsteroidal anti-inflammatory drug activated gene (NAG-1)]. In tumor cells, GDF15 can exhibit tumorigenic and antitumorigenic activity in different circumstances. GDF15 may also be protective in heart failure [64]. Due to elevated levels of GDF15 observed in patients with rheumatoid arthritis, it has been proposed to participate in inflammatory responses [65]. Johnen *et al.* described GDF15 as an appetite suppressor at high concentrations, promoting cachexia in patients with advanced cancers [62]. GDF15 also induces osteoclast formation *in vitro*, and osteoclast formation *in vivo* [66]. The effects upon appetite and bone metabolism may be worthy of additional studies in patients with thalassemia or related anemias. Overall, GDF15 expression seems to be that of a stress effector with subtle and protean functions that facilitate the host's response to or recovery from cellular damage or death. In cases of ineffective erythropoiesis, the incomplete suppression of hepcidin by GDF15 may occur when the host response to erythroblast death becomes exaggerated by the shear magnitude and chronicity of the disease.

Conclusion

GDF15 expression is usually associated with cellular stress or apoptosis. In patients with thalassemia and other diseases manifested by ineffective erythropoiesis, particularly high levels of GDF15 are present in the serum. It is proposed that the elevated levels of GDF15 contributes to the suppression of hepcidin and subsequent tissue iron overloading in those patients. A more complete understanding of GDF15 signal transduction and functions in health and disease are topics for future research.

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* Of special interest

** Of outstanding interest

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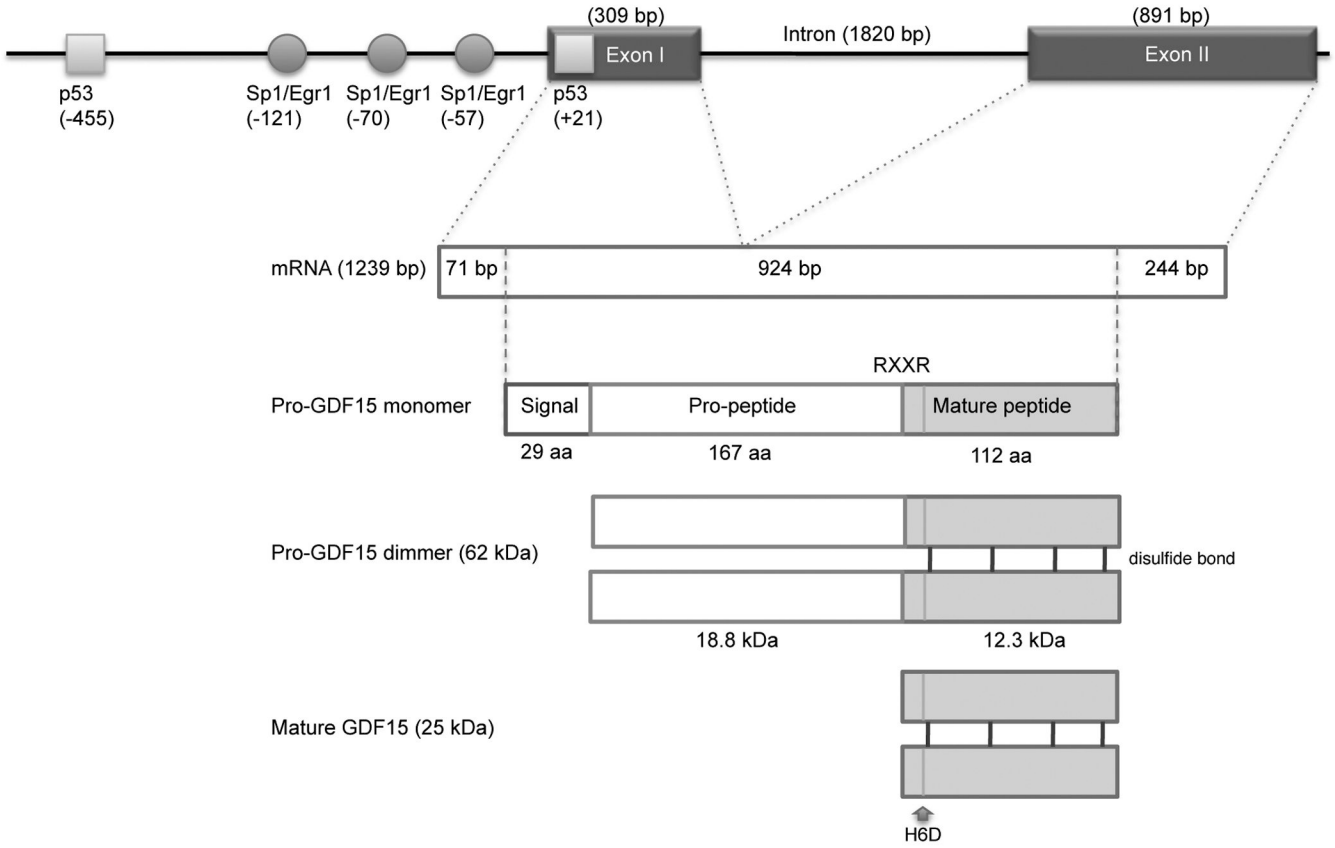

Figure 1. Genomic structure and transcription for protein production of matured GDF15

The diagram shows GDF15 gene structure with several transcription factors binding sites in the promoter region. GDF15 is synthesized from two exons, and dimerized with disulfide bonds, then cleaved at RXXR site to form C-terminal mature GDF15 protein. H6D denotes a single-nucleotide polymorphism at position 6 of the mature protein resulting in histidine to aspartic acid substitution.

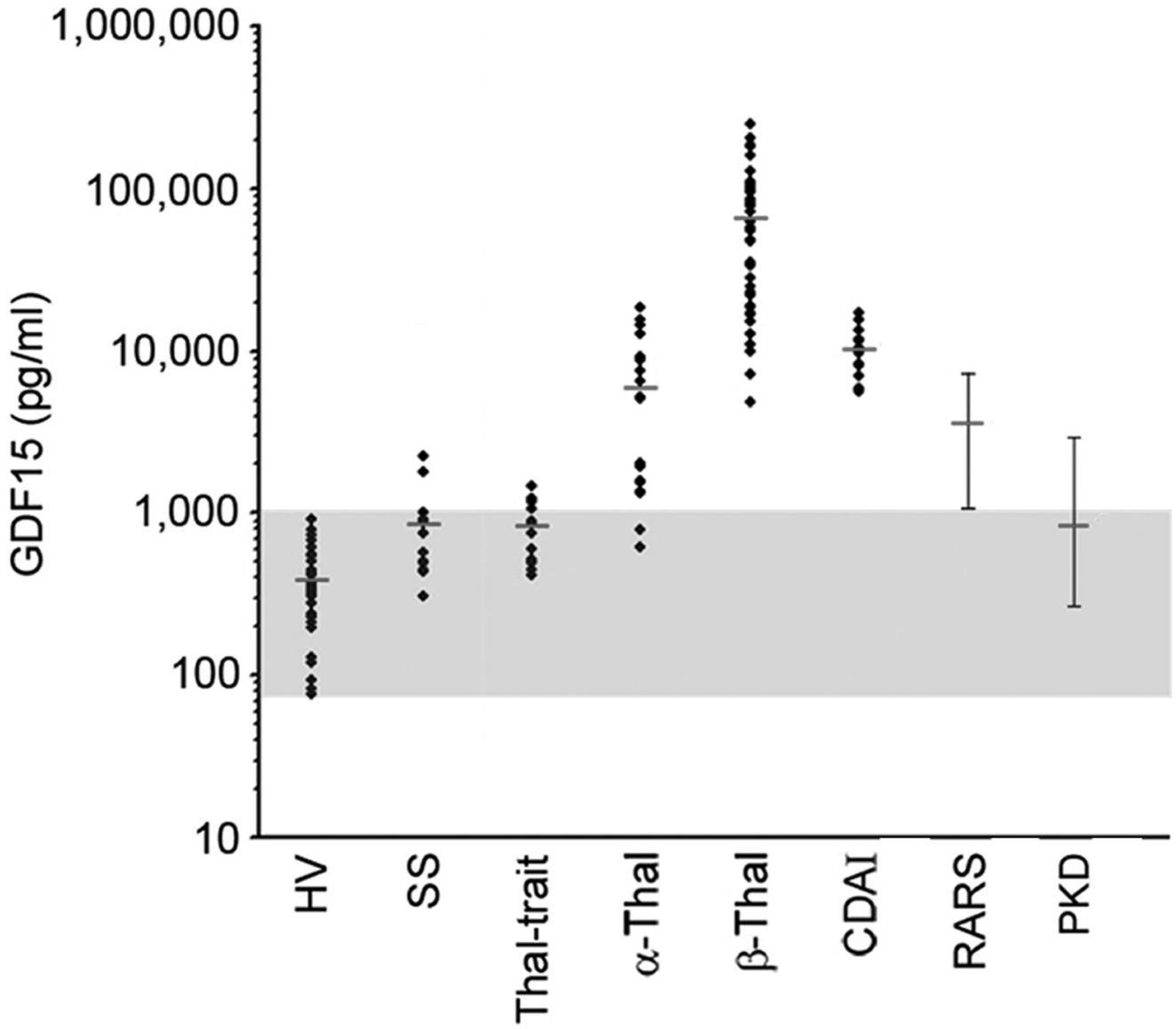

Figure 2. GDF15 concentrations in human blood from healthy volunteers and several hematological diseases

The individual data points represent GDF15 concentrations from healthy volunteers (HV; n = 37), sickle cell anemia (SS; n = 13), thalassemia trait (Thal-trait; n = 12), α-thalassemia (α-Thal; n = 20), β-thalassemia (β-Thal; n = 40) [23], and congenital dyserythropoietic anemia type I (CDAI; n = 17) [28]. The lines represent the maximum and minimum concentrations from patients with refractory anemia with ring sideroblasts (RARS, n = 20) [31] and pyruvate kinase deficiency (PKD, n = 22) [34]. Bars show the mean of GDF15 concentrations.

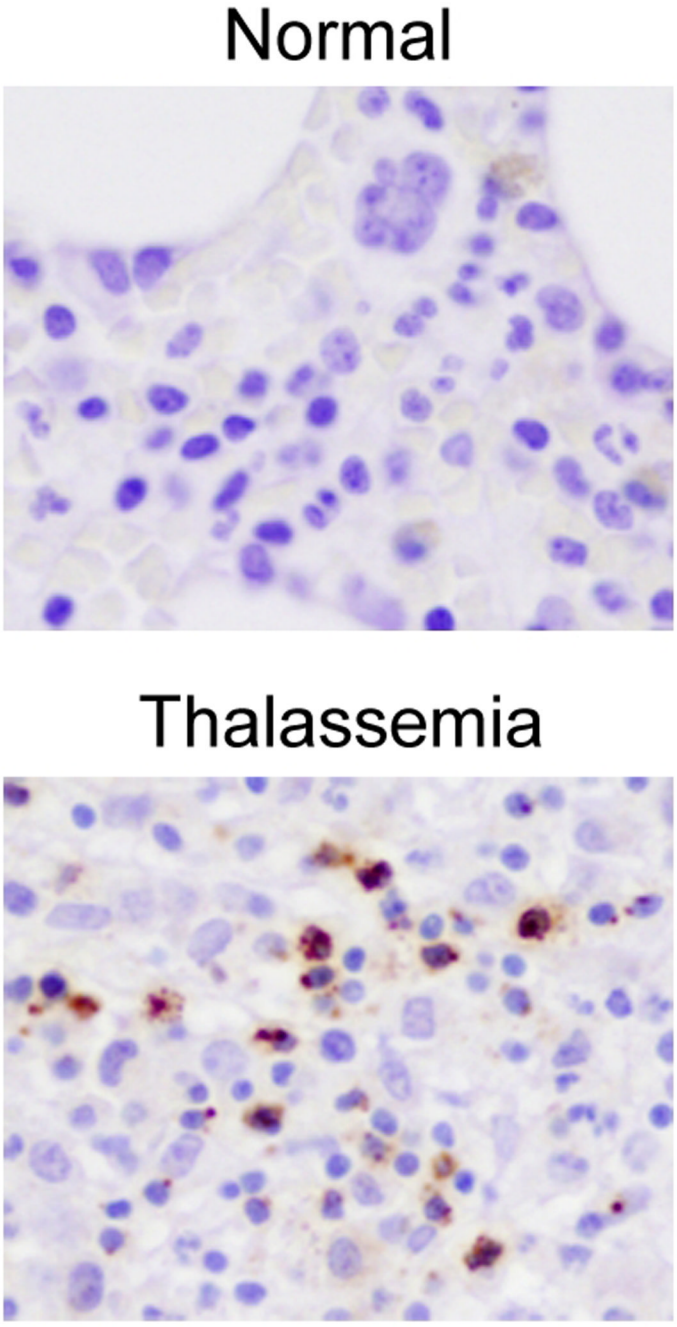

Figure 3. GDF15 expression in bone marrow from a patient with thalassemia

Brown coloration marks GDF15 present in late-stage erythroblasts in thalassemia bone marrow. No similar staining was noted in bone marrow from a healthy volunteer.

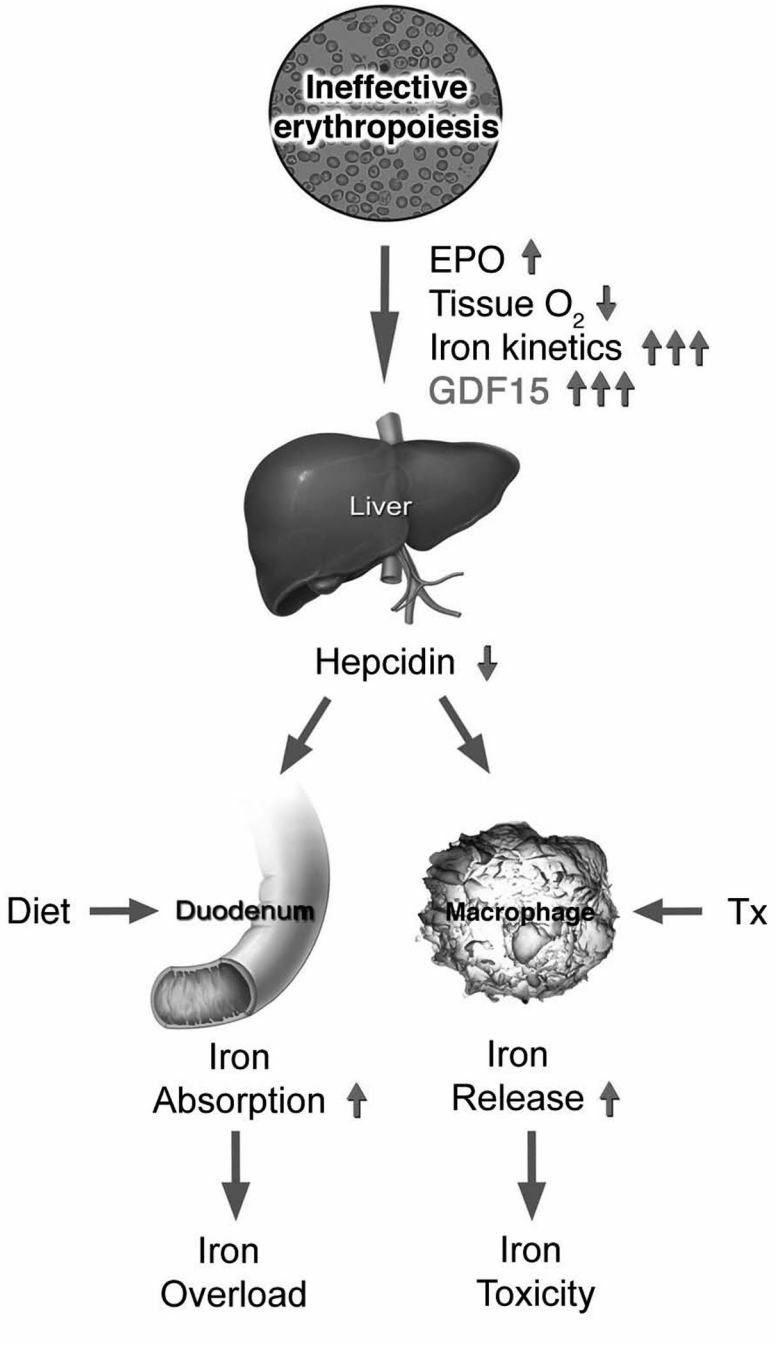

Figure 4. Model of iron homeostasis in patients with ineffective erythropoiesis
With permission from reference [23]