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What can we learn from ineffective erythropoiesis in thalassemia?

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Abstract

Erythropoiesis is a dynamic process regulated at multiple levels to balance proliferation, differentiation and survival of erythroid progenitors. Ineffective erythropoiesis is a key feature of various diseases, including β-thalassemia. The pathogenic mechanisms leading to ineffective erythropoiesis are complex and still not fully understood. Altered survival and decreased differentiation of erythroid progenitors are both critical processes contributing to reduced production of mature red blood cells. Recent studies have identified novel important players and provided major advances in the development of targeted therapeutic approaches. In this review, β-thalassemia is used as a paradigmatic example to describe our current knowledge on the mechanisms leading to ineffective erythropoiesis and novel treatments that may have the potential to improve the clinical phenotype of associated diseases in the future.

Keywords

ineffective erythropoiesis; red blood cells; thalassemia

1. Introduction

The production of adequate numbers of functional red blood cells (RBC) is necessary for tissue oxygenation and survival. In several diseases, multiple pathogenic processes result in ineffective erythropoiesis (IE) and anemia. Ongoing research continues to provide valuable

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insight into these pathways and their understanding drives the development of novel treatments to reverse IE and improve the anemia. In this review, we focus on β-thalassemia, which is characterized by IE, to discuss the pathological mechanisms and therapeutics that lead to IE.

2. Erythropoiesis: Steady state and stress

2.1 Normal Erythropoiesis

Erythropoiesis is a dynamic multistep process where erythroid progenitors differentiate to produce mature enucleated RBC. Hematopoietic stem cells, residing in the bone marrow (BM) niche, generate committed erythroid progenitors, which can be further functionally defined in colony assays in vitro [1–3]. The early stage burst forming unit erythroid (BFU-E) generate large erythroid colonies and progress to late stage colony forming unit erythroid (CFU-E), characterized by smaller erythroid colonies. This process occurs with a concomitant decrease in proliferative capacity and increase in erythropoietin (EPO) sensitivity, noted by the appearance of erythropoietin receptors (EPOR) on the cell surface [4, 5]. As erythropoiesis progresses, morphologically recognizable precursors emerge by successive mitoses [6, 7]. The earliest recognizable erythroblast is the proerythroblast, which undergoes cytoplasmic maturation and nuclear changes to generate basophilic, polychromatophilic and orthrochromatic erythroblasts. Finally, the latter extrude their nuclei to become reticulocytes and, ultimately, mature RBC, which circulate in the blood stream until senescence [8]. Maturation of erythroblasts is marked by progressive hemoglobinization, so that RBC contain adequate amounts of hemoglobin (Hb). Under steady state conditions homeostatic control mechanisms regulate proliferation, differentiation and destruction of erythroid precursors in order to maintain a balance between RBC production and death. This process ensures adequate oxygenation of tissues [9–11].

Multiple signaling molecules and cell-cell interactions control erythropoiesis. The master regulator is EPO, a hormone primarily produced by the interstitial cells in the renal cortex [12]. In fetal life, liver is the main source of EPO while, after birth, the kidney becomes the main producer [12]. EPO is released into circulation and signals through EPOR, which is downregulated following the basophilic erythroblast stage. Binding of EPO to EPOR induces the dimerization of EPOR monomers. In response to EPO stimulation and receptor reorientation, Janus kinase 2 (JAK2), found on the intracellular part of EPOR, is rapidly phosphorylated creating docking sites for the SH2 domains of several signal transduction proteins, including STAT5 (Table 1) [13, 14]. Activated STAT5 translocates to the nucleus where it controls the expression of a subset of genes involved in proliferation, differentiation and survival of erythroid progenitors [15–18]. Although the JAK/STAT pathway is critical in promoting erythropoiesis, another signaling cascade is also activated by EPO stimulation; the PI3K-AKT pathway. AKT kinase, the downstream target of PI3K, phosphorylates several transcription factors, including FOXOs, and regulates their activity [19, 20]. FOXOs play an important role in regulating cell growth, survival and differentiation [21, 22].

Expression of *EPO* is low under basal conditions. Specialized cells in the kidney and in the liver of the fetus and the newborn are sensitive to oxygen concentration in circulation. When local oxygen delivery to these organs decreases, *EPO* expression is upregulated. This effect

is mediated by the activation of hypoxia inducible transcription factors (HIFs), which stimulate an enhancer element of the *EPO* gene [23, 24]. In particular, HIF-2α is the major activator of hypoxia-induced *EPO* expression [24–27]. EPO production can be inappropriately low in several conditions causing severe anemia. This could be due to either destruction of the EPO producing cells, as in chronic kidney disease, or repression of EPO synthesis due to circulating inflammatory cytokines, as in rheumatoid arthritis, AIDS, chronic kidney disease or cancer [28–31]. On the other hand, inappropriately high EPO production may be caused by several tumors, such as renal or hepatocellular carcinoma, and results in polycythemia [32, 33].

2.2 Stress erythropoiesis

In a state of local decreased oxygen delivery (hypoxemia, anemia), a physiologic response is elicited, intended to increase oxygen delivery to tissues. This process, called stress erythropoiesis, is a uniquely defined state of erythropoiesis, that results in the production of large numbers of erythrocytes, and has distinct differences in mediators involved from steady state [34].This is a response to acute erythropoietic stress and increased EPO concentration, caused by multiple stimuli, including hemorrhage, hemolysis, high altitude and pharmacological treatment. EPO acts on EPOR and through STAT5 rapidly increases the expression of anti-apoptotic regulator *BCL-XL* in early erythroblasts, while suppressing the proapoptotic proteins BIM, NOXA and co-expressed tumor necrosis family receptor FAS, together with its ligand FASL [35–38]. Unique to stress erythropoiesis, the phosphorylation of STAT5 produces a high intensity, graded signal, which increases with rising EPO concentration [39]. The early erythroblast compartment is mainly EPO responsive, and therefore mostly affected by anti-apoptotic processes, predominantly in the spleen, where a reserve of splenic progenitors is widely expanded during hypoxia. Under steady state conditions, the majority of early erythroid progenitors in the spleen undergo apoptosis. In acute anemic stress, high EPO prevents apoptosis of this reserve erythroid progenitor population, by mechanisms mentioned earlier [40, 41]. This regulatory mechanism provides a transient survival signal on erythroblasts, and remains active until suppression pathways are activated [42].

Multiple other pathways have been identified in stress erythropoiesis. In response to acute anemia, bone morphogenetic protein 4 (Bmp4) modulates the activity of the transcription factor SMAD5 to induce the expansion of a specialized population of stress erythroid progenitors in the spleen and rapidly produce a large number of erythrocytes [43, 44] (Table 1). This pool of specialized progenitors is different from steady state progenitors residing in the mouse BM, and can rapidly produce great number of RBC [43, 45–47]. These cells originate from hematopoietic stem cells that migrate from the BM to the spleen. In the spleen, under stress erythropoiesis conditions, they become stress erythroid progenitors, which express different cell surface markers than steady state progenitors. These specialized progenitors acquire their characteristics due to the stress microenvironment in the spleen [40, 45, 46, 48].

Bmp4 requires hypoxia and SCF signaling, through its receptor c-KIT, to induce a response of splenic stress progenitors [44]. Hypoxia has a dual pathway of action; it maximizes sensitivity of progenitors to Bmp4 and SCF while regulating expression of *Bmp4* by Hif-2α. EPO has a critical role in this process, as immature splenic stress erythroid progenitors can

only differentiate if EPO synthesis is increased, while hypoxia potentiates the response to EPO [46]. In parallel, activation of the glucocorticoid receptor (GR) promotes self-renewal of erythroid progenitors, which is required for their rapid expansion [49]. Recently, a synergistic action of peroxisome proliferator-activated receptor alpha (PPARalpha) with GR was demonstrated, but the precise role of PPARalpha in erythropoiesis remains to be elucidated[50]. Hedgehog signaling is also implicated in stress erythropoiesis, but studies produced controversial results [45, 51]. The hedgehog pathway is an important signaling pathway for embryonic development, tissue homeostasis and stem cell biology. It can be assumed that some members of the hedgehog family induce erythroid progenitors to become stress progenitors, while others negatively regulate basal and stress erythropoiesis. Another important pathway is the Notch signaling pathway. In particular, NOTCH 2 receptor has a crucial role in erythroid differentiation and in recovery from stress erythropoiesis [52, 53].

Deficiency of NOTCH2 receptor in mice exposed to stress erythropoiesis resulted in decreased number of progenitors and inefficient stress response [52].

2.3 Erythroblastic islands

Erythroid development occurs in the presence of macrophages in structures called erythroblastic islands, which consist of a central machrophage surrounded by clusters of erythroblasts in different stages of differentiation. Adhesion interactions between erythroblasts and macrophages are necessary for erythroid development. Identification of the involved molecules has been intensively studied during the last decades in humans and mice. Some of these possible antigens include erythroblast macrophage protein (Emp), α_4, α_5, β_1, VCAM-1, ICAM-4 and SWAP70 [54, 55].

These niches are pivotal for erythropoiesis, as they create a microenvironment where cell to cell and cell to extracellular matrix interactions exert positive and negative regulatory mechanisms that control erythroid proliferation and differentiation [56]. Studies in mice provide valuable insight into these pathways. In fact, macrophage depletion in steady state erythropoiesis induces a mild iron deficiency anemia with reduced number of erythroblasts in the mouse BM [57, 58]. However, in stress erythropoiesis, lack of macrophages significantly impairs recovery from the anemia, which cannot be rescued by iron supplementation [54, 57, 58]. These observations indicate a crucial role of macrophages on erythroid expansion and reconstitution.

3. Ineffective erythropoiesis

IE can be characterized by abnormal differentiation or maturation of erythroid progenitors, as well as increased destruction of abnormal erythroblasts [59]. In IE, although proliferation and survival of erythroid progenitors is expanded, only a small proportion of cells mature, and a limited number of RBCs are produced, proportionally fewer than expected from the number of erythroblasts under normal conditions (Figure 1) [60]. The combination of increased proliferation and reduced differentiation results to a net increase of erythroid progenitors. This concept was first introduced when erythrokinetic studies in patients suffering from different types of anemia revealed an imbalance between BM activity and viable RBC released in peripheral blood circulation [61–63]. These observations led to the

discrimination between total and effective BM activity. In β-thalassemia, on which we focus for this review, IE represents a hallmark of the disease and has been extensively studied.

3.1 Thalassemia

Thalassemias are a hereditary group of disorders characterized by insufficient or absent production of α-or β-globin chains, which are the two major components of Hb [64]. Alpha thalassemia can present clinically with different phenotypes, from asymptomatic to lethal, where the severity of the anemia relates to the number of non-functional copies of the α-globin gene[65]. When three out of the four α-globin genes become inactivated, due to deletions with or without a nondeletion point mutation, excess β chains form β_4 tetramers called hemoglobin H (HbH) [66]. HbH disease is associated with moderate to severe anemia, hemolysis, splenomegaly and iron overload. Splenectomy is often recommended for these patients. The deletion of all four α-globin genes is the most severe form of the disease and usually results in hydrops fetalis during pregnancy and in-utero death of the affected embryo.

β-thalassemia is one of the most common causes of congenital anemias with a worldwide annual incidence of 1:100.000 [34]. It is caused by several hundred mutations in the β-globin gene or its promoter, which result in impaired production of β-globin chains [55, 67]. Decreased or absent β-globin synthesis correlates with different degrees of clinical severity ranging from asymptomatic carriers to severely anemic, transfuse-dependent patients. Thalassemia major (TM) patients are diagnosed early in life with severe anemia, hepatosplenomegaly and failure to thrive. They require chronic RBC transfusions for survival and iron chelation therapy to prevent or limit iron overload. The less severe form of the disease, called thalassemia intermedia (TI), presents later in life and is non-transfusion dependent. Although patients with TI do not require RBC transfusions early on, as the disease progresses the degree of the anemia might worsen, and splenomegaly develops over time. In that case, RBC transfusions may become necessary for their survival [19]. IE is a hallmark of the disease that results in progressive marrow expansion and extramedullary erythropoiesis (EMH), which occurs primarily in the spleen and the liver. The degree of expansion correlates with the severity of the anemia and can reach 30 times higher compared to normal volume [68]. As a result, patients with TM can develop characteristic bone deformities [69]. Finally, β-thalassemia trait patients may present with only a mild anemia and do not require any treatment [70].

The thalassemia syndromes can be alternatively classified phenotypically, based on the requirements for RBC transfusions, into two groups; transfusion-dependent thalassemias (TDTs) and not-transfusion dependent thalassemias (NTDTs). TDTs require regular RBC transfusions to survive and include TM and transfusion dependent HbH disease. NTDTs describe patients that do not require lifelong RBC transfusions and include TI and HbH disease.

Erythrokinetic and ferrokinetic studies in thalassemic patients provide an elaborative characterization of IE. Mildly to severe anemic β-thalassemia patients exhibit increased BM erythropoiesis ranging from 50% to 15%, respectively, in addition to shortened RBC survival [71]. Furthermore, studies of iron incorporation into RBC, by ^{59}Fe injections in

healthy and thalassemic individuals, demonstrate increased iron absorption but decreased output of ^{59}Fe in circulating RBC [72, 73]. As a matter of fact, in healthy subjects 75% to 90% of the injected ^{59}Fe is incorporated into newly formed RBC within 7 to 10 days, while in thalassemic patients this percentage is significantly lower, ranging from 15% to 20% [72, 74]. This difference is explained by iron sequestration in organs where erythroid precursors are destructed prematurely, such as the BM. In contrast, in HbH disease peripheral hemolysis is the major pathway of anemia and IE represents a minimal pathogenetic mechanism [75, 76].

4. Mechanisms leading to ineffective erythropoiesis

Different mechanisms are involved in the pathogenesis of IE in β-thalassemia. In the last decade, our understanding of the pathologic processes implicated has significantly improved. The use of murine models of β-thalassemia has contributed extensively to this purpose. These models present with a clinical phenotype similar to that of patients with β-thalassemia. However, it should be noted that mice cannot faithfully recapitulate all aspects of human erythropoiesis. These pathways, as well as important players associated with these processes are described below.

4.1 Increased apoptosis of thalassemic erythroid precursors

The early discovery of the discrepancy between increased proliferation of erythroid progenitors in the BM of thalassemic patients and low RBC counts in circulation led to the hypothesis of enhanced apoptosis of erythroid precursors. Indeed, multiple studies in TM, using human BM samples, verify this concept by demonstrating specific ladder patterns of DNA breakdown products or by using annexin V staining analyzed by Flow cytometry and TUNEL analyses [77, 78]. Moreover, two different quantitative measurements of apoptosis of erythroid progenitors, reflecting alterations in the plasma membrane and nucleus, revealed a 3- to 4-fold increase in programmed cell death in patients versus healthy controls [79]. A study using an in vitro system of erythropoiesis from human TM BM cells suggested the maturation of arrest at the polychromatophilic normoblast stage [78]. However, the cells were identified only morphologically and additional studies using more qualitative and quantitative assay may provide a more accurate description of this phenomenon and define at what stage thalassemic erythroid precursors undergo apoptosis.

4.2 Decreased differentiation of erythroid progenitors

Even though originally the main pathological mechanism responsible for the IE in β-thalassemia has been associated with increased apoptosis of erythroid precursors, recent observations highlight the contributing role of altered erythroid differentiation. A study using two different mouse models of β-thalassemia, i.e. TM and TI, demonstrates a significantly higher rate of proliferation of splenic erythroblasts in the presence of elevated EPO concentration, in vitro and in vivo[80]. Specifically, these are poorly differentiated cells expressing high levels of proteins involved in cell cycle such as EPOR, JAK2, BCL-XL, CDK2, KI-67 and CycA. Similar results were obtained when human TM peripheral hematopoietic stem cells were cultured to generate erythroblasts [81]. Thalassemic erythroid progenitors had a higher absolute expression of *JAK2, CYCA, BCL-XL and KI-67*. Immunohistochemical analysis of these erythroid progenitors at different stages of

maturation revealed upregulation of proliferative marker KI-67. In addition, spleen sections of TM patients had increased number of KI-67 positive erythroblasts [80]. All these results indicate a high rate of proliferation of thalassemic erythroid progenitors. In TM and TI mice, studies of erythroid progenitors at different stages of maturation in the BM and spleen, demonstrate an increased percentage of immature progenitors with a combined reduction in the mature subpopulation. Finally, no differences were noted in apoptosis, compared to controls, when assessed by three different methodologies.

Although the mechanisms leading to decreased differentiation have not yet been elucidated, transcription factor ID1 may be an important player. *ID1* is upregulated in erythroid precursors by the JAK/STAT pathway. High concentration of ID1 inhibits cell differentiation therefore continuous activation of JAK2 could hinder erythroid maturation [19, 68]. An additional mediator could be the transcription factor FOXO3. Indeed, loss of *Foxo3* in mice results in impaired antioxidant response and mitotic arrest in intermediate erythroid precursors, leading to decreased erythroid maturation [82]. Collectively these abnormalities resemble the process in IE. Moreover, murine absence of Foxo3 induces activation of JAK/AKT/mTOR signaling pathway in immature erythroblasts [83]. Hyperactivation of mTOR alters cell cycle progression and maturation rate (Figure 2). Different studies indicate the significance of this pathway in the pathogenesis of IE. Pharmacologic inhibition of mTOR signaling in thalassemic mice enhanced production of mature RBC and improved the anemia [83]. In another approach, administration of resveratrol, a natural polyphenolic stilbene, in thalassemic mice activated Foxo3 and inhibited Akt signaling. After treatment, the animals demonstrated amelioration of IE and increased RBC survival [84].

This model describes a vicious circle that contributes to IE and exacerbates the anemia. Hypoxia induces the production of EPO, which acts on an increasing number of erythroid progenitors through EPOR. As a result, persistent phosphorylation of JAK2 leads to survival and active proliferation and expansion of erythroid progenitors[19]. While the erythron increases, the spleen becomes a primary site of EMH [59, 60]. Consecutively, splenomegaly can result in splenic sequestration of RBC and deterioration of the anemia [85]. This mechanism is also supported by the observation that in thalassemic mice, the Hb concentration inversely correlates with weight of the spleen and degree of EMH [80].

4.3 Oxidative stress

In β-thalassemia, the defect in β-globin chain production results in the excess of α-globins. Free α-globins form aggregates that are unstable and may autoxidize, denature and precipitate as hemichromes within the cells. The result is a high production of reactive oxygen species (ROS), free heme and iron (Figure 3) [86]. Free iron generates ROS through the Fenton reaction [87]. In fact, increased production of ROS has been documented in RBC isolated from thalassemic patients [88, 89]. ROS can oxidize lipids, nucleic acids, cellular proteins and alter the activity of transcription factors, membrane channels and signaling pathways [90]. Furthermore, pathological free iron initiates self-amplifying redox reactions that induce free hemoglobin oxidation and membrane destabilization [91]. As a result, extensive oxidizing damage on RBC can promote their premature death by hemolysis, thus contributing to IE.

Oxidative stress can cause severe alterations in membrane stability, cellular hydration, and on cytoskeleton proteins [90]. Hemichromes bound to the cytoplasmic membrane of erythroid progenitors mediate the phosphorylation of the cytoplasmic domain of band-3, an anion transmembrane transport protein [92]. This results in reorientation of intramembrane and extracellular domains, band-3 aggregation and disruption of ankyrin binding [93]. The newly formed band-3 clusters act as an antigen and bind with high affinity to IgG anti-band 3 antibodies and complement fragments providing a signal for removal from macrophages [94–97]. Moreover, the disrupted function of band-3 results in decreased concentration of NADPH, an anti-oxidant agent and exacerbates the oxidative stress [86]. Another important pathway is the oxidation of protein 4.1, a member of the cytoskeleton in erythrocytes. Defective protein 4.1 results in its reduced ability to bind with spectrin, as well as in ineffective interactions between spectrin and actin, leading to unstable and fragile RBC [90, 98, 99]. α-globin aggregates form early in erythroid differentiation and colocalize with areas of defective association between spectrin and protein 4.1 in β-thalassemic erythroblasts. This could result in the oxidation of the cytoskeletal proteins, since α-globin aggregates tend to autoxidize.

4.4 Iron Metabolism

Iron overload is an important and potentially devastating complication of β-thalassemia as the excess free iron deposits in organs, particularly in the heart and liver, and contributes to the morbidity and mortality of the disease [100, 101]. Although regular RBC transfusions are the main cause of iron overload in TM, dysregulated iron absorption from the gastrointestinal tract also contributes to iron accumulation in both TM and TI [68, 102, 103]. In fact, studies in non-transfused patients demonstrate a 3-to 4-times fold increase in iron loading from the enterocytes than normal [59]. Research in the last years has shed light on the mechanisms controlling iron regulation and on the intertwined relation between iron loading and IE (Figure 4).

Iron enters in circulation either by absorption of dietary iron through the enterocytes or by recycling Hb from senescent RBC through macrophages. The main regulator of systemic iron homeostasis is hepcidin, a 25-aa peptide produced predominantly in the liver [100]. Under normal conditions hepcidin is released in response to plasma iron concentration and iron stores, and binds to ferroportin (FPN-1), the only currently known iron exporter found primarily on the surface of duodenal enterocytes, macrophages and hepatocytes. Binding with FPN-1 results in internalization and degradation of the latter which prevents iron export into the circulation [74, 104]. Thus, in presence of high iron concentration increased expression of hepcidin reduces the amount of iron exported from enterocytes and macrophages. Both intracellular and extracellular iron concentration play a key role in hepcidin regulation. In circulation, the serum protein transferrin (TF) binds to iron and transports it to the erythron or peripheral tissues. The uptake of iron bound to TF by transferrin receptor 1 (TFR1) is the only known pathway of iron delivery for erythropoiesis. Increased iron plasma concentration is sensed by the hemochromatosis protein (HFE)/TFR1 complex and transferrin receptor 2 (TFR2) which trigger hepicidin production [105–109]. Although the signaling pathways activated in this process are still not completely understood, bone morphogenetic protein (BMP)/SMAD signaling is involved. Bone morphogenetic protein 6 (BMP6) interacts with

the co-receptor hemojuvelin and BMP receptors type I/II to phosphorylate and activate the SMAD1/5/8-SMAD4 complex and induce hepcidin expression [110–114]. In the liver elevated intracellular iron concentration can also stimulate *BMP6* expression and increase hepcidin production [115, 116]. On the other hand, transmembrane protease serine 6 (TMPRSS6), an enzyme that cleaves hemojuvelin from the cell surface, is a negative regulator of hepcidin [117–120].

4.4.1 Iron Metabolism and erythropoiesis—Hepcidin expression is controlled by multiple stimuli including hypoxia, erythropoiesis and inflammation [121–125]. In several diseases, including chronic inflammatory conditions, malignancies or chronic infections, hepcidin concentration is increased, causing retention of iron in macrophages and enterocytes resulting in a type of iron restricted anemia called anemia of inflammation (Table 1). In presence of elevated EPO concentration, hypoxia and increased erythropoiesis, hepcidin expression is reduced resulting in increased iron availability for the production of RBC [59, 123, 126, 127]. Therefore, it is not surprising that several studies in both mice and patients affected by β-thalassemia detected decreased hepcidin concentration [103, 128–133]. Downregulation of hepcidin is essential in resolving acute stress, but chronically reduced concentration will increase iron load. In fact, inappropriately suppressed production of hepcidin causes systemic iron overload even in non-transfused thalassemic patients [68]. The suggested mechanisms that reduce hepcidin expression include the presence of molecules called erythroid regulators. A member of the transforming growth factor-beta superfamily (TGF-β) i.e. growth differentiation factor 15 (GDF15), and twisted-gastrulation 1 (TWSG1), a BMP binding protein, have been proposed as such molecules [106, 134–136]. Nonetheless, results are inconsistent, and participation of these molecules in suppression of hepcidin has not been definitively demonstrated [106, 137–140]. Recent studies suggest that the hormone erythroferrone (ERFE), whose expression is induced by EPO, may be an erythroid regulator. In a mouse model of TI high concentration of ERFE was found in the BM and spleen. Ablation of ERFE increased hepcidin concentration and decreased hepatic iron overload, suggesting that ERFE contributes to hepcidin suppression [139, 141].

In β-thalassemia, iron and IE are linked in a complicated relationship. A study using two different mouse models of β- thalassemia demonstrated that the different degrees of IE dictated the pattern of iron distribution [130]. In TM mice, hepcidin concentration was very low due to the extreme IE that prevented hepcidin from sensing the iron load. In contrast, in a TI model hepcidin expression was relatively low during the first months of life but increased as iron overload worsened. This indicates that, under conditions of milder IE, hepcidin can still respond partially to iron status. Moreover, when mice suffering from severe anemia received RBC transfusions, their hematopoietic parameters and IE were improved while hepcidin expression was increased [130]. This effect has also been demonstrated in human patients [103, 129, 142]. All the above suggest that the expression of hepcidin is determined by the relative levels of IE and iron load.

4.5 Erythroid iron metabolism and the role of classic iron related molecules in erythropoiesis

4.5.1 The role of TFR1 and TFR2 in erythropoiesis—Iron is essential for efficient erythropoiesis and for that reason must be readily available for erythroid differentiation.. A study disrupting the *Tfr1* gene in mice underlined the importance of this protein for erythropoiesis [143]. Homozygous deletion of the gene was lethal and embryos were severely anemic with signs of hydrops fetalis. In addition to its canonical function, TFR1 participates in another fundamental signaling pathway. Polymeric IgA1 (pIgA1) is produced by plasma cells in hypoxia and binds to TFR1 independent of TF, activating the Erk/Akt signaling pathway [144, 145]. This action of pIgA1 is additive to TF and potentiates erythroblast response to EPO, therefore promoting erythroid expansion. Recent data suggest that decreasing TFR1 concentration might ameliorate IE in β-thalassemia [146]. In this study, reduced TFR1 concentration in mice affected by TI improved hematopoietic parameters and decreased α-globin precipitation in circulating RBC. These beneficial effects will need to be confirmed with further studies but indicate that targeting TFR1 may be a novel promising approach in improving IE. Other classic iron related molecules, initially discovered in the liver, might also play a role in erythropoiesis. For instance, it has been suggested that HFE is also expressed on erythroid progenitors, forming complexes with TFR1 [147–151]. It is then possible that HFE may compete with the overlapping sites of TFR1 for TF, regulating erythroid iron uptake [105]. TFR2 is also important for normal erythropoiesis. *TFR2* is expressed during erythroid differentiation and forms a complex with EPOR, possibly altering sensitivity of erythroid precursors to EPO [107, 152–157]. In a recent study, lethally irradiated mice transplanted with *Tfr2* deficient hematopoietic cells had increased RBC in the periphery and differentiated erythroblasts in the BM [158]. The mice had normal serum EPO concentration, but erythroblasts had increased expression of EPO related genes i.e. *Epor*, *Erfe*, *Bcl-X*, concurring with the hypothesis of increased sensitivity to EPO. Thus, in addition to its direct role in the regulation of hepcidin in hepatocytes, Tfr2 might also contribute to the regulation of erythroid maturation.

4.5.2 The IRE/IRP regulatory system—Intracellular iron concentration regulates the expression of genes involved in iron import, export, transport, storage and usage at the post-transcriptional level. Several iron-related transcripts contain untranslated mRNA sequences called iron responsive elements (IREs) that are recognized and bound by cytoplasmic iron responsive proteins (IRPs) under low-iron conditions [159–163]. IRPs can act both as translational enhancers or silencers depending on the position of the IRE on the mRNA; they promote iron storage, export and usage in presence of high iron concentration and induce iron absorption and transport in iron deficiency [159, 161, 162, 164–170]. Recently, studies in mice demonstrated that deficiency of *Irp1* or *Irp2* resulted in different erythroid defects and revealed a new regulatory axis through Hif2α [171–173]. Deficiency of *Irp1* increased stabilization of *Hif2α* mRNA, resulting in increased EPO concentration and polycythemia. Lack of *Irp2* led to microcytosis, decreased *Tfr1* mRNA and increased ferritin and Alas2 concentrations [174, 175].

4.5.3 Heme and erythropoiesis—In addition to being an essential component of Hb, heme participates, as an important regulatory molecule, in erythropoiesis. It can interact with

IRP2, resulting in degradation of the latter, and directly induce the transcription of specific genes, by binding to the transcriptional repressor Btb And Cnc Homology 1 (BACH1) [176–181]. This pathway has been suggested to have a role in activating transcription of the globin genes [180, 182, 183]. Transcription of the β-globin gene can be repressed by BACH1 or induced by nuclear factor erythroid-derived 2 (NF-E2) [184–188]. In an in vitro system, inhibition of heme biosynthesis in human erythroleukemia cells decreased the concentration of α- and β-globin chains. Moreover, intracellular heme concentration was found to control BACH1 and NF-E2 recruitment [180, 182]. In a separate study, it was demonstrated that heme promoted the displacement of BACH1 from the β-globin locus control region [183].

At the translational level heme can affect globin protein synthesis through the heme-regulated eIF2α kinase (HRI), which is predominantly expressed in hemoglobinized erythroblasts [189, 190]. When heme concentration is low, HRI autohosphorylates and subsequently phosphorylates the α subunit of the eukaryotic translation initiation factor 2 (eIF2α) that inhibits protein synthesis [191]. On the contrary, during Hb synthesis heme binds to a heme binding domain on HRI, which as a result becomes inactive, allowing the synthesis of the β-globin genes [190–192]. Therefore, HRI balances the production of heme and globins to ensure that no excess globins are produced. In β-thalassemia HRI may have an effect on disease severity. In a mouse model of TI *Hri* deficiency was lethal; however, *Hri* haploinsufficency did not affect embryonic survival but led to a more severe phenotype with increased α-globin aggregates accumulation and reduced life span compared to their thalassemic counterparts [193]. These results point to a critical role of HRI for erythroid progenitor survival. Further studies are needed to elucidate the potential use of HRI as a therapeutic target in β-thalassemia to decrease α-globin accumulation and ameliorate disease severity.

4.6. Molecular Chaperones

4.6.1 HSP70—Complex mechanisms promote differentiation and maturation of erythroid progenitors. The erythroid transcription factor, or GATA-binding factor 1 (GATA-1), holds a pivotal role in this process, by regulating the expression of erythroid promoters and anti-apoptotic genes, such as *BCL-XL* [194]. In order to advance survival and differentiation of progenitors, it is necessary that specific cysteine-aspartic proteases, called caspases, are activated. As erythroid cells mature, caspases become transiently active and mediate crucial pathways of terminal differentiation, such as chromatin condensation and cell size reduction [90]. Caspases have paradoxical effects on erythropoiesis. In addition to their role in erythroid maturation, they are required for programmed cell death. Two different types of caspases (initiators and effectors) finally lead to DNA damage and apoptosis [195]. One of the effectors involved is caspase-3, which targets and cleaves GATA-1. It is evident that during erythroid differentiation protective mechanisms are needed to avoid cell death. Recently, the chaperone heat shock protein 70 (HSP70) was identified to protect GATA-1 in this process [11].

HSP70 is a ubiquitous chaperone that is constitutively expressed in human erythroblasts. During late stages of erythroid differentiation, when caspases become active at the basophilic stage, HSP70 colocalizes in the nucleus with GATA-1 and protects it from

cleavage. EPO has a central function in this pathway by regulating cellular localization of HSP70 [11, 196]. In presence of low EPO concentration reduced nuclear HSP70 translocation results in less GATA-1 bound to HSP70. Free GATA-1 is prone to cleavage by caspase-3. In β-thalassemia HSP70 may be an important player that promotes IE. In vitro studies demonstrated that erythroblasts derived from TM patients contain low concentration of HSP70 and GATA-1 in the nucleus, while HSP70 is mainly localized in the cytoplasm [197]. The same observations were noted in BM samples of TM patients. The decrease in concentration of nuclear HSP70 is due to interactions between HSP70 and free α-globin chains, but not Hb, in the cytoplasm. Concentration of HSP70 and GATA-1 in the nucleus decreases as maturation and hemoglobinization progress. It is hypothesized that as HSP70 forms complexes with excess free α-globin chains in the cytoplasm, GATA-1 remains unprotected and is cleaved by caspase-3. In fact, restoring nuclear concentration of HSP70 in TM erythroblasts, by lentiviral transduction, improves terminal maturation and decreases apoptosis [197]. Although further research is necessary, increasing nuclear concentration of HSP70, in order to protect GATA-1, may be an alternative approach to improve IE. To this end, targeting the interactions between HSP70 and α-globin chains in the cytoplasm or decreasing HSP70 nuclear export could be beneficial for patients.

4.6.2 AHSP—The α-Hb stabilizing protein (AHSP) is an important chaperone that protects the erythroid cells from free α-globin chain toxicity. It binds to free α-globin chains, but not β chains or HbA, stabilizes them and prevents their denaturation [196, 198]. AHSP is an erythroid specific chaperone primarily found in late stage erythroid precursors [196, 199]. Interactions between AHSP and free α-globin chains result in the formation of a stable complex that prevents aggregation, precipitation and autoxidation of α chains. In addition, AHSP binds to α-Hb (unmatched α-globin chains bound to heme) and stabilizes it, by inhibiting hemebound iron to participate in any reactions that generate ROS. Expression of this chaperone is induced by GATA-1. It is also affected by iron status, due to the presence of a functional iron response element located in the 3' untranslated region of the *AHSP* gene [200, 201].

In β-thalassemia, IE depends on the concentration of free α-Hb chains that induce oxidative stress and damage erythroid progenitors. Therefore, it can be postulated that AHSP concentration is associated with disease severity. In patients with β-thalassemia inadequate concentration of AHSP results in increased hemolysis and severe anemia that requires frequent RBC transfusions [196, 199]. Studies in mice provide important information on the significance of this chaperone and its association with disease. In normal mice loss of *Ahsp* results in increased production of ROS, abnormal morphology and decreased life span of erythrocytes. These AHSP deficient mice demonstrate an increased proportion of immature erythroid progenitors in the spleen, maturation arrest and increased apoptosis of erythroid precursors, reflecting the setting of IE [90, 202]. In a mouse model of TI, absence of *Ahsp* worsens the anemia and morphology of erythrocytes and exacerbates α-globin precipitation.

In contrast, in TI mice, overexpression of *Ahsp* failed to alleviate the severity of the disease and had no effect on hematopoietic phenotype [203]. In addition, several studies in thalassemic patients did not detect an association between polymorphisms in the *ASHP* gene and disease severity [91, 201, 204, 205]. Further research is necessary to address important questions

regarding the role of AHSP as a disease modifier in β-thalassemia. This patient population is heterogeneous and studies looking at the *ASHP* gene sequence in relation with the patient genome could advance our current knowledge. AHSP can be potentially used in the treatment of β-thalassemia to limit α-globin precipitation, formation of ROS and ameliorate IE. For instance, a potential approach could be to target the specific mechanisms that lead to α-Hb stabilization or to produce mimetic proteins [201].

5. The erythroblastic island in β-thalassemia

Since macrophages play an important role in maintaining erythropoiesis in conditions of acute stress, it is not unexpected that their depletion in β-thalassemia, a condition of chronic stress erythropoiesis, could limit sustained erythropoiesis. This concept was tested by using clodronate-loaded liposomes to deplete macrophages in a mouse model of the disease. Treatment rapidly ameliorated the anemia, noted by increased Hb and RBC number. In addition, treated animals had decreased numbers of immature erythroid progenitors and proportionally increased differentiated erythroblasts in the BM and spleen, indicating an improvement of erythropoiesis [57]. A concurrent reductive effect was also noted on spleen size and EMH. Furthermore, serum iron concentration was decreased and hepcidin expression was elevated, indicating improved iron status, which could also contribute to the amelioration of IE. Chronic use of clodronate-loaded liposomes sustained the beneficial effect in reducing IE and significantly improved the morphology and life span of RBC. In addition, α-globin chain aggregates were also decreased [57].

The effects promoted by macrophage depletion can be elucidated by better understanding macrophage-erythrocyte interactions. The effect that macrophages exert on erythroid development seems to be dependent on direct contact of the two different cell types. In vitro, when erythroblasts derived from human thalassemic patients are cultured in the presence of macrophages they proliferate in a higher rate than when cultured alone [57]. This response is accompanied by a higher percentage of cells cycling and reduced numbers of differentiated enucleated cells. On the contrary, in conditions were the erythroblasts do not come in contact with the macrophages the positive effect on proliferation is hindered. These observations point to a critical role of macrophages in erythroid proliferation and survival.

In a clinical setting depletion of macrophages could be detrimental for patients, as it could compromise the immune response and increase the risk of infections. Nonetheless, targeting the interactions between erythroblasts and macrophages in the erythroblastic island could be a novel therapeutic strategy to improve IE. This approach would aim at the specific molecules involved in cellular crosstalk, while immunologic function would remain unaffected.

6. Targeting Ineffective erythropoiesis: novel potential therapies

Managing IE still remains a difficult task. Current regiments can only partially alleviate the phenotype of the disease, allowing IE to have detrimental effects on physiologic processes. The discovery of new therapeutic agents that could substantially improve erythropoiesis would reduce the need for RBC transfusions and improve quality of life of patients.

Extensive research in the last years has focused on this aim, by targeting the specific mechanisms that mediate IE (Figure 5).

6.1 Targeting iron metabolism

Targeting iron could be beneficial for the improvement of IE. Decreased iron availability in the erythron would reduce the production of erythroid cells carrying abnormal Hb and therefore would limit the quantity of hemichromes and improve erythropoiesis. In this direction, several different therapeutic molecules have been studied. Synthetic small peptides called mini-hepcidins act like hepcidin agonists [206]. These molecules can bind to FPN-1 and decrease serum iron concenration. Administration of mini-hepcidins in mouse models of hemochromatosis, a disease characterized by abnormal iron accumulation in parenchymal organs, demonstrated promising results [206, 207]. The treated mice had reduced iron absorption and increased iron redistribution in splenic macrophages, which led to decreased iron concentration in the liver and heart. Furthermore, the beneficial effects of low iron on IE have been demonstrated in a mouse model of TI. Mice were either placed on low iron diet or were genetically engineered to moderately overexpress hepcidin. In both cases, limiting iron overload decreased the formation of α chain/heme aggregates and ROS while reversed IE and splenomegaly [208]. The mice demonstrated improvement of anemia and amelioration of RBC structure and lifespan. In accordance with these results, when mini-hepcidins were tested in the same mouse model the same positive effects were noted on hematopoietic parameters and IE [209, 210]. In addition, the mice had decreased total iron concentration in the liver, kidney and spleen.

Another promising approach is targeting the serine protease TMPRSS6, which physiologically attenuates expression of hepcidin. By decreasing the activity of TMPRSS6, iron concentration will reduce and IE should ameliorate. As a matter of fact, when *Tmprss6* was deleted from a mouse model of TI the outcome was an improvement in hematologic parameters, splenomegaly and IE [211]. Pharmacologic inhibition of *Tmprss6,* by second generation antisense oligonucleotides or lipid nanoparticle (LNP)-formulated siRNAs, demonstrated a beneficial effect in mice affected by TI. Treated mice had lower concentration of ROS, hemichromes and apoptosis along with improvement of IE, RBC survival and splenomegaly [212, 213]. Recent studies suggest that a combinational therapy of iron chelators and Tmprss6 inhibitors has a superior effect in decreasing liver iron overload than each monotherapy alone or dietary iron restriction, reflected in a lower degree of IE [214, 215].

Reducing the amount of iron delivered to the erythron by each molecule of TF could ameliorate the anemia and improve the degree of IE. A study using apotransferrin (apo-TF), i.e. TF not bound to iron, in thalassemic mice demonstrated that apo-Tf administration altered the proportion of erythroid precursors to more mature versus immature and decreased α-globin precipitation and apoptosis of mature erythroid precursors [216]. Moreover, chronic treatment with apo-TF normalized the anemia and iron content in the liver, heart and kidney [217]. Concomitantly iron regulation was modified, noted by decreased expression of ERFE and increased concentration of hepcidin. The proposed rationale behind these observations lies in difference in TFR1-mediated iron delivery via holo-TF (with two bound iron

molecules per Tf molecule) compared with monoferric-TF (with one). Not only does diferric Tf deliver twice the number of iron molecules per cycle, it binds TFR1 with greater affinity than does monoferric TF. Providing exogenous apo-TF leads to a fall in transferrin saturation and a shift in the relative population of TF molecules to the monoferric form. As such in treated mice there is decreased iron delivery to erythroid precursors, resulting in lower mean corpuscular hemoglobin (MCH) and decreased hemichrome formation [60].

All these results suggest that therapeutic strategies modulating iron metabolism can be beneficial in reducing oxidative stress and significantly improving IE. By aiming at the pathogenetic mechanism of iron overload, these treatments offer a novel approach that could produce better results than current regiments.

6.2 Use of JAK2 inhibitors

JAK2 activation is a critical process in sustaining IE and developing EMH in thalassemic patients. In addition, several studies indicate a potential role of JAK2 in iron regulation [68, 218]. For instance, STAT5 controls the expression of *IRP2* and *TFR1* [219, 220]. The data suggest that activation of the JAK/STAT5 pathway induces, both directly and indirectly, iron uptake. To that end, the use of JAK2 inhibitors (JAK2i) seems as a promising therapeutic tool. Administration of JAK2i in a mouse model of TI led to a significant decrease in spleen size and IE [80]. In human patients, this approach could translate to an efficient substitute of therapeutic splenectomy [19, 85]. Currently, splenectomy is often the best treatment option in patients with excessive RBC transfusion needs or massive splenomegaly that prevents sufficient control of iron concentration. However, splenectomized patients have a higher risk for certain complications, such as infections and pulmonary hypertension [19]. Therefore, treatment with JAK2i could be a useful alternative for this group of patients. Moreover, as spleen size decreases the degree of blood sequestration will reduce and the need for RBC transfusions will be limited [55]. Data of clinical trials using JAK2i in patients suffering from myeloproliferative syndromes demonstrate a beneficial effect in reducing spleen size [221]. These results have to be carefully evaluated since side effects, including anemia, were also documented [222]. Such an effect could be detrimental in thalassemic patients but may be potentially avoided by a short-term use of JAK2i or with the combination of RBC transfusions. Future studies will be required to evaluate the benefits and risks of JAK2i use.

6.3 TGF-β superfamily: a new role in erythropoiesis

Recently, an innovative approach was developed to improve IE by targeting a pathway that is distinct from the EPO/JAK/STAT. This novel method focuses on regulation of erythropoiesis by members of the TGF-β superfamily, which include BMPs and growth differentiation factors (GDFs). All these ligands bind to a type II receptor and consequently phosphorylate and activate a type I receptor. Complexes containing different combinations of type I and II receptors activate SMAD signaling transcription factors, which then accumulate in the nucleus and alter gene expression [223]. The interest in erythropoiesis lies in signaling through activin receptors type IIA (ActRIIA) and IIB (ActRIIB) that activate the SMAD2/3 proteins. High activation of phosphorylated SMAD2/3 has been documented in splenic erythroid progenitors of thalassemic mice, a finding that indicates a relation between activation of SMAD2/3 and IE [224–226].

Pharmacologic inhibition of this pathway has been achieved by the development of two different receptor fusion proteins that consist of the extracellular domain of either ActRIIA (Sotatercept, ACE-011 or its mouse version RAP-011) or ActRIIB (Luspatercect, ACE-536 or its mouse version RAP-536) linked to the Fc protein of IgG and act as ligand traps. Use of these two agents in mouse models of TI not only significantly improved the anemia and degree of IE, but also reduced complications of the disease [224, 226]. Treatment improved erythroid differentiation with decreased immature and increased later stage erythroblasts. Furthermore, administration of RAP-011 promoted apoptosis of immature erythroid cells by inducing expression of *Fas* and *FasL* in early erythroblasts [226]. It is therefore suggested that inhibition of activin receptors contributes to improvement of IE with a dual mechanism: stimulation of terminal erythroid differentiation and normalization between proliferation and differentiation. A positive effect was also noted on iron status, spleen size and oxidative stress [224, 226]. The drugs reduced α-globin aggregates, ROS concentration and abnormal erythroid morphology, indicated by reduced hemoglobin debris and degree of poikilocytosis, observed in peripheral blood smears of treated animals.

In search of the possible ligands for RAP-011 and RAP-536, growth differentiation factor 11 (GDF11) has been identified as a potential candidate. The data indicate that GDF11 exerts negative regulation on late stage erythroid differentiation [226–228]. Studies in mice and humans suggest that IE is associated with GDF11 overexpression in relatively immature erythroblasts mostly in the spleen where EMH takes place [226, 227]. In support of this hypothesis, patients with β-thalassemia have high concentration of GDF11 in the serum [233]. It is therefore proposed that these ligand traps act by binding with GDF11 and inhibiting SMAD2/3 phosphorylation. Furthermore, it is suggested that oxidative stress can induce expression of GDF11, which successively promotes ROS, resulting in an autocrine amplification loop innate to IE. These results combined, indicate that inhibiting the TGF-β signaling pathway can be an excellent therapeutic regiment for patients suffering from diseases associated with IE. Currently, ongoing phase 2 and 3 clinical trials are testing the efficacy of these agents in patients with β-thalassemia.

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Practice points

- IE is a major contributor to the pathogenesis of β-thalassemia.
- IE is characterized by increased numbers of erythroid progenitors in the BM and disproportionally decreased circulating RBC.
- Management of β-thalassemia includes RBC transfusions and iron chelation.
- Emerging treatments are under development to improve erythropoiesis and the consequent iron overload.

Research Agenda

- Assess the use of agents targeting iron metabolism such as mini-hepcidins and TMPRSS6-ASO in clinical studies.
- Target iron absorption in the duodenum as a therapeutic approach in mouse models of β-thalassemia.
- Better understand the role of erythroblast transferrin receptors in erythropoiesis and their potential for targeting in the treatment of β-thalassemia.
- Analyze *HSP70* gene sequence in a large heterogeneous patient population with different degrees of clinical severity to understand its role as a genetic modifier.
- Develop approaches to protect GATA1 from cleavage in erythroid cells.
- Further characterize the role of the TGF-β related molecules in erythropoiesis
- Identify the specific molecules involved in macrophage-erythroblast interactions.
- Evaluate the use of JAK2 inhibitors in combination with RBC transfusions.
- Better understand the role of HRI as a modifier of disease severity in β-thalassemia.

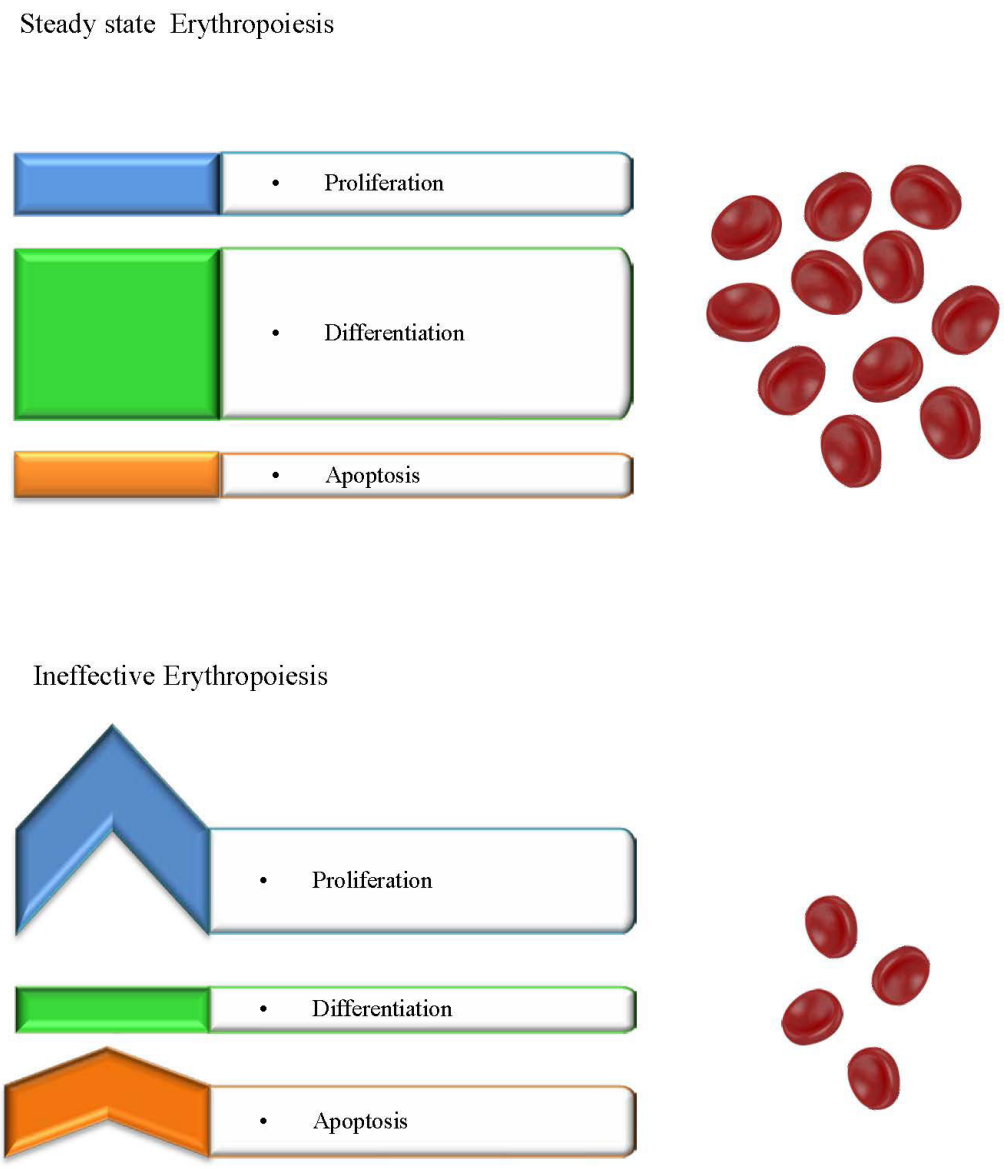

Figure 1. Steady state and Ineffective Erythropoiesis

Steady state erythropoiesis is homeostatic and results in the production of red blood cells in a constant rate. In IE, decreased differentiation combined with increased proliferation and apoptosis of progenitors results in the production of limited RBCs.

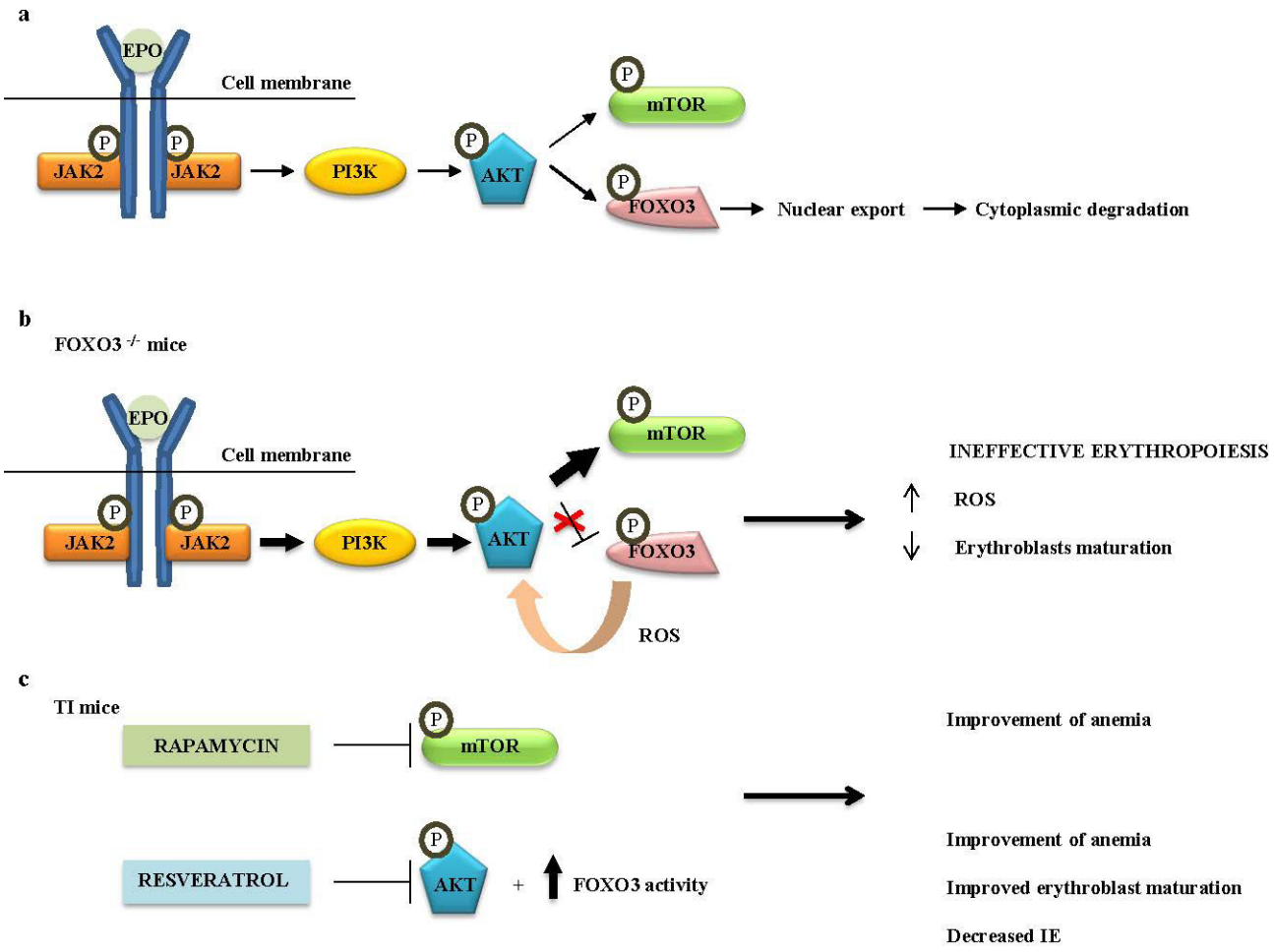

Figure 2. The role of FOXO3-mTOR cross talk in β-thalassemia

a. EPO stimulation promotes activation of phosphoinositide 3-kinase (PI3K). PI3K phosphorylates AKT kinase, which phosphorylates FOXO3 and induces its nuclear export, resulting in inhibition of FOXO3 transcriptional activity. AKT kinase also phosphorylates and regulates mammalian target of rapamycin (mTOR) kinase. **b.** In FOXO3 deficient mice (FOXO3 $^{-/-}$) lack of FOXO3 increases activation of JAK2/AKT/mTOR signaling. Impaired antioxidant response, due to FOXO3 deficiency, increases ROS that partially mediate hyperactivation of JAK2/AKT/mTOR signaling. FOXO3 deficient erythroblasts exhibit abnormalities similar to IE. **c.** Inhibition of mTOR signaling by rapamycin in TI mice, improved the anemia, implicating mTOR signaling in the pathogenesis of IE. Resveratrol inhibits AKT kinase and increases activation of FOXO3. Resveratrol treatment of TI mice accelerated erythroblast maturation and decreased IE.

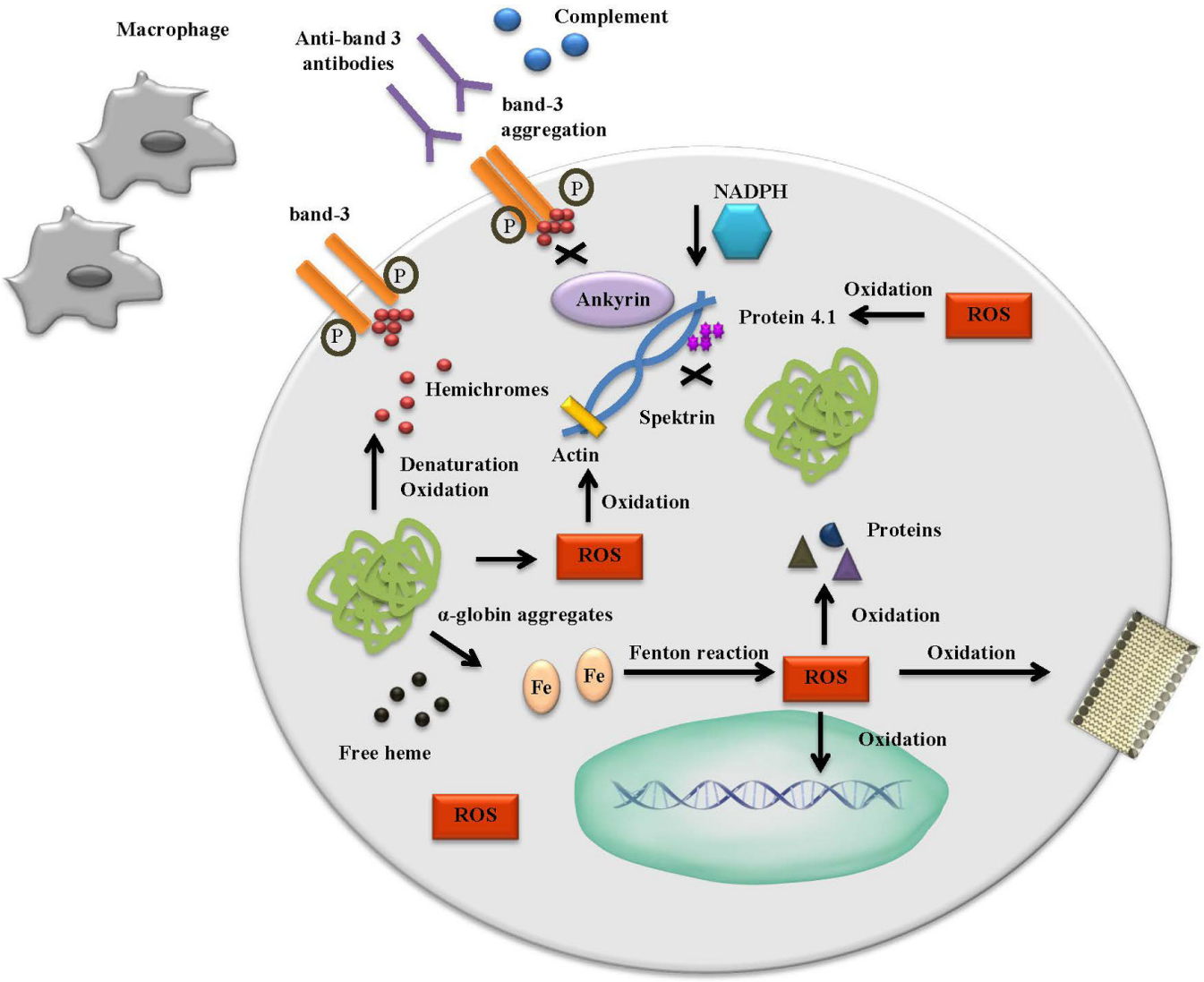

Figure 3. Oxidative Stress in β-thalassemia

Free α-globins form aggregates that autoxidize, denature and precipitate as hemichromes. As a result, ROS, free heme and iron are produced. ROS are also produced thought the Fenton reaction from free iron. ROS oxidize lipids, nucleic acids and cellular proteins. Hemichromes mediate the phosphorylation of the cytoplasmic domain of band-3, resulting in band-3 aggregation and disruption of ankyrin binding. Anti-band 3 antibodies, together with complement, recognize band-3/hemichrome clusters and mediate phagocytosis of erythrocytes by macrophages. Disruption of band-3 leads to decreased concentration of NADPH further inducing oxidative stress. ROS induce oxidation on protein 4.1, spektrin and actin leading to unstable and fragile RBC.

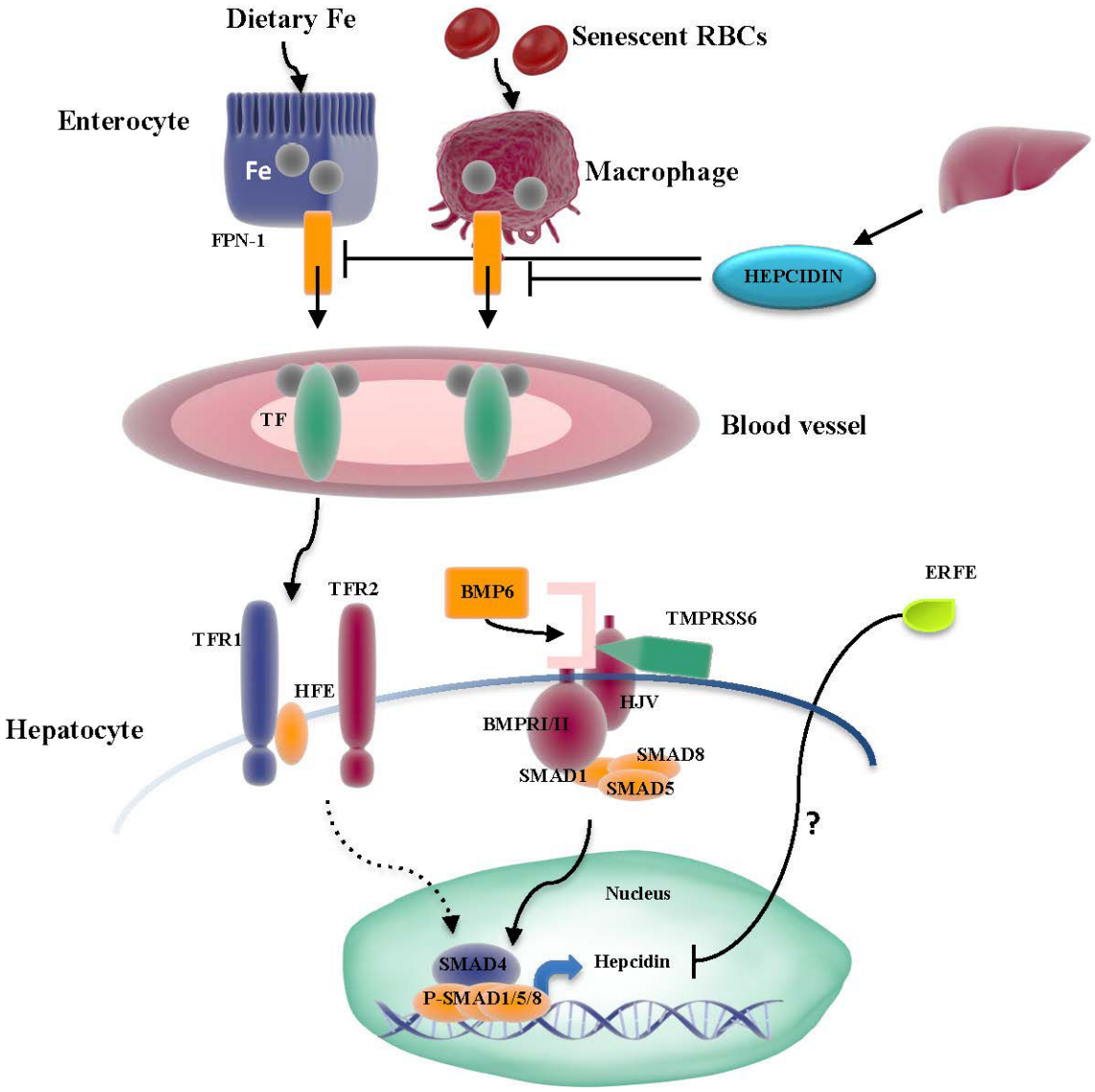

Figure 4. Important players in Iron Metabolism

Dietary iron absorbed by the enterocytes and iron recycled from senescent RBC through macrophages gets exported through FPN-1 and successively binds to TF in blood vessels. TF-Iron complexes get transferred to hepatocytes. In this process BMP6 has a critical role. Increased intracellular iron concentration trigger BMP6 expression. In turn, BMP6 binds to type I and II receptors and co-receptor HJV that consequently activate SMAD1/5/8. In association with SMAD 4 they upregulate hepcidin expression, which inhibits iron export in the circulation. Furthermore, in hepatocytes, the relative binding of TF to TFR1 versus the HFE/TFR2 complex modulates hepcidin expression, potentially through the SMAD pathway (dotted line). On the contrary, the enzyme TMPRSS6 cleaves HJV and negatively regulates hepcidin expression. High EPO levels induce expression of the erythroid regulator ERFE in erythroblasts, which decreases hepcidin expression by a mechanism not characterized yet (indicated by a question mark).

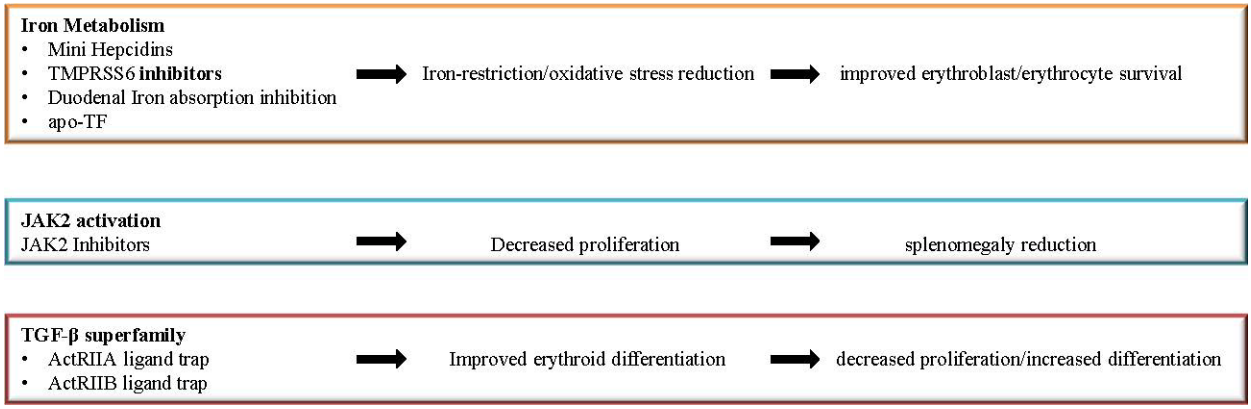

Figure 5. Novel treatments for β-thalassemia

Current management of β-thalassemia with RBC transfusions and iron chelation does not efficiently address the pathophysiology of IE. As described in the text, novel drugs are being developed to target proteins that modulate erythropoiesis, iron metabolism or the activity of some of the ligands of the TGF-β superfamily. These molecules have the potential to improve the phenotype of the disease and reduce the risk of complications.

Table 1

Important mediators of erythropoiesis under different conditions.

ERYTHROPOIESIS	ERYTHROID PATHWAYS	ERYTHROBLASTS	IRON METABOLISM	RED BLOOD CELLS
Steady-state	EPO-JAK2-STAT5	BM progenitors	Hepcidin	
Stress	EPO-JAK2-STAT5 BMP4-SMAD5-SCF/cKIT GR-PPARalpha? Hedgehog Notch2 pIgA1/TFR-Erk/Akt	Erythroid stress progenitors	↓ Hepcidin	
Anemia of Inflammation (iron-restricted)	EPO-JAK2-STAT5	BM progenitors	Inflammation ↑ Hepcidin