The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia

Stefano Rivella *

Weill Medical College of Cornell University, New York, NY, USA

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ABSTRACT

Ineffective erythropoiesis is the hallmark of beta-thalassemia that triggers a cascade of compensatory mechanisms resulting in clinical sequelae such as erythroid marrow expansion, extramedullary hematopoiesis, splenomegaly, and increased gastrointestinal iron absorption. Recent studies have begun to shed light on the complex molecular mechanisms underlying ineffective erythropoiesis and the associated compensatory pathways; this new understanding may lead to the development of novel therapies. Increased or excessive activation of the Jak2/STAT5 pathway promotes unnecessary disproportionate proliferation of erythroid progenitors, while other factors suppress serum hepcidin levels leading to dysregulation of iron metabolism. Preclinical studies suggest that Jak inhibitors, hepcidin agonists, and exogenous transferrin may help to restore normal erythropoiesis and iron metabolism and reduce splenomegaly; however, further research is needed.

1. Introduction

Ineffective erythropoiesis (IE), due to excess production of free alpha (α)-globin chains, is the hallmark of beta (β)-thalassemia. Ineffective erythropoiesis results in profound anemia and triggers a number of compensatory mechanisms responsible for the clinical sequelae associated with β-thalassemia such as erythroid marrow expansion, extramedullary hematopoiesis (EMH), splenomegaly, and increased gastrointestinal iron absorption (Fig. 1). Depending on the severity of IE, the erythroid marrow can expand to nearly 30 times normal volume, resulting in severe skeletal deformities and osteopenia. In addition, both the increase in plasma volume caused by marrow expansion and splenomegaly exacerbate anemia and increase transfusion requirements. This article explores the biologic mechanisms responsible for IE and potential future therapies.

2. Biological basis of ineffective erythropoiesis

The unbalanced synthesis of α- and β-globin chains in red blood cells (RBCs) is primarily responsible for hemolysis of RBCs and for the premature death (via apoptosis) of erythroid precursors in the bone marrow and at extramedullary sites, including the spleen. The excess of α-globin chains (and associated toxic heme) aggregate and form inclusion bodies (hemichromes) within the cell, which leads to formation of reactive oxygen species (ROS), resulting in oxidative stress and membrane damage within mature RBCs and immature developing erythroblasts. Together, this chain of events is primarily responsible for IE in patients with β-thalassemia.

3. Compensatory mechanisms

The anemia and resulting hypoxia associated with β-thalassemia lead to a dramatic increase in serum levels of erythropoietin (EPO) as the body attempts to compensate for the reduced oxygen-carrying capacity of the blood (Fig. 2). However, the thalassemic erythroid marrow is unable to respond adequately to EPO, resulting in compensatory erythroid hyperplasia and massive expansion of the erythroid marrow without a commensurate increase in the number of mature erythrocytes in the peripheral blood. Several intrinsic and extrinsic mechanisms are thought to inhibit erythroid cell differentiation in patients with β-thalassemia, further limiting the production of mature RBCs. In the absence of stoichiometric production of α- and β-globin chains and in the presence of elevated EPO levels, erythroid precursors continue to proliferate but fail to differentiate. As a result, the marrow and spleen become packed with immature erythroid precursors, which eventually undergo apoptosis before they reach the reticulocyte stage due to the burden of precipitated α-globin chains and oxidative stress. This vicious cycle continues to drive erythropoiesis to the extent that masses of extramedullary erythropoietic tissue form in various regions of the body including the chest, abdomen, and pelvis.

4. The role of Janus kinase (Jak)

Janus kinase-2 (Jak2) is an important signaling molecule that regulates proliferation, differentiation, and survival of erythroid progenitor cells in response to EPO. When EPO binds to its recep-
Fig. 1. Excess production of free α-globin chains causes ineffective erythropoiesis and a variety of clinical sequelae. Primary disease processes are shown in orange, and compensatory mechanisms in yellow. Reprinted with permission from Olivieri NF. N Engl J Med 1999;341:99–109. 1


Jak2 is phosphorylated and in turn phosphorylates and activates the signal transducer and activator of transcription-5 (STAT5). Upon activation, STAT5 migrates to the nucleus and activates genes crucial for proliferation and differentiation of erythroid progenitors. 5 In murine models and patients with β-thalassemia, erythroid precursors express elevated levels of the phosphorylated active form of Jak2 (pJak2), and other downstream signaling molecules that promote proliferation and inhibit differentiation of erythroid progenitor cells (Fig. 3). 5,6 A recent study showed that Jak2 activation upregulated the transcription factor ID1; 7 high levels of ID1 have been found to inhibit cellular differentiation. 6 Jak2 signaling also activates the phosphoinositol-3-kinase (PI3K)-AKT pathway, which plays an important role in regulating cell survival and the activity of the transcription factor Forkhead box O3 (FoxO3), which modulates oxidative stress during erythropoiesis. 8 Taken together, findings from these studies suggest a model in which persistent phosphorylation of Jak2 as a consequence of high EPO levels induces erythroid hyperplasia and massive EMH, and the early erythroid progenitors
that fail to differentiate colonize and proliferate predominantly in the spleen and liver,\textsuperscript{4} thus contributing to hepatosplenomegaly. Given the central role of Jak2 in the pathophysiology of IE, it has been hypothesized that Jak2 inhibitors may be effective in modulating some of these compensatory mechanisms that lead to the severe clinical complications associated with β-thalassemia.

5. Role of ineffective erythropoiesis in increased iron absorption

Recent studies have begun to shed light on how IE can contribute to increased gastrointestinal iron absorption.\textsuperscript{8} This will be discussed in more detail in the article titled “Iron overload in non-transfusion-dependent thalassemia: a clinical perspective”. Briefly, increased erythropoiesis in response to elevated EPO levels leads to increased iron absorption by decreasing serum levels of the peptide hormone hepcidin, which controls the concentration of ferroportin on the intestinal epithelium (Fig. 2).\textsuperscript{4,6,8} Low levels of hepcidin correlate with higher levels of ferroportin, resulting in increased intestinal iron absorption, and most of that excess iron is stored in the liver. One factor that appears to be important for regulating hepcidin levels is known as growth differentiation factor 15 (GDF15).\textsuperscript{9} Serum levels of GDF15 are elevated in patients with β-thalassemia, and GDF15 downregulates expression of hepcidin in vitro, although the precise mechanism is not known. Therefore, elevated levels of GDF15 may suppress serum hepcidin levels, resulting in increased gastrointestinal iron absorption.

Recent data suggest that Jak2 also may have both direct and indirect effects on iron metabolism. For example, in vitro studies suggest that Jak2 may regulate ferroportin degradation.\textsuperscript{10,11} In addition, STAT5 can upregulate expression of proteins that have an important role in regulating iron metabolism by erythrocytes. STAT5 stimulates expression of iron regulatory protein 2 (IRP-2) and transferrin receptor-1 (Tfr1).\textsuperscript{12,13} and Tfr1 is required for erythrocytes to uptake transferrin-bound iron. These findings suggest that suppressing Jak2 activity could decrease iron uptake by erythrocytes, thereby limiting oxidative damage and reducing hemolysis.\textsuperscript{6} Lastly, iron overload leads to an increase in non-transferrin-bound iron (NTBI), and NTBI is a catalyst for formation of ROS.\textsuperscript{10} Therefore, iron overload can contribute directly to the increased oxidative stress that characterizes IE.

Given the important role of GDF15 and hepcidin in regulating iron absorption, and evidence that the serum levels of these hormones are indicative of IE,\textsuperscript{14,15} they could be useful biomarkers in β-thalassemia. Recent work has shown that GDF15 levels correlate with serum ferritin, liver iron concentration, and the multiplicity of iron overload related complications in transfusion-independent patients with β-thalassemia intermedia (TI).\textsuperscript{15}

6. Potential future therapies

Jak2 inhibitors appear promising for the treatment of β-thalassemia based on the potential role of Jak2 in IE and iron metabolism.\textsuperscript{2} Preliminary studies in murine models of β-thalassemia have shown that Jak2 inhibitors can affect IE and decrease spleen size.\textsuperscript{3} Results from clinical studies in patients with myeloproliferative disorders that are characterized by activating Jak2 mutations suggest that Jak inhibitors may be an effective treatment option with a tolerable safety profile. For example, in a phase 1–2 study, the Jak inhibitor INCB018424 was shown to rapidly reduce splenomegaly in patients with myelofibrosis and palpable spleens.\textsuperscript{16} Based on the available preclinical evidence, it is anticipated that Jak2 inhibitors might reduce splenomegaly, transfusion requirements, and possibly iron overload in patients with β-thalassemia; however, clinical evidence is not yet available.

Reducing iron overload could have a range of positive effects in patients with β-thalassemia, particularly those with TI. Reducing erythroid iron intake might limit the synthesis of heme and the formation of hemichromes and ROS, and this might result in more effective erythropoiesis, increased circulating hemoglobin (Hb) levels, and decreased splenomegaly. This could potentially be achieved by restricting dietary iron or by increasing serum levels of hepcidin. In a murine model of TI, Hbb\textsuperscript{th1/th1} mice fed an iron-restricted diet or genetically engineered to express moderate levels of hepcidin did not develop iron overload.\textsuperscript{17} These studies in transgenic Hbb\textsuperscript{th1/th1} mice further demonstrated that modestly increasing expression of hepcidin increased Hb levels, reduced splenomegaly, and increased erythropoiesis, as evidenced by fewer erythroid progenitor cells in the spleen. Therefore, drugs that increase serum hepcidin levels or act as hepcidin agonists might be beneficial in β-thalassemia.\textsuperscript{18} However, although studies in mouse models have shown that moderate levels of hepcidin are beneficial, high levels can adversely affect iron recycling by macrophages, a factor that should be evaluated carefully if this strategy is utilized in transfusion-independent patients with TI.\textsuperscript{10,18}

Administration of exogenous transferrin may be another approach to normalize erythropoiesis in patients with β-thalassemia. Studies in murine models have shown that long-term administration of transferrin injections to Hbb\textsuperscript{th1/th1} mice increased Hb...
production, decreased reticulocytosis and serum EPO levels, reversed splenomegaly, and increased hepcidin expression. These findings suggest that exogenous transferrin administration may restore more normal erythropoiesis; this is consistent with the hypothesis that in patients with β-thalassemia, modulation of iron delivery to erythroid cells could profoundly ameliorate IE, increase hepcidin secretion, and correct dysregulation of iron absorption, distribution, and metabolism.19

7. Conclusions

Recent studies, primarily in murine models of TI, have begun to shed light on the complex molecular mechanisms underlying IE and the associated compensatory pathways in patients with β-thalassemia. The prominent role of the EPO receptor/Jak2/STAT5 signaling pathway in the regulation of erythroid development, oxidative stress, and iron metabolism suggests that Jak inhibitors could be an effective treatment strategy. Preclinical research also suggests that modulating serum levels of hepcidin or transferrin could serve to normalize iron absorption and uptake by erythrocytes and this could have profound effects on IE and splenomegaly. The challenge for the near future is to translate these promising preliminary results into effective treatment options for patients with β-thalassemia.

Conflict of interest statement

Dr. Rivella is a consultant for Novartis. In addition, he is a co-inventor for the patents US8058061 B2 C12N 20111115 and US7541179 B2 C12N 20090602. The consulting work and intellectual property of Dr. Rivella did not affect in any way the design, conduct, or reporting of this manuscript.

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