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REFERENCES

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Artuso et al, page 2286

Tfr2 suppression benefits β-thalassemic erythropoiesis

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Multiple studies in murine model systems of β-thalassemia have demonstrated that iron restriction improves the ineffective erythropoiesis characteristic of this disorder,1 the mechanisms of which have not yet been fully elucidated. In this issue of Blood, Artuso et al demonstrate that erythroid knockout of transferrin receptor 2 (Tfr2) also improves hematologic parameters in β-thalassemic mice.2 The authors invoke changes in erythropoietin (Epo) sensitivity rather than erythroid iron delivery per se as the underlying mechanism.

Tfr2 is a transmembrane glycoprotein homologous to the classical transferrin receptor Tfr1. Whereas Tfr1 is ubiquitously expressed and is the main mechanism for cellular iron uptake, Tfr2 has more restricted expression and appears to function as a sensor of circulating iron. Tfr2 is highly expressed in hepatocytes, where it participates in the regulation of hepcidin expression to modulate iron homeostasis. Loss-of-function mutations in the Tfr2 gene result in the inappropriate low hepcidin production, excess circulating iron, and hemochromatosis (type 3).3,4 A role for Tfr2 in erythroid cells was not initially apparent because murine models and human patients with hemochromatosis type 3 have no obvious abnormalities in erythroid parameters. It was later discovered that Tfr2 complexes with and stabilizes cell-surface Epo receptor (EpoR).5 In these studies, in vitro Tfr2 silencing in human erythroid progenitors resulted in a significant decrease in erythroid lineage commitment. Elevated Epo levels in Tfr2 knockout mice further supported a role for Tfr2 in upregulating EpoR-mediated signaling. As such, one might predict that loss of erythroid Tfr2 in vivo would lead to decreased Epo sensitivity and erythropoiesis. However, subsequent observations in iron-deficient Tfr2 knockout mice suggest the contrary.

Tmprss6 knockout mice with ubiquitous loss of Tfr2 have higher red blood cell count, more severe microcytosis, and greater iron deficiency than Tmprss6 knockout mice with hepatocellular-specific Tfr2 knockout.6,7 These findings reveal that the additional loss of erythroid Tfr2 is associated with increased erythropoiesis and suggest a role for erythroid Tfr2 that is particularly relevant during iron restriction to prevent excess erythropoiesis when hemoglobinization is limited by limited iron. To specifically examine the role of erythroid (independent of hepatocellular) Tfr2, Nai et al transplanted Tfr2 knockout bone marrow into wild-type recipient mice.8 Bone marrow–specific loss of Tfr2 resulted in more red blood cells, microcytosis, reduced apoptosis of erythroblasts, and evidence for increased Epo-mediated signaling, particularly in the setting of iron deficiency.8 In another model system, floxed Tfr2 mice crossed with Vav-Cre mice demonstrate an apparent block in erythroid differentiation during iron deficiency.9 The authors suggest that a greater severity of iron deficiency in the different model systems may account for their findings.8,9

In the current work, the authors propose that removing Tfr2 from erythroblasts would enhance Epo sensitivity, decrease erythroid precursor apoptosis, and improve erythropoiesis in β-thalassemia. At the same time, however, erythроferrone (ERFE), an erythroblast-derived regulator of hepcidin, is among the Epo-responsive genes upregulated in mice with erythroid loss of Tfr2.8,9 Because suppression of ERFE appears to be an important contributor to the improvements in iron status in β-thalassemic mice,10 it is unclear whether enhancing Epo sensitivity in this setting would be beneficial. Theoretically, loss of Tfr2 could enhance ERFE expression,
further suppress hepcidin, and potentially worsen iron overload in β-thalassemia.

The authors examine the consequences of knockout of erythroid Tfr2 on erythropoiesis in β-thalassemia by performing a bone marrow transplant of Tfr2−/−thalassemic (Hbbth3−/−b) cells into Hbbth3−/−mice.2 The results demonstrate significantly elevated hemoglobin in Tfr2−/−/Hbbth3−/−relative to Hbbth3−/−mice between 9 and 22 weeks following bone marrow transplant, with a decrease in serum Epo, fewer reticulocytes, and an increased proportion of mature erythroid precursors in the bone marrow. The increased hemoglobin is associated with a decrease in circulating Epo and modestly decreased expression of Epo-responsive genes (including ERFE). Spleen size is unchanged. Furthermore, the authors iron-restrict Tfr2−/−/Hbbth3−/−bone marrow–transplanted mice to inquire whether the mechanism of improved hematologic parameters is iron deficiency–driven or whether Tfr2 loss works by an alternative mechanism. The authors propose that the improvement in hematologic parameters in Tfr2−/−/Hbbth3−/−bone marrow–transplanted mice is not the result of limited available iron.

There are inherent complexities in the relationship between Tfr2 and Epor that require accounting for the circulating ligand for Tfr2 (ie, transferrin isofoms) and for Epor (ie, Epo concentration). Assessing Epo responsiveness in this setting is challenging, given the change in circulating Epo levels in the Tfr2−/−/Hbbth3−/−bone marrow–transplanted mice. Additional experiments are required to fully clarify the expected proportionality between circulating Epo levels and Epo-responsive gene expression. Although RNAseq analysis from spleen identify changes that might be expected with Epo-mediated increased erythropoietic activity, as pointed out by the authors, the analysis is confounded by differences in spleen iron. As such, the conclusion that erythroid parameter improvements in β-thalassemic mice with loss of erythroid Tfr2 are entirely the result of enhanced Epo-sensitivity will likely require further study.

Based on these interesting findings, the authors suggest a potentially translatable approach by manipulating Tfr2 in β-thalassemic erythroblasts. However, the therapeutic application of decreased Tfr2 in erythroblasts may prove to be challenging. The beneficial effect on erythropoiesis in β-thalassemic mice dissipates at 37 weeks posttransplant, possibly as a consequence of critical iron deficiency for erythropoiesis. A better understanding of the basis for this effect, and the effect of transferrin and Epo on the functional properties of erythroid Tfr2 are needed. Nonetheless, results of Tfr2 haplo-insufficient Hbbth3−/−mice suggest the possibility of partial Tfr2 inhibition using antisense oligonucleotide or small interfering RNA technology. Last, investigating the consequences of Tfr2 loss in mouse models of β-thalassemia major, rather than interim, would be informative.

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THROMBOSIS AND HEMOSTASIS

Comment on Amin et al, page 2298

Postthrombotic syndrome: simple prevention

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In this issue of Blood, Amin et al provide data showing early compression therapy post-venous thromboembolism (VTE) to be effective in reducing the incidence of postthrombotic syndrome (PTS) by achieving reduced residual vein obstruction (RVO) on follow-up ultrasound.1

PTS is a significant, disabling,2 and costly3 complication in up to 50%4 of patients with VTE. Given that other PTS treatment interventions, such as the use of elastic compression stockings,5 or early thrombolysis6 provide limited benefit or are controversial in reducing the incidence of this morbidity, the current findings are potentially of great clinical interest. The scale of benefit appears comparable to the effect of well-controlled anticoagulation therapy on risk reduction of the incidence of PTS.7

Variable in incidence, and in severity, PTS is a common and potentially disabling