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Transferrin and TfR1 in co-regulation of erythropoiesis and iron metabolism

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Take-home messages

- Apo-transferrin decreases TfR1 expression and normalizes enucleation in β-thalassemic erythroid precursors.
- Decreased TfR1 expression causes iron restriction specifically in β-thalassemic erythroid precursors.
- Decreased TfR1 enables endogenous mechanism(s) to upregulate hepcidin in an iron- and erythroferrone-independent manner, re-enforcing sustained iron-restricted erythropoiesis in β-thalassemic mice.

Introduction

Hemoglobin synthesis during erythropoiesis is intrinsically dependent on iron. Iron delivery for erythropoiesis is exclusively dependent on the iron transporter transferrin and iron uptake by erythroid precursors requires transferrin-Fe binding to transferrin receptor 1 (TfR1).[1,2] Erythroid precursors regulate iron metabolism in part by secreting factors, such as the recently identified erythroferrone (ERFE),[3] which suppresses the hormone hepcidin, a key inhibitor of dietary iron absorption, recycling from senescent RBCs, and mobilization from iron stores.[4] As a physiological erythroid regulator of hepcidin, ERFE is increased in stress (i.e. phlebotomy) and ineffective erythropoiesis (i.e. β-thalassemia).[3] In spite of these recent discoveries, the regulatory interactions between erythropoiesis and iron metabolism remain incompletely understood.

Current state of the art

Exogenous iron-free apo-transferrin ameliorates ineffective erythropoiesis in β-thalassemic mice,[5] resulting in increased hepcidin. In addition, apo-transferrin-treated β-thalassemic mice exhibit normalized *Erfe* expression in sorted erythroid precursors and reversal of systemic iron overload,[6] suggesting that iron depletion improves ineffective erythropoiesis and thus increases hepcidin expression *despite* relative iron deficiency (**Figure 1**). This finding is counter-intuitive as iron deficiency suppresses hepcidin expression. We hypothesized that the beneficial effect of apo-transferrin in β-thalassemic mice is a consequence of *more than* frank iron restriction. Furthermore, we hypothesized that because ERFE expression is STAT5 dependent [3] and thus decreased with decreasing serum erythropoietin, *Erfe* expression in apo-transferrin-treated β-thalassemic mice is an indirect consequence of improved erythroid maturation.[5]

- First, we compared the results from apo-transferrin-treated β-thalassemic mice with the effects of other systemic iron depleting approaches. Apo-transferrin-treated β-thalassemic mice demonstrate results in contrast to the effect of dietary iron restriction, which results in decreased liver hepcidin expression[7] without improving hemoglobin in β-thalassemic mice[8] and is thus only partially beneficial in diseases of ineffective erythropoiesis. Furthermore, iron chelator therapy does not change erythropoiesis or hepcidin expression despite improved liver iron overload in β-thalassemic mice.[9] On the other hand, multiple means of increasing hepcidin expression to decrease iron overload in β-thalassemic mice result in both improved erythropoiesis and iron overload.[7,10-12] However, one of these approaches, using homozygous inactivation of *Tmprss6* leads to excessive hepcidin production, impaired dietary iron absorption, and microcytic anemia in mice.[13,14] Furthermore, Tmprss6-/- β-thalassemic mice exhibit increased erythropoietin,[10], suggesting that excessive hepcidin upregulation causes iron restriction[7] and prevents complete reversal of ineffective erythropoiesis. Taken together, *moderately* increased hepcidin results in iron sequestration, decreasing iron absorption and recycling, and resulting in restricted availability of iron for erythropoiesis. Thus, inducing iron-restriction specifically in the erythroid compartment may provide endogenous mechanism(s) for increasing hepcidin to maximize benefit for erythropoiesis and iron overload in β-thalassemia.
- Second, we hypothesized that apo-transferrin's effect is mediated via decreased TfR1 expression, resulting in disproportional decrease in erythroid precursor iron uptake. We thus evaluated TfR1 expression in β-thalassemic mice *in vivo* and *in vitro* with and without added apo-transferrin. Our cur-

rent findings demonstrate that β-thalassemic erythroid precursors overexpress TfR1, an effect which can be reversed by the administration of exogenous apo-transferrin. We anticipate that the increased number of β-thalassemic erythroid precursors limit iron availability per cell and, in conjunction with increased serum erythropoietin concentration, results in increased TfR1 expression *in vivo*. *In vitro* experiments demonstrate that apo-transferrin inhibits TfR1 and induce *Erfe* expression independent of erythropoietin- and iron-related signaling, decreases TfR1 partitioning to reticulocytes during enucleation, and enhances enucleation of defective β-thalassemic erythroid precursors. These findings strongly suggest that overexpressed TfR1 may play a regulatory role contributing to iron overload and anemia in β-thalassemic mice. Specifically, our data demonstrates that apo-transferrin functions via an effect on TfR1 trafficking, increasing enucleation as evidence of improved erythropoiesis and modulating iron metabolism by decreasing *Erfe* expression. Taken together, our findings suggest that novel regulatory pathways for transferrin and TfR1 are central to the crosstalk between erythropoiesis and iron metabolism.

- Lastly, we hypothesize that the beneficial effect of exogenous apo-transferrin on ineffective erythropoiesis is a consequence of reduced TfR1 expression or altered TfR1 trafficking in erythroid precursors. TfR1 is characteristically expressed on erythroid precursors, progressively decreasing during erythroid differentiation.[15] TfR1 is lost both during enucleation and reticulocyte maturation, the latter a consequence of proteolytic cleavage, leading to soluble TfR1 in circulation.[16] TfR1 expression itself is upregulated in iron deficiency[17] and by increased erythropoiesis,[18] but its function in erythropoiesis beyond its canonical involvement in cellular iron uptake

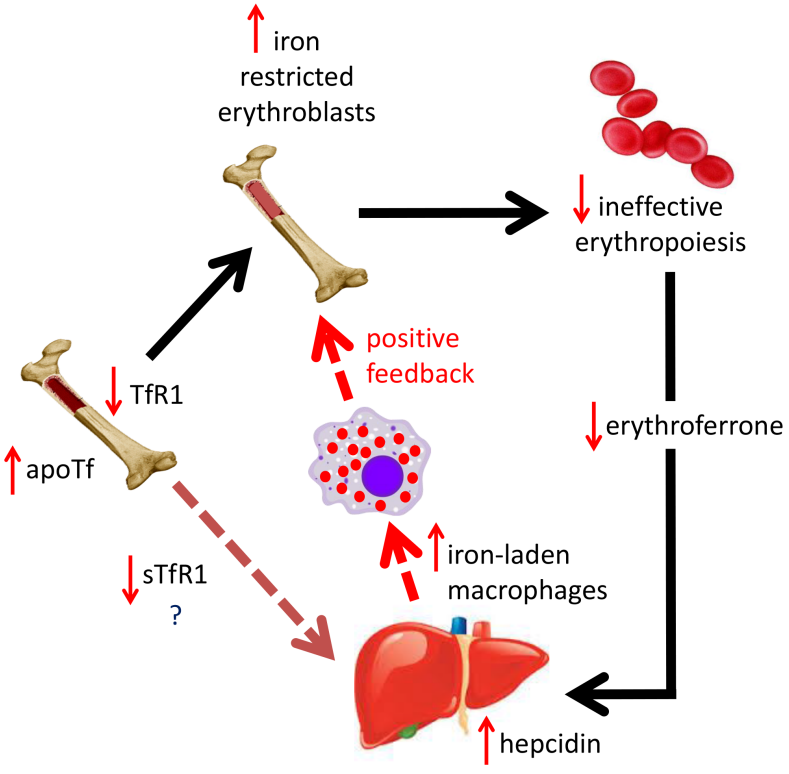

Figure 1. Working model of the effect of exogenous apo-transferrin and restricted TfR1 expression in β-thalassemia. The administration of exogenous apo-transferrin and restricted TfR1 expression both result in iron restriction disproportionately in erythroid precursors, in light of the highest concentration of TfR1 on these cells, and improve ineffective erythropoiesis in β-thalassemia. With increased hemoglobin, fewer erythroid precursors are required to maintain homeostasis, resulting in reversal of splenomegaly and a decrease of circulating erythroferrone and soluble TfR1 concentrations. As a consequence, hepcidin is de-repressed and re-enforces the sequestration of iron within macrophages and iron restricted erythropoiesis. We are currently exploring the role of soluble TfR1 in more directly regulating hepcidin expression. apoTf = apo-transferrin; TfR1 = transferrin receptor 1; sTfR1 = soluble transferrin receptor 1.

is incompletely understood, and hepcidin expression in TfR1 heterozygous mice (TfR1+/-) is surprisingly increased despite relative iron deficiency, iron-restricted erythropoiesis, and increased *Erfe* expression.[19] To evaluate further, we crossed TfR1+/- mice—themselves exhibiting iron-restricted erythropoiesis with increased hepcidin—with β-thalassemic mice. Resultant double-heterozygote mice demonstrate long-term improvement in ineffective erythropoiesis, hepcidin de-repression, and increased erythroid enucleation relative to β-thalassemic mice. Furthermore, hepcidin de-repression occurs in an iron- and erythroferrone-independent manner, suggesting that iron restriction within erythroblasts may de-repress hepcidin directly, resulting in a positive feedback to maintain iron restricted erythropoiesis. Taken together, the effects of apo-transferrin treatment and TfR1 haplo-insufficiency on β-thalassemic mice thus provide evidence of an additional mechanism for increasing hepcidin by *erythroid regulation*.

Future perspectives

Further studies are necessary to explore the potential use of exogenous apo-transferrin to reverse ineffective erythropoiesis in β-thalassemia and other diseases of concurrent anemia and iron overload. Our data present additional therapeutic targets in this pathway, and support our hypothesis that reversal of ineffective erythropoiesis and iron overload require parallel management in β-thalassemia. This data also raises numerous questions to explore. For instance, reticulocyte release of exosomal TfR1 is altered in apo-transferrin treated β-thalassemic mice. How is this effect related to improved erythropoiesis after exogenous apo-transferrin? What are the *regulatory* implications of altering TfR1 expression or localization? What is the role of exosomal or soluble TfR1 in regulating hepcidin expression in β-thalassemia? Are TfR1 antagonists—similar to the effect of exogenous apo-transferrin—potential therapeutic targets to simultaneously improve erythropoiesis and de-repress hepcidin? By exploring the impact on iron transport in circulation as well as erythroid precursor import and export, these results will extend our understanding of co-regulation of erythropoiesis and iron metabolism in iron overload anemias.

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First evaluation of hepcidin in TfR1+/- mice, suggesting a function of other erythroid regulators other than erythroferrone.