Deregulating iron-erythropoiesis regulation: transferrin receptor 2 as potential target for treating anemia in CKD

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Both insufficient kidney production of erythropoietin and inflammation-mediated reduction of transferrin-bound iron are major factors in anemia of chronic kidney disease. Improved therapies for anemia in chronic kidney disease may involve modifying regulators of erythropoiesis and iron availability. Olivari *et al.* show in a mouse model of chronic kidney disease that transferrin receptor 2 in hepatocytes, where it is required for hepcidin production, and in erythroid cells, where it downregulates erythropoietin receptor activity, is a potential therapeutic target.

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As chronic kidney disease (CKD) progresses, most patients become anemic due to the underproduction of erythrocytes.[1] Inflammation plays a major role in the pathogenesis of CKD anemia as it decreases erythropoiesis directly by reducing numbers of erythroid progenitor cells in the bone marrow and indirectly by suppressing kidney production of the glycoprotein hormone erythropoietin (EPO). EPO is required for erythroid progenitor survival, which is mediated by increased expression of anti-apoptotic proteins, including B-cell lymphoma-x (long isoform), also known as BCL-XL. Inflammatory cytokines also decrease plasma concentrations of transferrin-iron that erythroblasts use to synthesize hemoglobin. Two inflammatory cytokines that have major roles in reducing transferrin-iron are interleukin 6 (IL-6) via the Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3) pathway and activin B via the bone morphogenetic protein receptor–suppressor of mothers against decapentaplegic (BMPR-SMAD) pathway that induce liver production of hepcidin. BMPR-SMAD signaling in hepatocytes is also activated by increased iron stores and transferrin saturation. Hepcidin is the hormone that downregulates iron exporter ferroportin on all cells.[1] Elevated plasma hepcidin concentrations, as found in CKD, reduce iron transport into plasma from liver iron storage sites, duodenal cells that absorb dietary iron, and macrophages recycling iron from phagocytosed senescent erythrocytes. As a result, erythropoietic cells have inadequate iron to complete normal differentiation.

Recombinant human EPO or one of its long-acting derivatives (erythropoiesis-stimulating agents [ESAs]) in conjunction with iron supplementation has been a mainstay of anemia therapy in CKD. However, EPO replacement therapy, especially when used in supraphysiological doses, has been associated with increased cardiovascular risk.[1] In addition to these ESA safety concerns, acute reactions and chronic iron overload can occur with repeated iron administrations. Elevated plasma hepcidin accompanying the inflammation of CKD limits duodenal iron absorption and iron mobilization such that availability of transferrin-bound iron for uptake by erythroblasts is decreased for both orally and intravenous administered iron. These limitations of current ESAs and iron therapies in anemic patients with CKD may be overcome if iron absorption and availability can be increased while limiting ESA use.

A potential target that can improve iron absorption and availability without increasing endogenous EPO production is transferrin receptor 2 (TFR2). TFR2 is homologous to transferrin receptor 1 (TFR1) in the extracellular domain but not the cytoplasmic domain. Unlike TFR1's expression in all cell types, TFR2 expression is not regulated by intracellular iron and is restricted mainly to hepatocytes and erythroid cells.[2] TFR2 binds holotransferrin less avidly than TFR1.[2] TFR1's major function is importation of transferrin-bound iron into all cells, whereas TFR2's major function is sensing iron in hepatocytes, where it regulates hepcidin production. This iron-sensing function is demonstrated by the hemochromatosis phenotype in individuals with *TFR2* mutations. In hepatocytes, TFR2 is a component of the receptor complex that includes the hemochromatosis-associated protein

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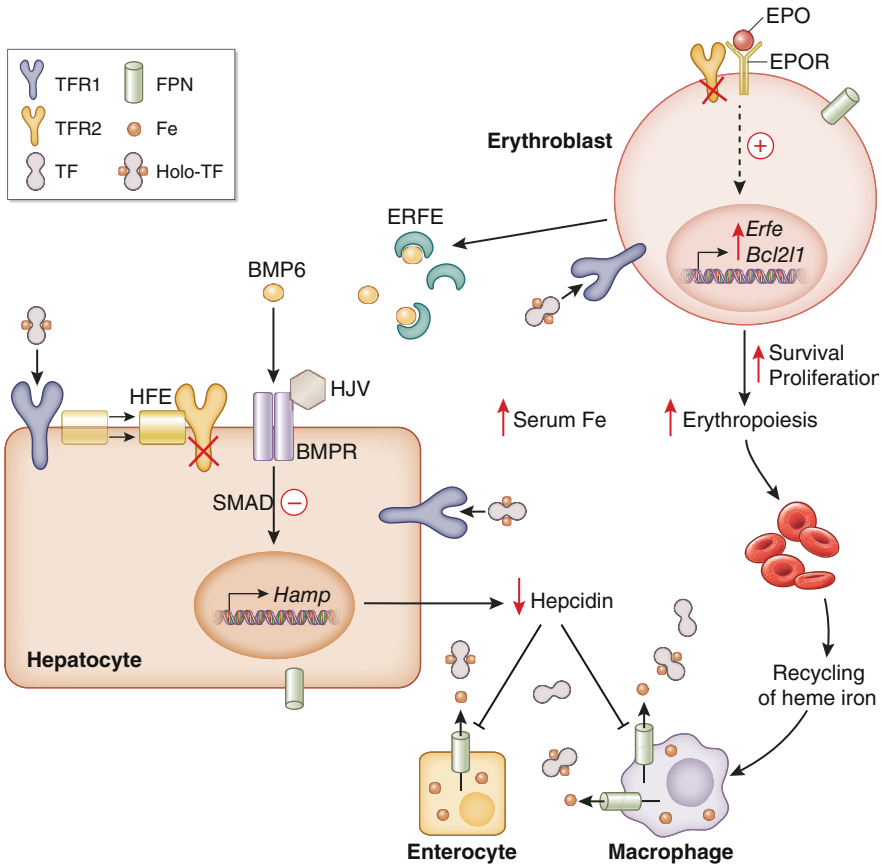

Figure 1 | Global transferrin receptor 2 (TFR2) inactivation stimulates erythropoiesis. Schematic of the underlying mechanisms proposed by Olivari *et al.*[3] in an animal model of experimental chronic kidney disease based on adenine feeding. The functional consequences of global and/or tissue-specific TFR2 inactivation (red X) are illustrated by red arrows and red circles. Low-affinity TFR2 is highly expressed in hepatocytes and erythroblasts. In the liver, TRF2 participates in iron sensing and regulates hepcidin production in an iron-dependent manner through its interaction with the hemochromatosis-associated protein homeostatic iron regulator (HFE [High Fe]), and the bone morphogenetic protein receptor (BMPR)/hemojuvelin (HJV) signaling complex. Under high-iron conditions, a large proportion of transferrin (TF) binds iron, and this iron-bound transferrin, which is termed holotransferrin (Holo-TF), releases HFE from a complex with transferrin receptor 1 (TFR1). The subsequent TFR2/HFE interaction potentiates suppressor of mothers against decapentaplegic (SMAD)–dependent signal transduction and stimulates hepcidin production. Hepcidin downregulates ferroportin (FPN) on all cells, including the macrophages that recycle senescent erythrocytes and duodenal enterocytes that absorb dietary iron. TFR2 mutations in humans have been associated with hereditary hemochromatosis type III, which is characterized by inappropriately low plasma hepcidin levels but not erythrocytosis. In erythroid precursors, TFR2 complexes with the erythropoietin (EPO) receptor (EPOR; a homodimer). Deficiency of TRF2 in erythroid cells enhances sensitivity to EPO, stimulating erythropoiesis. Stimulated erythropoiesis is associated with increased expression of erythroid survival factor B-cell lymphoma 2–like 1 (*Bcl2l1*), which encodes B-cell lymphoma-x (long isoform), also known as BCL-XL. Erythroferrone (ERFE) is produced by erythroid precursors when erythropoiesis is stimulated and suppresses hepcidin production by sequestering, bone morphogenetic protein 6 (BMP6). BMP6 is produced by liver endothelial cells and promotes the transcription of hepcidin antimicrobial peptide (*Hamp*), the gene encoding hepcidin.

homeostatic iron regulator (High Fe), hemojuvelin (HJV), and BMPR (Figure 1).[3] The BMPR binds bone morphogenetic protein 6 (BMP6; and BMP6/BMP2 heterodimers) produced by hepatic endothelial cells in response to iron loading and increased transferrin saturation. Signaling of this complex via the SMAD pathway induces hepcidin gene (*HAMP*) transcription. On the other hand, in erythroid progenitors/precursors, TFR2 downregulates signaling by the EPO receptor (EPOR), which promotes cell survival and differentiation.[2] When their populations are expanding, such as following ESA administration in CKD, erythroid cells produce erythroferrone (ERFE), a hormone that sequesters BMP6, thereby suppressing production of hepcidin by the hepatocyte. In an experimental model of transient anemia of inflammation (AI), ERFE hastened the recovery from anemia by decreasing hepcidin and increasing iron availability.[4] However, stable populations of erythroid cells do not produce ERFE. Thus, in terms of increasing iron availability to erythroid cells, TFR2 is a potential target. In hepatocytes, TFR2 deficiency reduces hepcidin production via decreased BMPR-SMAD activity and, in erythroid cells, TFR2 deficiency increases EPO sensitivity, leading to

increased erythropoiesis and ERFE, which decreases hepcidin production.

In this issue, Olivari *et al.* used 3 animal models of TFR2 deficiency previously shown to increase EPOR activity in erythroid cells and decrease hepcidin production in hepatocytes.[3] These models are based on (i) genetic *Tfr2* inactivation in the murine germline ($Tfr2^{-/-}$ mice) affecting all cell types, including hematopoietic cells and hepatocytes, (ii) marrow transplantation of hematopoietic stem cells from $Tfr2^{-/-}$ to wild-type mice, creating TFR2 deficiency only in hematopoietic cells, and (iii) anti-sense oligonucleotide approach to reduce *Tfr2* expression specifically in the liver. The effects of TFR2 deficiency in each animal model were examined in mice with adenine-induced nephropathy, a disease model of CKD associated with anemia, after 8 weeks of exposure to adenine. Compared with their respective controls, $Tfr2^{-/-}$ mice with adenine-induced nephropathy were characterized by less anemia and a statistically significant decrease in serum EPO levels. In contrast, mice with hepatic-only or hematopoietic-only TFR2 deficiency had only transient improvements in the anemia and similar serum EPO concentrations compared with their respective controls. The decrease in serum EPO levels in $Tfr2^{-/-}$ mice would suggest that hypoxia feedback mechanisms in the kidney were intact as the amelioration of the anemia is expected to improve kidney tissue oxygenation and, thereby, lower kidney EPO production. Thus, combined hematopoietic and hepatic TFR2 deficiency was required to sustain enhanced erythropoiesis in adenine-induced nephropathy, likely due to improved iron availability for the increased erythropoiesis. This result is interesting, as patients with homozygous mutations in *TFR2* develop hemochromatosis but not erythrocytosis and could indicate that iron overload may modulate EPOR signal transduction in the context of TRF2 deficiency.

Olivari *et al.* examined TFR2 deficiency in hematopoietic cells in an AI model induced by turpentine injections. In this AI model, the anemia degree and duration were less, but the amelioration pattern was similar to hematopoietic TFR2-deficient mice with anemia of CKD. Compared with controls with AI, hematopoietic TFR2-deficient mice had lower serum EPO and similar hepcidin levels.

These studies together suggest that targeted TFR2 deficiency can potentially provide improvement of anemia in CKD and inflammatory disorders without increasing endogenous EPO. However, the improvements in hemoglobin in each model were partial. Likewise, the effects of TFR2 deficiency on iron metabolism and hepcidin production are difficult to assess without further characterization of standard serum iron parameters and hepcidin levels or liver *Hamp* transcription in a time course–dependent manner. Olivari *et al.*[3] demonstrated that anti-sense oligonucleotide–induced inactivation of TFR2 decreased liver *Hamp* expression and increased serum iron levels. However, differences in liver *Hamp* expression and serum iron levels were not significantly different between $Tfr2^{-/-}$ mice, which displayed sustained improvements in anemia, and their respective control mice. In the hepatocyte, TFR2 functions as a sensor of iron status and not of inflammation. Therefore, TFR2 deficiency would not necessarily be expected to mitigate inflammation-related hepcidin induction by the IL-6-JAK2-STAT3 pathway, the continued stimulation of which may have been responsible for the only partial improvement of anemia. Furthermore, hepcidin is cleared by the kidney, and CKD results in elevated serum hepcidin levels, which provides a rationale for strategies that target hepcidin directly. Although combined hematopoietic and hepatic TFR2 deficiencies were most effective in ameliorating anemia after 8 weeks of adenine exposure, long-term effects were not investigated, and additional CKD models were not included.

The results reported by Olivari *et al.* suggest that as a future therapeutic target for CKD anemia, TFR2 would have to be nonfunctioning in both erythropoietic cells and hepatocytes to ameliorate anemia. Achieving such coordinated hepatic and erythropoietic deficiency may be challenging. The anti-sense oligonucleotide approach used in the studies reduced TFR2 in hepatocytes but not in erythroid cells. As a surface membrane protein, TFR2 may be targeted by monoclonal antibodies or inhibitory ligands, but TFR2's external domain homology with TFR1 will need to be considered to avoid inhibiting TFR1 activity. If EPOR hypersensitivity due to TFR2 deficiency can be achieved, then it will likely require tight control because erythrocytes produced by erythroid cells with EPOR hypersensitivity due to JAK2 mutation exacerbate vascular disease.[5]

Like TFR2 deficiency, other pharmacologic agents can modify expression of iron-erythropoiesis regulators.[1] Hypoxia-inducible factors (HIFs) regulate transcription of genes involved with iron-erythropoiesis interactions, including the genes encoding transferrin and EPO. Inhibitors of HIF–prolyl hydroxylases stabilize HIF α-subunits by preventing their proteasomal degradation. In clinical trials, HIF–prolyl hydroxylases improved anemia in CKD by stimulating endogenous EPO production, increasing unsaturated transferrin, and decreasing serum hepcidin levels.[1,6] These changes increase iron availability and utilization in erythropoiesis, as shown by uniform increases in erythrocyte size and hemoglobin content.[6] Inhibitors of sodium-glucose transport protein 2 decrease serum hepcidin and transferrin iron saturation while modestly increasing serum EPO levels.[7] In patients with CKD and type 2 diabetes, sodium-glucose transport protein 2 inhibitor therapy reduced risk for anemia-related events, including ESA use.[8] A clinical trial in patients with CKD of an anti–IL-6 monoclonal antibody, which potentially decreases hepcidin production by blocking the IL-6–JAK2–STAT3 pathway, showed increased

hemoglobin concentrations while decreasing serum hepcidin, increasing serum iron and transferrin, and decreasing ESA use.[9] In sum, clinical application of TFR2 deficiency to treat anemia in CKD could join other potential treatments under development that aim to minimize EPO exposure while improving iron availability. Each potential therapy involves deregulating the activity of a regulator (e.g., hepcidin or EPO) of the complex interactions between iron and erythropoiesis.

DISCLOSURE

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REFERENCES

1. Babitt JL, Eisenga MF, Haase VH, et al. Controversies in optimal anemia management: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) conference. *Kidney Int*. 2021;99:1280–1295.
2. Camaschella C, Pagani A, Silvestri L, Nai A. The mutual crosstalk between iron and erythropoiesis. *Int J Hematol*. 2022;116:182–191.
3. Olivari V, Di Modica SM, Lidonnici MR, et al. A single approach to targeting transferrin receptor 2 corrects iron and erythropoietic defects in murine models of anemia of inflammation and chronic kidney disease. *Kidney Int*. 2023;104:61–73.
4. Kautz L, Jung G, Nemeth E, Ganz T. Erythroferrone contributes to recovery from anemia of inflammation. *Blood*. 2014;124:2569–2574.
5. Liu W, Ostberg N, Yalcinkaya M, et al. Erythroid lineage Jak2V617F expression promotes atherosclerosis through erythrophagocytosis and macrophage ferroptosis. *J Clin Invest*. 2022;132:e155724.
6. Koury MJ, Agarwal R, Chertow GM, et al. Erythropoietic effects of vadadustat in patients with anemia associated with chronic kidney disease. *Am J Hematol*. 2022;97:1178–1188.
7. Ghanim H, Abuaysheh S, Hejna J, et al. Dapagliflozin suppresses hepcidin and increases erythropoiesis. *J Clin Endocrinol Metab*. 2020;105:e1056–e1063.
8. Oshima M, Neuen BL, Jardine MJ, et al. Effects of canagliflozin on anaemia in patients with type 2 diabetes and chronic kidney disease: a *post-hoc* analysis from the CREDENCE trial. *Lancet Diabetes Endocrinol*. 2020;8:903–914.
9. Pergola PE, Devalaraja M, Fishbane S, et al. Ziltivekimab for treatment of anemia of inflammation in patients on hemodialysis: results from a phase 1/2 multicenter, randomized, double-blind, placebo-controlled trial. *J Am Soc Nephrol*. 2021;32:211–222.

RORing toward immunoregulation in glomerulonephritis?

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CD4$^+$ T cells that express forkhead box protein 3 are important in maintaining tolerance and restraining effector responses. Herrnstadt *et al.* use a model of rapidly progressive glomerulonephritis to examine the nature and role of forkhead box protein 3–positive and retinoid acid–related orphan receptor γt–positive regulatory T cells. These cells are prominent in experimental glomerulonephritis, both locally and systemically, and are present in kidneys of people with anti-neutrophil cytoplasmic antibody–associated vasculitis. Functionally, despite their expression of retinoid acid–related orphan receptor γt, associated with T-helper cell 17 cells, they regulate cellular immunity, both systemically and within the kidney.

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Current treatments for immune-mediated kidney diseases are mostly nonspecific and tend to suppress a broad range of immune effectors.[1] Although improvements in the therapy of glomerulonephritis (GN) are likely to come from better use of current therapies, and targeting proinflammatory pathways upregulated in disease, enhancing regulatory (anti-inflammatory) components of immune responses is an alternative and potentially complementary strategy. Such an approach could not only suppress inflammation but also "reset" pathologic immune responses, particularly in autoimmune forms of glomerulonephritis. One way to promote immunoregulation is to enhance regulatory T-cell (Treg) number and/or function. Tregs are a specialized lineage of CD4$^+$ T lymphocytes that maintain tolerance, dampen inflammatory responses, and classically express the transcription factor forkhead box protein 3 (Foxp3). In this issue of *Kidney International*, Herrnstadt *et al.*[2] examine the role of CD4$^+$ Tregs that express not only Foxp3, typically associated with immunoregulation, but also the transcription factor retinoid acid–related orphan receptor γt (RORγt), classically expressed by proinflammatory T-helper cell 17 (Th17) cells.

Foxp3$^+$ Tregs are a well-known key immunosuppressive cell capable of restraining a wide range of immune responses to self and foreign antigens. *In vivo*, Foxp3$^+$ Tregs can be divided

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