

Toward a Novel Therapeutic Option for Polycythemia

By Francesca Vinchi

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Red blood cell (RBC) production is a finely tuned process that requires coordinated oxygen- and iron-dependent regulation of cell differentiation and iron homeostasis. Excess production of erythrocytes, referred to as erythrocytosis or polycythemia, occurs physiologically, as an adjustment to high altitude, or pathologically, because of intrinsic abnormalities in erythroid precursors or inappropriately high level of erythropoietin (EPO).

In response to hypoxia, RBC number is increased as a compensatory mechanism in an attempt to enhance oxygen availability and respiratory capacity. Hypoxia promotes EPO release by the kidney, which in turn stimulates RBC production. This process is orchestrated by key transcription factors, called hypoxia-inducible factors (HIFs). HIFs are increased in response to anemia as well as low oxygen and iron levels. Exposure to low oxygen levels causes the stabilization of the α subunits of HIFs (HIF1 α , HIF2 α), thus leading to the transcription of HIF targets, including EPO, and therefore erythrocytosis. HIF2 α is regulated at the posttranslational level by prolyl hydroxylases (PHDs) that use oxygen and iron as substrates to hydroxylate HIF2 α . Following hydroxylation, HIF2 α undergoes ubiquitination by the von Hippel-Lindau (VHL)-E3 ubiquitin ligase and degradation through the proteasomal pathway. Hence, HIF2 α can sense hypoxia and iron deficiency, and then increases EPO expression and drives RBC production. Missense mutations in VHL cause Chuvash polycythemia, an autosomal recessive disorder hallmarked by congenital erythrocytosis, due to excessive HIF2 α stabilization (Fig. 1).

The intracellular level of HIF α subunits are also regulated by iron regulatory proteins (IRP1, IRP2). IRPs act as cytosolic iron sensors and control the fate of messenger RNAs (mRNA) encoding proteins involved in iron metabolism. This role is dependent on their ability to bind specific RNA stem-loop structures, termed iron-responsive elements (IREs), on specific target mRNAs and control the translation of the encoded protein. IRP1 is a bifunctional protein that binds to IREs as an apo-protein in iron-deficient conditions (apo-IRP1), and it converts to cytosolic aconitase through the acquisition of an iron-sulfur [4Fe-4S] cluster in iron-replete conditions (holo-IRP1). IRP targets include transferrin receptor 1 (TfR1), divalent metal ion transporter 1 (DMT1), the iron exporter ferroportin (FPN) and the iron storage protein ferritin (Ft). The RNA binding activity of the IRPs is therefore regulated by cellular iron, being decreased in iron-replete cells and increased in iron-deplete ones. Hence, by regulating IRP activity, intracellular iron levels control the expression of IRP targets, adjusting iron handling (uptake, storage, and export) accordingly. As for iron, hypoxic conditions inactivate IRP1 by favoring holo-IRP1 formation and inhibiting IRE-binding activity, and relieve IRE-bearing transcripts from IRP1-mediated repression.

HIF2 α has been described as one of the targets of IRP1. In 2013, different groups showed that mice lacking IRP1 are hallmarked by elevated HIF2 α levels, increased EPO production and polycythemia.¹⁻³ These findings supported the concept that IRP1 has a crucial role in repressing HIF2 α translation, thus keeping in balance RBC production. Conversely, translational derepression of HIF2 α triggers hypoxia-like erythrocytosis. IRP1-deficient mice develop severe iron deficiency, likely caused by increased erythropoiesis that consumes large amounts of iron for RBC production, and consequently depletes circulating transferrin-bound iron as well as tissue iron stores. IRP1-mediated regulation of HIF2 α translation acts as a protective mechanism that prevents erythropoiesis from consuming too much iron, thus depleting systemic iron. While HIF2 α senses hypoxia and stimulates EPO expression and RBC production, IRP1 fine-tunes HIF2 α expression to ensure that there is enough iron available for iron-sulfur cluster synthesis. When too

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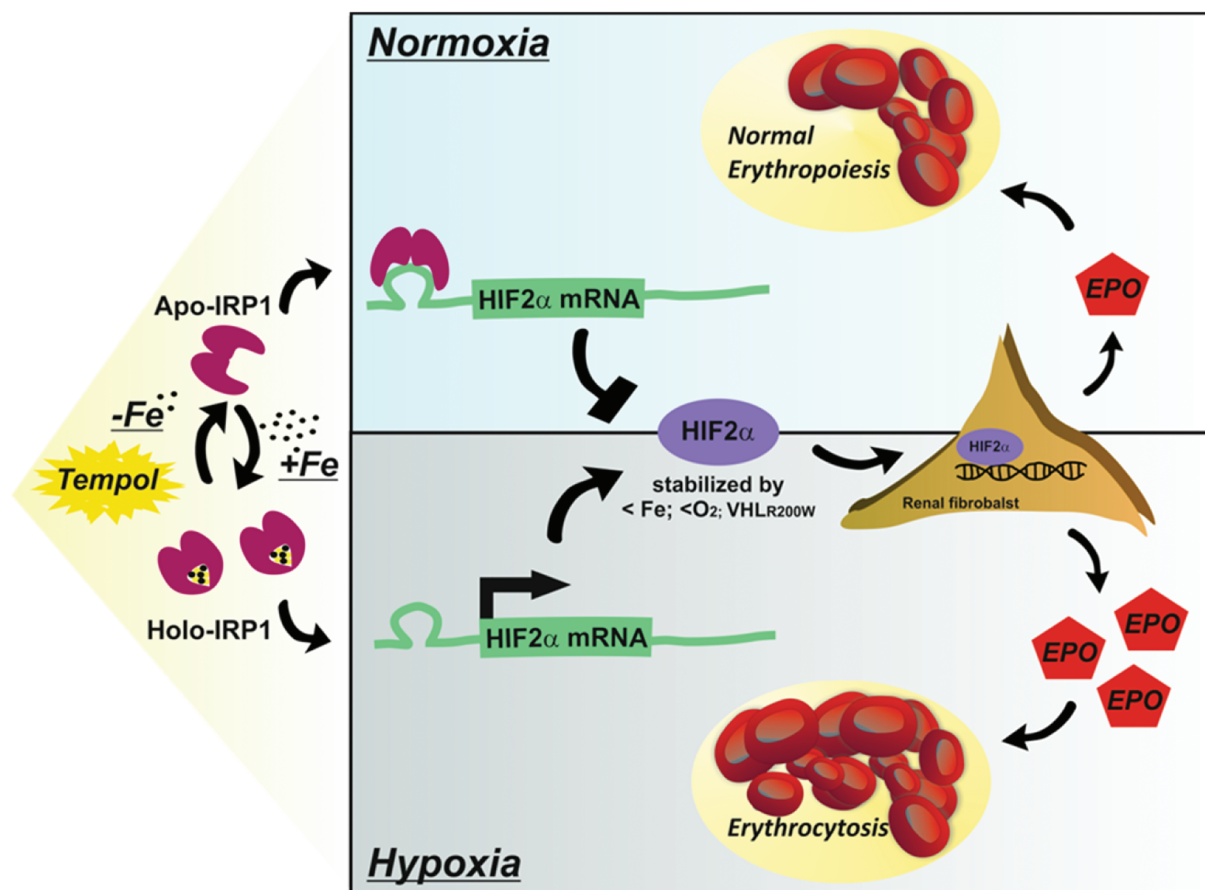


Figure 1. Tempol prevents hypoxia-induced erythrocytosis by inhibiting HIF2 α -mediated EPO production. In iron-replete conditions, IRP1 exerts cytosolic aconitase activity dependent on iron-sulfur cluster; in iron-deplete conditions, IRP1 loses the iron-sulfur cluster group and binds to the IRE sequence of target mRNAs, thus regulating the expression of iron-related genes. In renal fibroblasts, by binding HIF2 α IRE, IRP1 regulates translationally the expression of HIF2 α according to iron and oxygen status. In hypoxia or iron deficiency conditions, HIF2 α protein is stabilized due to the inactivation of PHD-VHL degradation pathway and translocates to the nucleus, where it transcriptionally activates EPO expression. EPO in turn acts on erythroblasts to stimulate RBC production. This process occurs under hypoxic conditions associated with high altitude and with pathological elevation of EPO (e.g., Chuvash mutation). Tempol, by activating the IRE binding activity of IRP1, mediates HIF2 α translational repression, which leads to reduced EPO production. This effect prevents hypoxia-induced erythrocytosis. Tempol can be applied as therapeutic strategy to counteract high altitude-triggered erythrocytosis as well as high HIF2 α -mediated polycythemia.

much iron is consumed, and systemic iron levels are low, iron-sulfur cluster synthesis is impaired and IRP1 is converted to the IRE-binding form, which represses HIF2 α translation, and thereby restricts RBC production. Through this feedback mechanism, IRP1 controls the balance between systemic iron homeostasis and erythropoiesis.³

Recently Ghosh et al⁴ showed that mice bearing the human missense mutation VHL^{R200W} develop Chuvash polycythemia, which closely reflects erythrocytosis observed in IRP1-deficient animals. VHL^{R200W}-mutant mice feature increased RBC counts, elevated hemoglobin levels, splenomegaly, and skin erythema. The authors used a stable nitroxide radical, Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), as a therapeutic approach to correct polycythemia in these animals.⁴ Tempol is a membrane-permeable free radical scavenger with antioxidant properties. It can degrade superoxide radicals in a superoxide dismutase (SOD) mimetic manner and suppress the formation of hydroxyl radicals by inhibiting Fenton's reaction. Importantly, Tempol has the ability to activate the IRE-binding activity of IRP1. Therefore the rationale for its administration in Chuvash polycythemia is based on the attempt to inhibit HIF2 α translation through enhanced HIF2 α IRE binding of IRP1. In the mouse model of Chuvash polycythe-

mia, Tempol ameliorates erythrocytosis by lowering HIF2 α expression and EPO levels (Fig. 1).⁴ The lack of hematocrit improvement in IRP1-deficient animals confirmed that Tempol-mediated amelioration of erythrocytosis requires IRP1 activation. Consistently, the authors elegantly showed that the therapeutic action of Tempol on Chuvash polycythemia is abrogated by the deletion of IRP1 in VHL^{R200W}-mutant mice.

These findings open a new perspective in the treatment of Chuvash polycythemia, for which phlebotomy is the primary standard therapy to date. Recurrent phlebotomies might result in a contradictory effect: phlebotomy-induced iron deficiency, by directly stabilizing HIF2 α , can further stimulate erythropoiesis, thus only temporarily and partially restoring hematocrit levels. The use of Tempol would overcome the limitation of the current therapeutic approach, by reducing HIF2 α levels. Interestingly, Tempol shows beneficial effects in other diseases hallmarked by elevated HIF2 α and/or VHL mutations, including VHL-deficient clear cell renal cell carcinoma (CCRCC), or neurodegeneration associated with IRP2 loss and altered cell iron homeostasis.⁵

Importantly, Ghosh et al demonstrated the even broader relevance of this therapeutic strategy for individuals exposed to hypoxic conditions in high altitude, who commonly develop

erythrocytosis. Wild-type mice exposed to prolonged hypoxia are in part protected from polycythemia development and show a longer life expectancy when treated with Tempol (Fig. 1).⁴ Thus Tempol represents a potentially valuable therapy to limit polycythemia triggered by high altitude and its associated side effects.

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