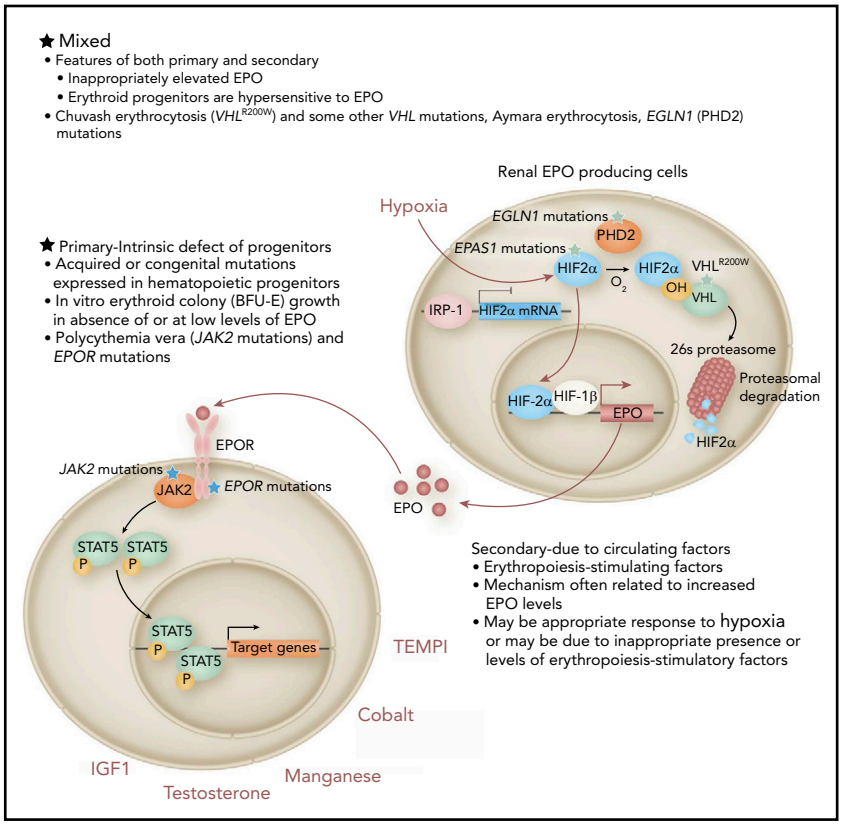
Comment on Ghosh et al, page 2509

HIF-2 inhibitor, erythrocytosis, and pulmonary hypertension

Josef T. Prchal[1,2] and Victor R. Gordeuk[3] | [1]University of Utah; [2]Huntsman Cancer Center at Salt Lake City; [3]University of Illinois at Chicago

In this issue of *Blood*, Ghosh et al[1] describe correction in animal models of 2 distinct types of erythrocytosis and their associated pulmonary hypertension by an inhibitor of 1 of the hypoxia inducible transcription factors (HIFs; ie, HIF-2).

Both erythrocytosis and polycythemia have been used to describe conditions associated with elevation of red cell mass, which often, but not always, is reflected by a concomitant elevation of hemoglobin and hematocrit. Because the latest publications use the term polycythemia only for polycythemia vera (PV) but not for all other forms of erythrocytosis, we will also use the same terminology here. Erythrocytoses are congenital or acquired, appropriate responses to tissue hypoxia or inappropriate (unrelated to tissue hypoxia), and primary or secondary. Primary erythrocytoses/polycythemia are exemplified by intrinsic hyperproliferation of erythroid progenitors caused by either *JAK2* somatic mutations of hemopoietic stem cells in PV or by germline gain-of-function mutations of the erythropoietin (EPO) receptor in primary familial erythrocytosis. In contrast, the secondary erythrocytoses, either acquired or inherited, are caused by circulating factors, most commonly EPO, and the erythroid progenitors are intrinsically normal[2] (see figure).



Erythrocytosis can be primary, secondary, or mixed. Pathways for each of these types of erythrocytosis are depicted. BFU-1, burst forming unit erythroid; *EGLN1*, gene encoding prolyl hydroxylase-PHD2; *EPAS1*, gene encoding HIF-2α; EPO, erythropoietin; EPOR, erythropoietin receptor; HIF, hypoxia-inducible transcription factor; IGF1, insulin-like growth factor-1; IRP1, iron regulatory protein 1; JAK2, Janus kinase 2; STAT 5, signal transducer and activator of transcription; TEMPI, telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting; VHL, von Hippel-Lindau protein.

Hypoxia regulates many physiologic processes, but these responses may also be misregulated and cause human diseases. Erythropoiesis, iron regulation, vasculogenesis, energy metabolism, and cancer may all be impacted by abnormal responses to hypoxia. Hypoxic responses are regulated by transcription factors HIF-1 and HIF-2. HIF-1 was discovered from studies of the hypoxic transcriptional regulation of *EPO*. In hypoxia, HIF-1 binds to the DNA hypoxia-responsive element oligomer of *EPO*, which greatly augments *EPO* transcription. A plethora of other genes are also regulated by HIFs via hypoxia-responsive elements. The impact of HIFs in medicine was recognized by the Nobel Prize awarded in 2019 to Kaelin, Ratcliffe, and Semenza.

HIF-1 is ubiquitously expressed, whereas HIF-2 has restricted expression, and these factors may differ in their specificity. HIF-1α and HIF-2α subunits share the same β subunit; only the dimers have transcriptional activity. The α-subunits of HIFs are rapidly degraded in normoxia. This is initiated by prolyl hydroxylation of α-subunits by iron-dependent prolyl hydroxylases. These hydroxylated α-subunits then bind the von Hippel-Lindau protein, and this complex is ubiquitinated and degraded in proteasomes.[2]

Although it is clear that HIF-2 is the principal regulator of *EPO* transcription, HIF-1 also exerts control of erythropoiesis. The deletion of *Epas1* (encoding Hif-2α) in the murine embryo results in pancytopenia with only a modest decrease in hematocrit. In contrast, the deletion of the *Hif-1a* gene causes a lethal defect of erythropoiesis.[2]

The first congenital disorder of hypoxia sensing to be described is Chuvash erythrocytosis (CE), an autosomal recessive disorder caused by the *VHL*^{R200W}

loss-of-function mutation leading to elevated levels of both HIF-1 and HIF-2.[3] This disorder is endemic in Chuvashia (Republic of Russia) and in the Italian island of Ischia; it is sporadic worldwide. Patients with CE not only have elevated EPO (a feature of secondary erythrocytosis) but also increased sensitivity of erythroid progenitors to EPO (a feature of primary erythrocytosis). Thromboses are the principal cause of morbidity and mortality in CE. However, thromboses are not related to increased hematocrit and are increased in phlebotomized patients.[4] Elevated pulmonary artery pressures may also occur in CE.[4]

Hypoxic regulation of iron availability and erythropoiesis are closely related as exemplified by the interaction of iron metabolism with erythropoiesis by hepcidin and erythroferrone.[5] Iron regulatory protein (IRP-1) binds to the iron-responsive element in the 5′ untranslated region of HIF-2α and represses the translation of HIF-2α protein.[6] Ghosh et al previously reported that deletion of *Irp1* in mice results in erythrocytosis, pulmonary hypertension, and cardiac fibrosis attributable to translational derepression of Hif-2α. The resultant high expression of Hif-2α targets EPO, endothelin-1, and a chemokine Cxcl12.[7] This year, 4 families from Iceland and the United Kingdom with erythrocytosis and mutations in IRP1 were described.[8] They represent a novel type of congenital erythrocytosis.

Ghosh et al now convincingly demonstrate the crucial role of HIF-2 in murine models of 2 congenital types of erythrocytosis: (1) CE and (2) erythrocytosis caused by mutation of *IRP1*. Specific inhibitors of HIF-2 have been developed based on a unique cavity in the structure of HIF-2α and have already been used in clinical trials of renal carcinoma. The authors of this paper used a second-generation inhibitor of HIF-2α (ie, MK-6482), and its oral administration corrected erythrocytosis and pulmonary hypertension in both Vhl^{R200W} and *Irp1* knockout mice and also normalized the expression of HIF-2–regulated genes.

Whether the use of the HIF-2α inhibitors will be clinically beneficial to correct hematocrit in patients with these and other types of erythrocytosis remains to be shown. HIF-2α inhibitors would likely correct the appropriate types of erythrocytosis, such as in pulmonary disease, Eisenmenger complex, and erythrocytosis because of high-affinity hemoglobins. However, this might not be clinically beneficial, as the resultant decrease of blood oxygen–carrying capacity would make tissue hypoxia worse. Similarly, HIF-2α inhibitors may not correct erythrocytosis of PV, a disease characterized by low EPO levels. Although correction of elevated hematocrit is a common practice, it is not universally accepted that reduction of hematocrit in PV reduces the predisposition to thromboses, PV's major morbidity and mortality risk.[9]

It is enticing to hypothesize that these results can be extended to other more common acquired disorders. Pulmonary hypertension is a devastating disorder that is generally acquired, but some patients have a congenital predisposition. The possible corrective use of HIF-2α inhibitors in pulmonary hypertension would be of immense value.

Elevated signaling by HIFs is present in the platelets and granulocytes of both CE and PV, augmenting transcription of prothrombotic genes such as tissue factor, which is a HIF-1–regulated gene.[10] Although it is likely that HIF-2α inhibitors will correct erythrocytosis in patients with CE, it is not clear that they will ameliorate thrombotic risk in either CE or PV if thrombosis is predominantly mediated by HIF-1–regulated genes. Furthermore, the increased erythroid sensitivity to EPO in CE may not be caused by HIF-2 but rather HIF-1; in our ongoing experiments, we can correct this by use of the specific HIF-1 inhibitor, digoxin,[1,10] but more work needs to be done in this area.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Ghosh MC, Zhang D-L, Ollivierre WH, et al. Therapeutic inhibition of HIF-2α reverses polycythemia and pulmonary hypertension in murine models of human diseases. *Blood*. 2021;137(18):2509-2519.
2. Prchal JT. Primary and secondary erythrocytoses/polycythemias. In: Kaushansky K, Lichtman MA, Prchal JT, Levi M, Burns LJ, Linch DC, eds. Williams Hematology, 10th ed. New York: McGraw Hill; 2020:941-959.
3. Ang SO, Chen H, Hirota K, et al. Congenital Chuvash polycythemia: altered oxygen homeostasis due to homozygosity for a hypomorphic *VHL* allele. *Nat Genet*. 2002;32(4):614-621.
4. Gordeuk VR, Miasnikova GY, Sergueeva AI, et al. Thrombotic risk in congenital erythrocytosis due to up-regulated hypoxia sensing is not associated with elevated hematocrit. *Haematologica*. 2020;105(3):e87-e90.
5. Ganz T. Iron metabolism. In: Kaushansky K, Lichtman MA, Prchal JT, Levi M, Burns LJ, Linch DC, eds. Williams Hematology, 10th ed. New York: McGraw Hill; 2020:6730683.
6. Percy MJ, Sanchez M, Swierczek S, et al. Is congenital secondary erythrocytosis/polycythemia caused by activating mutations within the HIF-2 α iron-responsive element? *Blood*. 2007;110(7):2776-2777.
7. Ghosh MC, Zhang DL, Jeong SY, et al. Deletion of iron regulatory protein 1 causes polycythemia and pulmonary hypertension in mice through translational derepression of HIF2α. *Cell Metab*. 2013;17(2):271-281.
8. Oskarsson GR, Oddsson A, Magnusson MK, et al. Predicted loss and gain of function mutations in ACO1 are associated with erythropoiesis. *Commun Biol*. 2020;3(1):189.
9. Gordeuk VR, Key NS, Prchal JT. Re-evaluation of hematocrit as a determinant of thrombotic risk in erythrocytosis. *Haematologica*. 2019;104(4):653-658.
10. Gangaraju R, Song J, Kim SJ, et al. Thrombotic, inflammatory, and HIF-regulated genes and thrombosis risk in polycythemia vera and essential thrombocythemia. *Blood Adv*. 2020;4(6):1115-1130.

DOI 10.1182/blood.2020010323