On either side of homeostasis: *EPAS1* gain- and loss-of-function mutations

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In this issue of *Haematologica*, Rosato and colleagues from the laboratory of Dr. Roberta Russo, and other prominent collaborators, report their studies of a boy with anemia referred to them by clinical hematologists from Ancona, Italy, who noted that the child had moderate anemia but did not have appropriately elevated erythropoietin (EPO) expression.[1] In fact, his EPO level was low, suggesting that it was the cause of his anemia. All the common causes of anemia were excluded. The presence of a similar phenotype in the father and his older sister suggested a genetic disorder with autosomal dominant inheritance.

The delivery of O_2 by red cells to all the other cells of the body is based on a beautiful homeostatic system in which hypoxia leads to increased EPO production, which binds to its receptor (EPOR) on erythroid progenitor cells and stimulates increased red blood cell formation, thereby ameliorating tissue hypoxia. The response to hypoxia is mediated by a short DNA sequence located downstream of the *EPO* gene that is known as the hypoxia response element, which contains a binding site for hypoxia-inducible factors (HIF).[2] HIF are heterodimeric transcription factors that consist of an O_2-regulated HIF-1α, HIF-2α or HIF-3α subunit and a constitutively expressed HIF-1β subunit.[3] HIF-1 is expressed in virtually all nucleated cells, whereas HIF-2 and HIF-3 have limited tissue expression. In the presence of O_2, HIF-α subunits are modified by a family of prolyl hydroxylase domain proteins (PHD1-3) and the hydroxylated proteins are bound by the von Hippel-Lindau (VHL) protein, which recruits a ubiquitin protein ligase, leading to the ubiquitination and proteasomal degradation of the HIF-α proteins. Under hypoxic conditions, the hydroxylation reaction is inhibited, and HIF-α subunits rapidly accumulate and regulate the expression of thousands of genes, including *EPO*, which mediate adaptive responses to hypoxia.

The key role of the HIF-PHD-VHL pathway in regulating erythropoiesis was underscored by the finding that familial erythrocytosis (i.e., abnormally increased red blood cell levels) is in some cases due to: a mutation in PHD2 that decreases hydroxylase activity; a mutation in VHL that decreases its binding to hydroxylated HIF; or a mutation in HIF-2α that protects it from hydroxylation (Table 1 lists all loci encoding HIF pathway components at which mutations have been identified; the references are available in the paper by Semenza[3]). However, the report by Rosato *et al*. provides the first example of the converse: that loss of HIF-2α expression results in anemia.[1] The authors of this paper found in the propositus and his anemic family members a novel *EPAS1* mutation, c.(61del), consisting of deletion of the 61st nucleotide of the coding sequence. This single nucleotide deletion alters the reading frame, resulting in the generation of a premature stop codon, such that the protein translated from the mutant mRNA would only contain the first 20 amino acids of HIF-2α. This severely truncated protein is likely degraded, resulting in a null allele and haploinsufficiency for HIF-2α, which leads to deficient EPO synthesis and anemia.

HIF-2, like HIF-1, has other systemic regulatory functions. *EPAS1* gain-of-function mutations have diverse pathophy-

Table 1. Identified causes of familial erythrocytosis.

ECYT*	Gene	Protein	Mutation§	Effect	Genetics
ECYT1	*EPOR*	EPOR	W439X	GOF	AD
ECYT2	*VHL*	VHL	R200W	LOF	AR
ECYT3	*EGLN1*	PHD2	P317R	LOF	AD
ECYT4	*EPAS1*	HIF-2α	G537W	GOF	AD
ECYT5	*EPO*	EPO	c.32delG	GOF	AD

*ECYT6-8 are not shown: these are due to mutations in β-globin, α-globin, and bis-phosphoglycerate mutase, respectively, which affect hemoglobin-O_2 affinity. §At each locus, multiple mutations have been identified in different affected individuals; only the first reported mutation is shown. All are missense mutations except ECYT1 (nonsense) and ECYT5 (single nucleotide deletion). ECYT: familial erythrocytosis; EPOR; erythropoietin receptor; GOF: gain-of-function; AD: autosomal dominant inheritance; VHL: von Hippel-Lindau; LOF: loss-of-function; AR: autosomal recessive inheritance; PHD2: prolyl hydroxylase domain protein 2; HIF-2α: hypoxia-inducible factor-2α; EPO: erythropoietin.

siological consequences in addition to erythrocytosis, including pulmonary hypertension[4] and thrombosis.[5] Whether there is impairment of any other HIF-2-regulated systemic function associated with the *EPAS1*c.(61del) genotype remains to be determined. One recent scientific advance may point to a potential therapy for affected individuals, which is the Food and Drug Administration's approval, just several months ago, of daprodustat, a selective inhibitor of the HIF prolyl hydroxylases, which increases HIF activity and thereby increases EPO production.[6] The drug has been approved for the treatment of anemia in patients with dialysis-dependent chronic kidney disease, which leads to a progressive loss of EPO production. It is likely that daprodustat would boost HIF-2α levels in *EPAS1*c.(61del) carriers, leading to increased EPO mRNA and protein expression. The close coupling of this drug approval and the report by Rosato *et al*. highlight the remarkably rapid progress that has been made since the discovery of HIF-1 three decades ago,[2] with the development of the HIF stabilizer daprodustat for the treatment of anemia[6] and the HIF-2α inhibitor belzutifan for the treatment of renal cell carcinoma.[7] This is just the beginning.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally.

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