Hypoxia. 5. Hypoxia and hematopoiesis

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Yoon D, Ponka P, Prchal JT. Hypoxia. 5. Hypoxia and hematopoiesis. Am J Physiol Cell Physiol 300: C1215–C1222, 2011. First published March 2, 2011; doi:10.1152/ajpcell.00044.2011.—Our understand- ing of organismal responses to hypoxia has stemmed from studies of erythropoietin regulation by hypoxia that led to the discovery of the master regulator of the hypoxic response, i.e., hypoxia-inducible factor (HIF). This is a transcription factor that is now known to induce the expression of a battery of genes in response to hypoxia. HIF-1 and HIF-2 regulate many genes that are involved in erythropoiesis and iron metabolism, which are essential for tissue oxygen delivery.

Hypoxia-inducible factor; erythropoiesis; oxygen delivery

HEMATOPOIESIS is the process by which pluripotent stem cells differentiate into mature erythroid (red blood cells), myeloid (neutrophils, eosinophils, basophils, and monocytes), megakaryocytic (platelets), mast, and lymphoid (B, T, and NK cells) cell types (Fig. 1). This results in the steady level of circulating blood cells with each cell type possessing unique function and distinct life spans. Each year, an average adult produces ~200–300 kg of blood cells equivalent to three to four times a person’s body weight. The large production numbers and fast turnover of blood cells indicate that hematopoietic cell proliferation and differentiation must be tightly controlled; even a small negative or positive imbalance in their production or life spans would cause anemia and other cytopenias or hyperviscosity due to erythrocytosis/polycythemia and specific complications from various types of leukocytosis and thrombocytosis. Hematopoiesis first appears in the yolk sac and then in the aorta-gonad-mesonephros region of the embryo, subsequently in the fetal liver and, finally, in the bone marrow of an adult. To support this process, stem cells and their progenies must be maintained in close contact with stromal cells, which are nonhematopoietic mesenchymal cells. Hematopoietic growth factors control the development of many blood cell types by sustaining cell viability, proliferation, or differentiation. To support these functions, hematopoietic growth factors must regulate hematopoietic stem cells and their differentiating progenies. These modifications include altering gene expression and interaction of transcription factors and/or signaling to regulate differentiation and proliferation. This review will primarily focus on the regulation of oxygen delivery to the tissues in response to changes in oxygen tension that is mediated by the modulation of red cell production in health and disease.

The body adapts to hypoxia (low oxygen tension) by numerous mechanisms. One of these adaptive responses is to enhance erythropoiesis and, consequently, increase circulating red blood cells to deliver more oxygen to tissues. The key regulators of the hypoxic response pathway are hypoxia-inducible factors (HIFs). HIFs are transcription factors composed of a hypoxia inducible α subunit and a constitutively expressed β subunit (also called aryl hydrocarbon receptor nuclear translocator, ARNT). The α subunits consist of three different isoforms: HIF-1α, HIF-2α, and HIF-3α. HIF-1α and HIF-2α share significant sequence identity and regulation. The HIF-1α subunit is ubiquitously expressed, whereas HIF-2α and HIF-3α subunits have tissue-specific expression. In the presence of oxygen, HIF-1α and HIF-2α are prolyl hydroxylated by iron requiring prolyl hydroxylase domain (PHD2) enzymes, and their hydroxylated products interact with von Hippel Lindau (VHL) protein, which leads to ubiquitinization and rapid destruction in proteasomes (47). In hypoxia, HIF-1α and HIF-2α subunits escape ubiquitination-dependent proteasomal protein degradation that takes place in normoxia. The accumulated HIF-1α and HIF-2α subunits are rapidly translocated into the nucleus and form heterodimers with the β subunit. Thus resultant hypoxia-induced stabilization of HIF α subunits permits the formation of the active HIF heterodimers that bind to the promoter or enhancer region of their target genes and modulate their transcription. However, since HIF-3α has no nuclear localizing sequence or coactivator binding domain, it may have no transcriptional activity (33). Historically, HIF-1α was identified as a main regulator for erythropoietin (EPO) gene (78). Subsequently, numerous targets of HIFs have been identified, including vascular endothelial growth factor (VEGF), transferrin (TF), and transferrin receptor (TFR) and an array of other genes. It is estimated that in endothelial cells as many as 3% of genes are hypoxia regulated (43).

ROLE OF ERYTHROPOIETIN IN ERYTHROPOIESIS

EPO plays a central role in the regulation of erythropoiesis by blocking apoptosis in the late erythroid progenitors (colony forming units-erythroid, CFU-Es) and enhancing proliferation and terminal differentiation of their progenies (36). It has also been reported that EPO contributes to the expansion of multipotent hematopoietic progenitors (75). Excessive EPO levels lead to an accumulation of red blood cells, i.e., polycythemia. During fetal development EPO is mainly produced in the liver; its primary site of production then changes to the kidney during late gestation but continues to a lesser extent in the liver. Renal EPO is produced by peritubular fibroblast-like type-1 interstitial cells located in the renal cortex and outer medulla (7, 46, 48, 89). Hepatic EPO is produced by hepatocytes (48) and contributes about 10% of the plasma EPO. However, the central nervous system also expresses EPO and its receptor (EPOR) wherein neurons and astrocytes in the cortex and the hippocampus of murine and human brains are the sources of
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Fig. 1. The diagram illustrates the development of blood cell lineages from pluripotent hematopoietic stem cells into mature cells. CFU, colony forming unit; GEMM, granulocyte, erythrocyte, monocyte, and megakaryocyte; EO, eosinophil; GM, granulocyte and macrophage; Meg, megakaryocyte; BFU-E, burst forming unit-erythrocyte; Bas, basophil; NK, denotes natural killer cells.

cerebral EPO (13, 44). Cerebral EPO may not contribute to the circulating levels of EPO due to the blood-brain barrier, thus the role of cerebral EPO does not directly modulate erythropoiesis; however, EPO likely plays a protective role against hypoxia and ischemia (88). It has been shown that the astrocyte-specific deletions of Hif-1α and Hif-2α in mouse do not cause anemia but reduce the acute erythropoietic response to hypoxia by ~50% (89). Besides the kidney, liver, and brain, hypoxia induced EPO transcripts were also found in several other tissues; i.e., spleen, lung, testis, and erythroid progenitors (21, 85).

**TISSUE-SPECIFIC REGULATORY ELEMENTS OF THE ERYTHROPOIETIN GENE**

Hypoxia is the main regulator of EPO levels. Acute exposure of mammals, including humans, to hypoxia at high altitude enhances plasma EPO more than 10-fold (1). This hypoxia-associated elevation of EPO levels is primarily caused by increased EPO expression in the kidney and the liver and is regulated by HIF-1 and HIF-2-mediated transcription of the EPO gene. Several laboratories identified nucleotide sequences (named hypoxia responsive element; HRE) responsible for hypoxia-inducible EPO gene transcription located immediately after the polyadenylation site of the human and mouse EPO gene (9, 64, 81). Additional HREs were identified 5′- and 3′-flanking regions of the EPO gene, and the tissue-specific EPO regulation was demonstrated to be mediated by these tissue-specific genomic regions (78–80). Transgenic mouse experiments using various deletions of 5′- and 3′-flanking regions of the human EPO gene identified the location of different tissue-specific EPO gene hypoxia regulatory elements; i.e., kidney inducible element (KIE) located between −14 kb and −6 kb of 5′flanking region of the human EPO gene and the negative regulatory element (NRE) located within −6 kb to 5′ of the human EPO gene. However, these reported elements were composed of too large DNA segments (>4 kb) and need to be delineated to smaller specific nucleotide sequences to properly identify transcription factors and further define their functional relevance and tissue specificity.

More recently, GATA binding motifs were identified in the proximal region (also called the core promoter region) of the mouse Epo gene, where GATA-2 and GATA-3 bind. This GATA binding element serves as a negative regulatory element and it is reported to have an important role in tissue-specific Epo production (29, 30, 48).

**TISSUE-SPECIFIC HIF-1 AND HIF-2 REGULATION OF ERYTHROPOIETIN**

As discussed above, hypoxia increases HIFs by stabilizing HIF-α subunits. HIF-1α−/− deficient mouse embryos are viable only up to day 11.5 and at embryonic stage 9–10 days have low levels of the Epo transcript, even though HIF-2α transcript levels are comparable to wild-type littermates (90). This demonstrates that HIF-1 plays the central role in Epo production during early mouse embryogenesis and that HIF-2 cannot compensate for the HIF-1 role during this developmental stage.

**Differential Effect of HIF-1 and HIF-2 on Renal and Hepatic EPO Production**

HIF-1 was first identified by studies of the regulation of EPO production in a hepatic cancer cell line. Yet kidney is the major site of EPO production, as witnessed by the fact that nonrenal EPO does not compensate for the loss of renal EPO production in patients with chronic kidney disease. The following evidence for the relative roles of HIF-1 and HIF-2 regulation of EPO transcription in the kidney and liver is available. In the adult stage, HIF-2 plays a key role in hepatic EPO production: 1) A mouse with inactivated Vhl (a negative regulator of HIF-1α and HIF-2α) and Hif-1α genes in hepatocytes had increased hepatic Epo production and polycythemia (67); 2) Conditional inactivation of Hif-2α in a pVhl-deficient mouse suppressed hepatic Epo transcripts and corrected polycythemia (66). Work from the same group examined the role of HIF-2α in renal Epo production using kidney cell-specific deletion of Hif-2α (32). Hif-2α-deficient mice had significantly decreased red blood cell counts and hematocrit levels. In these mice, plasma Epo protein levels were lower (116±12 pg/ml in kidney-specific Hif-2α-deficient mouse vs. 166±9 pg/ml in control) and Epo mRNA levels were ~12-fold less than control. There was increased HIF-1α protein and HIF-1 target gene transcripts in the kidney, but this could not correct for the Hif-2 deficiency-induced anemia. Furthermore, upon hypoxic exposure, the renal Epo transcript in Hif-2α-deficient mice was not induced in contrast to wild-type littermate control. In addition, these investigators also generated a kidney- and liver-specific Hif-2α-deficient mouse and showed that in hypoxia the kidney- and liver-specific Hif-2α-deficient mouse had
and have poor vascularization of the yolk sac. At embryonic day 9.5, Hif-1 decreased hemoglobin content in erythroid colonies from multilineage and committed erythroid progenitors, as well as Hif-2 deficient embryos. Hif-1-deficient embryos are embryonic lethal by day 10.5 and have decreased myeloid multilineage and committed erythroid progenitors, as well as decreased hemoglobin content in erythroid colonies from Hif-1α-deficient yolk sac progenitors (90). These Hif1-α-/- embryos had a significant decrease in EpoR mRNA levels in yolk sac, as well as Epo and EpoR mRNA in the embryo and a profound defect in iron homeostasis, as demonstrated by aberrant expression of hepcidin, Fpn1, Irp1, and Frascati. The erythropoietic defects in Hif-1α-deficient erythroid colonies could not be corrected by cytokines, including supraphysiologic concentrations of EPO and VEGF; the defects were ameliorated but not fully corrected by ferric salicylaldehyde isonicotinoyl hydrazone (Fe-SIH), a compound delivering iron into cells independently of iron transport proteins. These results demonstrated that HIF-1α is not essential for the formation of multipotent hematopoietic progenitors, but that HIF-1 is required for optimal erythropoiesis (90). These results suggest that HIF-regulated factor(s) besides EPO play an important role in HIF-1-dependent erythropoiesis.

Regulation of EPO in the Brain

Both mouse neurons and astrocytes express Epo gene following ischemic injury. The role of mouse cerebral Epo was investigated as a component of the systemic response to hypoxia using mice with astrocyte-specific deleted Hif-1α, Hif-2α, or Vhl. The mouse with astrocyte-specific Vhl deletion had polychemotaxis and elevated plasma Epo levels (89). To analyze the relative roles of Hif-1 and Hif-2 since Vhl deficiency causes both Hif-1α and -2α stabilization, double-deficient mice (Vhl+/Hif-1α−/− and Vhl+/Hif-2α−/−) were also examined. Whereas absence of VHL and HIF-1α in astrocytes does shorten its lifespan, loss of VHL and HIF-2α rescue the mouse up to normal lifespan with normal plasma Epo level (89). Deletion of Vhl in astrocytes increased cerebral Epo transcript, and this elevation was almost abolished by concurrent deletion of Hif-2α but not Hif-1α (89). In addition, plasma Epo elevation from astrocyte-specific deletion of Vhl or Vhl/Hif-1α resulted in reduction of renal Epo transcript with no changes in hepatic Epo transcript (89). The hypoxia induction of Epo transcript as well as plasma Epo in astrocytes-specific deleted Hif-2α mouse was significantly reduced, but double deletion of Hif-1α and Hif-2α in astrocytes showed further reduction (89). These combined results indicated that although Hif-2 is the major contributor for hypoxia-induced cerebral Epo, there was also a contribution of Hif-1. In these experiments not only the brain but also plasma Epo levels increased suggesting that brain directly participates in mouse hypoxia-induced erythropoiesis independently of renal function by a yet to be defined mechanism.

ERYTHROPOIESIS AND IRON METABOLISM

Red blood cells, which contain ~80% of organismal iron, have a particularly intimate relationship with this metal and its ligand oxygen. Since the ferrous iron of each heme group can bind a single oxygen molecule, the hemoglobin tetramer can reversibly bind and transport four molecules of oxygen. Heme is central for delivery of oxygen from erythrocytes to tissue. Additionally, the optimal delivery of iron is essential for heme and hemoglobin synthesis. Iron deficiency leads to impaired erythropoiesis resulting in hypochromic anemia, a testimony to the close link of iron metabolism and erythropoiesis and the optimal function of hemoglobin. HIFs play a central role in control of both iron metabolism and erythropoiesis. In turn, iron availability is essential for the activity of the negative regulators of α subunits levels of HIFs; i.e., PHD enzymes (59).

Hif-1α-/- Embryos

Important lessons were learned from Hif-1α-/- embryos (31). Hif-1α-deficient embryos are embryonic lethal by day 10.5 and have poor vascularization of the yolk sac. At embryonic day 9.5 these Hif-1α-/- embryos have decreased myeloid multilineage and committed erythroid progenitors, as well as decreased hemoglobin content in erythroid colonies from Hif-1α-deficient yolk sac progenitors (90). These Hif1-α-/- embryos had a significant decrease in EpoR mRNA levels in yolk sac, as well as Epo and EpoR mRNA in the embryo and a profound defect in iron homeostasis, as demonstrated by aberrant expression of hepcidin, Fpn1, Irp1, and Frascati. The erythropoietic defects in Hif-1α-deficient erythroid colonies could not be corrected by cytokines, including supraphysiologic concentrations of EPO and VEGF; the defects were ameliorated but not fully corrected by ferric salicylaldehyde isonicotinoyl hydrazone (Fe-SIH), a compound delivering iron into cells independently of iron transport proteins. These results demonstrated that HIF-1α is not essential for the formation of multipotent hematopoietic progenitors, but that HIF-1 is required for optimal erythropoiesis (90). These results suggest that HIF-regulated factor(s) besides EPO play an important role in HIF-1-dependent erythropoiesis.

Developing red blood cells are the most avid consumers of iron in the organism. Immature erythroid cells can obtain iron only from plasma TF following its binding to the membrane TFR and internalization of TF-TFR complexes (35). If hypoxia is unable to augment iron supply to erythroid progenitors, the iron availability could become a limiting factor for hypoxia-stimulated erythropoiesis. Hypoxia increases TF levels (69) and HIF-1 enhances the expression of TF (72). The TFR promoter region contains a functional HRE, which mediates transcriptional activation of TFR in response to hypoxia (40, 86). Moreover, a putative HRE has been identified in the promoter region of DMT1 (divalent metal transporter 1) (38) that exports TF-borne ferrous iron from endosomes and transports Fe²⁺ into duodenal enterocytes. Recent studies demonstrated that although HIF-1α is not necessary for iron absorption, HIF-2α plays an important role in regulating the transcription of DMT1 (45). Taken together, these reports support a model that hypoxia may augment the overall iron transport machinery, resulting in increasing iron uptake into the cells. The rescue of the erythropoietic differentiation defect in Hif-1α-/- yolk sacs by supplementation with Fe-SIH indicates that defects in iron metabolism significantly contribute to the phenotype seen in these animals (90).

HIFs and non-erythroid hematopoiesis

HIFs in The Early Hematopoietic Progenitors and Microenvironment

During mammalian embryogenesis, oxygen concentrations in the embryo are at hypoxic levels (22, 71). Vascular endothelial cells and hematopoietic stem cells originate from a common ancestor, i.e., the hemangioblast, and demonstrate hypoxia-induced cell proliferation (2, 60).

Arn−/− mouse. Unlike the HIF1-α subunits, the protein level of the HIF1-β subunit, also known as ARNT, is not affected by hypoxia. Yet the ARNT subunit is required to form functioning HIF heterodimers. Arn deficiency is embryonic lethal by day 10.5, and Arn−/− embryos exhibit various developmental defects, including defective angiogenesis and hematopoiesis (42). Arn−/− embryos also have decreased (CD34+) hematopoietic stem cells, and Arn−/− embryonic stem cells fail to respond to hypoxia (42). Colony-forming assays in various hematopoietic lineages from Arn−/− embryoid bodies exhibited only not significant reductions of hematopoietic progenitors but also did not respond to hypoxia.
(2). Similar defects were also found in a mouse with deleted VEGF receptor (Flk-1) wherein there was also a decrease of embryonic stem cells, and these defects were corrected by exogenous VEGF (2, 74). Collectively, these results demonstrated that hypoxia induced the proliferation and differentiation of hematopoietic progenitors and that this effect was at least in part mediated by HIF-induced VEGF. HIF-1 was reported to play an important role in the maturation of mesoderm into hemangioblast, and the lack of HIF-1 resulted in a reduction of hematopoietic and endothelial precursors (65). Since Arnt also forms heterodimer with the arylhydrocarbon receptor and Sim (42), studies of the Arnt¡/ ¡embryo must be interpreted with caution as a model for understanding the role of HIFs in hematopoiesis.

Hif-2α¡/ ¡mouse. The elegant work of Scortegagna and colleagues (76) demonstrated that HIF-2 has significant role in the bone marrow microenvironment. The Hif-2α¡/ ¡mouse was viable but with significant perinatal mortality. The transplantation studies with normal bone marrow and the Hif-2α¡/ ¡marrow to a wild-type recipient led to the conclusion that the diminished peripheral blood counts were due to the HIF-2 effect on the bone marrow microenvironment or on expression of systemic mediators.

Gain-of-function EPOR mutation. A human gain-of-function EPOR mutation causes dominantly inherited polycythemia (20, 84). The corresponding knock-in mouse exhibits an enhancement of in vitro differentiation of CD34+ progenitors into erythroid colonies and in humans is associated with an increase of circulating endothelial precursors (57). The gain-of-function EPOR knock-in mouse has enhanced erythropoiesis and megakaryopoiesis (28). These data suggest that Epo, one of the HIF targets, may play a role not only in early erythroid progenitors but also in earlier pluripotent hematopoietic progenitor, such as CD34-positive cells and/or hemangioblast.

HIFs and Lymphopoiesis

Lymphocyte proliferation depends on glycolysis-derived ATP (14) and HIF-1 regulates essential glycolysis genes including glucose transporter 1 and glycolytic enzymes (31, 68). To test a role of HIF in lymphopoiesis, Kojima et al. (34a) bypassed the embryonic lethality of HIF-1α deficiency by using the recombination activating gene-2 (RAG-2)-deficient blastocyst complementation system. The chimeric mouse Hif1α−/−→Rag2+/+ had reduced B cell progenitors (46% vs. 8%). These results indicate that HIF-1α deficiency impairs the proliferation of immature B cells and modulates the rate of B cell expansion. Whereas these mice had no changes in T cells, HIF-1 induces Foxp3, a member of the forkhead/winged-helix family of transcriptional regulators, in Jurkat T cells and mononuclear cells (10). Foxp3 mutation in the scurfy mouse resulted in a fatal lymphoproliferative disorder including regulatory T cell deficiency (11, 15). Foxp3 is highly expressed by regulatory T cells and is associated with regulatory T cell activity and phenotype (34). Overexpression of Hif-1α increases Foxp3 mRNA and the proportion of regulatory T cells in mouse splenocytes (10).

HIFs and Macrophages

HIF-1α and VEGF are also expressed in activated macrophages (16, 27, 87). Although there is no significant defect on myelopoiesis, the macrophage function in inflammation was impaired by the absence of HIF-1 (19).

CONGENITAL DISORDERS OF HYPOXIA SENSING

The defects of hypoxia sensing are best defined in polycythemic human diseases. However, one instance of HIF-1-related anemia has been described. HIF-1 regulates a pro-apoptotic protein Bcl-2 family member Nix/Bnip3L. Nix−/− mice are anemic. Circulating erythrocytes in Nix−/− mice retain mitochondria due to impaired Nix-dependent mitochondrial autophagy and have decreased survival as a result of mitochondria-derived, oxidant radical-dependent hemolysis (73).

Chuvash Polycythemia

Chuvash polycythemia (CP) is the only known endemic congenital polycythemia. CP is due to an abnormality in the oxygen-sensing pathway. The condition causes thrombotic and hemorrhagic vascular complications, which lead to early mortality (61, 82). The inheritance is autosomal recessive (82). A homozygous mutation of the VHL gene (598C>T) is the cause of this disease. This mutation impairs the interaction of pVHL with both HIF-1α and HIF-2α, thus reducing the rate of ubiquitin-mediated destruction of HIF-1α and HIF-2α. As a result, the level of HIF-1 and HIF-2 heterodimers increases and leads to an increased expression of target genes, including EPO, VEGF, and plasminogen activator inhibitor (PAI-1) (5, 6). The effect of this mutation on hypoxic sensing is depicted in Fig. 2. The role of circulating EPO in the CP phenotype is indisputable; however, there must be other factors associated with the CP VHL mutation that contribute to the polycythemic phenotype since the erythroid progenitors of CP patients are hypersensitive in vitro to extrinsic EPO; the mechanism of this observation remains unexplained (5, 6). Despite increased expression of HIF-1α, HIF-2α, and VEGF in normoxia, CP patients do not display a predisposition to tumor formation. Almost half of CP patients have often unsuspected cerebral ischemic lesions (24). The high prevalence of this disorder is not unique to the Chuvash Autonomous Russian Republic, and it has been also reported on the Italian island of Ischia (58). The Chuvash VHL 598C>T mutation has also been described sporadically in Caucasians in the United States and Europe and in people of Punjabi/Bangladeshi Asian ancestry (55). Some patients with congenital polycythemia have proved to be compound heterozygotes for the Chuvash VHL 598C>T mutation and other VHL mutations, including 562C>G, 574C>T, and 388C>G. Additionally, a Croatian boy was homozygous for VHL 571C>G, the first example of a homozygous VHL germline mutation other than VHL 598C>T causing polycythemia (12, 17, 18, 25, 49–51).

A small number of cases of congenital polycythemia that appear to have a mutation of only one VHL allele confound an obvious pathophysiological explanation. In a Ukrainian family, two children with polycythemia were heterozygotes for VHL 376G>T (D126Y), but the father, who had the same mutation, was not polycythemic (100). An English patient was heterozygote for VHL 598C>T (106); however, inheritance of a deletion of a VHL allele, or null VHL allele in a transposition was not excluded. Subsequently, two polycythemic VHL heterozygous patients were described in whom a null VHL
allele was more rigorously excluded (12, 17); the molecular mechanism of their polycythemic phenotype remains to be elucidated.

To address the question of whether the VHL 598C>T substitution occurred in a single founder or resulted from recurrent mutational events, haplotype analysis of eight highly informative single nucleotide polymorphisms spanning the VHL gene was performed on 101 subjects bearing the VHL 598C>T mutation and 447 normal, unrelated individuals from Chuvash, Southeast Asian, Caucasian, Hispanic, and African-American ethnic groups (39). Polymorphisms of the VHL locus in normal controls (having a wild-type VHL 598C allele) and subjects bearing CP VHL 598T were in strong linkage disequilibrium. These studies indicated that in most individuals, the VHL 598C>T mutation arose in a single ancestor about 50,000 years ago. However, this is not the case for a Turkish polycythemic family with a VHL 598C>T mutation wherein the VHL 598C>T mutation occurred independently (17).

CP homozygotes have decreased survival because of thrombotic complications, mostly venous (24), and thus are under negative selection pressure. The high frequency of the mutation in some areas may be due to a genetic drift, but it is also possible that the propagation of the VHL 598C>T mutation is the result of a survival advantage for heterozygotes. Such an advantage might be related to subtle improvement of iron metabolism, erythropoiesis, embryonic development, energy metabolism (77), or some other as yet unknown effect. A potential protective role of a mildly augmented hypoxic response is improved protection against bacterial infections, since the neutrophil and macrophage HIF-1-mediated response was reported to be essential for the bactericidal action of neutrophils (19).

Classic von Hippel Lindau Syndrome

VHL syndrome is an autosomal dominant genetic abnormality affecting the posttranslational control of HIF-1α and HIF-2α (23, 26, 37). This syndrome is characterized by a propensity for developing renal cell carcinomas, retinal hemangioblastomas, cerebellar and spinal hemangioblastomas, pancreatic cysts, and pheochromocytomas. The tumors result from a somatic mutation in addition to the germline mutation; i.e., loss of heterozygosity. Polycythemia is not part of VHL syndrome; however, hemangioblastomas of the central nervous system and, less commonly, pheochromocytoma and renal cancer have long been associated with polycythemia (37). Other patients with VHL syndrome also develop acquired polycythemia (24, 37, 70).

OTHER CONGENITAL DISORDERS OF HYPOXIA SENSING

Proline Hydroxylase Deficiency

A family with a PHD2 mutation (950C>G) was described, in which heterozygotes for this mutation have mild or borderline polycythemia (56). Since then, four additional patients with unexplained polycythemia who are heterozygote carriers of different mutations in PHD2 have been reported (two frameshift mutations, 606delG and 840_841insA, both located in exon 1, and two nonsense mutations, 1112G>A and 1129C>T, in exon 3) (4, 53). One of these patients had a major thrombotic event (thrombosis of the sagittal sinus). In both cases, heterozygotes for this mutation had mild or borderline polycythemia, which the authors referred to as erythrocytosis. Testing for EPO hypersensitivity of erythroid progenitors was not reported. Because of the small family size, the possibility


Fig. 3. The diagram summarizes the effects of hypoxia on hematopoiesis. EPO, erythropoietin; EPO-receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; TF, transferrin; TFR, TF receptor; HSC, hematopoietic stem cells; FOXP3, forehead box P3.
of a nonerythroid etiology for the erythrocytosis could not be excluded, but this work further supports the concept that abnormalities in factors involved in the regulation of HIF signaling can result in polycythemia.

**HIF-2α Gain-of-Function Mutations**

A family was described in which members with polycythemia were heterozygous carriers of a HIF-2α Gly537Tyr mutation that served to stabilize the HIF-2α protein (3, 54). The same group subsequently reported four additional polycythemic patients with heterozygous Met535Val or Gly537Arg mutations in the HIF-2α gene (52). These patients tend to present at a young age with elevated EPO. These findings support the importance of the proline hydroxylase, HIF-2α, VHL axis in human EPO regulation, and the pathogenesis of familial polycythemias due to abnormal hypoxia sensing; this is yet additional evidence that HIF-2α may be central to regulating EPO levels (54).

**EVOLUTIONARY AMELIORATION OF EXCESSIVE PHYSIOLOGICAL RESPONSE TO CHRONIC HYPOXIA ASSOCIATED WITH LIVING IN EXTREME HIGH ALTITUDES**

Certain high-altitude populations develop genetic adaptations permitting their successful existence in an extremely hypoxic environment. While in most nonadapted populations, chronic exposure to hypoxia leads to an elevation of hematocrit due to an increased number of erythrocytes, i.e., polycythemia, the majority of Tibetan highlanders, unlike their Andean counterparts, maintain comparable hematocrit levels to populations living at sea level (8). While increased hemoglobin concentration may be considered a beneficial adaptation to hypoxia, at certain levels the increased number of erythrocytes increases hematocrit resulting in high blood viscosity, which impairs tissue blood flow leading to impaired tissue oxygen delivery (62, 63). We recently performed a genome-wide scan of Tibetan highlanders and reported 10 haplotypes of selected alleles that were subject to strong positive selection in samples of unrelated Tibetans. Of these 10 haplotypes in strong linkage disequilibrium, 3 EPAS1 (HIF-2α), EGLN1 (PHD2), and PPARA are constituents of the HIF pathway or are regulated by HIF; mutations or dysregulation of each of these three genes or their products has been reported to be associated with anemia or polycythemia (63, 83). Of these three genes, the haplotypes of two (PHD2 and PPARA) were significantly associated with protection against polycythemia in Tibetan highlanders (83) and identified 2 missense mutations of PHD2 gene that had a significant effect on hemoglobin concentration (41).

**SUMMARY**

The response of an organism to hypoxia is crucial for optimal oxygen delivery to the tissues. This is accomplished by the regulation of erythropoiesis mainly mediated by EPO and augmentation of iron delivery to nascent erythrocytes. Furthermore, hypoxia affects other lineage of hematopoiesis to maintain oxygen homeostasis (Fig. 3). More needs to be learned about the details of these control mechanisms. Uncovering the molecular basis of congenital defects of erythropoiesis provides a unique opportunity to better understand these controlling mechanisms, as well as to develop targeted therapies for anemias and polycythemic disorders. As demonstrated by the evolutionary adaptations of Tibetans, some physiological responses may be harmful for populations living in extreme hypoxic environments. We submit that we can learn a great deal from the deciphering of these genetic determinants of the Tibetan adaptation.

**DISCLOSURES**

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