

REVIEW ARTICLE

MECHANISMS OF DISEASE

Oxygen Sensing, Homeostasis, and Disease

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HYPOXIA PLAYS CRITICAL ROLES IN THE PATHOBIOLOGY OF HEART DISEASE, cancer, stroke, and chronic lung disease, which are responsible for 60% of deaths in the United States. This review summarizes advances in our understanding of how cells sense and respond to changes in oxygen availability and the physiologic or pathologic consequences of these responses in the context of chronic diseases. The role of hypoxia in inflammatory disorders was recently reviewed in the *Journal*¹ and is therefore not discussed here.

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MECHANISMS OF SIGNAL TRANSDUCTION IN HYPOXIA

Humans have evolved complex circulatory, respiratory, and neuroendocrine systems to ensure that oxygen levels are precisely maintained, since an excess or deficiency may result in the death of cells, tissue, or the organism. As discussed below, oxygen homeostasis represents an organizing principle for understanding evolution, development, physiology, and disease. Historically, oxygen sensing was thought to be limited to specialized cells, such as the glomus cells of the carotid body, which depolarize within milliseconds in response to hypoxemia by means of incompletely understood mechanisms.² We now recognize that all nucleated cells in the body sense and respond to hypoxia. Under conditions of reduced oxygen availability, hypoxia-inducible factor 1 (HIF-1) regulates the expression of genes that mediate adaptive responses.^{3–6} In hypoxic cells, the transcription of several hundred messenger RNAs (mRNAs) is increased, and the expression of an equal number of mRNAs is decreased. The changes are dependent on HIF-1 in both cases, but HIF-1 binding is detected only at genes with increased expression. HIF-1 decreases mRNA expression indirectly by regulating transcriptional repressors and microRNAs.^{3–6}

HIF-1 was first identified in human cells as a regulator of erythropoietin, the hormone that controls red-cell production; vascular endothelial growth factor (VEGF), which stimulates angiogenesis; and glycolytic enzymes, which adapt cell metabolism to hypoxic conditions (Fig. 1A).⁶ HIF-1 is composed of a constitutively expressed HIF-1 β subunit and an oxygen-regulated HIF-1 α subunit.⁷ Under aerobic conditions, HIF-1 α is hydroxylated by prolyl hydroxylase domain proteins (PHDs), which use oxygen and α -ketoglutarate as substrates and contain Fe²⁺ in their catalytic center.⁸ Hydroxylated HIF-1 α interacts with the von Hippel–Lindau (VHL) protein, the substrate-recognition subunit of a ubiquitin-protein ligase that targets HIF-1 α for proteasomal degradation (Fig. 1B). Under hypoxic conditions, hydroxylation is inhibited and HIF-1 α accumulates. HIF-1 transcriptional activity is regulated by factor-inhibiting HIF-1 (FIH-1), an asparaginyl hydroxylase that blocks the interaction of HIF-1 α with the coactivator protein p300.^{6,8} Thus, HIF-1 α hydroxylation provides a mechanism for transducing changes in oxygen availability to the nucleus as changes in gene transcription.

HIF-1 α ^{-/-} mouse embryos, which lack HIF-1 α , are arrested in their development at midgestation and die from cardiac and vascular defects and decreased erythropoiesis, indicating that all three components of the circulatory system are dependent on

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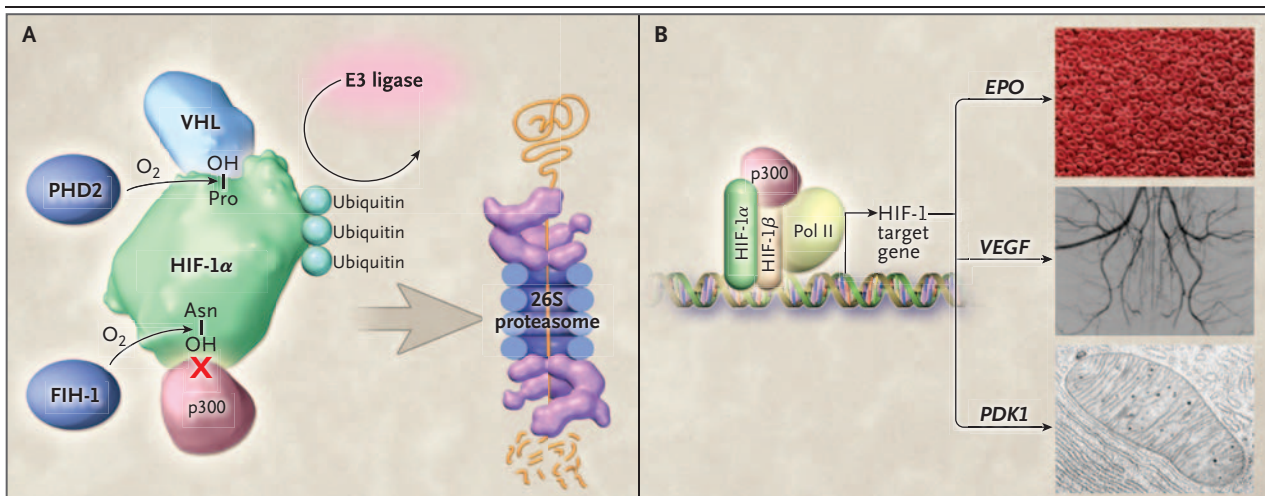


Figure 1. Oxygen Sensing, Gene Expression, and Adaptive Responses to Hypoxia.

In well-oxygenated cells (Panel A), prolyl hydroxylase domain 2 (PHD2) uses oxygen to hydroxylate hypoxia-inducible factor 1 (HIF-1 α) on a proline residue (Pro-OH). The von Hippel-Lindau (VHL) protein binds to HIF-1 α containing Pro-OH and recruits a ubiquitin E3 ligase. The polyubiquitination of HIF-1 α flags the protein for degradation by the 26S proteasome. Factor inhibiting HIF-1 (FIH-1) also uses oxygen to hydroxylate HIF-1 α on an asparagine residue (Asn-OH). HIF-1 α containing Asn-OH cannot be bound by the coactivator protein p300, thereby preventing HIF-1 α from activating gene transcription. Under hypoxic conditions (Panel B), the Pro and Asn hydroxylation reactions are inhibited, and HIF- α (i.e., either HIF-1 α or HIF-2 α) rapidly accumulates, dimerizes with HIF-1 β , recruits p300, binds to hypoxia response elements, and activates the transcription by RNA polymerase II (Pol II) of hundreds of target genes, such as the following: *EPO*, encoding erythropoietin, which is the hormone that stimulates red-cell production (photomicrograph at top); *VEGF*, encoding vascular endothelial growth factor, which is the angiogenic factor that stimulates blood-vessel formation (angiogram in middle); and *PDK1*, encoding pyruvate dehydrogenase kinase 1, which inhibits the conversion of pyruvate to acetyl coenzyme A for oxidation in the mitochondrion (electron micrograph at bottom).

HIF-1 for normal development.⁹⁻¹² Hypoxic responses are also mediated by HIF-2, a heterodimer composed of HIF-1 β and HIF-2 α (a paralogue of HIF-1 α that is also regulated by oxygen-dependent hydroxylation). HIF-1 α is present in all nucleated cells of all metazoan species, whereas HIF-2 α expression is restricted to certain cell types within vertebrate species and plays an important role in both erythropoiesis and vascularization.¹³

REGULATION OF CELLULAR METABOLISM BY HIF-1

Even the simple roundworm *Caenorhabditis elegans*, which consists of about 1000 cells and contains no specialized systems for oxygen delivery, expresses HIF-1, indicating that the primordial function of HIF-1 was to mediate adaptive responses that allow cells to survive oxygen deprivation. One way in which HIF-1 promotes cell survival under hypoxic conditions is by mediating a switch from oxidative to glycolytic metabolism. The glycolytic enzymes convert glucose to pyruvate, which can

be converted either to acetyl coenzyme A (CoA) for oxidation in the tricarboxylic acid cycle or to lactate as a glycolytic end product (Fig. 2). HIF-1 activates the expression of lactate dehydrogenase A and pyruvate dehydrogenase kinase 1 (PDK1), thus tipping the balance from oxidative to glycolytic metabolism.^{14,15}

As compared with glycolysis, oxidative metabolism yields 18 times as much ATP per mole of glucose consumed. Although it is the conventional wisdom that cells respire until oxygen is depleted, at which point they switch to glycolysis, we now know that this model of metabolic regulation is incorrect. HIF-1 $\alpha^{-/-}$ fibroblasts are incapable of switching from oxidative to glycolytic metabolism when shifted from aerobic conditions of 95% air and 5% carbon dioxide (20% oxygen, with a partial pressure of oxygen [P_O₂] of about 140 mm Hg) to hypoxic conditions (1% oxygen, with a P_O₂ of about 7 mm Hg).¹⁶ ATP levels are higher in HIF-1 $\alpha^{-/-}$ cells at 1% oxygen than in HIF-1 $\alpha^{+/+}$ cells at 20% oxygen, indicating that 1% oxygen does not limit ATP production.¹⁶ However, HIF-1 $\alpha^{-/-}$ fibroblasts

maintained at 1% oxygen or less will die owing to toxic levels of reactive oxygen species.^{14,16} Under aerobic conditions, electrons are transferred from NADH and flavin adenine dinucleotide (FADH₂) (generated by oxidation of acetyl CoA) to mitochondrial complex I or II, then to complex III, and finally to complex IV, where they react with oxygen to form water. Under hypoxic conditions, the release of electrons is increased before the transfer to complex IV, resulting in the formation of superoxide,¹⁷ which is then converted to hydrogen peroxide and other toxic reactive oxygen species. Thus, there is sufficient oxygen for oxidative phosphorylation to occur in hypoxic fibroblasts, but at the cost of a loss of redox homeostasis. The extent to which these findings apply to disease states, such as cancer and pulmonary hypertension, remains to be determined.

THE PHD–VHL–HIF AXIS IN HEREDITARY ERYTHROCYTOSIS

Hereditary erythrocytosis (congenital polycythemia) is a disorder characterized by excess red-cell production. At high hematocrit levels, capillary blood flow is impaired, placing affected persons at increased risk for cerebral and peripheral vascular thrombosis. Persons with Chuvash polycythemia are homozygous for a missense mutation that partially impairs binding of the VHL protein to hydroxylated HIF-1 α subunits, resulting in HIF activity that is inappropriately elevated at any given level of PO₂.¹⁸ Hypoxia-induced changes in respiration and pulmonary vascular tone occur at higher PO₂ levels in affected persons than in controls, reflecting a generalized defect in oxygen sensing.¹⁹ Cases of hereditary erythrocytosis due to a missense mutation in PHD protein 2 (PHD2) or HIF-2 α , which impairs the hydroxylation of HIF-1 α , HIF-2 α , or both, have also been described.^{18,20,21} Thus, the PHD–VHL–HIF pathway has been saturated with mutations, indicating its key role in erythropoiesis and other physiological systems designed to maintain oxygen homeostasis.

FAILED ADAPTATION TO HYPOXIA IN ISCHEMIC CARDIOVASCULAR DISEASE

Atherosclerotic stenosis of arteries in the heart and legs results in cardiac and limb ischemia, respectively, as blood flow downstream of the ste-

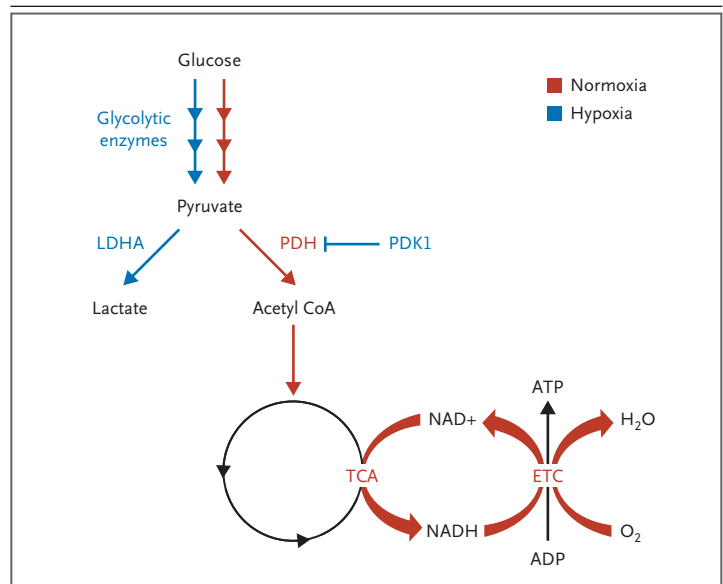


Figure 2. Regulation of Glucose Metabolism in Response to Changes in Cellular Oxygen Levels.

Glucose is converted to pyruvate by the action of the glycolytic enzymes. In well-oxygenated cells (red pathway), pyruvate dehydrogenase (PDH) converts pyruvate to acetyl coenzyme A (CoA), which is oxidized in the mitochondrial tricarboxylic acid (TCA) cycle, generating electrons that are transported through a series of protein complexes (ETC) and are eventually transferred to oxygen to form water. The proton gradient established by the ETC is used to synthesize ATP. Under hypoxic conditions (blue pathway), pyruvate dehydrogenase kinase 1 (PDK1) inactivates PDH, and lactate dehydrogenase A (LDHA) converts pyruvate to lactate. The expression of the glycolytic enzymes is also induced to increase flux through the pathway under hypoxic conditions.

nosis becomes restricted. In young, healthy mice, femoral-artery ligation results in the local induction of HIF-1, followed by the transcriptional activation of genes that encode VEGF and other angiogenic factors within the ischemic limb, and after several weeks, normal perfusion is restored.²² This adaptive vascular response is impaired by aging²² and diabetes,^{23,24} which are major risk factors for coronary artery disease and peripheral arterial disease. Whereas HIF-1 α ^{-/-} mice are subject to embryonic death, HIF-1 α ^{+/-} mice develop normally but have impaired recovery of perfusion and more severe tissue damage after arterial ligation.²²

VEGF and other secreted factors (Fig. 3) activate vascular cells to promote angiogenesis (the budding of new capillaries from existing vessels) and arteriogenesis (the remodeling of collateral blood vessels to accept increased flow, thus bypassing stenotic regions of the conduit arteries).^{25,26} Angiogenic factors also reach the circu-

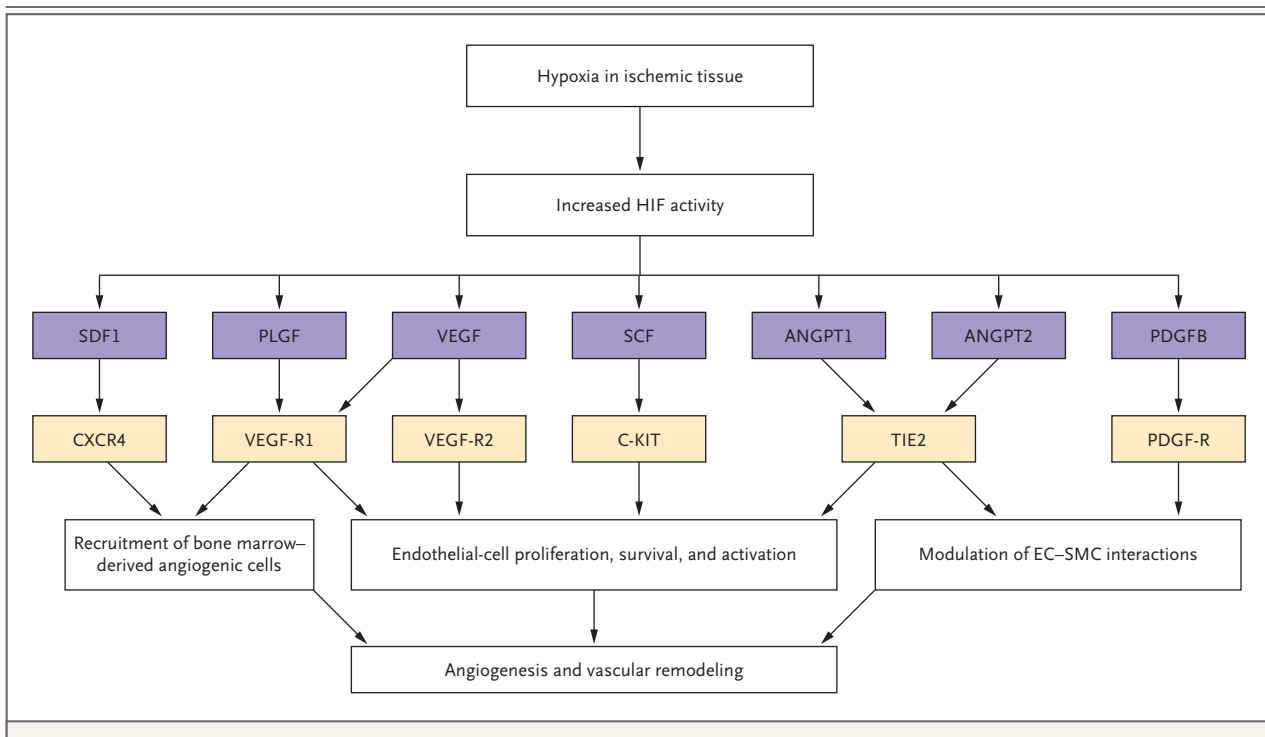


Figure 3. Regulation of Vascular Response to Hypoxia by HIF-1.

In ischemic tissue, hypoxia induces hypoxia-inducible factor 1 (HIF-1) activity, which activates the transcription of genes encoding multiple secreted factors (purple). These factors bind to cognate receptors (yellow) located on vascular endothelial cells, pericytes, and smooth-muscle cells, as well as on bone marrow–derived angiogenic cells (including endothelial progenitor cells, mesenchymal stem cells, and proangiogenic myeloid cells) that are recruited to the ischemic tissue and promote angiogenesis and vascular remodeling. ANGPT denotes angiopoietin, EC endothelial cell, PDGFB platelet-derived growth factor B, PLGF placental growth factor, SCF stem-cell factor (also known as c-kit ligand), SDF1 stromal-derived factor 1, SMC smooth-muscle cell, and VEGF vascular endothelial growth factor.

lation and stimulate the mobilization of bone marrow–derived angiogenic cells (BMDACs), which home to ischemic tissue and participate in angiogenesis and arteriogenesis.^{22,27} Although originally described as endothelial progenitor cells, most BMDACs are myeloid cells. Instead of differentiating into cells that are incorporated into the remodeling vessels, myeloid cells produce paracrine factors that stimulate vascular remodeling.^{28,29} To promote vascular remodeling, BMDACs must be mobilized from bone marrow and other tissues and enter the peripheral blood, home to ischemic tissue, be retained within the ischemic tissue by adherence to the vascular endothelium and extravasation from the vessel, and survive long enough within this tissue to initiate vascular responses.

With aging, the loss of ischemia-induced expression of angiogenic factors leads to a failure of BMDAC mobilization and homing.²² After arterial ligation in mice 2 to 8 months of age, intra-

muscular administration of a recombinant adenovirus encoding a form of HIF-1 α engineered to have constitutive activity (AdCA5) is sufficient to stimulate BMDAC mobilization and homing, improving the recovery of perfusion.²² AdCA5 also improves the outcome after arterial ligation in mice with a mutant leptin receptor (*Lep^r^{db/db}*), a model for type 2 diabetes.³⁰ AdCA5 is not sufficient to recover perfusion in mice 13 to 17 months of age; in these mice, AdCA5 gene therapy followed by intravenous administration of BMDACs results in recovery of blood flow but only when the BMDACs have been pretreated with dimethyloxallylglycine (DMOG), an α -ketoglutarate antagonist that induces HIF-1 activity by inhibiting prolyl hydroxylation.^{31,32} HIF-1 activation in BMDACs leads to the expression of β_2 integrins that promote vascular adherence and tissue retention,³¹ as well as metabolic enzymes, membrane transporters, and pH regulators that promote BMDAC survival in ischemic tissue.³²

HIF-1 also plays an important role in coronary artery disease. HIF-1 α induction occurs early in the course of myocardial infarction, as shown by examination of ventricular-biopsy specimens obtained from patients with acute infarction.³³ Transgenic mice with myocardial HIF-1 α overexpression have reduced infarct size, improved cardiac function, and increased capillary density after coronary-artery ligation.³⁴ Collateral vessels are identified on angiography in approximately two thirds of patients with critical coronary artery disease in which the degree of arterial stenosis ($\geq 70\%$) is sufficient to cause symptoms (angina). The factors that determine whether collaterals will develop in patients with critical stenosis have not been identified. However, when plaque rupture causes total coronary occlusion and myocardial infarction, patients with collateral vessels have smaller infarcts and are more likely to survive than those without collateral vessels.^{35,36} Single-nucleotide polymorphisms at the HIF-1 α locus are associated with an absence of coronary collateral vessels in patients with critical stenosis³⁷ and in patients with acute coronary syndromes who present with stable angina rather than infarction.³⁸ Thus, genetic variation may affect HIF-1 activity and thereby alter the presentation of coronary artery disease.

In mice, HIF-1 is required for ischemic preconditioning, a phenomenon in which exposure of the heart to several short (e.g., 5-minute) episodes of ischemia and reperfusion provides protection against the injurious effects of a subsequent prolonged (e.g., 30-minute) episode of ischemia.^{39,40} Mice with increased levels of cardiac HIF-1 α and HIF-2 α due to decreased PHD2 activity are protected against myocardial infarction in the absence of preconditioning.⁴⁰⁻⁴² These results suggest that HIF-1 not only regulates perfusion and oxygen delivery but also plays a critical role in the ability of the heart to survive episodes of oxygen deprivation, which may involve changes in energy metabolism (described above) and adenosine production.⁴³ In rodent stroke models, pretreatment with pharmacologic inducers of HIF activity results in a reduction in cerebral infarct size, and several HIF target genes are candidates for mediating neuroprotection.⁴⁴⁻⁴⁷ Among high-altitude residents in Switzerland, death from coronary artery disease or stroke is less common than it is in the general population, suggesting that living in chronic hypoxia provides some protection.⁴⁸

Preclinical data linking activation of the PHD-HIF system with ischemic adaptation provide a basis for therapeutic attempts to increase HIF or decrease PHD activity in patients with coronary artery disease and peripheral arterial disease. In a study of patients with critical limb ischemia (a condition in which perfusion is inadequate to maintain tissue viability), HIF-1 α levels were found to be increased in vascular endothelial cells but not in the ischemic muscle cells,⁴⁹ suggesting an impaired physiological response to ischemia. In phase 1 clinical trials, patients with peripheral arterial or coronary artery disease were given a recombinant adenovirus encoding a chimeric protein that contained the N-terminal half of HIF-1 α fused to the VP16 herpesvirus transactivator protein. There were no adverse consequences reported in these two studies in which intramuscular injections were administered to 34 patients with critical limb ischemia and in which intramyocardial injections were administered to 10 patients at the time of coronary-artery bypass grafting.^{50,51} However, there have been no reports of the efficacy of such treatment in patients with peripheral arterial or coronary artery disease, possibly because the chimeric protein does not retain all the activities of HIF-1 α ; gene therapy alone is insufficient to mediate a vascular response in such patients, who tend to be elderly; or the adenoviral proteins or VP16 induces a confounding inflammatory response.

An alternative strategy for treating peripheral arterial disease,⁵² cerebral ischemia,⁴⁴⁻⁴⁷ and other ischemic disorders would be the development of drugs that inhibit the prolyl hydroxylases and thereby activate HIF.^{47,53} These drugs either chelate iron, which is present in the catalytic center of the hydroxylases, or, like DMOG, compete with α -ketoglutarate for binding to the catalytic site. More than 60 different dioxygenases use iron and α -ketoglutarate,⁵⁴ and the development of inhibitors specific to a particular hydroxylase (e.g., one of the PHDs) would be highly desirable for clinical applications. However, in patients with Chuvash polycythemia, even modestly increased HIF signaling that is prolonged and systemic has pathologic effects.⁵⁵ Therefore, the clinical application of such agents must be approached in a careful and conservative manner.

Another alternative strategy would be the use of HIF target-gene products as therapeutic agents, which brings us back to where the story began,

with the cloning of the erythropoietin gene^{56,57} and the production of recombinant human erythropoietin for administration in patients with chronic renal failure whose kidneys do not make sufficient erythropoietin to sustain a normal red-cell mass.⁵⁸ Although erythropoietin is highly effective in stimulating erythropoiesis, the administration of a single angiogenic factor, such as VEGF, has failed as a means of stimulating vascular remodeling, a process that requires the coordinated action of multiple HIF-dependent angiogenic factors.²² One potential advantage of using downstream proteins as therapeutic agents is that they act immediately, whereas the effects of HIF-1 α gene therapy or PHD inhibitors are delayed because they require transcription and translation of target-gene products. In animal models, erythropoietin, which functions as a survival factor for many cell types, has improved recovery from an ischemic event when administered immediately before or after the event.⁵⁹⁻⁶² Positive results were reported in a small clinical trial of erythropoietin in patients with acute ischemic stroke,⁶³ but follow-up studies failed to demonstrate efficacy, and the use of erythropoietin in combination with tissue plasminogen activator resulted in increased mortality.^{64,65} PHD inhibitors, which induce the expression of multiple survival and angiogenic factors, may be more efficacious, but clinical trials are needed to confirm this hypothesis.

CO-OPTED ADAPTATION TO HYPOXIA IN CANCER

In ischemic cardiovascular disease, the induction of HIF activity is adaptive, and therapeutic efforts are directed toward augmenting the response. In cancer, co-optation of the physiologic responses to hypoxia plays a major role in disease progression, and therapeutic efforts are directed toward inhibiting HIF. Folkman established the critical role of angiogenesis in the growth of primary tumors and their metastasis.⁶⁶ Tumor vessels are structurally and functionally abnormal, and even highly vascularized cancers may contain areas of severe hypoxia. In breast cancer, the mean PO₂ is 10 mm Hg (as compared with >60 mm Hg in normal breast tissue), and a PO₂ of less than 10 mm Hg in the primary tumor is associated with increased risks of metastasis and death.⁶⁷ HIF-1 α overexpression in the primary tumor is also associated with increased mortality in breast cancer and

other common cancers (Fig. 4).⁶⁸ HIF-1 activates the transcription of genes that play critical roles in angiogenesis,⁶⁹ genetic instability,⁷⁰ immune evasion,⁷¹ metabolic reprogramming,⁷²⁻⁷⁴ invasion and metastasis,^{75,76} radiation resistance,⁷⁷ and stem-cell maintenance.⁷⁸ Although HIF-1 has the potential to regulate hundreds of target genes, only a subset will be regulated by HIF-1 in any given cancer. As with any protein implicated in cancer, one can find contradictory examples suggesting that HIF-1 is involved in limiting tumor growth or has no effect at all. The challenge is to understand the role of HIF-1 in a particular patient's cancer in order to determine whether it might be targeted therapeutically, and if so, how.

Intratumoral hypoxia is a major mechanism by which HIF-1 is activated in human cancers, but HIFs are also activated as a consequence of tumor-suppressor loss of function or oncogene gain of function.⁶⁸ The most dramatic example of this is VHL loss of function, which results in the constitutive overexpression of HIF-1 α and HIF-2 α .⁷⁹ Unlike persons with Chuvash polycythemia, who are homozygous for a missense mutation in VHL that partially impairs its ability to ubiquitinate HIFs, many persons with the VHL syndrome (type 1, 2A, or 2B) are heterozygous for a mutation that results in loss of function. Somatic mutation or epigenetic inactivation of the other allele blocks HIF ubiquitination and degradation. These patients are at high risk for tumors (particularly clear-cell renal carcinomas and cerebellar hemangioblastomas) that are highly vascularized owing to high levels of VEGF and other HIF-regulated angiogenic factors.⁷⁹

A growing number of chemotherapeutic agents have been shown to function as HIF-1 inhibitors through a variety of molecular mechanisms.^{68,80} Drugs such as topotecan are administered intermittently at maximum tolerated doses as cytotoxic agents, but when given at lower doses on a daily basis, they inhibit HIF-1 and downstream pathways such as angiogenesis in tumor xenograft models.⁸⁰ Several drugs that were used previously for the treatment of diseases other than cancer, including cardiac glycosides such as digoxin, have been shown to inhibit HIF-1 activity and to block tumor growth in mice,⁸¹ but whether they will do so within the limited dose range tolerated by humans is not known. Other HIF-1–blocking agents that are being investigated in clinical trials of cancer treatment (inhibitors of

histone deacetylases, heat-shock protein 90, and mammalian target of rapamycin [mTOR]) inhibit the activity of many other proteins; the extent to which inhibition of HIF-1 contributes to their therapeutic efficacy is unknown but may vary greatly from one patient to another. Including a drug that inhibits HIF-1 as part of a multiagent chemotherapeutic regimen will probably benefit a subset of patients with cancer, but the difficulty in matching patient populations with appropriate drug combinations remains the greatest obstacle to the effective treatment of metastatic disease.

MALADAPTIVE RESPONSES TO HYPOXIA IN PULMONARY HYPERTENSION

Hypoxic pulmonary hypertension is a progressive and often fatal complication of chronic lung disease. Unlike systemic arterioles, which dilate in response to hypoxemia in order to increase tissue perfusion, pulmonary arterioles constrict in order to shunt blood away from regions of the lung that are not ventilated. This is an adaptive response in patients with lobar pneumonia but not in those with chronic lung disease, in whom alveolar hypoxia occurs throughout the lungs, with pulmonary hypertension leading to right heart failure and progressive hypoxemia. HIFs regulate target genes that play key roles in the pathogenesis of pulmonary hypertension (Fig. 5).⁸²⁻⁸⁴ As compared with their wild-type littermates, HIF-1 α ^{+/-} or HIF-2 α ^{+/-} mice have less severe pulmonary hypertension when subjected to prolonged hypoxia.^{85,86} In contrast, persons with hereditary erythrocytosis due to mutations that stabilize the HIF-2 α protein have elevated pulmonary arterial pressures under normoxic conditions.⁸⁷ HIF-1-mediated alterations in the energy metabolism of pulmonary arterial smooth-muscle⁸⁸ and endothelial⁸⁹ cells may play an important pathogenic role in hypoxic pulmonary hypertension (World Health Organization [WHO] group III) and idiopathic pulmonary hypertension (WHO group I). Altered redox homeostasis also plays an important role in pulmonary hypertension.^{90,91} Epigenetic inactivation of superoxide dismutase 2 expression has been implicated in the induction of HIF-1 α expression in pulmonary arterial smooth-muscle cells from fawn-hooded rats, which are a model for idiopathic pulmonary hypertension.⁹⁰

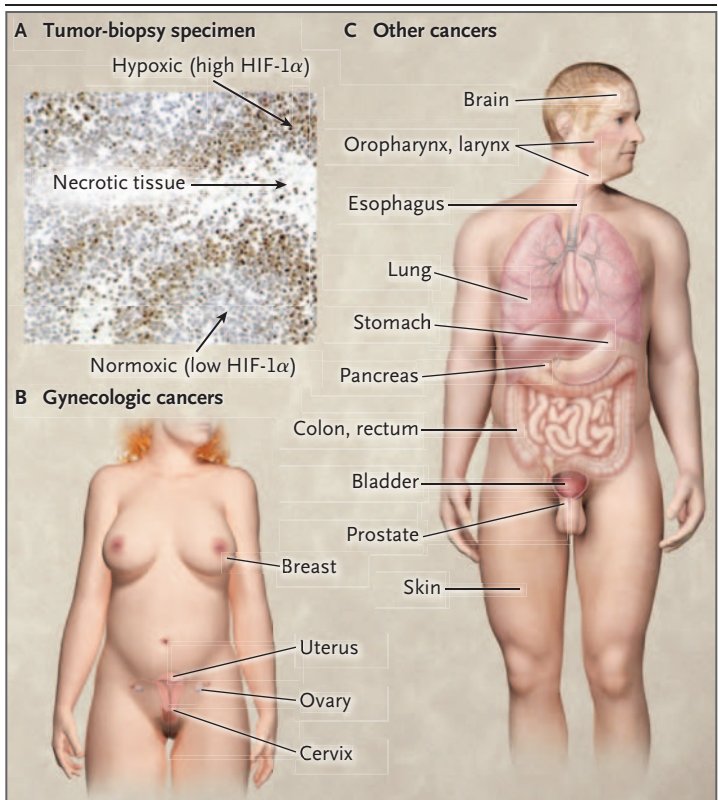
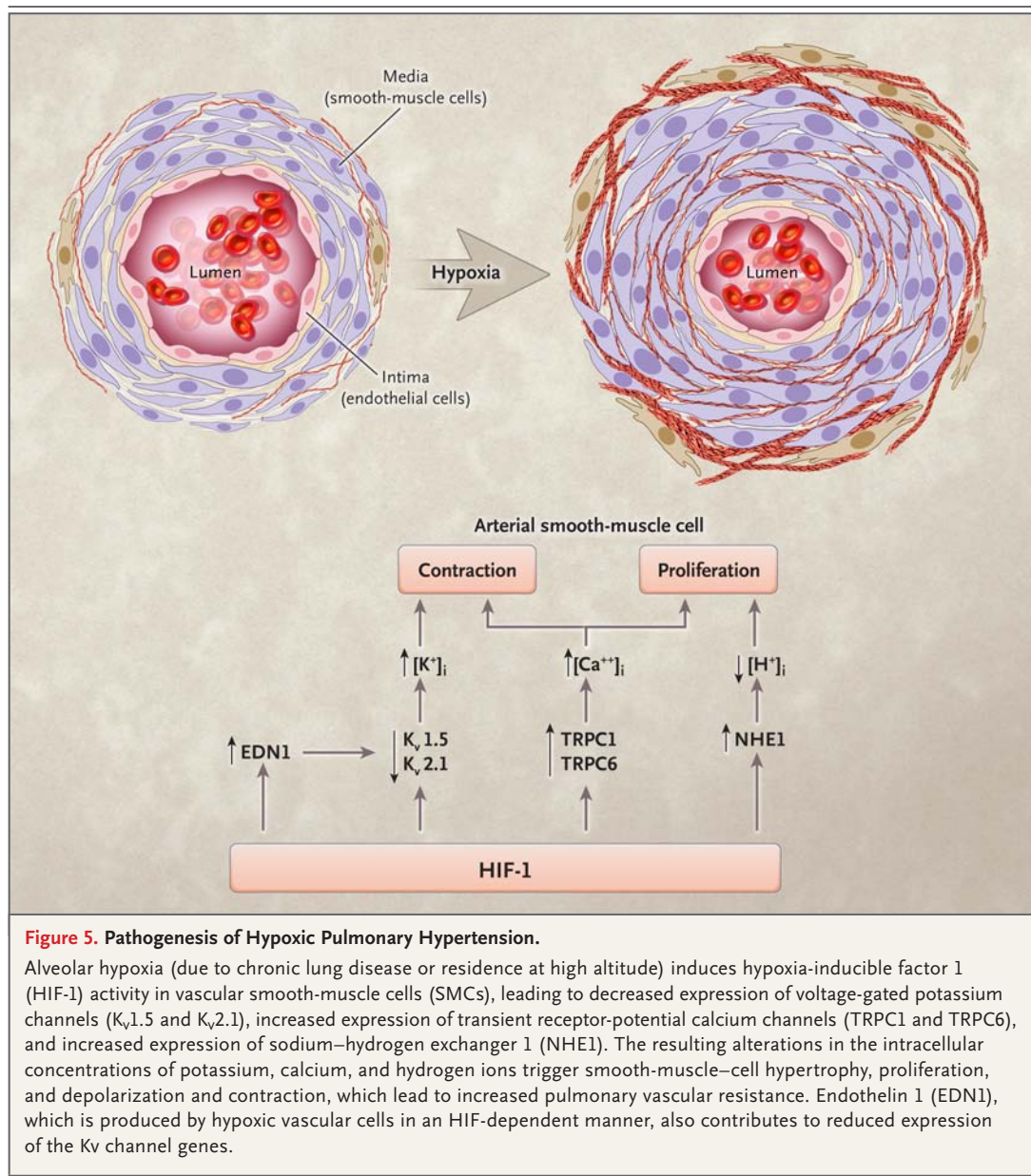


Figure 4. Hypoxia, Hypoxia-Inducible Factor 1 (HIF-1), and Cancer.

Panel A shows immunohistochemical staining with an antibody against HIF-1 α in a biopsy specimen from a tumor. The white areas represent necrotic tissue in which the cells have died because they are too distant from a blood vessel to receive adequate oxygen. The blue-gray areas represent cancer cells that are well oxygenated (normoxic). The brown staining indicates cells that express high levels of HIF-1 α , which are the viable cells farthest away from a blood vessel and are therefore the most hypoxic. Increased HIF-1 α protein levels in a diagnostic biopsy specimen are associated with an increased risk of death among patients with gynecologic cancers (Panel B) and other types of cancer (Panel C).

EVOLUTIONARY ADAPTATION OF TIBETANS TO HIGH ALTITUDE

For thousands of years, humans have lived on the Tibetan plateau at altitudes that exceed 3000 m. As compared with residents at sea level, where the atmospheric PO_2 is 150 mm Hg, residents of the Tibetan highlands, where the PO_2 is 110 mm Hg or less, live under conditions of chronic hypoxia and have evolved unique adaptations to their environment. Normally, when the lungs are subjected to high-altitude hypoxia, increased pulmonary vascular tone becomes a maladaptive response that leads to hypoxemia and heart fail-



ure. When hypoxemia is due to anemia, erythrocytosis is an adaptive response, whereas when it is due to ambient hypoxia, further increases in red-cell mass cannot correct the problem and may make it worse, owing to hyperviscosity associated with polycythemia. In adapted Tibetans, these responses to hypoxia are blunted, and many Tibetans have normal pulmonary arterial pressure and hemoglobin levels. Moreover, successfully adapted Tibetans have normal aerobic tissue metabolism despite hypoxemia.⁹²

With the advent of powerful DNA-sequencing methods, it became possible to identify loci at

which genetic selection has occurred in Tibetan highlanders relative to the lowland population from which they arose. Remarkably, the strongest evidence of selection was found at the locus encoding HIF-2 α .⁹³⁻⁹⁵ Evidence for selection at loci encoding PHD2, factor-inhibiting HIF-1 (FIH-1), and HIF target genes has also been reported.^{95,96} How the selected genetic variants alter the function of these proteins is not known. The variants may result in reduced activity of PHD2 and HIF-2 α , the net effect of which would be to alter the balance between HIF-2 α , which mediates vascular and erythropoietic responses to hypoxia, and

HIF-1 α , which mediates metabolic as well as vascular responses. In any case, these findings underscore the central role of the PHD–HIF system in maintaining oxygen homeostasis.

CONCLUSIONS

Oxygen-sensing and HIF-dependent responses to hypoxia are essential for prenatal development and postnatal physiology. They can play either protective or pathogenic roles in the chronic diseases that are the most common causes of death in the U.S. population, as well as in many other conditions (including ocular neovascularization^{97,98}

and organ transplantation^{99,100}) that cannot be discussed in this short review. The targeting of HIFs and PHDs represents a novel strategy for treating these diseases, provided that therapeutic effects can be obtained without untoward side effects. These conditions will be most easily met if the therapy is local, is of short duration, and is combined with other treatments that selectively amplify the consequences of modulating HIF activity in diseased tissue.

No potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

REFERENCES

- Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med* 2011; 364:656-65.
- Weir EK, López-Barneo J, Buckler KJ, Archer SL. Acute oxygen-sensing mechanisms. *N Engl J Med* 2005;353:2042-55.
- Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 2005;105:659-69.
- Mole DR, Blancher C, Copley RR, et al. Genome-wide association of hypoxia-inducible factor (HIF)-1 α and HIF-2 α DNA-binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem* 2009;284:16767-75.
- Xia X, Lemieux ME, Li W, et al. Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. *Proc Natl Acad Sci U S A* 2009;106:4260-5.
- Semenza GL. Oxygen homeostasis. *Wiley Interdiscip Rev Syst Biol Med* 2010;2:336-61.
- Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A* 1995;92:5510-4.
- Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008;30:393-402.
- Iyer NV, Kotch LE, Agani F, et al. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes Dev* 1998;12:149-62.
- Ryan HE, Lo J, Johnson RS. HIF-1 α is required for solid tumor formation and embryonic vascularization. *EMBO J* 1998; 17:3005-15.
- Compennolle V, Brusselmans K, Franco D, et al. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor 1 α . *Cardiovasc Res* 2003; 60:569-79.
- Yoon D, Pastore YD, Divoky V, et al. Hypoxia-inducible factor-1 deficiency results in dysregulated erythropoiesis signaling and iron homeostasis in mouse development. *J Biol Chem* 2006;281:25703-11.
- Patel SA, Simon MC. Biology of hypoxia-inducible factor-2 α in development and disease. *Cell Death Differ* 2008;15: 628-34.
- Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006;3:177-85.
- Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 2006;3:187-97.
- Zhang H, Bosch-Marcé M, Shimoda LA, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* 2008; 283:10892-903.
- Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate hypoxic signaling. *Curr Opin Cell Biol* 2009;21:894-9.
- Yoon D, Ponka P, Prchal JT. Hypoxia. 5. Hypoxia and hematopoiesis. *Am J Physiol Cell Physiol* 2011;300:C1215-C1222.
- Smith TG, Brooks JT, Balanos GM, et al. Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. *PLoS Med* 2006;3(7):e290.
- Percy MJ, Zhao Q, Flores A, et al. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. *Proc Natl Acad Sci U S A* 2006;103:654-9.
- Percy MJ, Furlow PW, Lucas GS, et al. A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. *N Engl J Med* 2008;358:162-8.
- Bosch-Marcé M, Okuyama H, Wesley JB, et al. Effects of aging and hypoxia-inducible factor-1 activity on angiogenic cell mobilization and recovery of perfusion after limb ischemia. *Circ Res* 2007; 101:1310-8.
- Botusan IR, Sunkari VG, Savu O, et al. Stabilization of HIF-1 α is critical to improve wound healing in diabetic mice. *Proc Natl Acad Sci U S A* 2008;105:19426-31.
- Thangarajah H, Vial IN, Grogan RH, et al. HIF-1 α dysfunction in diabetes. *Cell Cycle* 2010;9:75-9.
- Carmeliet P. Angiogenesis in life, disease and medicine. *Nature* 2005;438:932-6.
- van Weel V, van Tongeren RB, van Hinsbergh VW, van Bockel JH, Quax PH. Vascular growth in ischemic limbs: a review of mechanisms and possible therapeutic stimulation. *Ann Vasc Surg* 2008; 22:582-97.
- Dimmeler S. Regulation of bone marrow-derived vascular progenitor cell mobilization and maintenance. *Arterioscler Thromb Vasc Biol* 2010;30:1088-93.
- Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003;107:1164-9.
- Ziegelhoeffer T, Fernandez B, Kostin S, et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res* 2004;94:230-8.
- Sarkar K, Fox-Talbot K, Steenbergen C, Bosch-Marcé M, Semenza GL. Adenoviral transfer of HIF-1 α enhances vascular responses to critical limb ischemia in diabetic mice. *Proc Natl Acad Sci U S A* 2009;106:18769-74. [Erratum, *Proc Natl Acad Sci U S A* 2010;107:513.]
- Rey S, Lee K, Wang CJ, et al. Synergistic effect of HIF-1 α gene therapy and HIF-1-activated bone marrow-derived angiogenic cells in a mouse model of limb ischemia. *Proc Natl Acad Sci U S A* 2009;106:20399-404.
- Rey S, Luo W, Shimoda LA, Semenza GL. Metabolic reprogramming by HIF-1 promotes the survival of bone marrow-derived angiogenic cells in ischemic tissue. *Blood* 2011;117:4988-98.
- Lee SH, Wolf PL, Escudero R, Deutsch

- R, Jamieson SW, Thistlethwaite PA. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* 2000;342:626-33.
34. Kido M, Du L, Sullivan CC, et al. Hypoxia-inducible factor 1 α reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. *J Am Coll Cardiol* 2005;46:2116-24.
 35. Habib GB, Heibig J, Forman SA, et al. Influence of coronary collateral vessels on myocardial infarct size in humans: results of phase I Thrombolysis in Myocardial Infarction (TIMI) trial. *Circulation* 1991;83:739-46.
 36. Sabia PJ, Powers ER, Ragosta M, Sarembock LJ, Burwell LR, Kaul S. An association between collateral blood flow and myocardial viability in patients with recent myocardial infarction. *N Engl J Med* 1992;327:1825-31.
 37. Resar JR, Roguin A, Voner J, et al. Hypoxia-inducible factor 1 α polymorphism and coronary collaterals in patients with ischemic heart disease. *Chest* 2005;128:787-91.
 38. Hlatky MA, Quertermous T, Boothroyd DB, et al. Polymorphisms in hypoxia inducible factor 1 and the initial clinical presentation of coronary disease. *Am Heart J* 2007;154:1035-42.
 39. Cai Z, Zhong H, Bosch-Marcé M, et al. Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1 α . *Cardiovasc Res* 2008;77:463-70.
 40. Eckle T, Köhler D, Lehmann R, El Kasmi K, Eltzschig HK. Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. *Circulation* 2008;118:166-75.
 41. Hyvärinen J, Hassinen IE, Sormunen R, et al. Hearts of hypoxia-inducible factor prolyl 4-hydroxylase-2 hypomorphic mice show protection against acute ischemia-reperfusion injury. *J Biol Chem* 2010;285:13646-57.
 42. Hölscher M, Silter M, Krull S, et al. Cardiomyocyte-specific prolyl-4-hydroxylase domain 2 knock out protects from acute myocardial ischemic injury. *J Biol Chem* 2011;286:11185-94.
 43. Semenza GL. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim Biophys Acta* 2011;1813:1263-8.
 44. Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM, Sharp FR. Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol* 2000;48:285-96.
 45. Siddiq A, Ayoub IA, Chavez JC, et al. Hypoxia-inducible factor prolyl 4-hydroxylase inhibition: a target for neuroprotection in the central nervous system. *J Biol Chem* 2005;280:41732-43.
 46. Freret T, Valable S, Chazalviel L, et al. Delayed administration of deferoxamine reduces brain damage and promotes functional recovery after transient focal cerebral ischemia in the rat. *Eur J Neurosci* 2006;23:1757-65.
 47. Harten SK, Ashcroft M, Maxwell PH. Prolyl hydroxylase domain inhibitors: a route to HIF activation and neuroprotection. *Antioxid Redox Signal* 2010;12:459-80.
 48. Faeh D, Gutzwiller F, Bopp M. Lower mortality from coronary heart disease and stroke at higher altitudes in Switzerland. *Circulation* 2009;120:495-501.
 49. Ho TK, Rajkumar V, Ponticos M, et al. Increased endogenous angiogenic response and hypoxia-inducible factor 1 α in human critical limb ischemia. *J Vasc Surg* 2006;43:125-33.
 50. Rajagopalan S, Olin J, Deitcher S, et al. Use of a constitutively active hypoxia-inducible factor-1 α transgene as a therapeutic strategy in no-option critical limb ischemia patients: phase I dose-escalation experience. *Circulation* 2007;115:1234-43.
 51. Kilian EG, Sadoni S, Vicoli C, et al. Myocardial transfection of hypoxia inducible factor-1 α via an adenoviral vector during coronary artery bypass grafting: a multicenter phase I and safety study. *Circ J* 2010;74:916-24.
 52. Milkiewicz M, Pugh CW, Egginton S. Inhibition of endogenous HIF inactivation induces angiogenesis in ischemic skeletal muscles of mice. *J Physiol* 2004;560:21-6.
 53. Fraisl P, Aragonés J, Carmeliet P. Inhibition of oxygen sensors as a therapeutic strategy for ischemic and inflammatory disease. *Nat Rev Drug Discov* 2009;8:139-52.
 54. Nagel S, Talbot NP, Mecnović J, Smith TG, Buchan AM, Schofield CJ. Therapeutic manipulation of the HIF hydroxylases. *Antioxid Redox Signal* 2010;12:481-501.
 55. Gordeuk VR, Prchal JT. Vascular complications in Chuvash polycythemia. *Semin Thromb Hemost* 2006;32:289-94.
 56. Jacobs K, Shoemaker C, Rudersdorf R, et al. Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature* 1985;313:806-10.
 57. Lin FK, Suggs S, Lin CH, et al. Cloning and expression of the human erythropoietin gene. *Proc Natl Acad Sci U S A* 1985;82:7580-4.
 58. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. *N Engl J Med* 1987;316:73-8.
 59. Brines ML, Ghezzi P, Keenan S, et al. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci U S A* 2000;97:10526-31.
 60. Calvillo L, Latini R, Kajstura J, et al. Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. *Proc Natl Acad Sci U S A* 2003;100:4802-6.
 61. Abdelrahman M, Sharples EJ, McDonald MC, et al. Erythropoietin attenuates the tissue injury associated with hemorrhagic shock and myocardial ischemia. *Shock* 2004;22:63-9.
 62. Cai Z, Semenza GL. Phosphatidylinositol-3-kinase signaling is required for erythropoietin-mediated acute protection against myocardial ischemia/reperfusion injury. *Circulation* 2004;109:2050-3.
 63. Ehrenreich H, Hasselblatt M, Dembowski C, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med* 2002;8:495-505.
 64. Ehrenreich H, Weissenborn K, Prange H, et al. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke* 2009;40(12):e647-e656.
 65. Jia L, Chopp M, Zhang L, Lu M, Zhang Z. Erythropoietin in combination of tissue plasminogen activator exacerbates brain hemorrhage when treatment is initiated 6 hours after stroke. *Stroke* 2010;41:2071-6.
 66. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182-6.
 67. Vaupel P, Mayer A, Höckel M. Tumor hypoxia and malignant progression. *Methods Enzymol* 2004;381:335-54.
 68. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010;29:625-34.
 69. Liao D, Johnson RS. Hypoxia: a key regulator of angiogenesis in cancer. *Cancer Metastasis Rev* 2007;26:281-90.
 70. Bindra RS, Crosby ME, Glazer PM. Regulation of DNA repair in hypoxic cancer cells. *Cancer Metastasis Rev* 2007;26:249-60.
 71. Lukashev D, Ohta A, Sitkovsky M. Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues. *Cancer Metastasis Rev* 2007;26:273-9.
 72. Gillies RJ, Gatenby RA. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev* 2007;26:311-7.
 73. Kim JW, Gao P, Dang CV. Effects of hypoxia on tumor metabolism. *Cancer Metastasis Rev* 2007;26:291-8.
 74. Swietach P, Vaughan-Jones RD, Harris AL. Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev* 2007;26:299-310.
 75. Chan DA, Giaccia AJ. Hypoxia, gene expression, and metastasis. *Cancer Metastasis Rev* 2007;26:333-9.
 76. Sullivan R, Graham CH. Hypoxia-driven selection of the metastatic phenotype. *Cancer Metastasis Rev* 2007;26:319-31.

77. Moeller BJ, Richardson RA, Dewhirst MW. Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev* 2007;26:241-8.
78. Barnhart BC, Simon MC. Metastasis and stem cell pathways. *Cancer Metastasis Rev* 2007;26:261-71.
79. Kaelin WG Jr. Treatment of kidney cancer: insights provided by the VHL tumor-suppressor protein. *Cancer* 2009;115:Suppl:2262-72.
80. Melillo G. Targeting hypoxia cell signaling for cancer therapy. *Cancer Metastasis Rev* 2007;26:341-52.
81. Zhang H, Qian DZ, Tan YS, et al. Digoxin and other cardiac glycosides inhibit HIF-1 α synthesis and block tumor growth. *Proc Natl Acad Sci U S A* 2008;105:19579-86.
82. Shimoda LA, Fallon M, Pisarcik S, Wang J, Semenza GL. HIF-1 regulates hypoxic induction of NHE1 expression and alkalization of intracellular pH in pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L941-L949.
83. Wang J, Weigand L, Lu W, Sylvester JT, Semenza GL, Shimoda LA. Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca²⁺ in pulmonary arterial smooth muscle cells. *Circ Res* 2006;98:1528-37.
84. Whitman EM, Pisarcik S, Luke T, et al. Endothelin-1 mediates hypoxia-induced inhibition of voltage-gated K⁺ channel expression in pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L309-L310.
85. Yu AY, Shimoda LA, Iyer NV, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α . *J Clin Invest* 1999;103:691-6.
86. Brusselmans K, Compennolle V, Tjwa M, et al. Heterozygous deficiency of hypoxia-inducible factor-2 α protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. *J Clin Invest* 2003;111:1519-27.
87. Formenti F, Beer PA, Croft QP, et al. Cardiopulmonary function in two human disorders of the hypoxia-inducible factor (HIF) pathway: von Hippel-Lindau disease and HIF-2 α gain-of-function mutation. *FASEB J* 2011;25:2001-11.
88. Bonnet S, Michelakis ED, Porter CJ, et al. An abnormal mitochondrial-hypoxia inducible factor-1 α -Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. *Circulation* 2006;113:2630-41.
89. Fijalkowska I, Xu W, Comhair SA, et al. Hypoxia-inducible factor-1 α regulates the metabolic shift of pulmonary hypertensive endothelial cells. *Am J Pathol* 2010;176:1130-8.
90. Archer SL, Marsboom G, Kim GH, et al. Epigenetic attenuation of mitochondrial superoxide dismutase 2 in pulmonary arterial hypertension: a basis for excessive cell proliferation and a new therapeutic target. *Circulation* 2010;121:2661-71.
91. Bowers R, Cool C, Murphy RC, et al. Oxidative stress in pulmonary hypertension. *Am J Respir Crit Care Med* 2004;169:764-9.
92. Beall CM. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. *Proc Natl Acad Sci U S A* 2007;104:Suppl 1:8655-60.
93. Simonson TS, Yang Y, Huff CD, et al. Genetic evidence for high-altitude adaptation in Tibet. *Science* 2010;329:72-5.
94. Yi X, Liang Y, Huerta-Sanchez E, et al. Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* 2010;329:75-8.
95. Beall CM, Cavalleri GL, Deng L, et al. Natural selection on EPAS1 (HIF-2 α) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci U S A* 2010;107:11459-64.
96. Bigham A, Bauchet M, Pinto D, et al. Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLoS Genet* 2010;6(9):e1001116.
97. Morita M, Ohneda O, Yamashita T, et al. HLF/HIF-2 α is a key factor in retinopathy of prematurity in association with erythropoietin. *EMBO J* 2003;22:1134-46.
98. Yoshida T, Zhang H, Iwase T, Shen J, Semenza GL, Campochiaro PA. Digoxin inhibits retinal ischemia-induced HIF-1 α expression and ocular neovascularization. *FASEB J* 2010;24:1759-67.
99. Bernhardt WM, Gottmann U, Doyon F, et al. Donor treatment with a PHD-inhibitor activating HIFs prevents graft injury and prolongs survival in an allogeneic kidney transplant model. *Proc Natl Acad Sci U S A* 2009;106:21276-81.
100. Jiang X, Khan MA, Tian W, et al. Adenovirus-mediated HIF-1 α gene transfer promotes repair of mouse airway allograft vasculature and attenuates chronic rejection. *J Clin Invest* 2011;121:2236-49.

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