Leading Edge
Review

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Hypoxia-Inducible Factors in Physiology and Medicine

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Oxygen homeostasis represents an organizing principle for understanding metazoan evolution, development, physiology, and pathobiology. The hypoxia-inducible factors (HIFs) are transcriptional activators that function as master regulators of oxygen homeostasis in all metazoan species. Rapid progress is being made in elucidating homeostatic roles of HIFs in many physiological systems, determining pathological consequences of HIF dysregulation in chronic diseases, and investigating potential targeting of HIFs for therapeutic purposes.

Oxygen is central to biology because it is used during respiration. O_2 serves as the final electron acceptor in oxidative phosphorylation, which carries with it the risk of generating reactive oxygen species (ROS). ROS react with cellular macromolecules and alter their biochemical or physical properties, resulting in cell dysfunction or death. As a consequence, metazoan organisms have evolved elaborate cellular metabolic and systemic physiological systems that are designed to maintain oxygen homeostasis. This Review focuses on the role of hypoxia-inducible factors (HIFs) as master regulators of oxygen homeostasis and, in particular, on recent advances in understanding their roles in physiology and medicine. Due to space limitations and the remarkably pleiotropic effects of HIFs, the description of such roles will be illustrative rather than comprehensive.

O_2 and Evolution, Part 1

O_2 started accumulating in Earth's atmosphere ~2.5 billion years ago. Increased availability of atmospheric O_2 led to the evolution of an extraordinarily efficient system of oxidative phosphorylation that transfers chemical energy stored in carbon bonds of organic molecules to the high-energy phosphate bond in ATP, which is used to power physicochemical reactions in living cells. Energy produced by mitochondrial respiration is sufficient to power the development and maintenance of multicellular organisms, which could not be sustained by energy produced by glycolysis alone (Lane and Martin, 2010). The dimensions of primitive metazoan species were small enough that O_2 could diffuse from the atmosphere to all of the organism's thousand cells, as is the case for the worm *Caenorhabditis elegans*. To escape the constraints placed on organismal growth by diffusion, systems evolved that could conduct air to cells deep within the body, and these designs were sufficient for O_2 delivery to organisms with hundreds of thousands of cells, such as the fly *Drosophila melanogaster*. The final leap in body scale occurred in vertebrates, and it was associated with the evolution of complex respiratory, circulatory, and nervous systems designed to efficiently capture and distribute O_2 to hundreds of millions of millions of cells, such as the adult *Homo sapiens*.

Hypoxia-Inducible Factors

Hypoxia-inducible factor 1 (HIF-1) is expressed by all extant metazoan species analyzed to date (Loenarz et al., 2011). HIF-1 consists of the subunits HIF-1α and HIF-1β. Each subunit contains basic helix-loop-helix-PAS (bHLH-PAS) domains (Wang et al., 1995) that mediate heterodimerization and DNA binding (Jiang et al., 1996a). HIF-1β heterodimerizes with other bHLH-PAS proteins and is present in excess, such that HIF-1α protein levels determine HIF-1 transcriptional activity (Semenza et al., 1996).

Under well-oxygenated conditions, HIF-1α is bound by the von Hippel-Lindau (VHL) protein. VHL recruits an ubiquitin ligase that targets HIF-1α for proteasomal degradation (Kaelin and Ratcliffe, 2008). VHL binding is dependent upon hydroxylation of a specific proline residue in HIF-1α by the prolyl hydroxylase PHD2. PHD2 uses O_2 as a substrate, and thus, its activity is inhibited under hypoxic conditions (Epstein et al., 2001). In the reaction, one oxygen atom is inserted into the prolyl residue, and the other atom is inserted into the cosubstrate α-ketoglutarate, splitting it into CO_2 and succinate (Kaelin and Ratcliffe, 2008). Factor-inhibiting HIF-1 (FIH-1) represses HIF-1α transactivation function (Mahon et al., 2001). It hydroxylates an asparaginyl residue on HIF-1α, using O_2 and α-ketoglutarate as substrates, thereby blocking the association of HIF-1α with the p300 coactivator protein (Lando et al., 2002). Dimethyloxalylglycine (DMOG), which is a competitive antagonist of α-ketoglutarate, inhibits hydroxylases and induces HIF-1-dependent transcription (Epstein et al., 2001). HIF-1 activity is also induced by iron chelators (e.g., desferrioxamine) and cobalt chloride, which inhibit hydroxylases by displacing Fe(II) from the catalytic center (Epstein et al., 2001).

Studies in cultured cells (Jiang et al., 1996b) and isolated, perfused, and ventilated lung preparations (Yu et al., 1998) revealed an exponential increase in HIF-1α levels at O_2

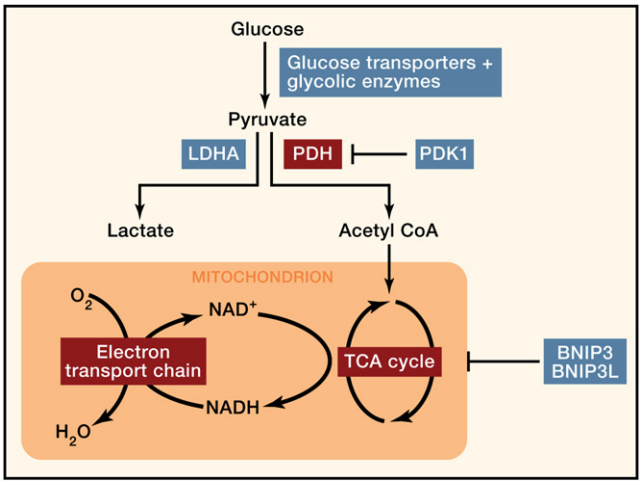

Figure 1. Regulation of Glucose Metabolism

Under hypoxic conditions, hypoxia-inducible factor 1 (HIF-1) activates the transcription of genes encoding the following: glucose transporters and glycolytic enzymes, which increase the flux of glucose to pyruvate; PDK1 (pyruvate dehydrogenase kinase), which inactivates PDH (pyruvate dehydrogenase), the mitochondrial enzyme that converts pyruvate to acetyl CoA for entry into the tricarboxylic acid (TCA/citric acid/Krebs) cycle; LDHA (lactate dehydrogenase), which converts pyruvate to lactate; and the mitochondrial proteins BNIP3 and BNIP3L, which induce mitochondrial-selective autophagy. The shunting of substrate away from the mitochondria reduces ATP production but prevents excess ROS production that occurs due to inefficient electron transport under hypoxic conditions.

concentrations <6% (~40 mm Hg), which cannot be explained by known biochemical properties of the hydroxylases. In most adult tissues, O_2 concentrations are in the range of 3%–5%, and any decrease occurs along the steep portion of the dose-response curve, allowing a graded response to hypoxia. Analyses of cultured human cells have revealed that expression of hundreds of genes was increased in response to hypoxia in a HIF-1-dependent manner (as determined by RNA interference) with direct binding of HIF-1 to the gene (as determined by chromatin immunoprecipitation [ChIP] assays). In addition, the expression of hundreds of genes was decreased in response to hypoxia in a HIF-1-dependent manner, but binding of HIF-1 to these genes was not detected (Mole et al., 2009). These results indicate that HIF-dependent repression occurs via indirect mechanisms, which include HIF-1-dependent expression of transcriptional repressors (Yun et al., 2002) and microRNAs (Kulshreshtha et al., 2007). ChIP-seq studies have revealed that only 40% of HIF-1-binding sites are located within 2.5 kb of the transcription start site (Schödel et al., 2011).

In vertebrates, HIF-2α is a paralog of HIF-1α that is also regulated by prolyl and asparaginyl hydroxylation. HIF-2α also dimerizes with HIF-1β, but it is expressed in a cell-restricted manner and plays important roles in erythropoiesis, vascularization, and pulmonary development. In *D. melanogaster*, the gene encoding the HIF-1α ortholog is designated *similar*, and its paralog is designated *trachealess* because inactivating mutations result in defective development of the tracheal tubes (Wilk et al., 1996). In contrast, *C. elegans* has only a single HIF-1α homolog (Epstein et al., 2001). Thus, in both invertebrates and vertebrates, the evolution of specialized systems for O_2 delivery was associated with the appearance of a HIF-1α paralog.

O_2 and Metabolism

The regulation of metabolism is a principal and primordial function of HIF-1. Under hypoxic conditions, HIF-1 mediates a transition from oxidative to glycolytic metabolism through its regulation of four factors: PDK1, LDHA, BNIP3, and BNIP3L. *PDK1* encodes pyruvate dehydrogenase (PDH) kinase 1, which phosphorylates and inactivates PDH, thereby inhibiting the conversion of pyruvate to acetyl coenzyme A for entry into the tricarboxylic acid cycle (Kim et al., 2006; Papandreou et al., 2006). *LDHA* encodes lactate dehydrogenase A, which converts pyruvate to lactate (Semenza et al., 1996). Finally, *BNIP3* (Zhang et al., 2008) and BNIP3L (Bellot et al., 2009) mediate selective mitochondrial autophagy (Figure 1). HIF-1 also mediates a subunit switch in cytochrome *c* oxidase that improves the efficiency of electron transfer under hypoxic conditions (Fukuda et al., 2007). An analogous subunit switch is also observed in *Saccharomyces cerevisiae*, although it is mediated by a completely different mechanism (yeast lack HIF-1). This conservation suggests that the subunit switch in cytochrome *c* oxidase may represent a fundamental response of eukaryotic cells to hypoxia.

It is conventional wisdom that cells switch to glycolysis for ATP production when O_2 becomes limiting. Yet, HIF-1α null mouse embryo fibroblasts, which do not downregulate respiration under hypoxic conditions, have higher ATP levels at 1% O_2 than wild-type cells at 20% O_2, demonstrating that under these conditions O_2 is not limiting for ATP production (Zhang et al., 2008). However, the HIF-1α null cells die under prolonged hypoxic conditions due to ROS toxicity (Kim et al., 2006; Zhang et al., 2008). These studies have led to a paradigm shift with regard to our understanding of the regulation of cellular metabolism (Semenza, 2010): the purpose of switching to glycolysis is to prevent excess mitochondrial generation of ROS that would otherwise occur due to the reduced efficiency of electron transfer under hypoxic conditions (Chandel et al., 1998). This may be particularly important in stem cells, in which avoidance of DNA damage is critical (Suda et al., 2011).

Role of HIFs in Development

Much of mammalian embryogenesis occurs at O_2 concentrations of 1%–5%, and O_2 functions as a morphogen (through HIFs) in many developmental systems (Dunwoodie, 2009). Mice that are homozygous for a null allele at the locus encoding HIF-1α die by embryonic day 10.5 with cardiac malformations, vascular defects, and impaired erythropoiesis. Thus, all three components of the circulatory system are dependent upon HIF-1 for normal development (Iyer et al., 1998; Yoon et al., 2011). Depending on the genetic background, mice lacking HIF-2α die by embryonic day 12.5 with vascular defects (Peng et al., 2000) or bradycardia due to deficient catecholamine production (Tian et al., 1998); die as neonates due to impaired lung maturation (Compernolle et al., 2002); or die several months after birth due to ROS-mediated multiorgan failure (Scortegagna et al., 2003). Thus, although vertebrate evolution was associated

with concomitant appearance of the circulatory system and HIF-2α, both HIF-1 and HIF-2 have important roles in circulatory system development. Conditional knockout of HIF-1α in specific types of cells has demonstrated important roles in chondrogenesis (the production of cartilage) (Schipani et al., 2001), adipogenesis (Yun et al., 2002), B lymphocyte development (Kojima et al., 2002), osteogenesis (Wang et al., 2007), hematopoiesis (Takubo et al., 2010), T lymphocyte differentiation (Dang et al., 2011), and innate immunity (Zinkernagel et al., 2007). Although knockout mouse experiments point to the adverse effects of HIF-1 loss of function on development, it is also possible that increased HIF-1 activity, induced by hypoxia in embryonic tissues as a result of abnormalities in placental blood flow, may also dysregulate development and result in congenital malformations. For example, HIF-1α has been shown to interact with and stimulate the transcriptional activity of Notch, which plays a key role in many developmental pathways (Gustafsson et al., 2005).

Diseases in which HIF-1 Mediates Protective Responses

Coronary Artery Disease

Heart disease is the major cause of mortality in the United States population. The formation of atherosclerotic plaques in the main coronary arteries leads to insufficient myocardial perfusion, especially when heart work and O_2 consumption are increased. A physiological response to myocardial ischemia (inadequate perfusion of the heart muscle) is the remodeling of collateral blood vessels to accept increased flow and thereby bypass stenotic regions, where lumenal diameter and blood flow are reduced. Two-thirds of patients with critical narrowing of a coronary artery (>70% reduction in diameter) have one or more collateral vessels. When rupture of atherosclerotic plaques results in complete coronary occlusion and myocardial infarction (MI; "heart attack"), patients with collaterals are likely to have smaller infarcts (areas of tissue necrosis) and are more likely to survive. In patients with critical coronary stenosis, the frequency of a single-nucleotide polymorphism (SNP) that changes proline to serine at residue 582 of HIF-1α was 5-fold greater among those without collaterals compared to those with collaterals (Resar et al., 2005). This SNP and two others at the *HIF1A* locus were associated with stable exertional angina (chest pain during exercise) rather than MI as the initial clinical presentation in patients with coronary artery disease (CAD) (Hlatky et al., 2007). Coronary stenosis and collaterals were not analyzed in this latter study, making interpretation of the data difficult. However, taken together these two clinical studies suggest that *HIF1A* is a major genetic modifier in CAD.

The remodeling of collateral vessels provides a means to increase O_2 delivery and thereby eliminate hypoxia. However, in the short-term, cells must adapt to survive O_2 deprivation, such as by shifting from oxidative to glycolytic metabolism. The powerful nature of these adaptations is illustrated by the preconditioning phenomenon, in which exposure of the heart to short periods (e.g., 5 min [I_5]) of ischemia, caused by occlusion of a coronary artery, followed by short periods (e.g., 5 min [R_5]) of reperfusion (recovery of blood flow) protect the heart against subsequent episodes of prolonged ischemia (e.g., 30 min [I_{30}]). Hearts from *Hif1a*$^{+/-}$ mice (heterozygous for a null allele at the *Hif1a* locus) show a complete loss of preconditioning (Cai et al., 2008). In addition to its effects on glucose metabolism, HIF-1 activates genes encoding enzymes that generate adenosine, a signaling molecule that mediates preconditioning (Eckle et al., 2008). The cardiac protection afforded by a preconditioning protocol lasting less than 1 hr (I_5-R_5-I_5-R_5-I_{30}) is dependent on HIF-1, indicating that HIF-1-dependent transcription and subsequent translation occur with remarkable rapidity.

Peripheral Arterial Disease

Atherosclerotic stenosis of major arteries in the legs results in ischemia that is manifested by intermittent claudication (leg pain during walking). This can progress to critical limb ischemia in which blood flow is not sufficient to maintain tissue viability, resulting in pain at rest and gangrene (tissue necrosis) that eventually necessitates limb amputation. The incidence of peripheral arterial disease (PAD) in the general population is ~5%, whereas ~20% of individuals over 70 years old have PAD, and 1%–2% of PAD patients develop critical limb ischemia. Limb ischemia induced in experimental animals by ligation of the femoral artery results in increased HIF-1α protein levels and HIF-1-dependent expression of downstream target genes, encoding multiple angiogenic growth factors. These factors include vascular endothelial growth factor (VEGF), stromal-derived factor 1 (SDF-1), placental growth factor, angiopoietin 1, angiopoietin 2, platelet-derived growth factor B, and stem cell factor. In addition, bone marrow-derived angiogenic cells (BMDACs) are recruited to the ischemic tissue and, together with the local effects of angiogenic factors, promote the recovery of tissue perfusion. All of these responses to limb ischemia are impaired in older mice and in *Hif1a*$^{+/-}$ mice (Bosch-Marce et al., 2007).

Clinical trials that target a single angiogenic factor (e.g., VEGF) have failed to promote recovery of patients with PAD. The observation that HIF-1 is a master regulator, which is responsible for the coordinated induction of multiple angiogenic factors, suggested that it may represent a better therapeutic target than a single angiogenic factor. AdCA5 is a recombinant adenovirus encoding an engineered form of HIF-1α that is constitutively active. A single intramuscular injection of AdCA5 into the ischemic limb was sufficient to overcome the impaired recovery of blood flow following femoral artery ligation in 8-month-old mice, but it was not effective in 13-month-old mice (Bosch-Marce et al., 2007; Rey et al., 2009). Small hairpin RNA (shRNA) targeting PHD2 or PHD3 also increased ischemic vascularization in 3-month-old mice (Loinard et al., 2009). Thirteen-month-old mice were rescued by a two-stage therapy, consisting of intramuscular administration of AdCA5 followed 24 hr later by intravenous administration of BMDACs, which were cultured for 4 days in the presence of angiogenic growth factors and DMOG.

The rationale for this staged approach with local and systemic delivery was as follows. First, AdCA5 induces production of angiogenic factors and thus provides a homing signal for BMDACs. Second, direct injection of a large number of cells into the ischemic tissue would increase cell death due to hypoxia, whereas systemic administration would select for the subpopulation of cells that were capable of homing to the ischemic tissue to participate in the vascular remodeling process. Combined therapy was ineffective if the BMDACs

were not treated with DMOG. At the molecular level, activation of HIF-1 in BMDACs had two important consequences. First, HIF-1 induced expression of β_2 integrins, which mediate increased adherence of circulating BMDACs to vascular endothelial cells, thereby increasing BMDAC retention within ischemic tissue (Rey et al., 2009). Second, metabolic reprogramming mediated by HIF-1 increased BMDAC survival within ischemic tissue (Rey et al., 2011). Combined therapy resulted in limb salvage even when both BMDAC donor and ischemic recipient mice were 17 months old, representing a model for autologous BMDAC therapy for elderly patients with critical limb ischemia.

A recombinant adenovirus, which encoded the HIF-1α bHLH-PAS domain fused to the herpes simplex virus VP16 transactivator protein, was administered to patients with critical limb ischemia by intramuscular injection without any adverse effects. However, the treatment had no significant therapeutic effect in a phase II trial (Creager et al., 2011). Although this trial involved administration of a potentially immunogenic non-native protein, which may have contributed to failure, the results also suggest that combined gene and cell therapy may be required in humans, as well as in mice.

Wound Healing

The principal vascular response to heart or limb ischemia involves arteriogenesis, the remodeling of collateral blood vessels. In contrast, cutaneous wound healing requires angiogenesis, the budding of new capillaries from existing vessels, and vasculogenesis, in which nonresident cells are recruited to participate in de novo blood vessel formation. Both local and systemic responses to wounding contribute to the reparative vascularization process. Endothelial cells in pre-existing vessels are activated to initiate angiogenesis in the injured tissue. In addition, the HIF-1-dependent release of cytokines from the wound elicits the mobilization and homing of BMDACs, which can participate in vasculogenesis or stimulate angiogenesis through paracrine mechanisms.

As in the case of CAD and PAD, impaired wound healing is associated with both aging and diabetes, with the combination having synergistic negative effects (Brem et al., 2007). Foot ulcers precede 85% of lower limb amputations in diabetic individuals. HIF-1α expression was markedly decreased in excisional skin wounds of young *db/db* mice when compared to nondiabetic littermates (Mace et al., 2007). HIF-1 activity was inhibited in skin fibroblasts from *db/db* mice exposed to high glucose concentrations, which could be reversed by treatment with DMOG or desferrioxamine. Topical application of desferrioxamine also improved wound vascularization and healing in *db/db* mice (Botusan et al., 2008; Thangarajah et al., 2009). Aging was associated with decreased expression of angiogenic factors and delayed wound healing in *db/db* mice (Liu et al., 2008). Expression of CA5 (Liu et al., 2008) or other constitutively active forms of HIF-1α (Mace et al., 2007; Botusan et al., 2008) resulted in increased angiogenic gene expression, BMDAC mobilization, wound vascularization, and an increased rate of wound healing. Intramuscular administration of AdCA5 also improved recovery of perfusion and limb salvage after femoral artery ligation in *db/db* mice (Sarkar et al., 2009).

Burn injuries represent a major health problem affecting ~1 million Americans annually. In wild-type mice subjected to burn wounding, HIF-1α protein levels in the wound, SDF-1 levels in plasma, and BMDACs circulating in blood were increased on day 2, leading to increased wound vascularization and perfusion on day 7. In *Hif1a*$^{+/-}$ mice, these angiogenic responses were impaired (Zhang et al., 2010). Aging also impaired HIF-1α and SDF-1 expression in response to burn wounding. When BMDACs from young donor mice were injected intravenously, homing to burn wound tissue was impaired in older recipients, whereas the age of the BMDAC donor had no effect on homing (Zhang et al., 2011b). In contrast, conditional knockout of HIF-1α in Tie2 lineage cells (which include BMDACs and endothelial cells) of either the donor or recipient inhibited BMDAC homing to burn wounds (Sarkar et al., 2012).

Organ Transplant Rejection

Chronic rejection following lung transplantation, which is manifested as obliterative bronchiolitis (airway fibrosis), accounts for the 50% 5 year survival of recipients, which is the worst of all organ allografts. The lung, which is perfused by the pulmonary arteries, pulmonary veins, and bronchial artery, is the only organ for which the major arterial blood supply is not re-established during transplant surgery (Wilkes, 2011). Autopsy data indicate that obliterative bronchiolitis is preceded by the loss of airway microvasculature (Luckraz et al., 2004). In an orthotopic tracheal transplantation model, analysis of knockout mice demonstrated that HIF-1-dependent recruitment of recipient *Tie2*$^{+}$ angiogenic cells and repair of airway microvasculature were critical determinants of graft survival. Furthermore, treatment of grafts with AdCA5 prior to transplantation increased graft perfusion, decreased fibrosis, and increased graft survival (Jiang et al., 2011). These results represent a paradigm shift in our understanding of chronic rejection from a purely immunological disease to one in which vascular responses to ischemia play a major role. Chronic rejection of renal transplants also involves destruction of the microvasculature, and in an allogenic kidney transplant model, treatment of donor rats with a prolyl hydroxylase inhibitor prior to nephrectomy improved survival of recipients (Bernhardt et al., 2009). Two major classes of immunosuppressive drugs, calcineurin and mTOR inhibitors, have been shown to block HIF-1 activity (Laughner et al., 2001; Liu et al., 2007) in cultured cells. Therefore, these drugs may exert unintended countertherapeutic effects. Finally, the potential role of aging-related impairment of HIF-1 activity in chronic rejection of solid organ transplants warrants investigation.

Colitis

The pathogenesis of chronic gastrointestinal inflammatory conditions such as Crohn's disease and ulcerative colitis includes microvascular abnormalities and tissue hypoxia. The severity of colitis induced by chemical (Karhausen et al., 2004) or bacterial (Hirota et al., 2010) toxins was increased in conditional knockout mice lacking HIF-1α expression in intestinal epithelial cells. Treatment of wild-type mice with DMOG reduced the severity of chemical (Cummins et al., 2008) or bacterial (Hirota et al., 2010) toxin-induced colitis.

Translational Prospects

Drug discovery programs have been initiated at many pharmaceutical and biotech companies to develop prolyl hydroxylase inhibitors (PHIs) that, as described above for DMOG, induce HIF activity for treatment of disorders in which HIF mediates

protective physiological responses. Local and short-term induction of HIF activity by PHIs, gene therapy, or other means are likely to be useful therapies for many of the diseases described above. In the case of ischemic cardiovascular disease, local therapy is needed to provide homing signals for the recruitment of BMDACs. Chronic systemic use of PHIs must be approached with great caution because individuals with genetic mutations that constitutively activate the HIF pathway (discussed below) have increased cardiovascular disease and mortality (Yoon et al., 2011). On the other hand, the profound inhibition of HIF activity and vascular responses to ischemia that are associated with aging suggest that systemic replacement therapy might be contemplated as a preventive measure for subjects for whom impaired HIF responses to hypoxia can be documented. In *C. elegans*, VHL loss of function increases life span in a HIF-1-dependent manner (Mehta et al., 2009), providing further evidence for a mutually antagonistic relationship between HIF-1 and aging.

Diabetes is an even more complicated condition. Impaired large-vessel responses to ischemia (CAD and PAD) and impaired small-vessel responses to cutaneous wounding are both common and due, at least in part, to loss of HIF activation. However, diabetes is also associated with excessive small-vessel proliferation (i.e., ocular neovascularization), and HIF-1 appears to play a key role in the pathogenesis of this complication (Yoshida et al., 2010).

Diseases in which HIF Activity Contributes to Pathogenesis

Hereditary Erythrocytosis

Individuals with excess red blood cell production due to germline mutations in the genes encoding VHL, PHD2, and HIF-2α have been identified, demonstrating the essential role of this pathway in regulating erythropoiesis (Yoon et al., 2011). These mutations impair hydroxylation and ubiquitination, thereby increasing the levels of HIF-1α and HIF-2α at any given partial pressure of O_2 (PO_2). Affected individuals manifest global physiologic changes that include altered ventilatory and pulmonary vascular responses to hypoxia (Smith et al., 2006) as well as altered metabolic responses to exercise (Formenti et al., 2010).

Cancer

Cancers contain hypoxic regions due to high rates of cell proliferation coupled with the formation of vasculature that is structurally and functionally abnormal. Increased HIF-1α or HIF-2α levels in diagnostic tumor biopsies are associated with increased risk of mortality in cancers of the bladder, brain, breast, colon, cervix, endometrium, head/neck, lung, ovary, pancreas, prostate, rectum, and stomach. Experimental manipulations that increase HIF-1α expression result in increased tumor growth, whereas loss of HIF activity results in decreased tumor growth (Semenza, 2010). HIFs are also activated by genetic alterations in human cancers, most notably VHL loss of function in clear cell renal carcinoma (Majmundar et al., 2010). HIFs activate transcription of genes that play key roles in critical aspects of cancer biology, including stem cell maintenance (Wang et al., 2011), cell immortalization, epithelial-mesenchymal transition (Mak et al., 2010), genetic instability (Huang et al., 2007), vascularization (Liao and Johnson, 2007), glucose metabolism (Luo et al., 2011), pH regulation (Swietach et al., 2007), immune evasion (Lukashev et al., 2007), invasion and metastasis (Chan and Giaccia, 2007), and radiation resistance (Moeller et al., 2007). Given the extensive validation of HIF-1 as a potential therapeutic target, drugs that inhibit HIF-1 have been identified and shown to have anticancer effects in xenograft models (Table 1) (Semenza, 2010).

Table 1. Drugs that Inhibit HIF-1

Process Inhibited	Drug Class	Prototype
HIF-1α protein synthesis	cardiac glycoside mTOR inhibitor microtubule-targeting agent topoisomerase I inhibitor	digoxin rapamycin 2-methoxyestradiol topotecan
HIF-1α protein stability	HDAC inhibitor HSP90 inhibitor calcineurin inhibitor guanylate cyclase activator	LAQ824 17-AAG cyclosporine YC-1
Heterodimerization	antimicrobial agent	acriflavine
DNA binding	anthracycline quinoxaline antibiotic	doxorubicin echinomycin
Transactivation	proteasome inhibitor antifungal agent	bortezomib amphotericin B
Signal transduction	BCR-ABL inhibitor cyclooxygenase inhibitor EGFR inhibitor HER2 inhibitor	imatinib ibuprofen erlotinib, gefitinib trastuzumab

More than 100 women die every day of breast cancer in the US. The mean PO_2 is 10 mm Hg in breast cancer as compared to >60 mm Hg in normal breast tissue, and cancers with PO_2 < 10 mm Hg are associated with increased risk of metastasis and patient mortality (Vaupel et al., 2004). Increased HIF-1α protein levels, as identified by immunohistochemical analysis of tumor biopsies, are associated with increased risk of metastasis and patient mortality in unselected breast cancer patients and in lymph node-positive, lymph node-negative, $HER2^+$, or estrogen $receptor^+$ subpopulations (Semenza, 2010). Metastasis is responsible for >90% of breast cancer mortality. The requirement for HIF-1 in breast cancer metastasis has been demonstrated for both autochthonous (spontaneously arising) tumors in transgenic mice (Liao et al., 2007) and orthotopic transplants (injection of human breast cancer cells into the mammary fat pad) in immunodeficient mice (Zhang et al., 2011a; Wong et al., 2011). Primary tumors direct the recruitment of bone marrow-derived cells to the lungs and other sites of metastasis (Kaplan et al., 2005). In breast cancer, hypoxia induces the expression of lysyl oxidase (LOX), a secreted protein that remodels collagen at sites of metastatic niche formation (Erler et al., 2009). In addition to LOX, breast cancers also express LOX-like proteins 2 and 4. *LOX, LOXL2,* and *LOXL4* are all HIF-1-regulated genes. HIF-1 inhibition blocks metastatic niche formation, regardless of which LOX/LOXL protein is expressed, but available LOX inhibitors are not effective against all LOXL proteins (Wong et al., 2011). These results again illustrate the role of HIF-1 as a master regulator that controls the expression of multiple genes involved in a single (patho)physiological process.

Table 2. HIF Target Genes Expressed in Pulmonary Arterial Smooth Muscle Cells in Pulmonary Hypertension

Target Gene(s)	Protein Product(s)	Consequence
EDN1	endothelin	contraction
KCNA5, *KCNB1*	voltage-gated K^+ channels	increased $[K^+]_i$
TRPC1, TRPC6	transient receptor potential Ca^{2+} channels	increased $[Ca^{2+}]_i$
NHE1	sodium-hydrogen exchanger	increased pH_i
PDK1	pyruvate dehydrogenase kinase	glycolysis

Traumatic Shock

Secondary lung injury is a major cause of death following severe abdominal trauma. Wild-type mice subjected to abdominal trauma/hemorrhagic shock develop intestinal villous necrosis and a severe pulmonary inflammatory response, whereas both gut and lung injury were attenuated in *Hif1a*$^{+/-}$ littermates (Feinman et al., 2010). Delineation of the mechanisms underlying maladaptive HIF-1-mediated responses to trauma, which are in contrast to the protective role of HIF-1 in the experimental colitis models described above, may identify novel therapeutic targets.

Pulmonary Arterial Hypertension

Prolonged exposure to alveolar hypoxia, which occurs in individuals with chronic lung disease, results in a remodeling of the pulmonary vasculature, leading to increased pulmonary arterial pressure and right ventricular hypertrophy. Multiple HIF-1 target genes that play key roles in the response of pulmonary artery smooth muscle cells to hypoxia have been identified (Table 2) (Shimoda and Semenza, 2011). *Hif1a*$^{+/-}$ and *Hif2a*$^{+/-}$ mice are both protected from hypoxic pulmonary hypertension, indicating that HIF-1α and HIF-2α play key pathogenic roles (Yu et al., 1999; Brusselmans et al., 2003). HIFs are also implicated in chemically induced and genetic forms of pulmonary arterial hypertension in which hypoxia is not an inciting factor (Bonnet et al., 2006).

Obstructive Sleep Apnea

In individuals with obstructive sleep apnea, pharyngeal soft tissue occludes the airway, resulting in decreased partial pressure of oxygen in the blood. This hypoxemia is sensed by carotid body (CB) chemoreceptors, leading to arousal, clearing of the airway, and reoxygenation. The cycle of hypoxia and reoxygenation is repeated dozens of times per night and results in increased ROS levels in the CB and brain. Sympathetic activation and increased plasma catecholamine levels lead to systemic hypertension. Obstructive sleep apnea is associated with both hypoxia and hypercarbia (abnormally high levels of carbon dioxide in the blood), but exposure of rodents to chronic intermittent hypoxia (CIH) is sufficient to elicit hypertension (Fletcher et al., 1992). Treatment of mice with the superoxide scavenger MnTMPyP blocks CIH-induced increases in HIF-1α, catecholamines, and blood pressure, indicating that HIF-1 is downstream of ROS. However, *Hif1a*$^{+/-}$ mice lack CIH-induced increases in ROS, catecholamines, and blood pressure, suggesting that HIF-1 is upstream of ROS (Peng et al., 2006). Recent data support the existence of a feedforward loop in which ROS trigger HIF-1α induction, leading to transcription of the *Nox2* gene encoding NADPH oxidase, which generates superoxide radicals (Yuan et al., 2011).

HIF-1α is expressed at low levels in the CB under normoxic conditions (normal PO_2) and is induced by CIH. In contrast, HIF-2α expression is high in the CB under normoxic conditions and decreased due to calpain-dependent degradation in response to CIH (Nanduri et al., 2009). Decreased HIF-2α levels are associated with decreased expression of the *Sod2* gene, which encodes the mitochondrial superoxide dismutase that converts superoxide to hydrogen peroxide. Treatment of CIH-exposed rats with a calpain inhibitor blocks HIF-2α degradation, restores SOD2 activity, and prevents oxidative stress and hypertension. Thus, disruption of the balance between HIF-1α and HIF-2α levels in the CB is central to the pathogenesis of CIH-induced hypertension. It is striking that continuous hypoxia induces HIF-1α and HIF-2α, resulting in pulmonary hypertension (Figure 2A), whereas CIH induces HIF-1α but inhibits HIF-2α, resulting in systemic hypertension (Figure 2B).

When isolated CBs from wild-type mice are superfused with a hypoxic gas mixture, increased depolarization of the O_2-sensing glomus cells and carotid sinus nerve activity are elicited, but these responses are absent in CBs from *Hif1a*$^{+/-}$ mice (Peng et al., 2006). In contrast, CBs from *Hif2a*$^{+/-}$ mice have augmented responses to acute hypoxia, and the mice manifest increased catecholamines and blood pressure under normoxic conditions (Peng et al., 2011). MnTMPyP treatment of *Hif2a*$^{+/-}$ mice normalizes blood pressure and CB responses. Thus, even under normoxic conditions, the balance between HIF-1α and HIF-2α controls CB function and cardiovascular homeostasis. Functional antagonism has also been reported in the regulation of nitric oxide (Takeda et al., 2010) and VEGF (Eubank et al., 2011) signaling by macrophages. These findings provide new insight into the logic underlying the acquisition of a HIF-1α paralog during vertebrate evolution.

Translational Prospects

Small-molecule inhibitors of HIF activity that have anticancer effects in mouse models have been identified (Table 1). Inhibition of HIF impairs both vascular and metabolic adaptations to hypoxia, which may decrease O_2 delivery and increase O_2 utilization. These drugs are likely to be useful (as components of multidrug regimens) in the treatment of a subset of cancer patients for whom high HIF activity is driving progression. As with all new cancer therapeutics, successful translation will require the development of methods for identifying the appropriate patient cohort. Effects of combination drug therapy also need to be considered. VEGF receptor tyrosine kinase inhibitors, which induce tumor hypoxia by blocking vascularization, have been reported to increase metastasis in mouse models (Ebos et al., 2009), which may be mediated by HIF-1. If so, combined use of HIF-1 inhibitors with these drugs may prevent unintended countertherapeutic effects.

HIF inhibitors may also be useful in the treatment of other diseases in which dysregulated HIF activity is pathogenic. Proof of principle has been established in mouse models of ocular neovascularization, a major cause of blindness in the developed world, in which systemic or intraocular injection of the HIF-1 inhibitor digoxin blocks excess blood vessel formation (Yoshida et al., 2010). Systemic administration of HIF inhibitors for cancer therapy

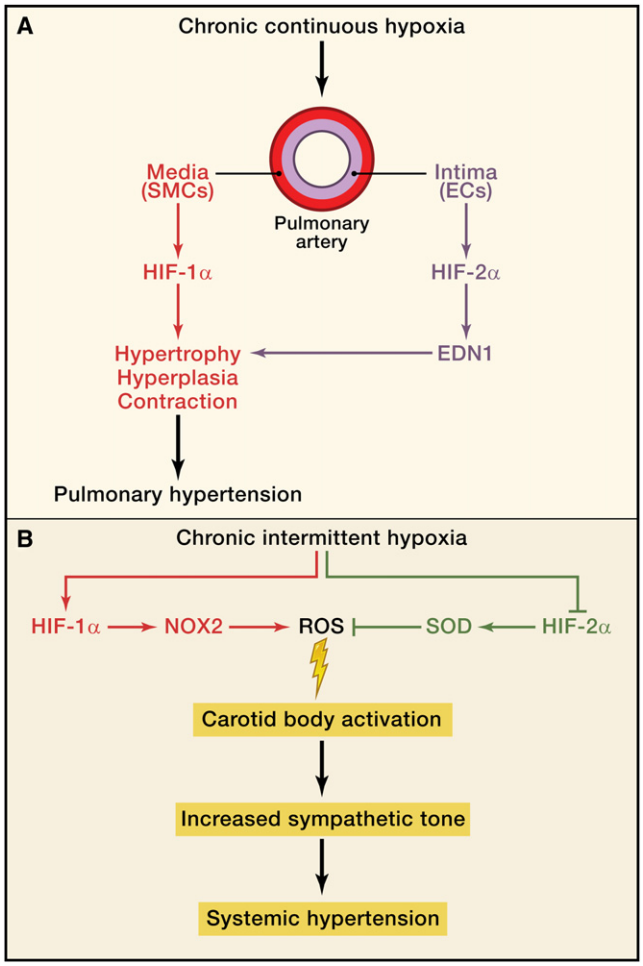

Figure 2. Vascular Effects of Continuous and Intermittent Hypoxia

(A) Chronic continuous alveolar hypoxia results in pulmonary arterial hypertension (increased blood pressure in the pulmonary artery). Activation of both HIF-1α and HIF-2α leads to changes in pulmonary arterioles that reduce lumenal diameter, thereby increasing pulmonary vascular resistance.

(B) Chronic intermittent hypoxia results in systemic arterial hypertension due to activation of HIF-1α and degradation of HIF-2α. Increased HIF-1α-dependent expression of NADPH oxidase 2 (NOX2), which generates superoxide anion, and decreased HIF-2α-dependent expression of superoxide dismutase 2 (SOD2), which consumes superoxide, result in increased ROS levels in the carotid body, leading to sympathetic nervous system activation and systemic hypertension.

would be contraindicated in patients who also have ischemic cardiovascular disease, in which HIF activity is protective.

O_2 and Evolution, Part 2

When lowlanders sojourn to high altitude, hypobaric hypoxia induces erythropoiesis. This is a relatively ineffective response because the problem is not insufficient red cells but rather insufficient ambient O_2. Chronic erythrocytosis increases the risk of heart attack, stroke, and fetal loss during pregnancy. Many high-altitude Tibetans maintain the same hemoglobin concentration as lowlanders, and yet, despite severe hypoxemia, they also maintain aerobic metabolism. The basis for this remarkable evolutionary adaptation appears to have involved the selection of genetic variants at multiple loci encoding components of the oxygen-sensing system, particularly HIF-2α (Beall et al., 2010; Simonson et al., 2010; Yi et al., 2010). Given that hereditary erythrocytosis is associated with modest HIF-2α gain of function, the Tibetan genotype associated with absence of an erythrocytotic response to hypoxia may encode reduced HIF-2α activity along with other alterations that increase metabolic efficiency. Delineating the molecular mechanisms underlying these metabolic adaptations may lead to novel therapies for ischemic disorders, illustrating the importance of oxygen homeostasis as a nexus where evolution, biology, and medicine converge.

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