

Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology

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Hypoxia-inducible factor 1 (HIF-1) activates transcription of genes encoding proteins that mediate adaptive responses to reduced oxygen availability. The HIF-1 β subunit is constitutively expressed, whereas the HIF-1 α subunit is subject to ubiquitination and proteasomal degradation, a process that is inhibited under hypoxic conditions. Recent data indicate that HIF-1 plays major roles in the prevention of myocardial and cerebral ischemia and in the pathogenesis of pulmonary hypertension and cancer. Modulation of HIF-1 activity by genetic or pharmacological means could provide a novel therapeutic approach to these common causes of mortality.

Although cells are cultured in the laboratory at an ambient oxygen concentration of 21%, which corresponds to a PO_2 of approximately 150 mmHg (torr) at sea level, cells in the human body are exposed to much lower oxygen concentrations ranging from 16% (100 torr) in the pulmonary alveoli to less than 6% (40 torr) in most other organs of the body. Tissue oxygenation represents a steady state based upon O_2 consumption, primarily by mitochondrial oxidative phosphorylation, and O_2 delivery via erythrocytes traveling through tissue capillary networks. This steady state represents a balance between the requirement for O_2 as an energy substrate and the inherent risk of oxidative damage to cellular macromolecules. As a result, O_2 homeostasis is precisely maintained and the disruption of this homeostasis is a crucial feature in the pathophysiology of heart disease, cancer, cerebrovascular disease and chronic lung disease, which represent the most common causes of mortality in western cultures. Recent studies have begun to delineate the molecular basis for a variety of cellular and systemic mechanisms of O_2 homeostasis. As might be expected, these mechanisms occur at every regulatory level, including gene transcription, protein translation and post-translational modification. The most global regulator of this process identified to date is the transcriptional activator hypoxia-inducible factor 1 (HIF-1). Although it has only been six years since the purification of HIF-1 and the isolation of cDNA sequences encoding the HIF-1 α and HIF-1 β subunits¹, remarkable progress has been made in understanding the structure and function of the protein and its role in development, physiology and disease pathogenesis.

HIF-1: a bHLH-PAS heterodimer
HIF-1 is a member of the basic helix-loop-helix (bHLH) superfamily of eukaryotic transcription

factors in which the HLH domains mediate subunit dimerization and the basic domains bind to DNA. A family of bHLH proteins that is found only in animals contains an auxiliary dimerization interface known as the PAS domain, an acronym that derives from the first three proteins in which it was discovered, PER, ARNT and SIM (Ref. 2). The bHLH-PAS proteins are heterodimers composed of a class I subunit, either ARNT (aryl hydrocarbon receptor nuclear translocator; also known as HIF-1 β), ARNT2, ARNT3 (also known as BMAL1/MOP3) or MOP9, and a Class II subunit, which includes AHR (aryl hydrocarbon receptor), CLOCK, HIF-1 α , HIF-2 α (EPAS1/HLF/HRF/MOP2), HIF-3 α , NPAS1 (MOP5), NPAS2 (MOP4), SIM1, or SIM2 (Refs 3,4).

Expression of HIF-1 α and HIF-1 β mRNA has been detected in all adult and embryonic mouse and human tissues that have been analyzed^{5,6}. By contrast, ARNT2 is expressed primarily within the brain and kidney^{5,7,8} whereas HIF-2 α is expressed in vascular endothelial cells, catecholamine-producing cells, kidney, and lung during embryogenesis^{5,9-12}. ARNT, HIF-1 α and HIF-2 α are all required for normal mouse development¹²⁻¹⁷.

HIF-1 α and HIF-2 α have a high degree of amino acid sequence identity within the bHLH-PAS and transactivation domains and, as a result, their ability to dimerize with ARNT or ARNT2 and bind to DNA, and to activate transcription are similar when tested *in vitro* or in cell transfection assays, respectively^{11,18}. A cysteine residue present in the basic domain of HIF-2 α but not HIF-1 α might provide a mechanism for specific redox regulation of DNA binding by HIF-2 α -ARNT heterodimers¹⁹. It is not clear whether HIF-2 α functions to augment HIF-1 α expression in certain cell types or whether heterodimers containing HIF-2 α activate target genes distinct from those regulated by HIF-1 as no examples of the latter have been described.

Hypoxia signal transduction: upstream and downstream of HIF-1

Three dozen HIF-1-regulated genes have been identified to date (Table 1). The protein products of these genes play key roles in angiogenesis, vascular reactivity and remodeling, glucose and energy

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Table 1. Genes that are transcriptionally regulated by HIF-1

Gene product	Function	Refs
Adenylate kinase 3	Nucleotide metabolism	66
α_{1B} -adrenergic receptor	Vascular tone	67
Adrenomedullin	Vascular tone, cell survival	68
Aldolase A	Glucose metabolism	13,17
Aldolase C	Glucose metabolism	13
Carbonic anhydrase 9	pH regulation	69
Ceruloplasmin	Iron metabolism	70
Endothelin-1	Vascular tone and remodeling	71
Enolase 1	Glucose metabolism	13
Erythropoietin	Erythropoiesis, cell survival	72
Glucose transporter 1	Glucose metabolism	13,17,66
Glucose transporter 3	Glucose metabolism	3
Glyceraldehyde-3-P-dehydrogenase	Glucose metabolism	13,17
Heme oxygenase-1	Vascular tone, cell survival	73
Hexokinase 1	Glucose metabolism	13
Hexokinase 2	Glucose metabolism	13
Insulin-like growth factor (IGF) 2	Cell proliferation and survival	74
IGF-binding protein 1	Cell proliferation and survival	64
IGF-binding protein 2	Cell proliferation and survival	74
IGF-binding protein 3	Cell proliferation and survival	74
Lactate dehydrogenase A	Glucose metabolism	13,17
Nitric oxide synthase 2	Vascular tone, cell survival	75
NIP3	Apoptosis	76
p21	Cell proliferation	46
p35srj	Regulation of HIF-1 activity	77
Phosphofructokinase L	Glucose metabolism	13
Phosphoglycerate kinase 1	Glucose metabolism	13,17,46
Plasminogen activator inhibitor 1	Angiogenesis	78
Prolyl-4-hydroxylase α (I)	Collagen metabolism	79
Pyruvate kinase M	Glucose metabolism	13
Transferrin	Iron metabolism	80
Transferrin receptor	Iron metabolism	81,82
Transforming growth factor β 3	Angiogenesis, cell proliferation	63
Triosephosphate isomerase	Glucose metabolism	13
Vascular endothelial growth factor	Angiogenesis, cell survival	13,17,46
VEGF receptor FLT-1	Angiogenesis	83

metabolism, cell proliferation and survival, erythropoiesis and iron homeostasis. HIF-1 binds to target genes at sites containing the core recognition sequence 5'-RCGTG-3' (Ref. 20). The presence of a HIF-1-binding site is necessary but not sufficient to direct gene expression in response to hypoxia²⁰, indicating that HIF-1 must interact with other transcription factors bound at adjacent sites²¹. Interactions between HIF-1 α and hepatocyte nuclear factor 4 (HNF4), which binds at an adjacent site in the hypoxia response element of the human erythropoietin gene, have been demonstrated²². HIF-1 α also interacts with coactivators such as CBP, p300, SRC-1 and TIF2 (Table 2) and this association is regulated by both oxygen concentration and redox state.

In addition to DNA-binding proteins and coactivators, HIF-1 α also interacts with proteins that regulate its half-life. The von Hippel-Lindau

Table 2. Proteins that interact with HIF-1 α

Protein	Interacting HIF-1 α residues	Refs
ARNT (HIF-1 β)	1-390	1,72
CBP	531-584, 786-826	84,85
HNF4	106-526	86
HSP90	ND	87,88
p53	ND	24,89
p300	786-826	90
REF-1	531-584, 776-826	84
SRC-1	776-826	84
TIF2	ND	84
VHL	549-582	84
ND, not determined		

(VHL) tumor suppressor protein plays an essential role in targeting HIF-1 α for degradation under non-hypoxic conditions²³ whereas p53 appears to function as a negative modulator of hypoxia-induced HIF-1 α expression²⁴. Recent studies provide convincing evidence that hypoxia results in decreased ubiquitination and proteasomal degradation of HIF-1 α (Refs 25-28) (Fig. 1) but the mechanism by which this occurs remains obscure. The oxygen signal might be converted to a redox signal, but results suggesting that increased²⁹ or decreased²⁷ hydrogen peroxide concentrations induce HIF-1-mediated gene expression have been reported. In addition, inhibitors of tyrosine kinases, serine/threonine phosphatases, diacylglycerol kinase, and the phosphoinositol-3-kinase pathway have been shown to block hypoxia-induced HIF-1 α expression²⁹⁻³³. However, it is not clear whether any of these molecules are directly involved in signaling hypoxia or whether they are only required to maintain the activity of some component of the system. Nitric oxide, which appears to play an important role in oxygen homeostasis in *Drosophila*³⁴, has the paradoxical effect of inducing expression of HIF-1 α under non-hypoxic conditions as well as inhibiting hypoxia-induced HIF-1 α expression^{35,36}.

HIF-1 as a mediator of disease prevention and disease pathogenesis

Myocardial ischemia and angiogenesis

Atherosclerosis is endemic to western cultures and results in narrowing of cerebral, coronary and peripheral arteries that deprives tissue downstream of the atheroma of adequate perfusion, a condition known as ischemia. Although ischemia involves both decreased levels of energy substrates (O₂ and glucose) and increased levels of toxic metabolites, hypoxia represents a sufficient signal to trigger a corrective response in the form of new blood vessel formation. A principal mechanism by which hypoxia induces angiogenesis is the HIF-1-mediated transcription of the *VEGF* gene encoding vascular endothelial growth factor³⁷. Myocardial hypoxia is a potent inducer of HIF-1 α protein and *VEGF* mRNA expression leading to increased vascularization in the fetal sheep heart³⁸.

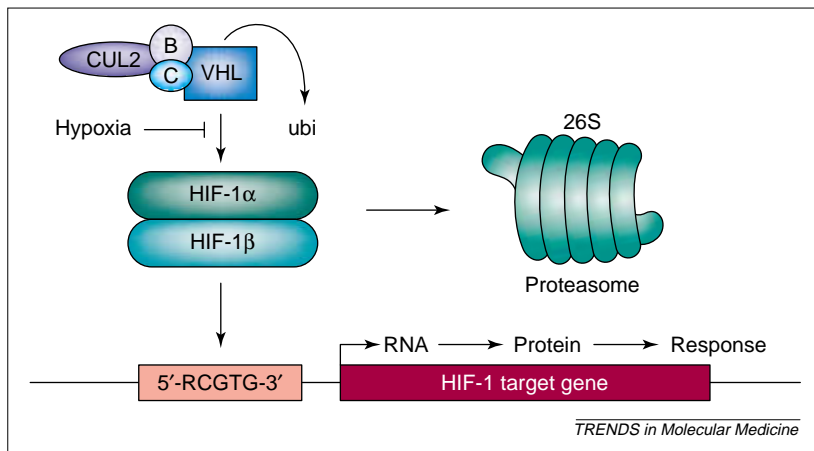


Fig. 1. Regulation of hypoxia-inducible gene expression by hypoxia inducible factor (HIF)-1. Under non-hypoxic conditions, the HIF-1 α subunit is bound by the von Hippel-Lindau tumor suppressor protein (VHL), which is the recognition component of a ubiquitin–protein ligase complex that also contains the proteins elongin B, elongin C, and cullin 2. Ubiquitination of HIF-1 α targets it for degradation by the 26S proteasome. Under hypoxic conditions, binding of VHL to HIF-1 α is inhibited, resulting in the accumulation of HIF-1 α , its dimerization with HIF-1 β , and binding to the core DNA recognition sequence 5'-RCGTG-3' which is contained within hypoxia response elements of target genes. The binding of HIF-1 activates increased transcription of these genes and ultimately results in the synthesis of proteins that mediate (patho)physiological responses to hypoxia.

Although ischemia in the adult human heart also results in the induction of HIF-1 α protein and *VEGF* mRNA expression³⁹, the resulting angiogenic response is often not sufficient to prevent infarction. Aged animals manifest both decreased ischemia-induced VEGF expression and a decreased endothelial cell response to exogenous VEGF (Ref. 40). The age-dependent impairment of VEGF expression is caused at least in part by impaired induction of HIF-1 activity in response to hypoxia or ischemia^{41,42}. The extent to which hypoxia induces VEGF expression in monocytes cultured from peripheral blood is predictive of the degree of coronary collateralization in patients with ischemic heart disease⁴³. Thus, it could be possible to identify individuals who would be particularly appropriate candidates for stimulation of myocardial angiogenesis by *VEGF* or *HIF-1 α* gene therapy⁴⁴.

Cerebral ischemia and preconditioning

In seven-day-old baby rats, left common carotid artery occlusion followed by exposure of the animal to ambient hypoxia (8% O₂ for 3 h) causes infarction in the cerebral hemisphere ipsilateral to the carotid occlusion. However, rats that are exposed to 8% O₂ for 3 h and then allowed to recover for 24 h before hypoxia/ischemia are protected from infarction. This protective phenomenon, which is known as delayed preconditioning, has been demonstrated in the brain, heart, kidney and other organs of the body. Exposure of baby rats to the hypoxic preconditioning stimulus results in a marked induction of HIF-1 α protein expression throughout the brain, whereas hypoxia/ischemia results in decreased HIF-1 α expression in the brain parenchyma and a dramatic increase in expression in blood vessels in the ipsilateral hemisphere⁴⁵. Cobalt chloride and

desferrioxamine, which induce HIF-1 α expression and HIF-1-DNA-binding activity, also induce cerebral protection when injected into baby rats 24 h before hypoxia/ischemia⁴⁵. Paradoxically, in the absence of preconditioning, HIF-1 might promote ischemia-induced neuronal death^{46,47}. The involvement of HIF-1 in either promoting or protecting against cell death depending on the context is similar to the case of inducible nitric oxide synthase (NOS2). *Nos2* knockout mice have reduced brain injury following middle cerebral artery occlusion⁴⁸ but have no delayed preconditioning response in the heart⁴⁹. This might be more than a coincidence, as *NOS2* is an HIF-1 target gene (Table 1). Although definitive studies remain to be performed, the available data suggest that HIF-1 might mediate delayed preconditioning.

Hypoxia-induced pulmonary vascular remodeling

In chronic obstructive lung disease, alveolar hypoxia induces pathological changes in the arterioles of the pulmonary circulation. When mice are exposed to 10% O₂ for three weeks, medial wall thickening significantly reduces the luminal diameter of these arterioles and increases pulmonary resistance, a condition known as pulmonary hypertension. In mice that are heterozygous for a knockout allele at the *Hif1a* locus, the development of hypoxia-induced pulmonary vascular remodeling is markedly impaired⁵⁰. Endothelin-1, which is produced by pulmonary vascular endothelial cells in response to hypoxia and induces contraction and proliferation of vascular smooth muscle cells (VSMC), is an HIF-1 target gene (Table 1). In addition, angiotensin II, platelet-derived growth factor, serotonin and thrombin, which all induce smooth muscle hypertrophy or proliferation, induce HIF-1 α expression in VSMC (Ref. 51), suggesting that HIF-1 might mediate both the induction of vasoactive cytokines and the responses induced by their binding in the pulmonary vasculature.

Cancer

Cancers must establish a vascular supply to grow beyond a volume of several cubic millimeters. Tumor vascularization results from decreased expression of anti-angiogenic factors and increased expression of angiogenic factors such as VEGF (Ref. 52). HIF-1 α expression, induced as a result of intratumoral hypoxia and genetic alterations that inactivate tumor suppressor genes or activate oncogenes, plays a critical role in this process⁵³. In breast cancer, HIF-1 α overexpression can be detected in ductal carcinoma *in situ*, the pre-invasive stage at which angiogenesis is first induced⁵⁴. By contrast, HIF-1 α is not overexpressed in benign fibroadenomas of the breast^{54,55}. HIF-1 α overexpression and vascularization are strongly correlated in brain tumors⁵⁶. In both breast and brain tumors, there is also a significant association between

Table 3. Immunohistochemical analysis of HIF-1α in human cancer^a

Brain tumor	Number analyzed	HIF-1α score ^b
Low-grade astrocytoma or mixed glioma	4	2.0
Anaplastic astrocytoma or oligodendroglioma	14	2.9
Glioblastoma multiforme	12	3.6
Breast tissue	HIF-1α expression ^c	% (+) Cells ^d
Normal	0/10	0
Hyperplasia	0/10	0
Ductal carcinoma <i>in situ</i> , well differentiated	11/20	3.5
Breast cancer, well differentiated	12/20	14.9
Ductal carcinoma <i>in situ</i> , poorly differentiated	17/20	11.8
Breast cancer, poorly differentiated	20/20	15.7

^aData from Refs 54, 56.
^bAll brain tumors that were analyzed overexpressed HIF-1α relative to surrounding normal brain tissue. Scoring: 1, nuclear staining in <1% of cells; 2, 1–10%; 3, 10–50%; 4, >50%. Mean score within each group is shown.
^cNumber of biopsies with detectable HIF-1α expression (>1% of cells)/total number of biopsies analyzed.
^dMean percentage of cells analyzed per biopsy with detectable HIF-1α expression.

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metastases⁵⁷. Thus, immunohistochemical analysis of HIF-1α expression in tumor biopsies could identify highly aggressive tumors that require intensive therapy to maximize the probability of patient survival. The association of HIF-1α overexpression with tumor progression, from the establishment of angiogenesis in pre-invasive lesions to the resistance of metastases to radiation therapy, suggests that inhibition of HIF-1 activity might be of therapeutic benefit, a hypothesis that is supported by animal models^{24,58–62}.

Involvement in other disease states

Recent studies implicate HIF-1 in the pathogenesis of pre-eclampsia⁶³ and intrauterine growth retardation⁶⁴, the leading causes of fetal and maternal morbidity and mortality. HIF-1 is also implicated in retinal neovascularization, a major cause of blindness⁶⁵.

Conclusion

HIF-1 plays critical roles in the pathophysiology of heart disease, cancer, cerebrovascular disease, and chronic lung disease, which are responsible for two-thirds of all deaths annually in the USA. Recent studies suggest that in each of these conditions HIF-1 could represent a novel therapeutic target. HIF-1α gene therapy could provide a means to stimulate angiogenesis in ischemic tissue. Small-molecule inducers of HIF-1 activity might provide protection against ischemia-induced cell death. Small-molecule inhibitors of HIF-1 activity might be of therapeutic benefit in the treatment of pulmonary hypertension and cancer. Whether strategies can be designed that will achieve the desired therapeutic effect without incurring undesired side effects is a major issue that will only be resolved by empirical data.

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Yellow fever vector live-virus vaccines: West Nile virus vaccine development

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By combining molecular-biological techniques with our increased understanding of the effect of gene sequence modification on viral function, yellow fever 17D, a positive-strand RNA virus vaccine, has been manipulated to induce a protective immune response against viruses of the same family (e.g. Japanese encephalitis and dengue viruses). Triggered by the emergence of West Nile virus infections in the New World afflicting humans, horses and birds, the success of this recombinant technology has prompted the rapid development of a live-virus attenuated candidate vaccine against West Nile virus.

Yellow fever (YF) virus is a positive-strand RNA virus widely used as an attenuated live-virus vaccine. Other RNA live-virus vaccines include measles, mumps, rubella and poliovirus, all of which have been in use for many years with remarkable safety and efficacy profiles^{1,2}, although this has been challenged by recent reports linking measles virus or measles vaccines with the occurrence of juvenile CROHN'S DISEASE (see Glossary) and autism^{3–5}. Recently, some of these RNA viruses have been explored as vectors to deliver foreign genes. A promising example is influenza virus, a negative-strand RNA virus commercialized as a nonliving-virus vaccine, first used in 1989 as a vaccine vector. Influenza vector technology, based on delivering defined EPITOPES by substitution of surface residues of

the influenza spike proteins, exploits influenza TROPISM to target generation of MUCOSAL IMMUNITY in the upper and lower respiratory tracts⁶, as well as systemic responses, which result in immunity in genital and intestinal tracts⁷. Poliovirus, a positive-strand RNA virus like YF, has been taken a step forward in preclinical studies and tested as a vaccine vector genetically modified to induce immunogenicity against tetanus toxin and simian immunodeficiency virus^{8,9}. Most advances regarding the use of RNA viruses as delivery systems rely on the significant progress of RT-PCR, REVERSE GENETICS, plasmid vectors and *in vitro* transcription systems. The first infectious animal RNA virus clone to be recovered from a full-length cDNA molecule was the 7.5-kb poliovirus in 1981¹⁰; only last year, a 27-kb porcine coronavirus, the longest viral RNA genome known, was successfully cloned using a bacterial artificial chromosome (BAC), a low-copy-number plasmid¹¹. The instability of bacterial vectors carrying viral cDNA was a gigantic hurdle for this technology, as experienced during the YF virus cloning. Rice *et al.*¹² were the first to generate YF virus RNA from a pair of cDNA clones ligated *in vitro* before RNA transcription. The same technology was

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