HIF-1: mediator of physiological and pathophysiological responses to hypoxia

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Semenza, Gregg L. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 88: 1474–1480, 2000.—All organisms can sense O₂ concentration and respond to hypoxia with adaptive changes in gene expression. The large body size of mammals necessitates the development of multiple complex physiological systems to ensure adequate O₂ delivery to all cells under normal conditions. The transcriptional regulator hypoxia-inducible factor 1 (HIF-1) is an essential mediator of O₂ homeostasis. HIF-1 is required for the establishment of key physiological systems during development and their subsequent utilization in fetal and postnatal life. HIF-1 also appears to play a key role in the pathophysiology of cancer, cardiovascular disease, and chronic lung disease, which represent the major causes of mortality among industrialized societies. Genetic or pharmacological modulation of HIF-1 activity in vivo may represent a novel therapeutic approach to these disorders.

angiogenesis; glycolysis; ischemia; pulmonary hypertension; vascular endothelial growth factor; hypoxia-inducible factor 1

PHYSIOLOGICAL RESPONSES TO HYPOXIA:
FUNDAMENTAL CONCEPTS

The ability to maintain O₂ homeostasis is essential to the survival of all invertebrate and vertebrate species. Physiological systems have evolved to ensure the optimal oxygenation of all cells in each organism. In simple invertebrates with few cell layers, direct diffusion of O₂ is sufficient for oxygenation. In Drosophila melanogaster, a series of branching tracheal tubes in the adult fly conduct air throughout the body, thus allowing O₂ to diffuse into individual cells. In humans and other vertebrates, the dramatic increase in body size is associated with the development of a complex physiological infrastructure for O₂ delivery that includes an intake and pumping station (lungs and diaphragm), transport vehicles (erythrocytes), vehicle propulsion device (heart), and highway system (vasculature). The precise establishment and regulation of these systems provide a major basis for O₂ homeostasis.

O₂ sensing was originally attributed solely to specialized chemoreceptor cells such as the carotid and neuroepithelial bodies that regulate cardiovascular and ventilatory rates, respectively (reviewed in Ref. 42). It is now appreciated that all nucleated cells in the human body sense O₂ concentration and respond to reduced O₂ availability (hypoxia) that is either acute or chronic in duration. As in other physiological systems, adaptive responses to acute changes in O₂ concentration (lasting from seconds or less to minutes) principally occur as a result of alterations (e.g., involving phosphorylation or redox state) of preexisting proteins, whereas chronic changes in O₂ concentration (lasting from minutes to hours or more) principally occur as a result of alterations in gene expression. The physiological mechanisms by which cells sense acute or chronic changes in O₂ concentration are only beginning to be understood at the molecular level (reviewed in Ref. 56), and several other mini-reviews in this series on Hypoxia Influences on Gene Expression will tackle various aspects of this important problem.

Not only is O₂ homeostasis essential for survival, but also hypoxia plays an important role in the pathogenesis of major causes of mortality, including cancer, cerebral and myocardial ischemia, and chronic heart and lung diseases. Investigating the molecular mechanisms of O₂ homeostasis therefore represents not only
an effort to delineate fundamental aspects of human physiology but also a means of gaining new insights about, and potentially new therapeutic approaches to, the most important public health problems of the present day. This review will specifically focus on responses to chronic hypoxia that involve changes in gene expression that are mediated by the transcriptional regulator hypoxia-inducible factor 1 (HIF-1).

HIF-1 IS A bHLH-PAS PROTEIN

HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β [also known as the aryl hydrocarbon nuclear translocator (ARNT)] subunits (68, 70). The amino-terminal half of each subunit contains basic helix-loop-helix (bHLH) and PER-aryl hydrocarbon nuclear translocator (ARNT)-SIM (PAS) domains (Fig. 1). The bHLH domain defines a large superfamily of dimeric eukaryotic transcription factors in which the HLH domain mediates dimerization, which is, in turn, required for DNA binding. Additional regulatory domains of HIF-1α include amino- and carboxy-terminal nuclear localization signals (NLS-N and NLS-C, respectively), the proline-serine-threonine-rich protein stabilization domain (PSTD), amino- and carboxy-terminal transactivation domains (TAD-N and TAD-C, respectively), and the transcriptional inhibitory domain (ID). Transcriptional coactivators CBP and p300 interact with TAD-C. See text for details and references.

HIF-1α and ARNT (HIF-1β) mRNA are expressed in most, if not all, human and rodent tissues (71, 72). In contrast, HIF-2α, HIF-3α, ARNT2, and ARNT3 show a more restricted pattern of expression. For example, mRNA encoding HIF-2α [also known as endothelial PAS domain protein 1 (EPAS1), HIF-1α-like factor, important functional domains in HIF-1α (bHLH, PAS, proline-serine-threonine-rich protein stabilization domain, amino-terminal transactivation domain, and carboxy-terminal transactivation domain; see Fig. 1) are highly conserved in HIF-2α (49, 67, 73). As for all the class I subunits, HIF-1α, -2α, and -3α each heterodimerize with one of the class II subunits, ARNT (HIF-1β), ARNT2, or ARNT3. It is not known whether heterodimers containing HIF-1α have different DNA-binding or transcriptional properties depending on the particular class I dimerization partner.

Table 1. Mammalian bHLH-PAS proteins

<table>
<thead>
<tr>
<th>Class</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Class I</td>
<td></td>
</tr>
<tr>
<td>ARNT (HIF-1β)</td>
<td>22</td>
</tr>
<tr>
<td>ARNT2</td>
<td>20</td>
</tr>
<tr>
<td>ARNT3 (BMAL1/MOP3)</td>
<td>23, 27, 62</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
</tr>
<tr>
<td>AHR</td>
<td>5</td>
</tr>
<tr>
<td>CLOCK</td>
<td>37</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>68</td>
</tr>
<tr>
<td>HIF-2α (EPAS1/HLF/HIF/MOP2)</td>
<td>11, 15, 23, 67</td>
</tr>
<tr>
<td>HIF-3α</td>
<td>18</td>
</tr>
<tr>
<td>NPAS1 (MOP5)</td>
<td>23, 80</td>
</tr>
<tr>
<td>NPAS2 (MOP4)</td>
<td>23, 80</td>
</tr>
<tr>
<td>SIM1</td>
<td>12</td>
</tr>
<tr>
<td>SIM2</td>
<td>12</td>
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</tbody>
</table>

bHLH, basic helix-loop-helix; PAS, PER-aryl hydrocarbon nuclear translocator (ARNT)-SIM; HIF-1; hypoxia-inducible factor 1; MOP, member of the PAS domain protein; AHR, aryl hydrocarbon receptor; EPAS1, endothelial PAS domain protein 1; HLF, HIF-1α-like factor; HRF, HIF-1α-related factor.
HIF-1α-related factor, and member of PAS domain family 2) is expressed in developing vascular endothelium, fetal lung, and catecholamine-producing cells (11, 15, 23, 30, 66, 67). It appears that HIF-1α plays a very general role by signaling the existence of hypoxia to the transcriptional machinery in the nucleus of all cells, whereas HIF-2α and HIF-3α play more limited or specialized roles in O2 homeostasis, a hypothesis that is supported by comparative analyses of HIF-1α and HIF-2α knockout mice as described below.

The aryl hydrocarbon receptor (AHR)/ARNT heterodimer was the first mammalian bHLH-PAS protein to be characterized (5, 22). On binding of aryl hydrocarbons, AHR translocates to the nucleus, dimerizes with ARNT, and activates transcription of genes encoding cytochrome P-450s involved in metabolism of these compounds. PAS domains are found in proteins expressed in organisms of all three kingdoms (Archaea, Bacteria, and Eucarya) and in many cases serve to bind cofactors (e.g., chromophore, heme, or FAD) that allow the sensing of light, O2 concentration, energy charge, or redox potential (reviewed in Ref. 64). Together, these findings suggest the possibility that HIF-1α (as well as HIF-2α, HIF-3α, and other class II bHLH-PAS proteins) may bind a cofactor through which the biological activity of the transcription factor could be modulated.

REGULATION OF HIF-1 ACTIVITY

The biological activity of HIF-1 is determined by the expression and activity of the HIF-1α subunit (32–34, 58). The regulation of HIF-1α expression and activity in vivo occurs at multiple levels, including mRNA expression (2, 72, 75), protein expression (25, 26, 33, 36, 52, 55, 68, 75), nuclear localization (35), and transactivation (10, 34, 35, 52). Among these, the most intensively studied has been the regulation of steady-state HIF-1α protein levels. Under nonhypoxic conditions, HIF-1α appears to be ubiquitinated and subject to proteosomal degradation (25, 26, 36, 55).Iron chelators that, like hypoxia, also induce HIF-1α expression (69) prevent ubiquitination of HIF-1α (36). Decreased ubiquitination of HIF-1α in hypoxic cells has also been demonstrated (C. H. Sutter, E. Laughner, and G. L. Semenza, unpublished observations).

Renal carcinoma cell lines, which lack expression of the von Hippel-Lindau (VHL) tumor suppressor protein, maximally express HIF-1α and HIF-2α under nonhypoxic conditions and O2-repressed regulated expression is restored in cells that have been transfected with a VHL expression vector (47). VHL functions as a component of a ubiquitin-protein ligase (28, 40, 60), suggesting that the constitutive expression of HIF-1α is due to a lack of ubiquitination under nonhypoxic conditions, but this has not been demonstrated. Iron chelators disrupt the association of VHL and HIF-1α, whereas these proteins remain associated in hypoxic cells (47). These data suggest a mechanism for the lack of ubiquitinated HIF-1α in cells treated with iron chelators (36) but do not provide the basis for regulation of HIF-1α protein stability by O2 concentration. In addition, there are no data addressing whether VHL is involved in the regulation of HIF-1α in cell types other than renal carcinoma lines.

HIF-1 TARGET GENES

To activate transcription of target genes, HIF-1α dimerizes with HIF-1β and the heterodimer binds to DNA at sites represented by the consensus sequence 5’-RCGTG-3’ (58). The HIF-1 binding site is present within a hypoxia response element, a cis-acting transcriptional regulatory sequence that can be located within 5’-flanking, 3’-flanking, or intervening sequences of target genes. The presence of an intact HIF-1 binding site is necessary, but not sufficient, for these elements to mediate transcriptional activation (58, 59). The number of target genes activated by HIF-1 continues to increase and includes genes whose protein products are involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling, and vasomotor responses (Table 2).

HIF-1 IS REQUIRED FOR EMBRYOGENESIS

Mice homozygous for a loss-of-function mutation in the gene encoding HIF-1α (29, 54) or HIF-1β (43) die at midgestation with vascular defects primarily involving the embryonic and extraembryonic circulations, respectively. In the case of Hif1a−/− mice, which lack HIF-1α, vasculogenesis initiates normally but by embryonic day 9 a marked regression of vascular endothelium in the cephalic region occurs (29). The vascular defect is preceded by the death of premigratory and postmigratory cephalic neural crest cells (29, 38). These mesenchy-

Table 2. HIF-1 target genes

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>References</th>
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<tbody>
<tr>
<td>Adenylate kinase 3</td>
<td>74</td>
</tr>
<tr>
<td>α2-Adrenergic receptor</td>
<td>9</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>7</td>
</tr>
<tr>
<td>AldolaseA</td>
<td>29, 54</td>
</tr>
<tr>
<td>Aldolase C</td>
<td>29</td>
</tr>
<tr>
<td>Endothelin-1 (ET-1)</td>
<td>24</td>
</tr>
<tr>
<td>Endostatin</td>
<td>29</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
<td>32</td>
</tr>
<tr>
<td>Glucose transporter 1</td>
<td>29, 54, 74</td>
</tr>
<tr>
<td>Glucose transporter 3</td>
<td>29</td>
</tr>
<tr>
<td>Glyceroldehyde phosphate dehydrogenase</td>
<td>29, 74</td>
</tr>
<tr>
<td>Heme oxygenase-1</td>
<td>39</td>
</tr>
<tr>
<td>Hexokinase 1</td>
<td>29</td>
</tr>
<tr>
<td>Hexokinase 2</td>
<td>29</td>
</tr>
<tr>
<td>Insulin-like growth factor II (IGF-II)</td>
<td>13</td>
</tr>
<tr>
<td>IGF binding protein 1</td>
<td>65</td>
</tr>
<tr>
<td>IGF factor binding protein 3</td>
<td>13</td>
</tr>
<tr>
<td>Lactate dehydrogenase A</td>
<td>29, 54</td>
</tr>
<tr>
<td>Nitric oxide synthase 2 (NOS2)</td>
<td>48, 51</td>
</tr>
<tr>
<td>p21</td>
<td>6</td>
</tr>
<tr>
<td>p33srj</td>
<td>3</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>29</td>
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<tr>
<td>Phosphoglycerate kinase 1</td>
<td>6, 29, 54</td>
</tr>
<tr>
<td>Pyruvate kinase M</td>
<td>29</td>
</tr>
<tr>
<td>Transferrin</td>
<td>53</td>
</tr>
<tr>
<td>Transferrin receptor</td>
<td>41, 61</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>6, 29, 54</td>
</tr>
<tr>
<td>VEGF receptor FLT-1</td>
<td>17</td>
</tr>
</tbody>
</table>
nal cells are progenitors of pericytes that are required for maintenance of blood vessel integrity at this stage of development, suggesting that mesenchymal cell death contributes to the vascular defect in Hif1a−/− mice. Hif1a−/− mice also have defects in cardiac morphogenesis and neural tube closure (29, 38). In contrast to the global and early effects of HIF-1α deficiency, Epas1−/− mice, which lack expression of HIF-2α, die later in development due to a specific defect in catecholamine production (66). Despite the prominent expression of HIF-2α in the vascular endothelium of wild-type mice, Epas1−/− mice have no obvious vascular defects, suggesting that HIF-1α expression in these cells (75) may be sufficient for normal development. However, the role of HIF-2α in vascular development within specific organs, such as the lung (11), warrants further study.

**INVolvEMENT OF HIF-1 IN THE PATHOPHYSIOLOGY OF HYPOXIC PULMONARY HYPERTENSION**

In contrast to Hif1a−/− mice, Hif1a+/− mice develop normally and are indistinguishable from wild-type littermates. However, when exposed to 10% O₂ for 1–6 wk, classic (patho)physiological responses to hypoxia, such as increases in hematocrit, right ventricular mass, and right ventricular pressure, are impaired in Hif1a−/− mice relative to their wild-type littermates (76). To investigate the effects of HIF-1α deficiency on remodeling of pulmonary arterioles, histological sections of lungs from Hif1a−/− and Hif1a+/− mice exposed to 10% O₂ for 3 wk were prepared for morphometric analysis. The proportion of nonmuscularized, partially muscularized, and completely muscularized pulmonary arterioles with an external diameter of ≤100 µm in Hif1a+/− and Hif1a−/− mice was significantly different by χ² analysis (P = 0.00001), with fewer completely muscularized and more nonmuscularized arterioles in the lungs of the Hif1a−/− mice (76). The wall thickness of completely muscularized pulmonary arterioles with a diameter of ≤100 µm was also significantly reduced in Hif1a+/− mice (P < 0.001). These results indicate that not only did chronically hypoxic Hif1a+/− mice have fewer completely muscularized pulmonary arterioles but the degree of muscularization in such vessels was reduced. Thus HIF-1 plays a major role in mediating pulmonary vascular remodeling in response to chronic hypoxia. Several known HIF-1 target genes (e.g., erythropoietin [EPO], endothelin-1 [ET-1], insulin-like growth factor 2 [IGF-2], nitric oxide synthase 2 [NOS2], and vascular endothelial growth factor [VEGF]), see Table 2, may be involved in these responses, and others will likely be identified in future studies. Local inhibition of HIF-1 activity in the lung by inhalation therapy may provide a means of preventing or retarding the development of this lethal complication of chronic lung disease in at-risk individuals. A dominant-negative form of HIF-1α that might be suitable for gene therapy applications has been described (16, 32). However, a specific small-molecule inhibitor of HIF-1 activity would also have great potential therapeutic utility.

**INVolvEMENT OF HIF-1 IN ISCHEMIC NEOVASCULARIZATION**

The role of HIF-1 in the activation of VEGF gene transcription in hypoxic cells is well established (6, 16, 29, 54) as is the role of VEGF in mediating ischemic neovascularization (reviewed in Ref. 14). In near-term fetal sheep subjected to isovolemic hemorrhage in utero, the development of cardiac hypertrophy was associated with increased expression of HIF-1α protein as well as VEGF mRNA and protein and increased myocardial vascularization (44). A similar correlation between HIF-1α protein and VEGF mRNA expression was established in a mouse model of ischemic retinopathy (50). Clinical trials are currently evaluating the safety and efficacy of administration of VEGF protein or DNA as a means of promoting increased vascularization within ischemic tissue. However, the expression of multiple VEGF isoforms and other angiogenic factors such as the angiopoietins may be required for optimal vascular development. HIF-1α gene therapy has the theoretical benefit of inducing the expression of multiple factors that promote angiogenesis and/or myocardial cell survival. Preclinical studies are currently underway to examine the therapeutic potential of this approach.

**INVolvEMENT OF HIF-1α IN PROMOTING OR PREVENTING ISCHEMIC CELL DEATH**

Several recent studies have provided evidence in support of the hypothesis that HIF-1α mediates the death of cultured cells subjected to O₂ and/or glucose deprivation (6, 19), possibly by associating with, and preventing the degradation of, p53 (1). One study, which utilized mouse embryonic stem cells, implied that HIF-1α has a proapoptotic role in tumor cells (6), a conclusion that appears unfounded especially in advanced cancers with p53 loss of function (see below). Another study, which utilized cultured cortical neurons, implied that HIF-1α promotes cell death in the context of cerebral ischemia (19). However, after middle cerebral artery occlusion in rats, increased expression of mRNA encoding HIF-1α and glycolytic enzymes was induced in the penumbra, which is the viable cortical tissue surrounding the infarct (2). Further studies are required to determine whether this response contributed to the survival of these cells. The presently available data are not sufficient to draw any final conclusions, and studies of Hif1a−/− mice may provide a means to determine the net effect of HIF-1α expression on cell viability during acute cerebral or myocardial ischemia.

Finally, second-window (delayed) models of cerebral and myocardial ischemic preconditioning have been described that involve de novo gene expression, suggesting a possible role for HIF-1 in this process. The implication of NOS2 gene expression in myocardial preconditioning (63) and the ability of HIF-1 to activate transcription of this gene (48, 51) are particularly provocative. Nuclear factor (NF)κB has also been
implicated in preconditioning and NOS2 gene expression (45), suggesting the possibility that HIF-1 and NF-κB may act synergistically to activate NOS2 transcription.

INVolVEMENT OF HIF-1 IN CANCER

Tumor progression to the lethal phenotype in which cells become capable of invasion and metastasis is associated with adaptation to hypoxia, and there is an inverse correlation between tumor oxygenation and clinical outcome (4, 21). Tumor cells lacking HIF-1 expression are markedly impaired in their growth and vascularization when injected into nude mice (31, 46). Among prostate cancer cell lines, the level of HIF-1α expression is correlated with the biological behavior of the cells in xenograft assays (78). Mutations that activate oncogenes (e.g., v-src) or inactivate tumor suppressor genes (e.g., VHL) are associated with increased expression of HIF-1α protein and HIF-1 DNA binding and transcriptional activity and the expression of downstream genes encoding glycolytic enzymes and VEGF (31, 46, 47). Increased HIF-1 expression in tumors can also result from activation of autocrine growth factor stimulation. IGF-2 induces expression of HIF-1α, which is in turn required for IGF-2 gene expression (13). IGF-2 is the most highly upregulated gene in colon cancer (77), thus providing a mechanism for the increased HIF-1α expression, which has been observed in this neoplasm (79). Thus HIF-1α is overexpressed in tumors as a result of physiological signals (hypoxia) and genetic alterations. These data suggest that inhibition of HIF-1 activity may represent a novel therapeutic approach to cancer therapy, especially in combination with angiogenesis inhibitors, which would further increase intratumoral hypoxia and thus provide an even greater therapeutic window for use of an HIF-1 inhibitor.

CONCLUSION

HIF-1 plays major roles in development, physiology, and pathophysiology. Modulation of HIF-1 activity may be of therapeutic utility in patients with cancer, chronic lung disease, and/or ischemic cardiovascular disease.

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In vivo expression of mRNAs encoding hypoxia-inducible factor 1.


