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Hypoxia-Inducible Factor 1: Control of Oxygen Homeostasis in Health and Disease

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ABSTRACT

Hypoxia-inducible factor 1 (HIF-1) is a transcriptional activator that mediates changes in gene expression in response to changes in cellular oxygen concentrations. HIF-1 is a heterodimer consisting of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit. In mice, complete HIF-1α deficiency results in embryonic lethality at midgestation because of cardiac and vascular malformations. Analyses of animal and cell culture models as well as human tissue have provided evidence that HIF-1 plays important roles in the pathophysiology of preeclampsia, intrauterine growth retardation, hypoxia-mediated pulmonary hypertension, and cancer. HIF-1 promotes neovascularization in response to myocardial or retinal ischemia by activating transcription of the gene encoding vascular endothelial growth factor. HIF-1 may also mediate the protective response to cerebral ischemia known as late-phase preconditioning. **(*Pediatr Res* 49: 614–617, 2001)**

Abbreviations:
HIF-1, hypoxia-inducible factor 1
VEGF, vascular endothelial growth factor

In humans, complex cardiovascular, hematopoietic, and respiratory systems develop to maintain oxygen homeostasis. Heart disease, cancer, cerebrovascular disease, and chronic obstructive lung disease are the most common causes of mortality in the United States, accounting for two thirds of all deaths annually. In these disorders, disruption of oxygen homeostasis represents a major aspect of disease pathophysiology. HIF-1 is a transcriptional activator that mediates changes in gene expression in response to changes in oxygen concentration. HIF-1 plays important roles in normal development, physiologic responses to hypoxia, and the pathophysiology of common human diseases.

MOLECULAR BIOLOGY

HIF-1 is a dimeric transcription factor composed of HIF-1α and HIF-1β subunits (1, 2). Under nonhypoxic conditions the HIF-1α subunit is subjected to ubiquitination and proteasomal degradation (3). In response to hypoxia, ubiquitination and degradation of HIF-1α are inhibited, resulting in rapid accumulation of the protein (4). In addition, the activity of the HIF-1α transactivation domains is induced by hypoxia (5, 6). HIF-1α dimerizes with HIF-1β, which is constitutively expressed, resulting in the formation of active HIF-1 protein that binds to the core sequence 5'-RCGTG-3' present in target genes, several dozen of which have been identified thus far (Table 1). New target genes continue to be identified and it is likely that the total number of HIF-1-regulated genes in the human genome is an order of magnitude greater than what is presently known.

DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY

Analysis of knockout mice has demonstrated that HIF-1α is required for embryonic development and survival. HIF-1α-deficient mouse embryos arrest in their development by d 9 of gestation (E9.0) and die by E10.5 with severe cardiovascular and neural tube defects and massive cell death, especially in the branchial and cephalic regions (7–9). Mice that are heterozygous for the knockout allele and thus partially HIF-1α deficient develop normally. However, when these mice are subjected to long-term hypoxia (10% O_2 for 3 wk), the development of erythrocytosis and pulmonary vascular remodeling is significantly impaired (10). The impaired development of medial wall hypertrophy in small pulmonary arterioles, which is the hallmark of hypoxia-induced pulmonary hypertension, indicates that HIF-1α is essential for this process. These results suggest that partial pharmacologic inhibition of HIF-1 activity might provide a means to prevent pulmonary vascular remod-

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Table 1. *HIF-1 target genes*

Gene product	References
Adenylate kinase 3	41
α_{1B}-Adrenergic receptor	42
Adrenomedullin	43
Aldolase A	7, 9
Aldolase C	7
Ceruloplasmin	44
Endothelin-1	45
Enolase 1	7
Erythropoietin	46
Glucose transporter 1	7, 9,41
Glucose transporter 3	7
Glyceraldehyde-3-phosphate dehydrogenase	7, 9
Heme oxygenase-1	47
Hexokinase 1	7
Hexokinase 2	7
IGF-II	48
IGF binding protein 1	40
IGF binding protein 2	48
IGF binding protein 3	48
Lactate dehydrogenase A	7, 9
Nitric oxide synthase 2	49,50
p21	11
p35srj	51
Phosphofructokinase L	7
Phosphoglycerate kinase 1	7, 9,11
Plasminogen activator inhibitor 1	52
Prolyl-4-hydroxylase α(I)	53
Pyruvate kinase M	7
Transferrin	54
Transferrin receptor	55,56
Transforming growth factor β_3	39
VEGF	7, 9,11
VEGF receptor FLT-1	57

eling without causing untoward side effects in at-risk patients with chronic obstructive lung disease.

MYOCARDIAL ISCHEMIA-INDUCED VASCULARIZATION

HIF-1α is also essential for angiogenesis in ischemic tissue. When HIF-1α-deficient embryonic stem cells are subjected to hypoxia, expression of mRNA encoding VEGF is not induced (7, 9, 11). In near-term fetal sheep, myocardial hypoxia-ischemia results in the induction of HIF-1α protein, VEGF mRNA and protein, and increased myocardial vascularization (12). The impaired angiogenic response to ischemia in older animals is caused in part by decreased VEGF production as a result of impaired HIF-1 DNA-binding activity (13–15). Preclinical trials of HIF-1α gene therapy for ischemia indicate that this strategy for therapeutic angiogenesis is at least as effective as VEGF gene therapy (16).

RETINAL VASCULARIZATION AND ISCHEMIC RETINOPATHY

Expression of HIF-1α protein and VEGF mRNA are spatially and temporally correlated during normal retinal development (17). These data are consistent with other studies indicating that hypoxia is an essential stimulus for retinal vascularization (18). In a mouse model of oxygen-induced ischemic retinopathy similar to retinopathy of prematurity, 1-wk-old (P7) mice are exposed to hyperoxia from P7 to P12, which blocks VEGF expression in the retina (19). When the mice are returned to normoxic conditions, retinal ischemia develops, which induces VEGF expression. Analysis of HIF-1α expression revealed a temporal and spatial correlation with VEGF mRNA expression, both with regard to the hyperoxic repression and ischemic induction (17), indicating that HIF-1-mediated VEGF expression may play a major role in the development of retinopathy of prematurity and other ischemic retinal disorders such as diabetic retinopathy. Retinal neovascularization can be prevented by blocking VEGF (20), suggesting that inhibition of HIF-1 activity may be of therapeutic utility in these conditions.

CEREBRAL ISCHEMIA AND DELAYED PRECONDITIONING

HIF-1 may also play a role in cerebral ischemia. Cerebral infarction can be induced in P7 rat pups by permanent ligation of the left common carotid artery and exposure to 8% O_2 for 3 h. Seven days later the pups are killed and analyzed, revealing an approximately 40% reduction in hemispheric weight ipsilateral to the carotid occlusion. In contrast, P7 rats that are subjected to 8% O_2 for 3 h and then 24 h later are subjected to carotid occlusion and hypoxia are dramatically protected against cerebral infarction (21), a phenomenon known as delayed (late-phase) preconditioning. The 3-h hypoxic preconditioning exposure was shown to induce HIF-1α protein expression throughout the brain (22). Analysis of HIF-1α expression in the brains of P7 rats subjected to carotid occlusion and hypoxia for 3 h and then killed immediately revealed induction of HIF-1α protein expression throughout the hemisphere contralateral to the occlusion, whereas in the ipsilateral hemisphere HIF-1α expression was decreased in the brain parenchyma and dramatically up-regulated in the cerebral microvasculature (22).

HIF-1α protein expression, HIF-1 DNA-binding and transcriptional activity, and expression of target genes can also be induced by exposing cultured cells to cobalt chloride or iron chelators such as desferrioxamine (1, 5, 23). A single injection of cobalt chloride or desferrioxamine induced HIF-1α expression in the brain and protected against the development of cerebral infarction after carotid occlusion and hypoxia (22). The ability of these agents to induce HIF-1α expression was correlated with their ability to induce protection (hypoxia $>$ $CoCl_2$ $>$ desferrioxamine). The basis for this protective effect is unknown. HIF-1 has been shown to induce the expression of erythropoietin and VEGF (Table 1), each of which has been shown to function as a neuronal survival factor (24, 25). In addition, HIF-1 coordinately regulates the expression of genes encoding at least 13 different glucose transporters and glycolytic enzymes (7). After middle cerebral artery occlusion, there is a spatial and temporal correlation between induction of HIF-1α mRNA and of mRNAs encoding aldolase A, glucose transporter 1, lactate dehydrogenase A, phosphofructokinase L, and pyruvate kinase M (Table 1) in the penumbra, which is the viable tissue surrounding the infarction (26). The induction of

glycolytic metabolism by HIF-1 may contribute to the protective effect of preconditioning with cobalt, desferrioxamine, or hypoxia. Whether the net effect of HIF-1 expression in the ischemic state is to protect against or promote infarction is unclear, as cell-based studies suggest that HIF-1 mediates hypoxia-induced apoptosis (27) *via* induction of p53 (11, 28, 29).

CANCER

In contrast to the potentially protective effect of HIF-1 expression in the context of cerebral and myocardial ischemia, HIF-1 plays an important role in promoting tumor progression (30). Mutations that inactivate tumor suppressor genes or activate oncogenes have, as one of their consequences, upregulation of HIF-1 activity, either through an increase in HIF-1α protein expression, HIF-1 transcriptional activity, or both (Table 2). Increased HIF-1 activity results in increased expression of target genes with important roles in tumor progression such as induction of tumor vascularization by VEGF (the angiogenic switch) and metabolic adaptation to hypoxia *via* increased glucose transporter and glycolytic enzyme activity (the Warburg effect). Immunohistochemical analysis of 40 human brain tumors revealed a significant correlation between HIF-1α protein expression, tumor grade, and tumor vascularization (31). HIF-1α is overexpressed in the majority of common human cancers, including breast, colon, lung, and prostate carcinoma (32).

The relationship between HIF-1α and the tumor suppressor p53 is of particular significance. Tumor cells subjected to hypoxia undergo p53-mediated apoptosis, which represents a powerful selection for cells that have sustained mutations that result in p53 loss of function (33). In unstimulated cells p53 is bound by MDM2, a ubiquitin-protein ligase that targets p53 for degradation by the proteasome (34, 35). In response to hypoxia, HIF-1α is induced and binds to p53, an interaction that protects p53 from degradation (28). Instead, MDM2 targets HIF-1α for degradation (36). Thus, two major consequences of p53 loss-of-function are the prevention of hypoxia-induced apoptosis and increased expression of HIF-1α. Increased HIF-1-mediated *VEGF* gene transcription results in increased vascularization of p53-nonexpressing as opposed to p53-expressing tumors (36). The von Hippel-Lindau tumor suppressor is also a ubiquitin-protein ligase that specifically targets HIF-1α for degradation under nonhypoxic conditions (37, 38). Von Hippel-Lindau loss-of-function in renal cell carcinomas and cerebellar hemangioblastomas results in constitutive overexpression of HIF-1α protein and VEGF mRNA, resulting in tumors that are among the most highly vascularized human cancers (31, 32, 37, 38).

Table 2. *Alterations in human cancer that increase HIF-1 activity*

Function	References
Tumor suppressor loss-of-function	
p53	32, 36
PTEN	58, 59
VHL	37, 38
Oncogene gain-of-function	
AKT	58, 59
FRAP (mTOR)	58
PI-3-kinase	58, 59
RAF/MEK/ERK (MAPK)	60, 61
RAS	62
SRC	63
Autocrine growth factor stimulation	
EGF	48, 58
FGF2	48
IGF-1/IGF-2/IGF-1R	48,64,65

CONCLUSION

HIF-1 is a master regulator of oxygen homeostasis, which is a fundamental requirement for survival. It orchestrates a multitude of biologic processes starting in early embryonic development and extending into adult life. Recent data suggest that HIF-1 may play an important role in the pathophysiology of preeclampsia (39) and intrauterine fetal growth retardation (40). In diseases that represent the most common causes of mortality in western societies (ischemic cardiovascular disease, cancer, and chronic lung disease), there is growing evidence suggesting that modulation of HIF-1 activity, using a pharmacologic or DNA-based approach, may have therapeutic effects.

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