

## MINIREVIEW

# Hypoxia-Inducible Factor-1 (HIF-1)

Qingdong Ke and Max Costa

*Nelson Institute of Environmental Medicine, New York University, School of Medicine, Tuxedo, New York*

Received May 25, 2006; accepted August 2, 2006

### ABSTRACT

Adaptation to low oxygen tension (hypoxia) in cells and tissues leads to the transcriptional induction of a series of genes that participate in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation/survival. The primary factor mediating this response is the hypoxia-inducible factor-1 (HIF-1), an oxygen-sensitive transcriptional activator. HIF-1 consists of a constitutively expressed subunit HIF-1 $\beta$  and an oxygen-regulated subunit HIF-1 $\alpha$  (or its paralogs HIF-2 $\alpha$  and HIF-3 $\alpha$ ). The stability and activity of the  $\alpha$  subunit of HIF are regulated by its post-translational modifications such as hydroxylation, ubiquitination, acetylation, and phosphorylation. In normoxia, hy-

droxylation of two proline residues and acetylation of a lysine residue at the oxygen-dependent degradation domain (ODDD) of HIF-1 $\alpha$  trigger its association with pVHL E3 ligase complex, leading to HIF-1 $\alpha$  degradation via ubiquitin-proteasome pathway. In hypoxia, the HIF-1 $\alpha$  subunit becomes stable and interacts with coactivators such as cAMP response element-binding protein binding protein/p300 and regulates the expression of target genes. Overexpression of HIF-1 has been found in various cancers, and targeting HIF-1 could represent a novel approach to cancer therapy.

The transcription factor hypoxia-inducible factor-1 (HIF-1) is a key regulator responsible for the induction of genes that facilitate adaptation and survival of cells and the whole organism from normoxia (~21% O<sub>2</sub>) to hypoxia (~1% O<sub>2</sub>) (Wang et al., 1995; Semenza, 1998). Since the identification of HIF, 2 decades ago, our knowledge of it has grown exponentially. Because of the realization that hypoxia has a strong impact, via gene expression, on cell biology and mammalian physiology, there has been enormous growing interest in the biology of the HIF-1 pathway and its role in human diseases such as cancer. Therefore, this review considers what has been learned about HIF-1: its discovery, its regulation, its target gene, its role in development and disease, and its implication for therapy.

### The Discovery of HIF-1

**HIF-1 $\alpha$ .** HIF-1 was discovered by the identification of a hypoxia response element (HRE; 5'-RCGTG-3') in the 3' enhancer of the gene for erythropoietin (EPO), a hormone that stimulates erythrocyte proliferation and undergoes hypoxia-induced transcription (Goldberg et al., 1988; Semenza et al., 1991). Subsequent studies have revealed the protein that binds to the HRE under hypoxic conditions as HIF-1, a heterodimeric complex consisting of a hypoxically inducible subunit HIF-1 $\alpha$  and a constitutively expressed subunit HIF-1 $\beta$  (Wang et al., 1995). HIF-1 $\beta$  is also known as the aryl hydrocarbon nuclear translocator (ARNT), which was originally identified as a binding partner of the aryl hydrocarbon receptor (Reyes et al., 1992), whereas HIF-1 $\alpha$  was newly discovered. These proteins belong to the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) protein family (Fig. 1) (Wang et al., 1995). The bHLH and PAS motifs are required for heterodimer formation between the HIF-1 $\alpha$  and HIF-1 $\beta$  subunits, and the downstream basic region affords specific bind-

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.  
doi:10.1124/mol.106.027029.

**ABBREVIATIONS:** HIF-1, hypoxia-inducible factor-1; HRE, hypoxia response element; ARNT, aryl hydrocarbon nuclear translocator; PAS, Per-ARNT-Sim; bHLH, basic helix-loop-helix; TAD, transactivation domains; CBP/p300, cAMP response element-binding protein binding protein; ODDD, oxygen-dependent degradation domain; 2-OG, 2-oxoglutarate; PHD, prolyl hydroxylase domain; VHL, von Hippel-Lindau; FIH-1, factor inhibiting HIF-1; ARD1, arrest-defective-1; MAPK, mitogen-activated protein kinase; VEGF, vascular endothelial cell growth factor; PTEN, phosphatase and tensin homolog deleted on chromosome 10; LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; PD98095, 2-(2-amino-3-methoxyphenyl)-oxanaphthalen-4-one; PI3K, phosphoinositide-3 kinase; YC-1, 3-(5'-hydroxy-methyl-2'-furyl)-1-benzylindazole.

ing to the HRE DNA sequence (Crews, 1998). Two transactivation (stimulation of transcription) domains, N-terminal (N-TAD) and C-terminal (C-TAD), in the C-terminal half of the HIF-1 $\alpha$  protein were identified later (Ruas et al., 2002). The C-TAD in particular has been shown to interact with coactivators such as CBP/p300 to activate gene transcription (Lando et al., 2002b). HIF-1 $\alpha$  also contains an oxygen-dependent degradation domain (ODDD) that mediates oxygen-regulated stability (Pugh et al., 1997). Later work revealed that HIF-1 $\alpha$  is ubiquitously expressed in human and mouse tissues and has a general role in multiple physiological responses to hypoxia, such as erythropoiesis and glycolysis, which quickly counteract oxygen deficiency, and angiogenesis, which provides a long-term solution (Semenza, 1998).

**HIF-2 $\alpha$ .** Shortly after the cloning of HIF-1 $\alpha$ , a closely related protein, HIF-2 $\alpha$  [also termed endothelial PAS protein, HIF-like factor (HLF), HIF-related factor (HRF), and member of the PAS superfamily 2 (MOP2)] was identified and cloned (Ema et al., 1997; Flamme et al., 1997; Hogenesch et al., 1997; Tian et al., 1997). HIF-2 $\alpha$  shares 48% amino acid sequence identity with HIF-1 $\alpha$  and accordingly shares a number of structural and biochemical similarities with HIF-1 $\alpha$  (for instance, heterodimerization with HIF-1 $\beta$  and binding HREs). In contrast to ubiquitously expressed HIF-1 $\alpha$ , though, HIF-2 $\alpha$  is predominantly expressed in the lung, endothelium, and carotid body (Ema et al., 1997; Tian et al., 1997, 1998).

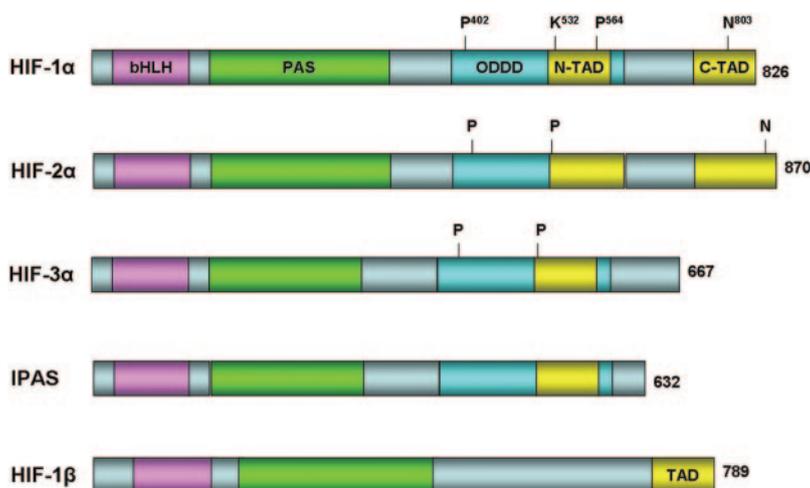
**HIF-3 $\alpha$ .** HIF-3 $\alpha$ , which was discovered later, is also expressed in a variety of tissues, dimerizes with HIF-1 $\beta$ , and binds to HREs (Gu et al., 1998). In addition, a splice variant of HIF-3 $\alpha$ , inhibitory PAS (IPAS), which is predominantly expressed in the Purkinje cells of the cerebellum and corneal epithelium, was subsequently discovered (Makino et al., 2001). IPAS possesses no endogenous transactivation activity; rather, it interacts with the amino-terminal region of HIF-1 $\alpha$  and prevents its DNA binding, acting as a dominant-negative regulator of HIF-1 (Makino et al., 2001). However, IPAS can also be induced by hypoxia in the heart and lung, contributing to a negative feedback loop for HIF-1 activity in these tissues (Makino et al., 2002). HIF-1 $\alpha$  and HIF-2 $\alpha$  have been more extensively studied, whereas research on HIF-3 $\alpha$  and other HIF isoforms is relatively scarce.

## The Regulation of HIF-1

Although HIF-1 $\beta$  is constitutively expressed and its mRNA and protein are maintained at constant levels regardless of oxygen availability (Kallio et al., 1997), HIF-1 $\alpha$  protein has a short half-life ( $t_{1/2} \sim 5$  min) and is highly regulated by oxygen (Salceda and Caro, 1997) (Fig. 2). The transcription and synthesis of HIF-1 $\alpha$  are constitutive and seem not to be affected by oxygen (Wang et al., 1995; Kallio et al., 1997; Wiesener et al., 1998). However, in normoxia, the HIF-1 $\alpha$  proteins are rapidly degraded, resulting in essentially no detectable HIF-1 $\alpha$  protein (Wang et al., 1995). During hypoxia, HIF-1 $\alpha$  becomes stabilized and translocates from the cytoplasm to the nucleus, where it dimerizes with HIF-1 $\beta$ , and the HIF complex formed becomes transcriptionally active (Huang et al., 1996; Kallio et al., 1997). The activated HIF complex then associates with HREs in the regulatory regions of target genes and binds the transcriptional coactivators to induce gene expression (Lando et al., 2002b). Tight regulation of the stability and subsequent transactivational function of HIF-1 $\alpha$  is chiefly controlled by its post-translational modifications, such as hydroxylation, ubiquitination, acetylation, and phosphorylation (Brahimi-Horn et al., 2005).

The modification of HIF-1 $\alpha$  occurs within several domains. In normoxia, hydroxylation of two proline residues and acetylation of a lysine residue in its ODDD promote interaction of HIF-1 $\alpha$  with the von Hippel-Lindau (pVHL) ubiquitin E3 ligase complex (Srinivas et al., 1999; Masson et al., 2001). pVHL complex tags HIF-1 $\alpha$  with ubiquitin and thereby marks it for degradation by the 26S proteasome. In addition, hydroxylation of an asparagine residue in the C-TAD inhibits the association of HIF-1 $\alpha$  with CBP/p300 and thus inhibits its transcriptional activity (Lando et al., 2002a).

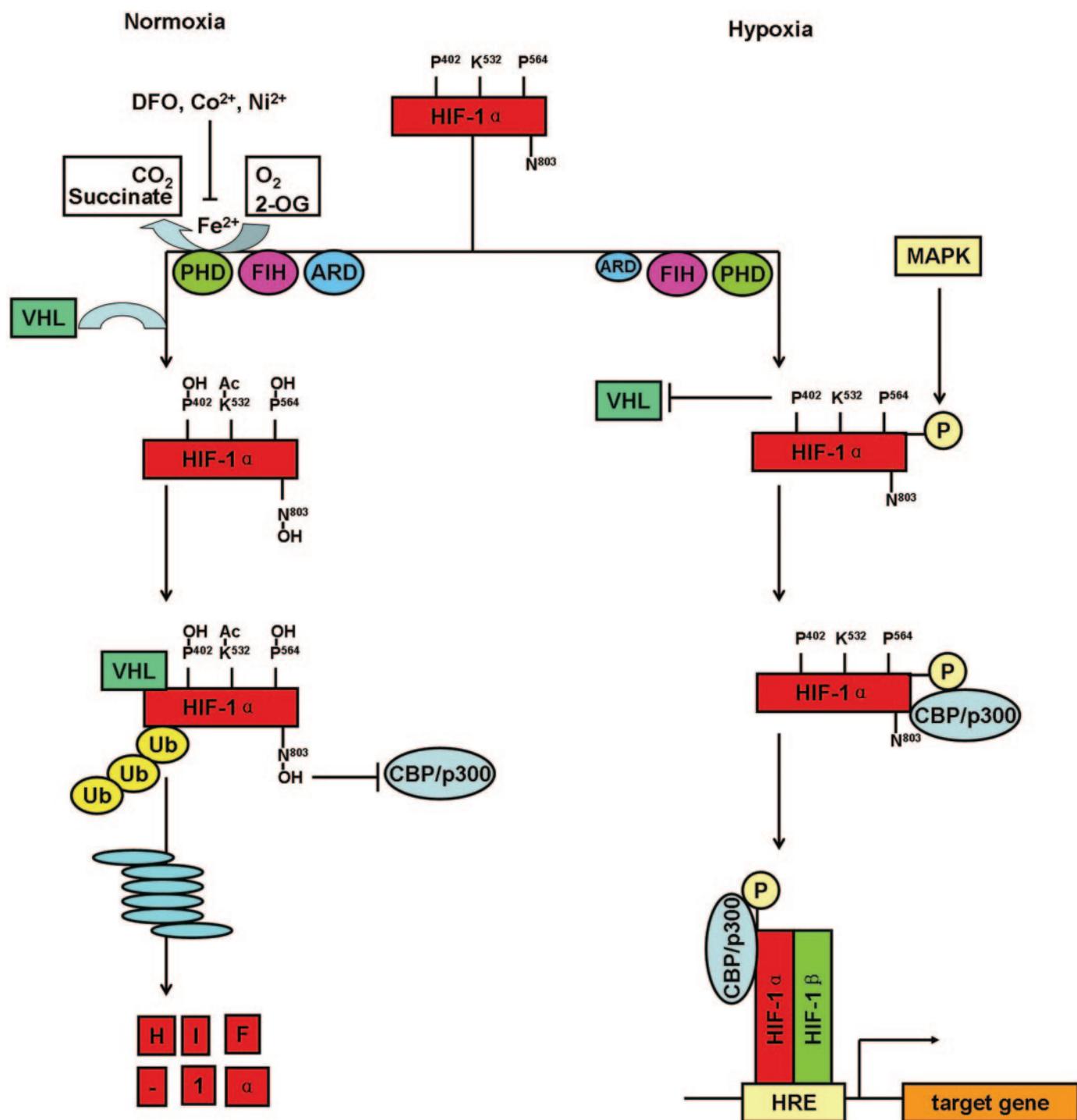
**Prolyl Hydroxylation by PHDs—Signaling for Polyubiquitination.** De novo synthesized cytoplasmic HIF-1 $\alpha$  is rapidly hydroxylated by a family of 2-oxoglutarate (2-OG)-dependent dioxygenases on proline 402 (Pro402) and 564 (Pro564) located within ODDD (Srinivas et al., 1999; Masson et al., 2001; Masson and Ratcliffe, 2003). The proline residues are conserved in HIF-2 $\alpha$  (Pro405 and Pro530) and HIF-3 $\alpha$ . They are also part of a consensus sequence, LXXLAP, that is conserved for the two sites of all the isoforms except for the second proline of HIF-3 $\alpha$ , which has the sequence LXXLHP



**Fig. 1.** Domain structure of human HIF- $\alpha$  and HIF-1 $\beta$ . HIF- $\alpha$  (HIF-1 $\alpha$ , HIF-2 $\alpha$ , HIF-3 $\alpha$ , IPAS) and HIF-1 $\beta$  belong to the bHLH and PAS protein family. HIF- $\alpha$  contains an ODDD that mediates oxygen-regulated stability through the hydroxylation of two proline (P) residues and the acetylation of a lysine (K). The proline residues are conserved in HIF-2 $\alpha$  and HIF-3 $\alpha$ . HIF-1 $\alpha$  and HIF-2 $\alpha$  also contain two transactivation domains (C-TAD and N-TAD), whereas HIF-1 $\beta$  has only one TAD. The total number of amino acids of each subunit is marked at the end of the domain structure.

(Bruick and McKnight, 2001; Masson et al., 2001). Mutation of both proline residues disrupted the interaction of HIF-1 $\alpha$  with pVHL and increased its stability in the presence of normal oxygen levels, whereas mutation of either proline alone only partially stabilized HIF-1 $\alpha$  (Masson et al., 2001).

Human HIF- $\alpha$  dioxygenase was termed prolyl hydroxylase domain (PHD), HIF-prolyl hydroxylase (HPH), or Egg-laying Nine (EGLN), and three isoforms have been reported: PHD1/HPH3/EGLN2, PHD2/HPH2/EGLN1, and PHD3/HPH1/EGLN3 (Bruick and McKnight, 2001; Epstein et al., 2001;



**Fig. 2.** Oxygen-dependent regulation of HIF-1 stabilization and transactivation. In normoxia (left), two proline residues of HIF-1 $\alpha$  (P<sup>402</sup> and P<sup>564</sup>) and asparagine (N<sup>803</sup>) are hydroxylated by PHDs and FIH-1, respectively, in an O<sub>2</sub>, 2-OG, and Fe<sup>2+</sup>-dependent manner. Hydroxylated HIF-1 $\alpha$  proteins bind to the E3 ubiquitin ligase VHL complex, leading to its degradation by the proteasome. Acetylation of lysine (K<sup>532</sup>) by ARD1 favors the interaction of HIF-1 $\alpha$  with VHL. Hydroxylated N<sup>803</sup> blocks the recruitment of transcriptional coactivator CBP/p300. In hypoxia (right), the activities of PHDs and FIH-1 are inhibited due to lack of O<sub>2</sub>, resulting in no proline and asparagine hydroxylation. Therefore, there is no VHL binding and HIF-1 $\alpha$  is stabilized. Stabilized HIF-1 $\alpha$  proteins translocate to the nucleus and bind to HIF-1 $\beta$ . HIF-1 $\beta$  may bind preferentially to the MAPK-induced phosphorylated form of HIF-1 $\alpha$ . Nonhydroxylated N<sup>803</sup> of HIF-1 $\alpha$  allows CBP/p300 recruitment to the target genes, resulting in gene transcription. In addition, the expression of ARD1 is decreased under hypoxia, causing less acetylated HIF-1 $\alpha$ .

Huang et al., 2002). The biochemical characteristics of the PHDs are similar to the collagen prolyl-4-hydroxylases, which are also 2-OG-dependent dioxygenases and require oxygen ( $O_2$ ) for hydroxylation as well as  $Fe^{2+}$  and ascorbate as cofactors (Schofield and Zhang, 1999). The collagen prolyl-4-hydroxylases, however, are not able to catalyze HIF-1 $\alpha$ /HIF-2 $\alpha$  proline hydroxylation (Jaakkola et al., 2001). The hydroxylation process splits  $O_2$ ; one oxygen atom is transferred to the proline residue and the other reacts with 2-OG to generate succinate and  $CO_2$  (Bruick and McKnight, 2001; Masson and Ratcliffe, 2003). Inactivation of the PHDs by 2-OG analogs can increase the half-life of HIF-1 $\alpha$  (Jaakkola et al., 2001; Ivan et al., 2002).  $Fe^{2+}$  at the active site of the PHDs is loosely bound by two histidine residues and one aspartic acid, forming a 2-histidine-1-carboxylate coordination motif. The requirement of  $Fe^{2+}$  for PHDs was demonstrated in the observation that iron chelators and metal ions (such as  $Co^{2+}$ ,  $Ni^{2+}$ , and  $Mn^{2+}$ ) are able to stabilize HIF-1 $\alpha$ , probably by diminishing the availability of  $Fe^{2+}$  for the enzyme or substituting  $Fe^{2+}$  from the  $Fe^{2+}$ -binding site (Masson and Ratcliffe, 2003; Yuan et al., 2003). Ascorbate helps to maintain Fe in the ferrous ( $Fe^{2+}$ ) state and is important in maintaining and achieving full activity of the PHDs (Bruick and McKnight, 2001; Epstein et al., 2001).

All three PHDs have the potential to hydroxylate HIF- $\alpha$  in vitro with their relative activities as PHD2  $\gg$  PHD3 > PHD1, and PHD2 was shown to be the key limiting enzyme that controls the HIF-1 $\alpha$  turnover in vivo (Huang et al., 2002; Berra et al., 2003). Knockdown of PHD2 by its specific small interfering RNA is sufficient to stabilize HIF-1 $\alpha$  levels under normoxia, whereas small interfering RNA silencing of either PHD1 or PHD3 is unable to produce similar effects on HIF-1 $\alpha$  (Berra et al., 2003). In addition, it was found that the mRNA and protein of PHD2 were induced during a hypoxia challenge, and the mRNA of PHD3 was up-regulated as well, whereas that of PHD1 remained unaffected (Epstein et al., 2001; Metzzen et al., 2003a). This may represent a way by which HIF-1 $\alpha$  self-regulates its expression. Furthermore, Siah1 and -2, the specific E3 ligases of PHD1 and -3, are transcriptionally up-regulated during hypoxia, thereby elevating the degradation of these PHDs by the proteasome (Nakayama and Ronai, 2004). When overexpressed as tagged proteins in transfected cells, PHD2 was found to be localized primarily in the cytoplasm, whereas PHD1 localized in the nucleus and PHD3 was in both compartments (Metzzen et al., 2003a). Despite its primary localization in the cytoplasm, PHD2 is able to shuttle between the cytoplasm and the nucleus, thereby contributing to HIF-1 $\alpha$  degradation in both compartments. Although all three enzymes are widely expressed in many tissues, they exhibit tissue-specific overexpression. PHD2 was abundant in adipose tissue (Oehme et al., 2002), PHD3 in the heart and placenta (Lieb et al., 2002; Oehme et al., 2002), and PHD1 in the testis (Lieb et al., 2002). The differences of the enzyme activity of PHDs, subcellular localization, and tissue distribution may enable a graded or tissue-specific response to hypoxia. Some proteins, such as OS-9, the protein product of a widely expressed gene with unclear function, have been shown to promote prolyl hydroxylation by interacting with both HIF-1 $\alpha$  and PHDs, thereby promoting the  $O_2$ -dependent degradation of HIF-1 $\alpha$  (Baek et al., 2005). In addition, several second messengers

have also been shown to modify the activity of PHDs (Appelhoff et al., 2004; Hirota et al., 2004; Temes et al., 2005).

In the presence of oxygen, the PHDs are active and hydroxylate the prolines of HIF-1 $\alpha$ , constituting a recognition signal for binding of pVHL and subsequent ubiquitination, followed by degradation of HIF-1 $\alpha$  (Ivan et al., 2001; Jaakkola et al., 2001). The absence of oxygen causes no enzyme activity, no modification of proline, and no pVHL/HIF binding, resulting in HIF-1 $\alpha$  stabilization and accumulation in the cell. The absolute requirement of oxygen as a cosubstrate suggests PHDs as the oxygen sensor in cells (Epstein et al., 2001).

**Polyubiquitination by pVHL—Signaling for Degradation.** Once the two proline residues of HIF-1 $\alpha$  are converted to hydroxyproline, pVHL then captures HIF-1 $\alpha$ . X-ray crystallographic studies of the pVHL/HIF-1 $\alpha$  complex have revealed that pVHL has a surface pocket into which the hydroxyproline fits accurately, and the overall binding configuration is highly specific (Hon et al., 2002; Min et al., 2002). The pVHL associates with the proteins elongin C, elongin B, cullin-2, and Rbx1 to form the VCB-Cul2 E3 ligase complex (Ivan and Kaelin, 2001). Binding of HIF-1 $\alpha$  to this multiprotein E3 complex causes polyubiquitination of HIF-1 $\alpha$ , ultimately leading to its degradation by the proteasome (Kamura et al., 2000). However, the exact lysine residue(s) ubiquitinated have not yet been identified.

The pVHL was first described in von Hippel-Lindau (VHL) disease, an inherited human cancer syndrome that is characterized by the development of multiple tumors, such as clear-cell renal carcinomas, pheochromocytomas, and hemangioblastomas in the retina and central nervous system (Ivan and Kaelin, 2001). The *VHL* gene encodes a full-length (213 amino acids) and an N-terminally truncated protein (amino acids 54–213). Because both proteins show similar functions, they are often collectively referred to as pVHL. Mutations in the *VHL* gene, the product of which functions as a tumor suppressor, were found in the diseases discussed above (Iliopoulos et al., 1998; Schoenfeld et al., 1998). In cells lacking wild-type pVHL, HIF-1 $\alpha$  and HIF-2 $\alpha$  are stable and active under normoxia, resulting in the over-production of hypoxia-inducible genes (Iliopoulos et al., 1996). Restoration of pVHL function by stable transfection reversed normoxic HIF- $\alpha$  protein stability and the aberrant increase of genes (Iliopoulos et al., 1996). Therefore, one mechanism by which mutations in pVHL might cause tumor formation is by permitting the stability and activity of HIF- $\alpha$  under normal oxygen tensions, resulting in the subsequent expression of genes encoding angiogenic factors even before cells are exposed to a hypoxic stress.

The pVHL E3 ligase complex is ubiquitously expressed in different tissues and predominantly localized to the cytoplasm. Its shuttling between the cytoplasm and nucleus enables HIF-1 $\alpha$  degradation in both compartments (Berra et al., 2001; Groulx and Lee, 2002).

However, the pVHL-dependent pathway may not be the only pathway leading to degradation of HIF-1 $\alpha$ . In addition to pVHL, a number of other proteins have been reported to affect HIF-1 $\alpha$  ubiquitination and stability. For example, the oncogenic E3 ubiquitin ligase murine double minute 2 (MDM2) has been suggested to bring about ubiquitination of HIF-1 $\alpha$  in a p53-dependent fashion (Ravi et al., 2000). The protein Jab1, a transcriptional coactivator of c-Jun and Jun

D, has also been shown to increase HIF-1 $\alpha$  levels under hypoxia, probably by competing with p53 for binding to HIF-1 $\alpha$  (Bae et al., 2002). In addition, pVHL has also been shown to interact with other proteins involved in the HIF-1 signaling pathway and thus may regulate more than just HIF-1 $\alpha$  stability.

**Lysine Acetylation by ARD1—Facilitating pVHL Binding.** Lysine residue 532 (Lys532) located in the ODDD domain of HIF-1 $\alpha$  was reported to be acetylated by an acetyltransferase named arrest-defective-1 (ARD1) (Jeong et al., 2002). ARD1 was originally identified in the yeast *Saccharomyces cerevisiae*, and its name came from the defective yeast mutants in the mitotic cell cycle (Whiteway and Szostak, 1985). Acetylation of Lys532 favors the interaction of HIF-1 $\alpha$  with pVHL, and thus destabilizes HIF-1 $\alpha$  (Jeong et al., 2002). Mutation of Lys532 to arginine resulted in increased stability of HIF-1 $\alpha$  (Tanimoto et al., 2000). In addition, artificial maintenance or increase of the acetylated state of HIF-1 $\alpha$  by butyric acid, a global inhibitor of deacetylases, caused decreased HIF-1 $\alpha$  protein levels (Kim et al., 2001).

Because the activity of acetyltransferases is not influenced by oxygen, ARD1 may be active and acetylate HIF-1 $\alpha$  regardless of oxygen conditions. But the mRNA and protein levels of ARD1 were decreased under hypoxia, which may cause less acetylated HIF-1 $\alpha$  in hypoxia than that in normoxia (Jeong et al., 2002).

**Asparagine Hydroxylation by FIH-1—Preventing CBP/p300 Binding.** The post-translational modifications of HIF-1 $\alpha$  described above regulate the stabilization of HIF-1 $\alpha$  protein. But the stabilization alone is not sufficient for full transcriptional activation of HIF-1. The second major mechanism controlling HIF activity is through the modulation of its transactivation domains N-TAD and C-TAD. These domains function by recruiting transcriptional coactivators such as CBP/p300, SRC-1, and TIF2 (Arany et al., 1996; Ebert and Bunn, 1998; Kallio et al., 1998; Ema et al., 1999; Carrero et al., 2000). Under normal oxygen tension, hydroxylation of the asparagines residue 803 (Asn803) in the C-TAD of HIF-1 $\alpha$  (Asn851 in HIF-2 $\alpha$ ) by factor inhibiting HIF-1 (FIH-1) prevented the interaction of HIF-1 $\alpha$  with CBP/p300 (Hewitson et al., 2002; Lando et al., 2002b; Sang et al., 2002). Hypoxia abrogated asparagine hydroxylation, which allowed the C-TAD of HIF-1 $\alpha$  to efficiently interact with CBP/p300 therein, activating the transcription of the respective target genes (Lando et al., 2002a). Replacement of Asn803 with alanine permitted coactivator binding with HIF-1 $\alpha$  at normoxia (Lando et al., 2002b). In addition, it has been reported that FIH-1 binds pVHL, forming a ternary complex with the HIF-1 $\alpha$  (Mahon et al., 2001). Although interaction with pVHL was not required for FIH-1 activity, histone deacetylases recruited by pVHL interfered with the transcription processes, facilitating FIH-1 to modulate HIF-1 $\alpha$  transactivation (Hewitson et al., 2002; Sang et al., 2002). FIH-1 is mainly located in the cytoplasm, but some fraction is likely to reside in the nucleus as well (Metzen et al., 2003a). The transcription of FIH-1 is independent of the oxygen concentration, and it does not influence HIF-1 $\alpha$  stability (Metzen et al., 2003a).

Like the PHDs, the asparaginyl hydroxylase FIH-1 is a 2-OG-dependent dioxygenase that also requires Fe<sup>2+</sup> and ascorbate as cofactors (Lando et al., 2002a). Utilization of

oxygen as a substrate allows FIH-1 to serve as a second oxygen sensor.

**Phosphorylation by MAPK—Enhancing Transactivation.** Despite the central importance of hydroxylases in sensing oxygen tension and regulating HIF-1 activity, there are other mechanisms that contribute to the control of HIF-1. Phosphorylation is well known to be crucial in controlling protein activities. Direct phosphorylation of HIF-1 $\alpha$  has been reported, and the mitogen-activated protein kinase (MAPK) pathway seems to play a role (Richard et al., 1999; Sodhi et al., 2000; Minet et al., 2001). It has been shown that p42/44 and p38 kinase phosphorylated HIF-1 $\alpha$ /HIF-2 $\alpha$  in vitro (Richard et al., 1999; Sodhi et al., 2000). In addition, inhibitors of p42/44 and p38 blocked HIF-1 $\alpha$ -mediated reporter gene expression (Hur et al., 2001). Transfection with active forms of p42/44 kinase stimulated HIF-1 $\alpha$  transcription activity without affecting HIF-1 $\alpha$  stability. Moreover, HIF-1 $\alpha$ /HIF-2 $\alpha$  transactivation during hypoxia required p42/44 MAPKs (Conrad et al., 1999; Minet et al., 2000; Hofer et al., 2001; Hur et al., 2001). It seems that phosphorylation does not affect stability or DNA binding of HIF-1 $\alpha$ ; rather, it increases the transcriptional activity of HIF-1 (Richard et al., 1999). One explanation could be that HIF-1 $\beta$  binds preferentially to the phosphorylated form of HIF-1 $\alpha$  (Suzuki et al., 2001). Although the functionally relevant phosphorylation sites remain to be identified, threonine 796 in HIF-1 $\alpha$  and 844 in HIF-2 $\alpha$  are candidate sites (Gradin et al., 2002).

In addition to the post-translational modification of HIF-1 $\alpha$  described above, SUMOylation of HIF-1 $\alpha$  has also been reported to contribute to repressing transactivation (Brahimi-Horn et al., 2005). Moreover, S-nitrosation on cysteine 800 of HIF-1 $\alpha$  has been shown to increase its transactivation through its interaction with CBP/p300 (Yasinska and Sumbayev, 2003).

Besides hypoxia, HIF-1 is also regulated in an oxygen-independent manner. Cytokines, growth factors, environmental stimuli, and other signaling molecules have been implicated in controlling HIF-1 under nonhypoxic condition (Table 1) (Feldser et al., 1999; Hellwig-Burgel et al., 1999; Richard et al., 2000; Salnikow et al., 2000; Gorchach et al., 2001; Haddad and Land, 2001; Stiehl et al., 2002; Li et al., 2004). Although complex and cell-type dependent, some have been shown to stimulate HIF-1 $\alpha$  transactivation or synthesis by activation of the MAPK or the phosphatidylinositol 3-kinase (PI3K) signaling pathways (Zelzer et al., 1998; Li et al., 2004).

## The Target Gene of HIF-1

Given that cells and organs need to adapt to changes in oxygen supply, it would not be surprising to find that a significant variety of the HIF-1 target genes are regulated in a tissue-specific manner. To date, there are more than 100 HIF-1 downstream genes identified with varying functions (Table 2). HIF-1 activates the expression of these genes by binding to a 50-base pair *cis*-acting HRE located in their enhancer and promoter regions (Semenza et al., 1991). Moreover, by using DNA microarrays, it has recently been reported that more than 2% of all human genes are regulated by HIF-1 in arterial endothelial cells, directly or indirectly (Manalo et al., 2005).

**Erythropoiesis/Iron Metabolism.** In response to hypoxia, the capacity of red blood cells to transport oxygen is

up-regulated by the expression of genes involved in erythropoiesis and iron-metabolism. Hypoxia increases the expression of EPO, which is required for the formation of red blood cells (Semenza et al., 1991). An increase in the number of erythrocytes enhances the delivery of oxygen to tissues. Products of iron-metabolizing genes control the major erythropoietic rate-limiting step of heme production. Hypoxia up-regulates transferrin (Tf), which transports  $\text{Fe}^{3+}$  into cells (Rolfs et al., 1997); the transferrin receptor (Tfr), which binds Tf and enables cellular transferrin uptake (Bianchi et al., 1999; Lok and Ponka, 1999; Tacchini et al., 1999); and ceruloplasmin (also known as a ferroxidase), which is required to oxidize ferrous ( $\text{Fe}^{2+}$ ) to ferric ( $\text{Fe}^{3+}$ ) iron (Lok and Ponka, 1999; Mukhopadhyay et al., 2000). Increasing of these genes supports iron supply to erythroid tissues (Rolfs et al., 1997).

**Angiogenesis.** Angiogenesis is a complex process that involves multiple gene products expressed by different cell types (Conway et al., 2001). A large number of genes involved in different steps of angiogenesis have been shown to increase by hypoxia challenge (Levy et al., 1995; Bunn and Poyton, 1996; Forsythe et al., 1996; Berra et al., 2000; Giordano and Johnson, 2001; Semenza, 2002). Among them, the vascular endothelial cell growth factor (VEGF) is the most potent endothelial-specific mitogen, and it directly participates in angiogenesis by recruiting endothelial cells into hypoxic and avascular area and stimulates their proliferation (Neufeld et al., 1999; Josko et al., 2000; Conway et al., 2001). Therefore, the induction of VEGF and various other

proangiogenic factors leads to an increase in the vascular density and hence a decrease in the oxygen diffusion distance. In addition, HIF-1 regulates genes involved in governing the vascular tone such as nitric oxide synthase (NOS2) (Melillo et al., 1995), heme oxygenase 1 (Lee et al., 1997), endothelin 1 (ET1) (Hu et al., 1998), adrenomedullin (ADM) (Nguyen and Claycomb, 1999), and the  $\alpha_{1B}$ -adrenergic receptor (Eckhart et al., 1997). Moreover, hypoxia induces genes involved in matrix metabolism and vessel maturation such as matrix metalloproteinases (MMPs) (Ben-Yosef et al., 2002), plasminogen activator receptors and inhibitors (PAIs) (Kietzmann et al., 1999), and collagen prolyl hydroxylase (Takahashi et al., 2000).

**Glucose Metabolism.** Under low oxygen supply, cells switch their glucose metabolism pathway away from the oxygen-dependent tricarboxylic acid (TCA) cycle to the oxygen-independent glycolysis (Dang and Semenza, 1999; Seagroves et al., 2001). With only 2 ATP molecules from each glucose molecule produced by glycolysis, instead of 38 ATP provided by TCA cycle, hypoxic cells elevate their ability to generate ATP by increasing the glucose uptake. This is achieved by up-regulating the expression of glycolytic enzymes and glucose transporters (Wenger, 2002). Hypoxia and HIF-1 increase virtually all the enzymes in the glycolytic pathway, as well as the glucose transporters 1 and 3 (GLU1, GLU3) (Chen et al., 2001). Furthermore, the glycolysis metabolic products, such as lactate and pyruvate, have been reported to cause HIF-1 $\alpha$  accumulation under normoxia and

TABLE 1  
Environmental regulator of HIF-1

Regulator	Function/Pathway Involved	Consequence	Reference
Nickel ( $\text{Ni}^{2+}$ )	Decreases cellular Fe level	Increased HIF-1 $\alpha$	Davidson et al., 2005
	Inhibits PHDs		Ke et al., 2005
	Down-regulates the expression of FIH-1 and ARD1		Salnikow et al., 2004
	Depletes intracellular ascorbate		Li et al., 2004
Cobalt ( $\text{Co}^{2+}$ )	PI3K/Akt	Increased HIF-1 $\alpha$	Yuan et al., 2003
	Replaces Fe		Ke et al., 2005
	Down-regulates the expression of FIH-1 and ARD1		Salnikow et al., 2004
Arsenite	Depletes intracellular ascorbate	Increased HIF-1 $\alpha$	Gao et al., 2004
	PI3K		Duyndam et al., 2001
	ROS		Duyndam et al., 2003
Chromium	p38 MAPK	Increased HIF-1 $\alpha$	Gao et al., 2004
	p38 MAPK, ROS		Gao et al., 2002
Vanadate	PI3K/Akt, ROS	Increased HIF-1 $\alpha$	Hwang et al., 2004
	AMP-activated protein kinase (AMPK)		
Desferrioxamine (DFO)	Fe chelator	Increased HIF-1 $\alpha$	Wang and Semenza, 1993
Insulin	PI3K	Increased HIF-1 $\alpha$	Stiehl et al., 2002
Interleukin-1 (IL-1)	ROS	Increased HIF-1 $\alpha$	Feldser et al., 1999
Insulin-like growth factor (IGF)-1, IGF-2			
Fetal calf serum	ROS	Increased HIF-1 $\alpha$	Richard et al., 2000
Angiotensin II (Ang II)	ROS	Increased HIF-1 $\alpha$	Gorlach et al., 2001
Thrombin, Platelet-derived growth factor (PDGF)			
Nitric oxide (NO)	MAPK, PI3K	Increased HIF-1 $\alpha$	Kasuno et al., 2004
	Inhibits PHDs		Metzen et al., 2003b
	Inhibits mitochondrial $\text{O}_2$ consumption		Hagen et al., 2003
Nitric oxide (NO) under hypoxia	Increases intracellular Fe and PHDs activity	Decreased HIF-1 $\alpha$	Callapina et al., 2005
TGF- $\alpha$	ROS	Increased HIF-1 $\alpha$ translocation and activity	Haddad and Land, 2001
			Increased DNA binding

regulate hypoxia-inducible gene expression, hence establishing a potential positive feedback loop (Lu et al., 2002).

**Cell Proliferation/Survival.** Hypoxia and HIF-1 induce growth factors, such as insulin-like growth factor-2 (IGF2) and transforming growth factor- $\alpha$  (TGF- $\alpha$ ) (Feldser et al., 1999; Krishnamachary et al., 2003). Binding of such growth factors to their cognate receptors activates signal transduction pathways that lead to cell proliferation/survival and stimulates the expression of HIF-1 $\alpha$  itself (Semenza, 2003). As mentioned above, cytokines and growth factors, as well as hypoxia in some cell types, can activate signaling pathways MAPK and PI3K, which promote cell proliferation/survival as well as contribute to HIF-1 activity. This leads to increased HIF-1 transcriptional activity of target genes, including those encoding IGF2 and TGF- $\alpha$ , thereby contributing to autocrine-signaling pathways that are crucial for cancer progression (Semenza, 2003).

**Apoptosis.** Paradoxically, cell adaptation to hypoxia leads not only to cell proliferation/survival but also to cell death in some circumstances. Hypoxia has been shown to induce apoptosis, where HIF-1 plays a complex role (Carmeliet et al., 1998). Genetic studies using embryonic stem cells harboring a deletion of HIF-1 $\alpha$  showed decreased apoptosis compared with wild type when challenged with low oxygen (Carmeliet et al., 1998). Activation of caspase-3 and Apaf-1-mediated caspase-9, and the release of cytochrome *c*, have been reported in several cell types under hypoxic conditions (Brunelle and Chandel, 2002; McClintock et al., 2002). It has also been demonstrated that the expression of HIF-1 $\alpha$  and HIF-1 $\beta$  significantly correlated with apoptosis and the proapoptotic factors, such as caspase-3, Fas, and Fas ligand

(Volm and Koomagi, 2000). Moreover, hypoxia depressed the antiapoptotic protein Bcl-2 (Carmeliet et al., 1998), whereas the proapoptotic protein Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNip3) and its homolog Nip3-like protein X (NIX) were up-regulated in a HIF-dependent manner (Bruick, 2000). Some genes involved in cell cycle control, such as p53 and p21, were also found to be HIF-dependent (Carmeliet et al., 1998). In addition, p53 has been implicated in regulating hypoxia-induced apoptosis through induction of apoptosis-related genes such as *Bax*, *NOXA*, *PUMA*, and *PERP* (Schuler and Green, 2001).

In addition to the above classes of genes, HIF-1 also regulated many other target genes implicated in diverse processes such as adipogenesis (Yun et al., 2002), carotid body formation (Kline et al., 2002), B lymphocyte development (Kojima et al., 2002), and immune reactions (Hellwig-Burgel et al., 2005).

Although there are some studies showing a role of HIF-2 $\alpha$  in the VEGF induction (Akeno et al., 2001; Compornolle et al., 2002), no bona fide target genes have yet been identified for HIF-2 $\alpha$  or HIF-3 $\alpha$ . However, a recent study using a genetic “knock-in” strategy has shown that targeted replacement of HIF-1 $\alpha$  with HIF-2 $\alpha$  results in expanded expression of HIF-2 $\alpha$ -specific target genes (i.e., Oct-4, a transcription factor essential for maintaining stem cell pluripotency) (Covello et al., 2006).

## The Role of HIF-1 in Development and Diseases

Hypoxia and the HIF pathway have been linked to the embryonic development and pathophysiology of numerous

TABLE 2  
HIF-1 target genes

Function	Gene (abbreviation)	Reference
Erythropoiesis/ iron metabolism	Erythropoietin (EPO)	Semenza et al., 1991
	Transferrin (Tf)	Rolfs et al., 1997
	Transferrin receptor (Tfr)	Bianchi et al., 1999
Angiogenesis	Ceruloplasmin	Lok and Ponka, 1999
	Vascular endothelial growth factor (VEGF)	Levy et al., 1995
	Endocrine-gland-derived VEGF (EG-VEGF)	LeCouter et al., 2001
	Leptin (LEP)	Grosfeld et al., 2002
Vascular tone	Transforming growth factor- $\beta$ 3 (TGF- $\beta$ 3)	Scheid et al., 2002
	Nitric oxide synthase (NOS2)	Melillo et al., 1995
	Heme oxygenase 1	Lee et al., 1997
	Endothelin 1 (ET1)	Hu et al., 1998
	Adrenomedullin (ADM)	Nguyen and Claycomb, 1999
Matrix metabolism	$\alpha_{1B}$ -Adrenergic receptor	Eckhart et al., 1997
	Matrix metalloproteinases (MMPs)	Ben-Yosef et al., 2002
	Plasminogen activator receptors and inhibitors (PAIs)	Kietzmann et al., 1999
Glucose metabolism	Collagen prolyl hydroxylase	Takahashi et al., 2000
	Adenylate kinase-3	O'Rourke et al., 1996
	Aldolase-A,C (ALDA,C)	Semenza et al., 1996
	Carbonic anhydrase-9	Wykoff et al., 2000
	Enolase-1 (ENO1)	Semenza et al., 1996
	Glucose transporter-1,3 (GLU1,3)	Chen et al., 2001
	Glyceraldehyde phosphate dehydrogenase (GAPDH)	Graven et al., 1999
	Hexokinase 1,2 (HK1,2)	Mathupala et al., 2001
	Lactate dehydrogenase-A (LDHA)	Semenza et al., 1996
	Pyruvate kinase M (PKM)	Semenza et al., 1994
	Phosphofructokinase L (PFKL)	Semenza et al., 1994
Cell proliferation/survival	Phosphoglycerate kinase 1 (PGK1)	Semenza et al., 1994
	6-phosphofructo-2-kinase/gructose-2,6-bisphosphate-3 (PFKFB3)	Minchenko et al., 2002
	Insulin-like growth factor-2 (IGF2)	Feldser et al., 1999
	Transforming growth factor- $\alpha$ (TGF- $\alpha$ )	Krishnamachary et al., 2003
	Adrenomedullin (ADM)	Cormier-Regard et al., 1998
	Bcl-2/adenovirus E1B 19kD-interacting protein 3 (BNip3)	Carrero et al., 2000
	Nip3-like protein X (NIX)	Bruick, 2000

human diseases. In order for solid tumors to grow, an increase of oxygen delivery to cells via angiogenesis and activation of glycolysis have been observed and named the Warburg effects (Seagroves et al., 2001). Given the importance of HIF-1 in the activation of genes essential to these processes, it is not surprising that both HIF-1 $\alpha$  and HIF-2 $\alpha$  have been strongly implicated in tumor progression and grade, hence conferring a selective advantage to tumor cells.

**Development.** Components of the HIF-1 system play essential roles in embryonic development. Knockout of either HIF-1 $\alpha$  (Iyer et al., 1998; Ryan et al., 1998; Kotch et al., 1999), HIF-2 $\alpha$  (Tian et al., 1998; Peng et al., 2000), or HIF-1 $\beta$  (Maltepe et al., 1997) resulted in abnormal vascular development and lethality in mice. HIF-1 $\alpha$  expression increased between embryonic days 8.5 and 9.5 in normal mouse embryos (Iyer et al., 1998). Embryos deficient in HIF-1 $\alpha$  (HIF-1 $\alpha$ <sup>-/-</sup>) died by embryonic day 11 as a consequence of lack of blood vessel formation, defective formation of the neural fold, and cardiovascular malformation (Iyer et al., 1998; Ryan et al., 1998). Global hypoxia was also observed. In addition, the rate of cell proliferation and the expression of hypoxia-inducible genes were decreased in HIF-1 $\alpha$ <sup>-/-</sup> cells, compared with those of wild-type cells (Iyer et al., 1998). Although heterozygous mice carrying a single HIF-1 $\alpha$  gene (HIF-1 $\alpha$ <sup>+/-</sup>) developed normally, they displayed impaired physiological responses when challenged by chronic hypoxia (Yu et al., 1999; Kline et al., 2002).

Targeted inactivation of HIF-2 $\alpha$  (HIF-2 $\alpha$ <sup>-/-</sup>) in mice resulted in rather different and variable phenotypes. HIF-2 $\alpha$ <sup>-/-</sup> mouse embryos died by E12.5-embryonic day 16.5 as a result of inadequate blood vessel fusion and remodeling, impaired fetal lung maturation, and a very slow heart rate because of insufficient catecholamine production (Tian et al., 1998; Peng et al., 2000). Thus, it seems that HIF-1 $\alpha$  and HIF-2 $\alpha$  have nonredundant functions in the regulation of gene expression during development, despite their close similarity in terms of amino acid sequence, domain architecture, DNA-binding capacity, and hypoxic activation.

HIF-1 $\beta$ <sup>-/-</sup> embryos died by embryonic day 10.5 and showed defect in blood vessel formation, defective angiogenesis of the yolk sac and branchial arches, stunted development, and embryo wasting (Maltepe et al., 1997). In addition, HIF-1 $\beta$ <sup>-/-</sup> cells failed to activate genes that normally respond to hypoxia and low glucose concentration (Maltepe et al., 1997).

**Cancer.** Overexpression of HIF-1 $\alpha$  and HIF-2 $\alpha$  was found in various human cancers, probably as a consequence of intratumoral hypoxia or genetic alteration (Zhong et al., 1999; Talks et al., 2000). The interior of the tumor mass becomes progressively hypoxic as its size increases until adequate blood vessels are obtained by tumors. Hypoxic conditions within tumors can result in increased HIF-1 stability and activity. Immunohistochemical analyses demonstrated that there are detectable levels of HIF-1 $\alpha$  protein in benign tumors, elevated levels in primary malignant tumors, and a marked amount in tumor metastases, in contrast to its absence in normal tissues (Zhong et al., 1999; Harris, 2002). Expression of HIF target genes is generally consistent with the levels of HIF-1 $\alpha$ . In addition, injection of HIF-1 $\alpha$  (or HIF-1 $\beta$ ) positive and deficient cells to immunocompromised mice revealed that HIF-1 $\alpha$  is a positive factor to tumorigenesis (Maxwell et al., 1997; Ryan et al., 2000).

There is a remarkable frequency of common genetic alterations in cancer cells associated with increased HIF-1 $\alpha$  expression. As mentioned in VHL disease, for example, loss of function of VHL resulted in constitutively expressed HIF-1 $\alpha$  (Iliopoulos et al., 1996). In addition, loss of function of wild-type p53, which is inactivated in most of human cancers, increased HIF-1 $\alpha$  levels and enhanced HIF-dependent transcription in tumors (Ravi et al., 2000). Loss of function of tumor suppressor gene PTEN in glioblastoma-derived cell line resulted in increased HIF-1 $\alpha$  levels and HIF-1-mediated gene expression, probably via activating of the PI3K/AKT signaling cascade (Zundel et al., 2000). The transforming potential of the v-Src oncogene is thought to be due in part to its induction of HIF and gain of function of v-SRC increased expression of HIF-1 $\alpha$  and HIF-dependent genes (Jiang et al., 1997). Moreover, enhanced HER2 receptor tyrosine kinase signaling has been shown to increase the rate of synthesis of HIF-1 $\alpha$  (Laughner et al., 2001). Increased activity of the HER2 receptor tyrosine kinase is a prevalent and important genetic alteration in breast cancer, correlating with tumor aggressiveness and decreased patient survival. Therefore, it seems that HIF-1 $\alpha$  overexpression confers selective advantages to tumor cells. A correlation between HIF-1 overexpression and patient mortality, poor prognosis, or treatment resistance has been noted in many studies (Semenza, 2003).

**Ischemic Disease.** Activation of HIF activity has also been demonstrated in a broad range of physiological responses to ischemic, hypoxic, and inflammatory conditions, where it plays a positive role to respond to the damage to organs or tissues. For example, the levels of HIF-1 $\alpha$  and VEGF were increased in the myocardium when patients developed acute coronary artery occlusion (Lee et al., 2000). Effective vascular remodeling after ischemic injury depended on an integrated program of HIF-dependent gene expression. Increasing of HIF-1 $\alpha$  expression and HIF-inducible genes was also observed in sheep and rat models of myocardial and cerebral ischemia (Martin et al., 1998; Bergeron et al., 1999). In addition, induction of HIF-1 $\alpha$  or HIF-2 $\alpha$  and their target genes has been shown in the pre-eclamptic placenta (Rajakumar et al., 2003), by macrophages in rheumatoid synovia (Hollander et al., 2001), in the ischemic retina (Ozaki et al., 1999; Grimm et al., 2002), as well as from wound healing (Elson et al., 2000).

## The Implication of HIF-1 in Therapy

The importance of HIF-1 as a transcription factor and the broad spectrum of processes influenced by HIF suggest that it could have important clinical implications. The many aspects of HIF-1 regulation provide a variety of possibilities for therapeutic intervention.

**Cancer Therapy—Inhibiting HIF-1 Activity.** First, immunohistochemical analysis of HIF-1 $\alpha$  expression in tumor biopsies may provide the prognostic information and thereby identify subsets of patients requiring aggressive therapy (Harris, 2002). To achieve high specificity to hypoxic tumor regions or cells, HREs from hypoxia-responsive genes can be used to express marker therapeutic genes selectively expressed in tumor cells that are hypoxic and overexpress HIF-1 (Dachs et al., 1997).

It has been suggested that disruption of the HIF-1 pathway might be effective in the treatment of pancreatic cancer

(Chen et al., 2003). The study demonstrated that dominant-negative HIF-1 $\alpha$  reduced the tumorigenicity of pancreatic cancer cells through the suspension of glucose metabolism (Chen et al., 2003). It also rendered the cancer cells sensitive to apoptosis and growth inhibition induced by hypoxia (Chen et al., 2003). A HIF-1 $\alpha$  C-TAD polypeptide that competes for CBP/p300 binding has been shown to decrease the expression of VEGF and tumor growth in mice (Kung et al., 2000).

Many novel therapeutic agents targeting signal-transduction pathways have shown to block HIF-1 $\alpha$  function and have antiangiogenic effects (Harris, 2002). These agents include trastuzumab (Herceptin) and gefitinib (Iressa); calphostin C (inhibitor a protein kinase C); wortmannin and LY294002 (inhibitors of PI3K); PD98095 (inhibitor of a MAPK); rapamycin [inhibitor of a FKBP12-rapamycin-associated protein or mammalian target of rapamycin (FRAP/mTOR)]; diphenylene iodonium (a redox signaling blocker), and mannoheptulose (inhibitor of a glucokinase). Several small molecular inhibitors of the HIF-1 transcriptional activation pathway have also been identified and shown to decrease HIF-1 $\alpha$  levels, inhibit the expression of VEGF and other HIF-1 target genes, impair xenograft growth and vascularization, and inhibit angiogenesis (Rapisarda et al., 2002). These molecules include topoisomerase I inhibitors YC-1, 17-allyl-aminogeldanamycin (inhibitor of the 90-kDa heat shock protein); inhibitors of the redox regulator thioredoxin-1; and the newly identified 2-methoxyestradiol that disrupts tumor microtubules.

**Ischemic Disease Therapy—Promoting HIF-1 Activity.** In contrast to the inhibition of HIF-1 activation in cancer therapy, promoting its activation could be advantageous in ischemic diseases (Vincent et al., 2000; Elson et al., 2001). Ischemic diseases such as stroke and heart attack are caused by localized hypoxia manifested as cerebral and myocardial ischemia, respectively. Increase of the VEGF expression by HIF-1 $\alpha$  or HIF-2 $\alpha$  could induce the formation of new blood vessels of the target area in the brain and heart, thereby providing an increased blood flow and oxygen supply and reduce harmful response to ischemia (Semenza, 1998). Transgenic mice overexpressing HIF-1 $\alpha$  in epidermis showed increased expression of VEGF and marked induction of hypervascularity without induction of edema, inflammation, or vascular leakage (Elson et al., 2001). The macrophage-derived peptide PR39 has been shown to stabilize HIF-1 $\alpha$  by decreasing its degradation, resulting in accelerated formation of vascular structures in vitro and increased myocardial vasculature in mice (Li et al., 2000). Direct induction of HIF-1 has been achieved by using the N- or C-terminal of ODDD polypeptides that block VHL-mediated degradation (Maranchie et al., 2002). Targeting of proline and asparaginyl hydroxylases could also be potential strategies for increasing HIF activity (Ivan et al., 2002).

## Conclusion

Despite recent rapid advance in understanding the molecular mechanisms of the HIF pathway in response to hypoxia, many important questions remain to be answered. For example, the distinct role of enzymes modifying HIF-1 $\alpha$  post-translationally, the interplay among the HIF-1 $\alpha$  post-translational modifications, the identity of additional target genes of HIF-1, the function of the paralogs of HIF-1 $\alpha$  (such as

HIF-2 $\alpha$  and HIF-3 $\alpha$ ), the link between HIF-1 activation and other oncogenic or tumor suppressor pathways, the mechanism by which the HIF-1 pathway contribute to tumor growth and other pathological responses. Unraveling such questions should provide new insights into cellular adaptation to hypoxia and aid to discover new therapeutic approaches to diverse human diseases.

## References

- Akeno N, Czyzyk-Krzyszka MF, Gross TS, and Clemens TL (2001) Hypoxia induces vascular endothelial growth factor gene transcription in human osteoblast-like cells through the hypoxia-inducible factor-2 $\alpha$ . *Endocrinology* **142**:959–962.
- Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, and Gleadle JM (2004) Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Biol Chem* **279**:38458–38465.
- Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, and Livingston DM (1996) An essential role for p300/CBP in the cellular response to hypoxia. *Proc Natl Acad Sci USA* **93**:12969–12973.
- Bae MK, Ahn MY, Jeong JW, Bae MH, Lee YM, Bae SK, Park JW, Kim KR, and Kim KW (2002) Jab1 interacts directly with HIF-1 $\alpha$  and regulates its stability. *J Biol Chem* **277**:9–12.
- Baek JH, Mahon PC, Oh J, Kelly B, Krishnamachary B, Pearson M, Chan DA, Giaccia AJ, and Semenza GL (2005) OS-9 interacts with hypoxia-inducible factor 1 $\alpha$  and prolyl hydroxylases to promote oxygen-dependent degradation of HIF-1 $\alpha$ . *Mol Cell* **17**:503–512.
- Ben-Yosef Y, Lahat N, Shapiro S, Bitterman H, and Miller A (2002) Regulation of endothelial matrix metalloproteinase-2 by hypoxia/reoxygenation. *Circ Res* **90**:784–791.
- Bergeron M, Yu AY, Solway KE, Semenza GL, and Sharp FR (1999) Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain. *Eur J Neurosci* **11**:4159–4170.
- Berra E, Benizri E, Ginouvès A, Volmat V, Roux D, and Pouyssegur J (2003) HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1 $\alpha$  in normoxia. *EMBO (Eur Mol Biol Organ) J* **22**:4082–4090.
- Berra E, Milanini J, Richard DE, Le Gall M, Vinals F, Gothie E, Roux D, Pages G, and Pouyssegur J (2000) Signaling angiogenesis via p42/p44 MAP kinase and hypoxia. *Biochem Pharmacol* **60**:1171–1178.
- Berra E, Roux D, Richard DE, and Pouyssegur J (2001) Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) escapes O<sub>2</sub>-driven proteasomal degradation irrespective of its subcellular localization: nucleus or cytoplasm. *EMBO Rep* **2**:615–620.
- Bianchi L, Tacchini L, and Cairo G (1999) HIF-1-mediated activation of transferrin receptor gene transcription by iron chelation. *Nucleic Acids Res* **27**:4223–4227.
- Brahimi-Horn C, Mazure N, and Pouyssegur J (2005) Signaling via the hypoxia-inducible factor-1 $\alpha$  requires multiple posttranslational modifications. *Cell Signal* **17**:1–9.
- Bruick RK (2000) Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. *Proc Natl Acad Sci USA* **97**:9082–9087.
- Bruick RK, and McKnight SL (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. *Science (Wash DC)* **294**:1337–1340.
- Brunelle JK and Chandel NS (2002) Oxygen deprivation induced cell death: an update. *Apoptosis* **7**:475–482.
- Bunn HF and Poyton RO (1996) Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* **76**:839–885.
- Callapina M, Zhou J, Schnitzer S, Metzén E, Lohr C, Deitmer JW, and Brune B (2005) Nitric oxide reverses desferrioxamine- and hypoxia-evoked HIF-1 $\alpha$  accumulation—implications for prolyl hydroxylase activity and iron. *Exp Cell Res* **306**:274–284.
- Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, et al. (1998) Role of HIF-1 $\alpha$  in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature (Lond)* **394**:485–490.
- Carrero P, Okamoto K, Coumilleau P, O'Brien S, Tanaka H, and Poellinger L (2000) Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1 $\alpha$ . *Mol Cell Biol* **20**:402–415.
- Chen C, Pore N, Behrooz A, Ismail-Beigi F, and Maity A (2001) Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J Biol Chem* **276**:9519–9525.
- Chen J, Zhao S, Nakada K, Kuge Y, Tamaki N, Okada F, Wang J, Shindo M, Higashino F, Takeda K, et al. (2003) Dominant-negative hypoxia-inducible factor-1 $\alpha$  reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. *Am J Pathol* **162**:1283–1291.
- Compennolle V, Brusselmans K, Acker T, Hoet P, Tjwa M, Beck H, Plaisance S, Dor Y, Keshet E, Lupu F, et al. (2002) Loss of HIF-2 $\alpha$  and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med* **8**:702–710.
- Conrad PW, Freeman TL, Beitner-Johnson D, and Millhorn DE (1999) EPAS1 trans-activation during hypoxia requires p42/p44 MAPK. *J Biol Chem* **274**:33709–33713.
- Conway EM, Collen D, and Carmeliet P (2001) Molecular mechanisms of blood vessel growth. *Cardiovasc Res* **49**:507–521.
- Cormier-Regard S, Nguyen SV, and Claycomb WC (1998) Adrenomedullin gene expression is developmentally regulated and induced by hypoxia in rat ventricular cardiac myocytes. *J Biol Chem* **273**:17787–17792.
- Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, and Keith B (2006) HIF-2 $\alpha$  regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev* **20**:557–570.

- Crews ST (1998) Control of cell lineage-specific development and transcription by bHLH-PAS proteins. *Genes Dev* **12**:607–620.
- Dachs GU, Patterson AV, Firth JD, Ratcliffe PJ, Townsend KM, Stratford IJ, and Harris AL (1997) Targeting gene expression to hypoxic tumor cells. *Nat Med* **3**:515–520.
- Dang CV and Semenza GL (1999) Oncogenic alterations of metabolism. *Trends Biochem Sci* **24**:68–72.
- Davidson T, Chen H, Garrick MD, D'Angelo G, and Costa M (2005) Soluble nickel interferes with cellular iron homeostasis. *Mol Cell Biochem* **279**:157–162.
- Duyndam MC, Hulscher ST, van der Wall E, Pinedo HM, and Boven E (2003) Evidence for a role of p38 kinase in hypoxia-inducible factor 1-independent induction of vascular endothelial growth factor expression by sodium arsenite. *J Biol Chem* **278**:6885–6895.
- Duyndam MC, Hulscher TM, Fontijn D, Pinedo HM, and Boven E (2001) Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1 $\alpha$  protein by the oxidative stressor arsenite. *J Biol Chem* **276**:48066–48076.
- Ebert BL and Bunn HF (1998) Regulation of transcription by hypoxia requires a multiprotein complex that includes hypoxia-inducible factor 1, an adjacent transcription factor, and p300/CREB binding protein. *Mol Cell Biol* **18**:4089–4096.
- Eckhart AD, Yang N, Xin X, and Faber JE (1997) Characterization of the  $\alpha$ 1B-adrenergic receptor gene promoter region and hypoxia regulatory elements in vascular smooth muscle. *Proc Natl Acad Sci USA* **94**:9487–9492.
- Elson DA, Ryan HE, Snow JW, Johnson R, and Arbeit JM (2000) Coordinate up-regulation of hypoxia inducible factor (HIF)-1 $\alpha$  and HIF-1 target genes during multi-stage epidermal carcinogenesis and wound healing. *Cancer Res* **60**:6189–6195.
- Elson DA, Thurston G, Huang LE, Ginzinger DG, McDonald DM, Johnson RS, and Arbeit JM (2001) Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1 $\alpha$ . *Genes Dev* **15**:2520–2532.
- Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, Poellinger L, and Fujii-Kuriyama Y (1999) Molecular mechanisms of transcription activation by HLF and HIF1 $\alpha$  in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO (Eur Mol Biol Organ) J* **18**:1905–1914.
- Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, and Fujii-Kuriyama Y (1997) A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1 $\alpha$  regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci USA* **94**:4273–4278.
- Epstein AC, Gleadow JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzzen E, Wilson MI, Dhanda A, et al. (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**:43–54.
- Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, and Semenza GL (1999) Reciprocal positive regulation of hypoxia-inducible factor 1 $\alpha$  and insulin-like growth factor 2. *Cancer Res* **59**:3915–3918.
- Flamme I, Frohlich T, von Reutern M, Kappel A, Damert A, and Risau W (1997) HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 $\alpha$  and developmentally expressed in blood vessels. *Mech Dev* **63**:51–60.
- Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, and Semenza GL (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* **16**:4604–4613.
- Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi X, and Jiang BH (2002) Vanadate-induced expression of hypoxia-inducible factor 1 $\alpha$  and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. *J Biol Chem* **277**:31963–31971.
- Gao N, Shen L, Zhang Z, Leonard SS, He H, Zhang XG, Shi X, and Jiang BH (2004) Arsenite induces HIF-1 $\alpha$  and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. *Mol Cell Biochem* **255**:33–45.
- Giordano FJ and Johnson RS (2001) Angiogenesis: the role of the microenvironment in flipping the switch. *Curr Opin Genet Dev* **11**:35–40.
- Goldberg MA, Dunning SP, and Bunn HF (1988) Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. *Science (Wash DC)* **242**:1412–1415.
- Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, Kietzmant T, and Busse R (2001) Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. *Circ Res* **89**:47–54.
- Gradin K, Takasaki C, Fujii-Kuriyama Y, and Sogawa K (2002) The transcriptional activation function of the HIF-like factor requires phosphorylation at a conserved threonine. *J Biol Chem* **277**:23508–23514.
- Graven KK, Yu Q, Pan D, Roncarati JS, and Farber HW (1999) Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene. *Biochim Biophys Acta* **1447**:208–218.
- Grimm C, Wenzel A, Groszer M, Maysner H, Seeliger M, Samardzija M, Bauer C, Gassmann M, and Reme CE (2002) HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat Med* **8**:718–724.
- Grosfeld A, Andre J, Hauguel-De Mouzon S, Berra E, Pouyssegur J, and Guerre-Millo M (2002) Hypoxia-inducible factor 1 transactivates the human leptin gene promoter. *J Biol Chem* **277**:42953–42957.
- Groulx I, and Lee S (2002) Oxygen-dependent ubiquitination and degradation of hypoxia-inducible factor requires nuclear-cytoplasmic trafficking of the von Hippel-Lindau tumor suppressor protein. *Mol Cell Biol* **22**:5319–5336.
- Gu YZ, Moran SM, Hogenesch JB, Wartman L, and Bradford CA (1998) Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3 $\alpha$ . *Gene Expr* **7**:205–213.
- Haddad JJ and Land SC (2001) A non-hypoxic, ROS-sensitive pathway mediates TNF-alpha-dependent regulation of HIF-1 $\alpha$ . *FEBS Lett* **505**:269–274.
- Hagen T, Taylor CT, Lam F, and Moncada S (2003) Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1 $\alpha$ . *Science (Wash DC)* **302**:1975–1978.
- Harris AL (2002) Hypoxia—a key regulatory factor in tumour growth. *Nat Rev Cancer* **2**:38–47.
- Hellwig-Burgel T, Rutkowski K, Metzzen E, Fandrey J, and Jelkmann W (1999) Interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$  stimulate DNA binding of hypoxia-inducible factor-1. *Blood* **94**:1561–1567.
- Hellwig-Burgel T, Stiehl DP, Wagner AE, Metzzen E, and Jelkmann W (2005) Review: hypoxia-inducible factor-1 (HIF-1): a novel transcription factor in immune reactions. *J Interferon Cytokine Res* **25**:297–310.
- Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, Elkins JM, Oldham NJ, Bhattacharya S, Gleadow JM, et al. (2002) Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. *J Biol Chem* **277**:26351–26355.
- Hirota K, Fukuda R, Takabuchi S, Kizaka-Kondoh S, Adachi T, Fukuda K, and Semenza GL (2004) Induction of hypoxia-inducible factor 1 activity by muscarinic acetylcholine receptor signaling. *J Biol Chem* **279**:41521–41528.
- Hofer T, Desbaillets I, Hopfl G, Gassmann M, and Wenger RH (2001) Dissecting hypoxia-dependent and hypoxia-independent steps in the HIF-1 $\alpha$  activation cascade: implications for HIF-1 $\alpha$  gene therapy. *FASEB J* **15**:2715–2717.
- Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH, and Bradford CA (1997) Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. *J Biol Chem* **272**:8581–8593.
- Hollander AP, Corke KP, Freemont AJ, and Lewis CE (2001) Expression of hypoxia-inducible factor 1 $\alpha$  by macrophages in the rheumatoid synovium: implications for targeting of therapeutic genes to the inflamed joint. *Arthritis Rheum* **44**:1540–1544.
- Hon WC, Wilson MI, Harlos K, Claridge TD, Schofield CJ, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DI, and Jones EY (2002) Structural basis for the recognition of hydroxyproline in HIF-1 $\alpha$  by pVHL. *Nature (Lond)* **417**:975–978.
- Hu J, Discher DJ, Bishopric NH, and Webster KA (1998) Hypoxia regulates expression of the endothelin-1 gene through a proximal hypoxia-inducible factor-1 binding site on the antisense strand. *Biochem Biophys Res Commun* **245**:894–899.
- Huang J, Zhao Q, Mooney SM, and Lee FS (2002) Sequence determinants in hypoxia-inducible factor-1 $\alpha$  for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. *J Biol Chem* **277**:39792–39800.
- Huang LE, Arany Z, Livingston DM, and Bunn HF (1996) Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. *J Biol Chem* **271**:32253–32259.
- Hur E, Chang KY, Lee E, Lee SK, and Park H (2001) Mitogen-activated protein kinase inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of hypoxia-inducible factor-1 $\alpha$ . *Mol Pharmacol* **59**:1216–1224.
- Hwang JT, Lee M, Jung SN, Lee HJ, Kang I, Kim SS, and Ha J (2004) AMP-activated protein kinase activity is required for vanadate-induced hypoxia-inducible factor 1 $\alpha$  expression in DU145 cells. *Carcinogenesis* **25**:2497–2507.
- Iliopoulos O, Levy AP, Jiang C, Kaelin WG Jr and Goldberg MA (1996) Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc Natl Acad Sci USA* **93**:10595–10599.
- Iliopoulos O, Ohh M, and Kaelin WG Jr (1998) pVHL19 is a biologically active product of the von Hippel-Lindau gene arising from internal translation initiation. *Proc Natl Acad Sci USA* **95**:11661–11666.
- Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K, Yang H, Sorokina I, Conaway RC, Conaway JW, et al. (2002) Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA* **99**:13459–13464.
- Ivan M, and Kaelin WG Jr (2001) The von Hippel-Lindau tumor suppressor protein. *Curr Opin Genet Dev* **11**:27–34.
- Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, and Kaelin WG Jr (2001) HIF1 $\alpha$  targeted for VHL-mediated destruction by proline hydroxylation: implications for O<sub>2</sub> sensing. *Science (Wash DC)* **292**:464–468.
- Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, et al. (1998) Cellular and developmental control of O<sub>2</sub> homeostasis by hypoxia-inducible factor 1 $\alpha$ . *Genes Dev* **12**:149–162.
- Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, et al. (2001) Targeting of HIF-1 $\alpha$  to the von Hippel-Lindau ubiquitylation complex by O<sub>2</sub>-regulated prolyl hydroxylation. *Science (Wash DC)* **292**:468–472.
- Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, and Kim KW (2002) Regulation and destabilization of HIF-1 $\alpha$  by ARD1-mediated acetylation. *Cell* **111**:709–720.
- Jiang BH, Agani F, Passaniti A, and Semenza GL (1997) V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. *Cancer Res* **57**:5328–5335.
- Josko J, Gwozdz B, Jedrzejowska-Szypulka H, and Hendryk S (2000) Vascular endothelial growth factor (VEGF) and its effect on angiogenesis. *Med Sci Monit* **6**:1047–1052.
- Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, and Poellinger L (1998) Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1 $\alpha$ . *EMBO (Eur Mol Biol Organ) J* **17**:6573–6586.
- Kallio PJ, Pongratz I, Gradin K, McGuire J, and Poellinger L (1997) Activation of hypoxia-inducible factor 1 $\alpha$ : posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. *Proc Natl Acad Sci USA* **94**:5667–5672.
- Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, and Conaway JW (2000) Activation of HIF1 $\alpha$  ubiquitination by a reconstituted von Hippel-

- Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci USA* **97**:10430–10435.
- Kasuno K, Takabuchi S, Fukuda K, Kizaka-Kondoh S, Yodoi J, Adachi T, Semenza GL, and Hirota K (2004) Nitric oxide induces hypoxia-inducible factor 1 activation that is dependent on MAPK and phosphatidylinositol 3-kinase signaling. *J Biol Chem* **279**:2550–2558.
- Ke Q, Kluz T, and Costa M (2005) Down-regulation of the expression of the FIH-1 and ARD-1 genes at the transcriptional level by nickel and cobalt in the human lung adenocarcinoma A549 cell line. *Int J Environ Res Public Health* **2**:10–13.
- Kietzmann T, Roth U, and Jungermann K (1999) Induction of the plasminogen activator inhibitor-1 gene expression by mild hypoxia via a hypoxia response element binding the hypoxia-inducible factor-1 in rat hepatocytes. *Blood* **94**:4177–4185.
- Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, et al. (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat Med* **7**:437–443.
- Kline DD, Peng YJ, Manalo DJ, Semenza GL, and Prabhakar NR (2002) Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 alpha. *Proc Natl Acad Sci USA* **99**:821–826.
- Kojima H, Gu H, Nomura S, Caldwell CC, Kobata T, Carmeliet P, Semenza GL, and Sitkovsky MV (2002) Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha-deficient chimeric mice. *Proc Natl Acad Sci USA* **99**:2170–2174.
- Kotch LE, Iyer NV, Laughner E, and Semenza GL (1999) Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol* **209**:254–267.
- Krishnamachary B, Berg-Dixon S, Kelly B, Agani F, Feldser D, Ferreira G, Iyer N, LaRusch J, Pak B, Taghavi P, et al. (2003) Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res* **63**:1138–1143.
- Kung AL, Wang S, Kleo JM, Kaelin WG, and Livingston DM (2000) Suppression of tumor growth through disruption of hypoxia-inducible transcription. *Nat Med* **6**:1335–1340.
- Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, and Bruick RK (2002a) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* **16**:1466–1471.
- Lando D, Peet DJ, Whelan DA, Gorman JJ, and Whitelaw ML (2002b) Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science (Wash DC)* **295**:858–861.
- Laughner E, Taghavi P, Chiles K, Mahon PC, and Semenza GL (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* **21**:3995–4004.
- LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, Frantz G, Rangel L, DeGuzman L, Keller GA, et al. (2001) Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature (Lond)* **412**:877–884.
- Lee PJ, Jiang BH, Chin BY, Iyer NV, Alam J, Semenza GL, and Choi AM (1997) Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. *J Biol Chem* **272**:5375–5381.
- Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, and Thistlethwaite PA (2000) Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* **342**:626–633.
- Levy AP, Levy NS, Wegner S, and Goldberg MA (1995) Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J Biol Chem* **270**:13333–13340.
- Li J, Davidson G, Huang Y, Jiang BH, Shi X, Costa M, and Huang C (2004) Nickel compounds act through phosphatidylinositol-3-kinase/Akt-dependent, p70(S6k)-independent pathway to induce hypoxia inducible factor transactivation and Cap43 expression in mouse epidermal Cl41 cells. *Cancer Res* **64**:94–101.
- Li J, Post M, Volk R, Gao Y, Li M, Metais C, Sato K, Tsai J, Aird W, Rosenberg RD, et al. (2000) PR39, a peptide regulator of angiogenesis. *Nat Med* **6**:49–55.
- Lieb ME, Menzies K, Moschella MC, Ni R, and Taubman MB (2002) Mammalian EGLN genes have distinct patterns of mRNA expression and regulation. *Biochem Cell Biol* **80**:421–426.
- Lok CN, and Ponka P (1999) Identification of a hypoxia response element in the transferrin receptor gene. *J Biol Chem* **274**:24147–24152.
- Lu H, Forbes RA, and Verma A (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* **277**:23111–23115.
- Mahon PC, Hirota K, and Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* **15**:2675–2686.
- Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, Cao Y, Berkenstam A, and Poellinger L (2001) Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature (Lond)* **414**:550–554.
- Makino Y, Kanopka A, Wilson WJ, Tanaka H, and Poellinger L (2002) Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus. *J Biol Chem* **277**:32405–32408.
- Maltepe E, Schmidt JV, Baunoch D, Bradford CA, and Simon MC (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature (Lond)* **386**:403–407.
- Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, and Semenza GL (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* **105**:659–669.
- Maranchie JK, Vasselli JR, Riss J, Bonifacio JS, Linehan WM, and Klausner RD (2002) The contribution of VHL substrate binding and HIF1-alpha to the phenotype of VHL loss in renal cell carcinoma. *Cancer Cell* **1**:247–255.
- Martin C, Yu AY, Jiang BH, Davis L, Kimberly D, Hohimer AR, and Semenza GL (1998) Cardiac hypertrophy in chronically anemic fetal sheep: Increased vascularization is associated with increased myocardial expression of vascular endothelial growth factor and hypoxia-inducible factor 1. *Am J Obstet Gynecol* **178**:527–534.
- Masson N and Ratcliffe PJ (2003) HIF prolyl and asparaginyl hydroxylases in the biological response to intracellular O(2) levels. *J Cell Sci* **116**:3041–3049.
- Masson N, Willam C, Maxwell PH, Pugh CW, and Ratcliffe PJ (2001) Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. *EMBO (Eur Mol Biol Organ) J* **20**:5197–5206.
- Mathupala SP, Rempel A, and Pedersen PL (2001) Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. *J Biol Chem* **276**:43407–43412.
- Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, Hankinson O, Pugh CW, and Ratcliffe PJ (1997) Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci USA* **94**:8104–8109.
- McClintock DS, Santore MT, Lee VY, Brunelle J, Budinger GR, Zong WX, Thompson CB, Hay N, and Chandel NS (2002) Bcl-2 family members and functional electron transport chain regulate oxygen deprivation-induced cell death. *Mol Cell Biol* **22**:94–104.
- Melillo G, Musso T, Sica A, Taylor LS, Cox GW, and Varesio L (1995) A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. *J Exp Med* **182**:1683–1693.
- Metzen E, Berchner-Pfannschmidt U, Stengel P, Marxsen JH, Stolze I, Klinger M, Huang WQ, Wotzlaw C, Hellwig-Burgel T, Jellmann W, et al. (2003a) Intracellular localization of human HIF-1 alpha hydroxylases: implications for oxygen sensing. *J Cell Sci* **116**:1319–1326.
- Metzen E, Zhou J, Jellmann W, Fandrey J, and Brune B (2003b) Nitric oxide impairs normoxic degradation of HIF-1alpha by inhibition of prolyl hydroxylases. *Mol Biol Cell* **14**:3470–3481.
- Min JH, Yang H, Ivan M, Gertler F, Kaelin WG Jr and Pavletich NP (2002) Structure of an HIF-1alpha-pVHL complex: hydroxyproline recognition in signaling. *Science (Wash DC)* **296**:1886–1889.
- Minchenko A, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead V, and Caro J (2002) Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. *J Biol Chem* **277**:6183–6187.
- Minet E, Arnold T, Michel G, Roland I, Mottet D, Raes M, Remacle J, and Michiels C (2000) ERK activation upon hypoxia: involvement in HIF-1 activation. *FEBS Lett* **468**:53–58.
- Minet E, Michel G, Mottet D, Raes M, and Michiels C (2001) Transduction pathways involved in Hypoxia-Inducible Factor-1 phosphorylation and activation. *Free Radic Biol Med* **31**:847–855.
- Mukhopadhyay CK, Mazumder B, and Fox PL (2000) Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. *J Biol Chem* **275**:21048–21054.
- Nakayama K, and Ronai Z (2004) Siah: new players in the cellular response to hypoxia. *Cell Cycle* **3**:1345–1347.
- Neufeld G, Cohen T, Gengrinovitch S, and Poltorak Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* **13**:9–22.
- Nguyen SV and Claycomb WC (1999) Hypoxia regulates the expression of the adrenomedullin and HIF-1 genes in cultured HL-1 cardiomyocytes. *Biochem Biophys Res Commun* **265**:382–386.
- O'Rourke JF, Pugh CW, Bartlett SM, and Ratcliffe PJ (1996) Identification of hypoxically inducible mRNAs in HeLa cells using differential-display PCR. Role of hypoxia-inducible factor-1. *Eur J Biochem* **241**:403–410.
- Oehme F, Ellinghaus P, Kolkhof P, Smith TJ, Ramakrishnan S, Hutter J, Schramm M, and Flamme I (2002) Overexpression of PH-4, a novel putative proline 4-hydroxylase, modulates activity of hypoxia-inducible transcription factors. *Biochem Biophys Res Commun* **296**:343–349.
- Ozaki H, Yu AY, Della N, Ozaki K, Luna JD, Yamada H, Hackett SF, Okamoto N, Zack DJ, Semenza GL, et al. (1999) Hypoxia inducible factor-1alpha is increased in ischemic retina: temporal and spatial correlation with VEGF expression. *Investig Ophthalmol Vis Sci* **40**:182–189.
- Peng J, Zhang L, Drysdale L, and Fong GH (2000) The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. *Proc Natl Acad Sci USA* **97**:8386–8391.
- Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, and Ratcliffe PJ (1997) Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. *J Biol Chem* **272**:11205–11214.
- Rajakumar A, Doty K, Daftary A, Harger G, and Conrad KP (2003) Impaired oxygen-dependent reduction of HIF-1alpha and -2alpha proteins in pre-eclamptic placenta. *Placenta* **24**:199–208.
- Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, and Melillo G (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res* **62**:4316–4324.
- Ravi R, Mookerjee B, Bhujwala ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL, and Bedi A (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev* **14**:34–44.
- Reyes H, Reisz-Porszasz S, and Hankinson O (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science (Wash DC)* **256**:1193–1195.
- Richard DE, Berra E, Gothie E, Roux D, and Pouyssegur J (1999) p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. *J Biol Chem* **274**:32631–32637.
- Richard DE, Berra E, and Pouyssegur J (2000) Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. *J Biol Chem* **275**:26765–26771.
- Rolfs A, Kvietikova I, Gassmann M, and Wenger RH (1997) Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. *J Biol Chem* **272**:20055–20062.

- Ruas JL, Poellinger L, and Pereira T (2002) Functional analysis of hypoxia-inducible factor-1 alpha-mediated transactivation. Identification of amino acid residues critical for transcriptional activation and/or interaction with CREB-binding protein. *J Biol Chem* **277**:38723–38730.
- Ryan HE, Lo J, and Johnson RS (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO (Eur Mol Biol Organ) J* **17**:3005–3015.
- Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, and Johnson RS (2000) Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res* **60**:4010–4015.
- Salceda S, and Caro J (1997) Hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem* **272**:22642–22647.
- Salnikow K, Blagosklonny MV, Ryan H, Johnson R, and Costa M (2000) Carcinogenic nickel induces genes involved with hypoxic stress. *Cancer Res* **60**:38–41.
- Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, and Kasprzak KS (2004) Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. *J Biol Chem* **279**:40337–40344.
- Sang N, Fang J, Srinivas V, Leshchinsky I, and Caro J (2002) Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel-Lindau protein-independent, hydroxylation-regulated association with p300/CBP. *Mol Cell Biol* **22**:2984–2992.
- Scheid A, Wenger RH, Schaffer L, Camenisch I, Distler O, Ferenc A, Cristina H, Ryan HE, Johnson RS, Wagner KF, et al. (2002) Physiologically low oxygen concentrations in fetal skin regulate hypoxia-inducible factor 1 and transforming growth factor-beta3. *FASEB J* **16**:411–413.
- Schoenfeld A, Davidowitz EJ, and Burk RD (1998) A second major native von Hippel-Lindau gene product, initiated from an internal translation start site, functions as a tumor suppressor. *Proc Natl Acad Sci USA* **95**:8817–8822.
- Schofield CJ and Zhang Z (1999) Structural and mechanistic studies on 2-oxoglutarate-dependent oxygenases and related enzymes. *Curr Opin Struct Biol* **9**:722–731.
- Schuler M and Green DR (2001) Mechanisms of p53-dependent apoptosis. *Biochem Soc Trans* **29**:684–688.
- Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, and Johnson RS (2001) Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. *Mol Cell Biol* **21**:3436–3444.
- Semenza GL (1998) Hypoxia-inducible factor 1: master regulator of O<sub>2</sub> homeostasis. *Curr Opin Genet Dev* **8**:588–594.
- Semenza GL (2002) Involvement of hypoxia-inducible factor 1 in human cancer. *Intern Med* **41**:79–83.
- Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* **3**:721–732.
- Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, and Giallongo A (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* **271**:32529–32537.
- Semenza GL, Neufeldt MK, Chi SM, and Antonarakis SE (1991) Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc Natl Acad Sci USA* **88**:5680–5684.
- Semenza GL, Roth PH, Fang HM, and Wang GL (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* **269**:23757–23763.
- Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri EA, and Gutkind JS (2000) The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. *Cancer Res* **60**:4873–4880.
- Srinivas V, Zhang LP, Zhu XH, and Caro J (1999) Characterization of an oxygen/redox-dependent degradation domain of hypoxia-inducible factor alpha (HIF-alpha) proteins. *Biochem Biophys Res Commun* **260**:557–561.
- Stiehl DP, Jelkmann W, Wenger RH, and Hellwig-Burgel T (2002) Normoxic induction of the hypoxia-inducible factor 1alpha by insulin and interleukin-1beta involves the phosphatidylinositol 3-kinase pathway. *FEBS Lett* **512**:157–162.
- Suzuki H, Tomida A, and Tsuruo T (2001) Dephosphorylated hypoxia-inducible factor 1alpha as a mediator of p53-dependent apoptosis during hypoxia. *Oncogene* **20**:5779–5788.
- Tacchini L, Bianchi L, Bernelli-Zazzera A, and Cairo G (1999) Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. *J Biol Chem* **274**:24142–24146.
- Takahashi Y, Takahashi S, Shiga Y, Yoshimi T, and Miura T (2000) Hypoxic induction of prolyl 4-hydroxylase  $\alpha$  (I) in cultured cells. *J Biol Chem* **275**:14139–14146.
- Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, and Harris AL (2000) The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* **157**:411–421.
- Tanimoto K, Makino Y, Pereira T, and Poellinger L (2000) Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. *EMBO (Eur Mol Biol Organ) J* **19**:4298–4309.
- Temes E, Martin-Puig S, Acosta-Iborra B, Castellanos MC, Feijoo-Cuaresma M, Olmos G, Aragones J, and Landazuri MO (2005) Activation of HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase. *J Biol Chem* **280**:24238–24244.
- Tian H, Hammer RE, Matsumoto AM, Russell DW, and McKnight SL (1998) The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev* **12**:3320–3324.
- Tian H, McKnight SL, and Russell DW (1997) Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev* **11**:72–82.
- Vincent KA, Shyu KG, Luo Y, Magner M, Tio RA, Jiang C, Goldberg MA, Akita GY, Gregory RJ, and Isner JM (2000) Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1alpha/VP16 hybrid transcription factor. *Circulation* **102**:2255–2261.
- Volm M and Koomagi R (2000) Hypoxia-inducible factor (HIF-1) and its relationship to apoptosis and proliferation in lung cancer. *Anticancer Res* **20**:1527–1533.
- Wang GL, Jiang BH, Rue EA, and Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O<sub>2</sub> tension. *Proc Natl Acad Sci USA* **92**:5510–5514.
- Wang GL, and Semenza GL (1993) Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: implications for models of hypoxia signal transduction. *Blood* **82**:3610–3615.
- Wenger RH (2002) Cellular adaptation to hypoxia: O<sub>2</sub>-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O<sub>2</sub>-regulated gene expression. *FASEB J* **16**:1151–1162.
- Whiteway M and Szostak JW (1985) The ARD1 gene of yeast functions in the switch between the mitotic cell cycle and alternative developmental pathways. *Cell* **43**:483–492.
- Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, Wood SM, Gatter KC, Harris AL, Pugh CW, et al. (1998) Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. *Blood* **92**:2260–2268.
- Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, et al. (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* **60**:7075–7083.
- Yasinska IM and Sumbayev VV (2003) S-nitrosation of Cys-800 of HIF-1alpha protein activates its interaction with p300 and stimulates its transcriptional activity. *FEBS Lett* **549**:105–109.
- Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, et al. (1999) Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest* **103**:691–696.
- Yuan Y, Hilliard G, Ferguson T, and Millhorn DE (2003) Cobalt inhibits the interaction between hypoxia-inducible factor- $\alpha$  and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor- $\alpha$ . *J Biol Chem* **278**:15911–15916.
- Yun Z, Maecker HL, Johnson RS, and Giaccia AJ (2002) Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. *Dev Cell* **2**:331–341.
- Zelzer E, Levy Y, Kahana C, Shilo BZ, Rubinstein M, and Cohen B (1998) Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *EMBO (Eur Mol Biol Organ) J* **17**:5085–5094.
- Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, and Simons JW (1999) Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res* **59**:5830–5835.
- Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, et al. (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* **14**:391–396.

**Address correspondence to:** Dr. Max Costa, Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987. E-mail: costam01@nyu.edu