

MOL #27029

Hypoxia-inducible Factor - 1 (HIF-1)

Qingdong Ke, Max Costa*

Nelson Institute of Environmental Medicine, New York University

School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987

MOL #27029

Running title: HIF-1, a new target for therapy

***Corresponding author: Max Costa**

Corresponding author mail address:

Dr. Max Costa

Nelson Institute of Environmental Medicine,

New York University School of Medicine,

57 Old Forge Road, Tuxedo, NY 10987

Tel: 845-731-3515

Fax: 845-351-2118

E-mail: costam01@nyu.edu

Number of text pages: 24 (main text) + 14 (references)

Number of tables: 2

Number of figures: 2

Number of references: 183

Number of words in the *Abstract*: 166

Number of words in the *Introduction*: 124

Number of words in the *Discussion*: 116

Abbreviations: HIF-1, hypoxia-inducible factor 1; HRE, hypoxia response element; EPO, erythropoietin; ODDD, oxygen-dependent degradation domain; IPAS, inhibitory PAS; pVHL, von Hippel-Lindau; 2-OG, 2-oxoglutarate; PHD, prolyl hydroxylase domain; ARD1, arrest-defective-1; FIH-1, factor inhibiting HIF-1; MAPK, mitogen-activated protein kinase; VEGF, vascular endothelial cell growth factor.

MOL #27029

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 β and an oxygen-regulated subunit HIF-1 α (or its paralogs HIF-2 α and HIF-3 α). The stability and activity of the α subunit of HIF are regulated by its posttranslational modifications such as hydroxylation, ubiquitination, acetylation, and phosphorylation. In normoxia, hydroxylation of two proline residues and acetylation of a lysine residue at the oxygen-dependent degradation domain (ODDD) of HIF-1 α trigger its association with pVHL E3 ligase complex, leading to HIF-1 α degradation via ubiquitin-proteasome pathway. In hypoxia, the HIF-1 α subunit becomes stable and interacts with co-activators such as CBP/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.

MOL #27029

Contents

Introduction

1. The discovery of HIF-1

1.1. HIF-1 α

1.2. HIF-2 α

1.3. HIF-3 α

2. The regulation of HIF-1

2.1. Prolyl hydroxylation by PHDs --- signaling for polyubiquitination

2.2. Polyubiquitination by pVHL --- signaling for degradation

2.3. Lysine Acetylation by ARD1 --- facilitating pVHL binding

2.4. Asparagine hydroxylation by FIH-1 --- preventing CBP/p300 binding

2.5. Phosphorylation by MAPK --- enhancing transactivation

3. The target gene of HIF-1

3.1. Erythropoiesis/iron metabolism

3.2. Angiogenesis

3.3. Glucose metabolism

3.4. Cell proliferation/survival

3.5. Apoptosis

4. The role of HIF-1 in development and disease

4.1. Development

4.2. Cancer

4.3. Ischemic disease

5. The implication of HIF-1 in therapy

MOL #27029

5.1. Cancer therapy --- inhibiting HIF-1 activity

5.2. Ischaemic disease therapy --- promoting HIF-1 activity

Conclusion

MOL #27029

Introduction

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% O₂) to hypoxia (~1% O₂) (Semenza, 1998; Wang et al., 1995). Since its identification two decades ago, what we know about HIF has grown exponentially. Due to the realization that hypoxia has a strong impact, via gene expression, on cell biology and mammalian physiology, there has been enormous growing interests in the biology of the HIF-1 pathway and its role in human diseases such as cancer. Accordingly, 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.

1. The discovery of HIF-1

1.1. HIF-1 α

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 α and a constitutively expressed subunit HIF-1 β (Wang et al., 1995). HIF-1 β is also known as ARNT, the aryl hydrocarbon nuclear translocator, which was originally identified as a binding partner of the aryl hydrocarbon receptor (AhR) (Reyes et al., 1992); whereas HIF-1 α was newly discovered. These proteins belong to the basic-Helix-Loop-Helix-Per-ARNT-Sim

MOL #27029

(bHLH-PAS) protein family (figure 1) (Wang et al., 1995). The bHLH and PAS motifs are required for heterodimer formation between the HIF-1 α and HIF-1 β subunits while the downstream basic region affords specific binding 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 α protein have been identified later (Ruas et al., 2002). The C-TAD in particular has been shown to interact with co-activators such as CBP/p300 to activate gene transcription (Lando et al., 2002b). HIF-1 α also contains an oxygen-dependent degradation domain (ODDD) that mediates oxygen-regulated stability (Pugh et al., 1997). Later work has revealed that HIF-1 α is ubiquitously expressed in human and mouse tissues and has a general role in multiple physiological responses to hypoxia, such as erythropoiesis and glycolysis that quickly counteract oxygen deficiency; and angiogenesis which provides a long-term solution (Semenza, 1998).

1.2. HIF-2 α

Shortly after the cloning of HIF-1 α , a closely related protein, HIF-2 α (also termed endothelial Per-ARNT-Sim (PAS) protein (EPAS), 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 α shares 48% amino acid sequence identity with HIF-1 α and accordingly shares a number of structural and biochemical similarities with HIF-1 α , for instance, heterodimerization with HIF-1 β and binding HREs. In contrast to ubiquitously expressed HIF-1 α , though, HIF-2 α is predominantly expressed in the lung, endothelium, and carotid body (Ema et al., 1997; Tian et al., 1998; Tian et al., 1997).

MOL #27029

1.3 HIF-3 α

HIF-3 α , which was discovered later, is also expressed in a variety of tissues, dimerizes with HIF-1 β , and binds to HREs (Gu et al., 1998). Additionally, a splice variant of HIF-3 α , 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, but rather interacts with the amino-terminal region of HIF-1 α 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 feed-back loop for HIF-1 activity in these tissues (Makino et al., 2002).

HIF-1 α and HIF-2 α have been more extensively studied, whereas research on HIF-3 α and other HIF isoforms is relatively scarce.

2. The regulation of HIF-1

While HIF-1 β is constitutively expressed and its mRNA and protein are maintained at constant levels regardless of oxygen availability (Kallio et al., 1997), HIF-1 α protein has a short half-life ($t_{1/2} \sim 5$ min) and is highly regulated by oxygen (Salceda and Caro, 1997) (figure 2). The transcription and synthesis of HIF-1 α are constitutive and seemly not to be affected by oxygen (Kallio et al., 1997; Wang et al., 1995; Wiesener et al., 1998). However, in normoxia, the HIF-1 α proteins are rapidly degraded, resulting in essentially no detectable HIF-1 α protein (Wang et al., 1995). During hypoxia, HIF-1 α becomes stabilized and translocates from the cytoplasm to the nucleus, where it dimerizes with HIF-1 β 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

MOL #27029

regulatory regions of target genes and binds the transcriptional co-activators to induce gene expression (Lando et al., 2002b). Tight regulation of the stability and subsequent transactivational function of HIF-1 α are chiefly controlled by its posttranslational modifications such as hydroxylation, ubiquitination, acetylation, and phosphorylation (Brahimi-Horn et al., 2005).

The modification of HIF-1 α 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 α with the von Hippel-Lindau (pVHL) ubiquitin E3 ligase complex (Masson et al., 2001; Srinivas et al., 1999). PVHL complex tags HIF-1 α 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 α with CBP/p300 and thus inhibits its transcriptional activity (Lando et al., 2002a).

2.1. Prolyl hydroxylation by PHDs --- signaling for polyubiquitination

De novo synthesized cytoplasmic HIF-1 α is rapidly hydroxylated by a family of 2-oxoglutarate (2-OG)-dependent dioxygenases on proline 402 (P402) and 564 (P564) located within ODDD (Masson and Ratcliffe, 2003; Masson et al., 2001; Srinivas et al., 1999). The proline residues are conserved in HIF-2 α (P405 and P530) and HIF-3 α . 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 α , which has the sequence LXXLHP (Bruick and McKnight, 2001; Masson et al., 2001). Mutation of both proline residues disrupted the interaction of HIF-1 α with pVHL and increased its stability in the presence of normal oxygen levels, whereas mutation of either proline alone only partially stabilized HIF-1 α (Masson et al., 2001).

MOL #27029

Human HIF- α 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; 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 though are not able to catalyze HIF-1 α /HIF-2 α proline hydroxylation (Jaakkola et al., 2001). The hydroxylation process splits O_2 , with one oxygen atom being transferred to the proline residue and the other reacting with 2-OG to generate succinate and CO_2 (Bruick and McKnight, 2001; Masson and Ratcliffe, 2003). Inactivation of the PHDs by 2-OG analogues can increase the half-life of HIF-1 α (Ivan et al., 2002; Jaakkola et al., 2001). 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 α , 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- α *in vitro* with their relative activities as PHD2>>PHD3>PHD1, and PHD2 was shown to be the key limiting enzyme that controls the HIF-1 α turnover *in vivo* (Berra et al., 2003; Huang et al., 2002).

MOL #27029

Knockdown of PHD2 by its specific small-interfering RNA (siRNA) is sufficient to stabilize HIF-1 α levels under normoxia, whereas siRNA silencing of either PHD1 or PHD3 is unable to produce similar effects on HIF-1 α (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 upregulated as well, while that of PHD1 remained unaffected (Epstein et al., 2001; Metzen et al., 2003a). This may represent a way by which HIF-1 α self-regulates its expression. Furthermore, Siah1 and 2, the specific E3 ligases of PHD1 and 3, are transcriptionally upregulated 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, while PHD1 localized in the nucleus and PHD3 was in both compartments (Metzen et al., 2003a). Despite its primary localization in the cytoplasm, PHD2 is able to shuttle between the cytoplasm and the nucleus, thereby contribute to HIF-1 α 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 α and PHDs, therein, promoting the O₂-dependent degradation of HIF-1 α (Baek et al., 2005). Additionally,

MOL #27029

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 α , constituting a recognition signal for binding of pVHL and subsequent ubiquitination, followed by degradation of HIF-1 α (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 α stabilization and accumulation in the cell. The absolute requirement of oxygen as a co-substrate suggests PHDs as the oxygen sensor in cells (Epstein et al., 2001).

2.2. Polyubiquitination by pVHL --- signaling for degradation

Once the two proline residues of HIF-1 α are converted to hydroxyproline, pVHL then captures HIF-1 α . X-ray crystallographic studies of the pVHL/ HIF-1 α 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 α to this multiprotein E3 complex causes polyubiquitination of HIF-1 α , ultimately leading to its degradation by the proteasome (Kamura et al., 2000). However, the exact lysine residue(s) that is(are) ubiquitinated have not as 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

MOL #27029

retina and central nervous system (Ivan and Kaelin, 2001). The *VHL* gene encodes a full length (213 amino acids) and an N-terminal truncated protein (amino acids 54–213). Since both proteins show similar functions, they are often collectively referred to as pVHL. Mutations in the *VHL* gene that 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 α and HIF-2 α 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- α 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- α 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 α 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 α . In addition to pVHL, a number of other proteins have been reported to affect HIF-1 α 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 α in a p53-dependent fashion (Ravi et al., 2000). The protein Jab1, a transcriptional co-activator of c-Jun and Jun D, has also been shown to increase HIF-1 α

MOL #27029

levels under hypoxia, probably by competing with p53 for binding to HIF-1 α (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 α stability.

2.3. Lysine Acetylation by ARD1 --- facilitating pVHL binding

Lysine residue 532 (K532) located in the ODDD domain of HIF-1 α 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 K532 favors the interaction of HIF-1 α with pVHL, and thus destabilizes HIF-1 α (Jeong et al., 2002). Mutation of K532 to arginine resulted in increased stability of HIF-1 α (Tanimoto et al., 2000). In addition, artificial maintenance or increase of the acetylated state of HIF-1 α by butyric acid, a global inhibitor of deacetylases, caused decreased HIF-1 α protein levels (Kim et al., 2001).

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

2.4. Asparagine hydroxylation by FIH-1 --- preventing CBP/p300 binding

The posttranslational modifications of HIF-1 α described above regulate the stabilization of HIF-1 α protein. But the stabilization alone is not sufficient for full transcriptional activation of HIF-1. The second major mechanism controlling HIF activity

MOL #27029

is through the modulation of its transactivation domains N-TAD and C-TAD. These domains function by recruiting transcriptional co-activators such as CBP/p300, SRC-1, and TIF2 (Arany et al., 1996; Carrero et al., 2000; Ebert and Bunn, 1998; Ema et al., 1999; Kallio et al., 1998). Under normal oxygen tension, hydroxylation of the asparagines residue 803 (N803) in the C-TAD of HIF-1 α (N851 in HIF-2 α) by Factor Inhibiting HIF-1 (FIH-1) prevented the interaction of HIF-1 α 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 α to efficiently interact with CBP/p300, activating the transcription of the respective target genes (Lando et al., 2002a). Replacement of N803 with alanine permitted co-activator binding with HIF-1 α 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 α (Mahon et al., 2001). Although interaction with pVHL was not required for FIH-1 activity, histone deacetylases (HDACs) recruited by pVHL interfered with the transcription processes, therein, facilitating FIH-1 to modulate HIF-1 α transactivation (Hewitson et al., 2002; Sang et al., 2002). FIH-1 is mainly located in the cytoplasm, with some fraction likely residing 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 α stability (Metzen et al., 2003a).

Like the PHDs, the asparaginyl hydroxylase FIH-1 is a 2-OG-dependent dioxygenase which also requires Fe²⁺ and ascorbate as cofactors (Lando et al., 2002a). Utilization of oxygen as a substrate allows FIH-1 to serve as a second oxygen sensor.

2.5. Phosphorylation by MAPK --- enhancing transactivation

MOL #27029

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. It has been well-known that phosphorylation is crucial in controlling protein activities. Direct phosphorylation of HIF-1 α has been reported and the Mitogen-Activated Protein Kinase (MAPK) pathway appears to play a role (Minet et al., 2001; Richard et al., 1999; Sodhi et al., 2000). It has been shown that p42/44 and p38 kinase phosphorylated HIF-1 α /HIF-2 α in vitro (Richard et al., 1999; Sodhi et al., 2000). Additionally, inhibitors of p42/44 and p38 blocked HIF-1 α -mediated reporter gene expression (Hur et al., 2001). Transfection with active forms of p42/44 kinase stimulated HIF-1 α transcription activity without affecting HIF-1 α stability. Moreover, HIF-1 α /HIF-2 α transactivation during hypoxia required p42/44 MAPKs (Conrad et al., 1999; Hofer et al., 2001; Hur et al., 2001; Minet et al., 2000). It seems that phosphorylation does not affect stability or DNA binding of HIF-1 α , but rather increases the transcriptional activity of HIF-1 (Richard et al., 1999). One explanation could be that HIF-1 β binds preferentially to the phosphorylated form of HIF-1 α (Suzuki et al., 2001). Although the functionally relevant phosphorylation sites remain to be identified, threonine 796 in HIF-1 α and 844 in HIF-2 α are candidate sites (Gradin et al., 2002).

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

MOL #27029

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 non-hypoxic condition (table 1) (Feldser et al., 1999; Gorlach et al., 2001; Haddad and Land, 2001; Hellwig-Burgel et al., 1999; Li et al., 2004; Richard et al., 2000; Salnikow et al., 2000; Stiehl et al., 2002). Although complex and cell-type dependent, some have been shown to stimulate HIF-1 α transactivation or synthesis by activation of the MAPK or the phosphatidylinositol 3-kinase (PI3K) signaling pathways (Li et al., 2004; Zelzer et al., 1998).

3. 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 one hundred 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).

3.1. Erythropoiesis/iron metabolism

In response to hypoxia, the capacity of red blood cells to transport oxygen is upregulated 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

MOL #27029

erythropoietic rate-limiting step of heme production. Hypoxia upregulates transferrin (Tf) which transports Fe^{3+} into cells (Rolfes 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 (Fe^{2+}) to ferric (Fe^{3+}) iron (Lok and Ponka, 1999; Mukhopadhyay et al., 2000). Increasing of these genes supports iron supply to erythroid tissues (Rolfes et al., 1997).

3.2 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 (Berra et al., 2000; Bunn and Poyton, 1996; Forsythe et al., 1996; Giordano and Johnson, 2001; Levy et al., 1995; 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 (Conway et al., 2001; Josko et al., 2000; Neufeld et al., 1999). Therefore, the induction of VEGF and various other pro-angiogenic 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 α_{1B} -adrenergic receptor (Eckhart et al., 1997). Moreover, hypoxia induces genes involved in matrix metabolism and vessel maturation such as

MOL #27029

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).

3.3 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 upregulating 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 α accumulation under normoxia and regulate hypoxia-inducible gene expression, hence establishing a potential positive feedback loop (Lu et al., 2002).

3.4. Cell proliferation/survival

Hypoxia and HIF-1 induce growth factors such as insulin-like growth factor-2 (IGF2) and transforming growth factor- α (TGF- α) (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 α itself (Semenza, 2003). As mentioned above, cytokines, growth factors, as well as hypoxia in some cell types, can activate signaling pathways MAPK

MOL #27029

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- α , therefore, contributing to autocrine-signaling pathways that are crucial for cancer progression (Semenza, 2003).

3.5. Apoptosis

Paradoxically, cell adaptation to hypoxia not only leads 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 α showed decreased apoptosis, compared to 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 α and HIF-1 β significantly correlated with apoptosis and the pro-apoptotic factors such as caspase-3, Fas, and Fas ligand (Volm and Koomagi, 2000). Moreover, hypoxia depressed the anti-apoptotic protein Bcl-2 (Carmeliet et al., 1998), while the pro-apoptotic protein Bcl-2/adenovirus E1B 19kD-interacting protein 3 (BNip3) and its homologue Nip3-like protein X (NIX) were upregulated 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).

MOL #27029

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), carotide 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 α in the VEGF induction (Akeno et al., 2001; Compernelle et al., 2002), no *bone fide* target genes have yet been identified for HIF-2 α or HIF-3 α . However, a recent study using a genetic "knock-in" strategy has shown that targeted replacement of HIF-1 α with HIF-2 α results in expanded expression of HIF-2 α -specific target genes (*i.e.* Oct-4, a transcription factor essential for maintaining stem cell pluripotency) (Covello et al., 2006).

4. 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 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 α and HIF-2 α have been strongly implicated in tumor progression and grade, hence conferring a selective advantage to tumor cells.

4.1. Development

Components of the HIF-1 system play essential roles in embryonic development. Knockout of either HIF-1 α (Iyer et al., 1998; Kotch et al., 1999; Ryan et al., 1998), HIF-2 α (Peng et al., 2000; Tian et al., 1998), or HIF-1 β (Maltepe et al., 1997) resulted in abnormal vascular development and lethality in mice. HIF-1 α expression increased

MOL #27029

between embryonic day 8.5 and 9.5 (E8.5 and E9.5) in normal mouse embryos (Iyer et al., 1998). Embryos deficient in *HIF-1 α* (*HIF-1 α ^{-/-}*) died by E11 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 α ^{-/-}* cells, compared to those of wild-type cells (Iyer et al., 1998). Although heterozygous mice carrying a single *HIF-1 α* gene (*HIF1 α ^{+/-}*) developed normally, they displayed impaired physiological responses, when challenged by chronic hypoxia (Kline et al., 2002; Yu et al., 1999).

Targeted inactivation of *HIF-2 α* (*HIF-2 α ^{-/-}*) in mice resulted in rather different and variable phenotypes. *HIF-2 α ^{-/-}* mouse embryos died by E12.5-E16.5 due to inadequate blood vessel fusion and remodeling, impaired fetal lung maturation, a very slow heart rate because of insufficient catecholamine production (Peng et al., 2000; Tian et al., 1998). Thus, it appears that *HIF-1 α* and *HIF-2 α* have non-redundant 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 β ^{-/-} embryos died by E10.5 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 β ^{-/-}* cells failed to activate genes that normally respond to hypoxia and low glucose concentration (Maltepe et al., 1997).

4.2. Cancer

MOL #27029

Overexpression of HIF-1 α and HIF-2 α was found in various human cancers, probably as a consequence of intratumoral hypoxia or genetic alteration (Talks et al., 2000; Zhong et al., 1999). The interior of the tumor mass becomes progressively hypoxic as its size increases until there are adequate blood vessels 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 α 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 (Harris, 2002; Zhong et al., 1999). Expression of HIF target genes is generally consistent with the levels of HIF-1 α . In addition, injection of HIF-1 α (or HIF-1 β) positive and deficient cells to immunocompromised mice revealed that HIF-1 α 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 α expression. As mentioned in VHL disease, for example, loss of function of VHL resulted in constitutively expressed HIF-1 α (Iliopoulos et al., 1996). In addition, loss of function of wild type p53, which is inactivated in most of human cancers, increased HIF-1 α 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 α 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 in part due to its induction of HIF and gain of function of v-SRC increased expression of HIF-1 α and HIF-dependent genes (Jiang et al., 1997). Moreover, enhanced HER2 receptor tyrosine kinase

MOL #27029

signaling has been shown to increase the rate of synthesis of HIF-1 α (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 appears that HIF-1 α 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).

4.3. Ischemic disease

Activation of HIF activity has also been demonstrated in a broad range physiological response 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 α and VEGF were increased in the myocardium when patients develop acute coronary artery occlusion (Lee et al., 2000). Effective vascular remodeling following ischemic injury depended on an integrated program of HIF-dependent gene expression. Increasing of HIF-1 α expression and HIF-inducible genes was also observed in sheep and rat models of myocardial and cerebral ischaemia (Bergeron et al., 1999; Martin et al., 1998). In addition, induction of HIF-1 α or HIF-2 α 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 (Grimm et al., 2002; Ozaki et al., 1999), as well as from wound healing (Elson et al., 2000).

5. 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.

MOL #27029

The many aspects of HIF-1 regulation provide a variety of possibilities for therapeutic intervention.

5.1. Cancer therapy --- inhibiting HIF-1 activity

First, immunohistochemical analysis of HIF-1 α expression in tumor biopsies may provide the prognostic information, and thereby, identify subsets of patients requiring aggressive therapy (Harris, 2002). In order to achieve high specificity to hypoxic tumor regions or cells, HREs from hypoxia-responsive genes can be used to express marker therapeutic genes that 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 α 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 α 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 α function and have anti-angiogenic effects (Harris, 2002). These agents include Herceptin, Iressa, Herbinycline (inhibitors of tyrosine kinase); calphostin C (inhibitor a protein kinase c, PKC); wortmannin and LY294002 (inhibitors of PI3K); PD98095 (inhibitor of a MAPK); rapamycin (inhibitor of a 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

MOL #27029

also been identified and shown to decrease HIF-1 α levels, inhibit the expression of VEGF and other HIF-1 target genes, impair xenograft growth and vascularization, as well as inhibit angiogenesis (Rapisarda et al., 2002). These molecules include topoisomerase I inhibitors, YC-1 (3-(5'-hydroxy-methyl-2'-furyl)-1-benzylindazole), 17-AAG (17-allyl-aminogeldanamycin, inhibitor of HSP90); inhibitors of the redox regulator thioredoxin-1; and the newly identified α -methoxyestradiol (2ME2) that disrupts tumor microtubules (MTs).

5.2 Ischaemic 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 ischaemic diseases (Elson et al., 2001; Vincent et al., 2000). Ischaemic 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 α or HIF-2 α 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 α 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 α 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 N- or C-terminal of ODDD polypeptides that block VHL-mediated degradation (Maranchie et al., 2002). Targeting of

MOL #27029

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, there are many important questions that yet remain to be answered. For example, the distinct role of enzymes modifying HIF-1 α posttranslationally, the interplay among the HIF-1 α posttranslational modifications, the identity of additional target genes of HIF-1, the function of the paralogs of HIF-1 α such as HIF-2 α and HIF-3 α , 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. Unravel of such questions should provide new insights into cellular adaptation to hypoxia and aid to discover new therapeutic approaches to diverse human diseases.

MOL #27029

References

- Akeno N, Czyzyk-Krzeska 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-2alpha. *Endocrinology* **142**(2):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**(37):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 U S A* **93**(23):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-1alpha and regulates its stability. *J Biol Chem* **277**(1):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 1alpha and prolyl hydroxylases to promote oxygen-dependent degradation of HIF-1alpha. *Mol Cell* **17**(4):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**(7):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**(12):4159-4170.
- Berra E, Benizri E, Ginouves 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-1alpha in normoxia. *Embo J* **22**(16):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**(8):1171-1178.
- Berra E, Roux D, Richard DE and Pouyssegur J (2001) Hypoxia-inducible factor-1 alpha (HIF-1 alpha) escapes O(2)-driven proteasomal degradation irrespective of its subcellular localization: nucleus or cytoplasm. *EMBO Rep* **2**(7):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**(21):4223-4227.
- Brahimi-Horn C, Mazure N and Pouyssegur J (2005) Signalling via the hypoxia-inducible factor-1alpha requires multiple posttranslational modifications. *Cell Signal* **17**(1):1-9.
- Bruick RK (2000) Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. *Proc Natl Acad Sci U S A* **97**(16):9082-9087.
- Bruick RK and McKnight SL (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **294**(5545):1337-1340.
- Brunelle JK and Chandel NS (2002) Oxygen deprivation induced cell death: an update. *Apoptosis* **7**(6):475-482.

MOL #27029

- Bunn HF and Poyton RO (1996) Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* **76**(3):839-885.
- Callapina M, Zhou J, Schnitzer S, Metzen E, Lohr C, Deitmer JW and Brune B (2005) Nitric oxide reverses desferrioxamine- and hypoxia-evoked HIF-1alpha accumulation--implications for prolyl hydroxylase activity and iron. *Exp Cell Res* **306**(1):274-284.
- Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D and Keshet E (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* **394**(6692):485-490.
- Carrero P, Okamoto K, Coumailleau 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 1alpha. *Mol Cell Biol* **20**(1):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**(12):9519-9525.
- Chen J, Zhao S, Nakada K, Kuge Y, Tamaki N, Okada F, Wang J, Shindo M, Higashino F, Takeda K, Asaka M, Katoh H, Sugiyama T, Hosokawa M and Kobayashi M (2003) Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. *Am J Pathol* **162**(4):1283-1291.
- Compernelle V, Brusselmans K, Acker T, Hoet P, Tjwa M, Beck H, Plaisance S, Dor Y, Keshet E, Lupu F, Nemery B, Dewerchin M, Van Veldhoven P, Plate K, Moons L, Collen D and Carmeliet P (2002) Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med* **8**(7):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**(47):33709-33713.
- Conway EM, Collen D and Carmeliet P (2001) Molecular mechanisms of blood vessel growth. *Cardiovasc Res* **49**(3):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**(28):17787-17792.
- Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC and Keith B (2006) HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev* **20**(5):557-570.
- Crews ST (1998) Control of cell lineage-specific development and transcription by bHLH-PAS proteins. *Genes Dev* **12**(5):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**(5):515-520.
- Dang CV and Semenza GL (1999) Oncogenic alterations of metabolism. *Trends Biochem Sci* **24**(2):68-72.

MOL #27029

- Davidson T, Chen H, Garrick MD, D'Angelo G and Costa M (2005) Soluble nickel interferes with cellular iron homeostasis. *Mol Cell Biochem* **279**(1-2):157-162.
- Duynham 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**(9):6885-6895.
- Duynham MC, Hulscher TM, Fontijn D, Pinedo HM and Boven E (2001) Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1alpha protein by the oxidative stressor arsenite. *J Biol Chem* **276**(51):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**(7):4089-4096.
- Eckhart AD, Yang N, Xin X and Faber JE (1997) Characterization of the alpha1B-adrenergic receptor gene promoter region and hypoxia regulatory elements in vascular smooth muscle. *Proc Natl Acad Sci U S A* **94**(17):9487-9492.
- Elson DA, Ryan HE, Snow JW, Johnson R and Arbeit JM (2000) Coordinate up-regulation of hypoxia inducible factor (HIF)-1alpha and HIF-1 target genes during multi-stage epidermal carcinogenesis and wound healing. *Cancer Res* **60**(21):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-1alpha. *Genes Dev* **15**(19):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 HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *Embo J* **18**(7):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 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci U S A* **94**(9):4273-4278.
- Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ and Ratcliffe PJ (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**(1):43-54.
- Feldser D, Agani F, Iyer NV, Pak B, Ferreira G and Semenza GL (1999) Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res* **59**(16):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**(1):51-60.

MOL #27029

- 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**(9):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**(35):31963-31971.
- Gao N, Shen L, Zhang Z, Leonard SS, He H, Zhang XG, Shi X and Jiang BH (2004) Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. *Mol Cell Biochem* **255**(1-2):33-45.
- Giordano FJ and Johnson RS (2001) Angiogenesis: the role of the microenvironment in flipping the switch. *Curr Opin Genet Dev* **11**(1):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* **242**(4884):1412-1415.
- Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, Kietzmann 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**(1):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**(26):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**(2-3):208-218.
- Grimm C, Wenzel A, Groszer M, Mayser 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**(7):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**(45):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**(15):5319-5336.
- Gu YZ, Moran SM, Hogenesch JB, Wartman L and Bradfield CA (1998) Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. *Gene Expr* **7**(3):205-213.
- Haddad JJ and Land SC (2001) A non-hypoxic, ROS-sensitive pathway mediates TNF-alpha-dependent regulation of HIF-1alpha. *FEBS Lett* **505**(2):269-274.
- Hagen T, Taylor CT, Lam F and Moncada S (2003) Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. *Science* **302**(5652):1975-1978.
- Harris AL (2002) Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer* **2**(1):38-47.

MOL #27029

- Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J and Jelkmann W (1999) Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. *Blood* **94**(5):1561-1567.
- Hellwig-Burgel T, Stiehl DP, Wagner AE, Metzen E and Jelkmann W (2005) Review: hypoxia-inducible factor-1 (HIF-1): a novel transcription factor in immune reactions. *J Interferon Cytokine Res* **25**(6):297-310.
- Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, Elkins JM, Oldham NJ, Bhattacharya S, Gleadle JM, Ratcliffe PJ, Pugh CW and Schofield CJ (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**(29):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**(40):41521-41528.
- Hofer T, Desbaillets I, Hopfl G, Gassmann M and Wenger RH (2001) Dissecting hypoxia-dependent and hypoxia-independent steps in the HIF-1alpha activation cascade: implications for HIF-1alpha gene therapy. *Faseb J* **15**(14):2715-2717.
- Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH and Bradfield 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**(13):8581-8593.
- Hollander AP, Corke KP, Freemont AJ and Lewis CE (2001) Expression of hypoxia-inducible factor 1alpha by macrophages in the rheumatoid synovium: implications for targeting of therapeutic genes to the inflamed joint. *Arthritis Rheum* **44**(7):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* **417**(6892):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**(3):894-899.
- Huang J, Zhao Q, Mooney SM and Lee FS (2002) Sequence determinants in hypoxia-inducible factor-1alpha for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. *J Biol Chem* **277**(42):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**(50):32253-32259.
- Hur E, Chang KY, Lee E, Lee SK and Park H (2001) Mitogen-activated protein kinase kinase inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of hypoxia-inducible factor-1alpha. *Mol Pharmacol* **59**(5):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 1alpha expression in DU145 cells. *Carcinogenesis* **25**(12):2497-2507.

MOL #27029

- 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 U S A* **93**(20):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 U S A* **95**(20):11661-11666.
- Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K, Yang H, Sorokina I, Conaway RC, Conaway JW and Kaelin WG, Jr. (2002) Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci U S A* **99**(21):13459-13464.
- Ivan M and Kaelin WG, Jr. (2001) The von Hippel-Lindau tumor suppressor protein. *Curr Opin Genet Dev* **11**(1):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) HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* **292**(5516):464-468.
- Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY and Semenza GL (1998) Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes Dev* **12**(2):149-162.
- Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW and Ratcliffe PJ (2001) Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* **292**(5516):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 α by ARD1-mediated acetylation. *Cell* **111**(5):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**(23):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**(5):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 α . *Embo J* **17**(22):6573-6586.
- Kallio PJ, Pongratz I, Gradin K, McGuire J and Poellinger L (1997) Activation of hypoxia-inducible factor 1 α : posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. *Proc Natl Acad Sci U S A* **94**(11):5667-5672.
- Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC and Conaway JW (2000) Activation of HIF1 α ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci U S A* **97**(19):10430-10435.

MOL #27029

- 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**(4):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**(1):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**(12):4177-4185.
- Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW and Kim KW (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat Med* **7**(4):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 U S A* **99**(2):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 U S A* **99**(4):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**(2):254-267.
- Krishnamachary B, Berg-Dixon S, Kelly B, Agani F, Feldser D, Ferreira G, Iyer N, LaRusch J, Pak B, Taghavi P and Semenza GL (2003) Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res* **63**(5):1138-1143.
- Kung AL, Wang S, Klco JM, Kaelin WG and Livingston DM (2000) Suppression of tumor growth through disruption of hypoxia-inducible transcription. *Nat Med* **6**(12):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**(12):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* **295**(5556):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**(12):3995-4004.
- LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, Frantz G, Rangell L, DeGuzman L, Keller GA, Peale F, Gurney A, Hillan KJ and Ferrara N (2001)

MOL #27029

- Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature* **412**(6850):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**(9):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**(9):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**(22):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**(1):94-101.
- Li J, Post M, Volk R, Gao Y, Li M, Metais C, Sato K, Tsai J, Aird W, Rosenberg RD, Hampton TG, Sellke F, Carmeliet P and Simons M (2000) PR39, a peptide regulator of angiogenesis. *Nat Med* **6**(1):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**(4):421-426.
- Lok CN and Ponka P (1999) Identification of a hypoxia response element in the transferrin receptor gene. *J Biol Chem* **274**(34):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**(26):23111-23115.
- Mahon PC, Hirota K and Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* **15**(20):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* **414**(6863):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-3 α locus. *J Biol Chem* **277**(36):32405-32408.
- Maltepe E, Schmidt JV, Baunoch D, Bradfield CA and Simon MC (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* **386**(6623):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**(2):659-669.
- Maranchie JK, Vasselli JR, Riss J, Bonifacino JS, Linehan WM and Klausner RD (2002) The contribution of VHL substrate binding and HIF1- α to the phenotype of VHL loss in renal cell carcinoma. *Cancer Cell* **1**(3):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

MOL #27029

- is associated with increased myocardial expression of vascular endothelial growth factor and hypoxia-inducible factor 1. *Am J Obstet Gynecol* **178**(3):527-534.
- Masson N and Ratcliffe PJ (2003) HIF prolyl and asparaginyl hydroxylases in the biological response to intracellular O₂ levels. *J Cell Sci* **116**(Pt 15):3041-3049.
- Masson N, Willam C, Maxwell PH, Pugh CW and Ratcliffe PJ (2001) Independent function of two destruction domains in hypoxia-inducible factor- α chains activated by prolyl hydroxylation. *Embo J* **20**(18):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**(46):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 U S A* **94**(15):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**(1):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**(6):1683-1693.
- Metzen E, Berchner-Pfannschmidt U, Stengel P, Marxsen JH, Stolze I, Klinger M, Huang WQ, Wotzlaw C, Hellwig-Burgel T, Jelkmann W, Acker H and Fandrey J (2003a) Intracellular localisation of human HIF-1 α hydroxylases: implications for oxygen sensing. *J Cell Sci* **116**(Pt 7):1319-1326.
- Metzen E, Zhou J, Jelkmann W, Fandrey J and Brune B (2003b) Nitric oxide impairs normoxic degradation of HIF-1 α by inhibition of prolyl hydroxylases. *Mol Biol Cell* **14**(8):3470-3481.
- Min JH, Yang H, Ivan M, Gertler F, Kaelin WG, Jr. and Pavletich NP (2002) Structure of an HIF-1 α -pVHL complex: hydroxyproline recognition in signaling. *Science* **296**(5574):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**(8):6183-6187.
- Minet E, Arnould 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**(1):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**(7):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**(28):21048-21054.
- Nakayama K and Ronai Z (2004) Siah: new players in the cellular response to hypoxia. *Cell Cycle* **3**(11):1345-1347.

MOL #27029

- Neufeld G, Cohen T, Gengrinovitch S and Poltorak Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. *Faseb J* **13**(1):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**(2):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**(2):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**(2):343-349.
- Ozaki H, Yu AY, Della N, Ozaki K, Luna JD, Yamada H, Hackett SF, Okamoto N, Zack DJ, Semenza GL and Campochiaro PA (1999) Hypoxia inducible factor-1alpha is increased in ischemic retina: temporal and spatial correlation with VEGF expression. *Invest Ophthalmol Vis Sci* **40**(1):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 U S A* **97**(15):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**(17):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 placentae. *Placenta* **24**(2-3):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**(15):4316-4324.
- Ravi R, Mookerjee B, Bhujwalla 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**(1):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* **256**(5060):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**(46):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**(35):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**(32):20055-20062.

MOL #27029

- 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**(41):38723-38730.
- Ryan HE, Lo J and Johnson RS (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *Embo J* **17**(11):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**(15):4010-4015.
- Salceda S and Caro J (1997) Hypoxia-inducible factor 1alpha (HIF-1alpha) 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**(36):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**(1):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**(39):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**(9):2984-2992.
- Scheid A, Wenger RH, Schaffer L, Camenisch I, Distler O, Ferenc A, Cristina H, Ryan HE, Johnson RS, Wagner KF, Stauffer UG, Bauer C, Gassmann M and Meuli M (2002) Physiologically low oxygen concentrations in fetal skin regulate hypoxia-inducible factor 1 and transforming growth factor-beta3. *Faseb J* **16**(3):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 U S A* **95**(15):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**(6):722-731.
- Schuler M and Green DR (2001) Mechanisms of p53-dependent apoptosis. *Biochem Soc Trans* **29**(Pt 6):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**(10):3436-3444.
- Semenza GL (1998) Hypoxia-inducible factor 1: master regulator of O₂ homeostasis. *Curr Opin Genet Dev* **8**(5):588-594.
- Semenza GL (2002) Involvement of hypoxia-inducible factor 1 in human cancer. *Intern Med* **41**(2):79-83.
- Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* **3**(10):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**(51):32529-32537.

MOL #27029

- Semenza GL, Nejfelt 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 U S A* **88**(13):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**(38):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**(17):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**(2):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**(1-3):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**(41):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**(34):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**(19):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**(2):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 J* **19**(16):4298-4309.
- Temes E, Martin-Puig S, Acosta-Iborra B, Castellanos MC, Feijoo-Cuaresma M, Olmos G, Aragonés J and Landazuri MO (2005) Activation of HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase. *J Biol Chem* **280**(25):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**(21):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**(1):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

MOL #27029

- hindlimb ischemia by naked DNA encoding an HIF-1 α /VP16 hybrid transcription factor. *Circulation* **102**(18):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**(3A):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 O₂ tension. *Proc Natl Acad Sci U S A* **92**(12):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**(12):3610-3615.
- Wenger RH (2002) Cellular adaptation to hypoxia: O₂-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O₂-regulated gene expression. *Faseb J* **16**(10):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**(2 Pt 1):483-492.
- Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, Wood SM, Gatter KC, Harris AL, Pugh CW, Ratcliffe PJ and Maxwell PH (1998) Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1 α . *Blood* **92**(7):2260-2268.
- Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ and Harris AL (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* **60**(24):7075-7083.
- Yasinska IM and Sumbayev VV (2003) S-nitrosation of Cys-800 of HIF-1 α protein activates its interaction with p300 and stimulates its transcriptional activity. *FEBS Lett* **549**(1-3):105-109.
- Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT and Semenza GL (1999) Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α . *J Clin Invest* **103**(5):691-696.
- Yuan Y, Hilliard G, Ferguson T and Millhorn DE (2003) Cobalt inhibits the interaction between hypoxia-inducible factor- α and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor- α . *J Biol Chem* **278**(18):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**(3):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-1 α /ARNT. *Embo J* **17**(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 1 α in common human cancers and their metastases. *Cancer Res* **59**(22):5830-5835.

MOL #27029

Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D and Giaccia AJ (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* **14**(4):391-396.

MOL #27029

Figure legends

Figure 1. Domain structure of human HIF- α and HIF-1 β .

HIF- α (HIF-1 α , HIF-2 α , HIF-3 α , IPAS) and HIF-1 β belong to the basic-Helix-Loop-Helix (bHLH) and Per-ARNT-Sim (PAS) protein family. HIF- α contains an oxygen-dependent degradation domain (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 α and HIF-3 α . HIF-1 α and HIF-2 α also contain two transactivation domains (C-TAD and N-TAD), while HIF-1 β has only one TAD. The total number of amino acids of each subunit is marked at the end of the domain structure.

Figure 2. Oxygen-dependent regulation of HIF-1 stabilization and transactivation.

In normoxia (left), two proline residues of HIF-1 α (P⁴⁰² and P⁵⁶⁴) and asparagine (N⁸⁰³) are hydroxylated by PHDs and FIH-1, respectively, in an O₂, 2-oxoglutarate (2-OG), and Fe²⁺-dependent manner. Hydroxylated HIF-1 α proteins bind to the E3 ubiquitin ligase VHL complex, leading to its degradation by the proteasome. Acetylation of lysine (K⁵³²) by ARD1 favors the interaction of HIF-1 α with VHL. Hydroxylated N⁸⁰³ blocks the recruitment of transcriptional coactivator CBP/p300.

In hypoxia (right), the activities of PHDs and FIH-1 are inhibited due to lack of O₂, resulting in no proline and asparagine hydroxylation. Consequently, there is no VHL binding and HIF-1 α is stabilized. Stabilized HIF-1 α proteins translocate to the nucleus and bind to HIF-1 β . HIF-1 β may bind preferentially to the MAPK-induced phosphorylated form of HIF-1 α . Non-hydroxylated N⁸⁰³ of HIF-1 α allows CBP/p300

MOL #27029

recruitment to the target genes, resulting in gene transcription. In addition, the expression of ARD1 is decreased under hypoxia, causing less acetylated HIF-1 α .

MOL #27029

Table 1. Environmental regulator of HIF-1.

regulator	Function/pathway involved	consequence	reference
Nickel (Ni ²⁺)	Decreases cellular Fe level	Increased HIF-1 α	(Davidson et al., 2005)
	Inhibits PHDs		(Ke et al., 2005)
	Down-regulates the expression of FIH-1 and ARD1		(Salnikow et al., 2004)
Cobalt (Co ²⁺)	Depletes intracellular ascorbate		(Li et al., 2004)
	PI3K/Akt	Increased HIF-1 α	(Yuan et al., 2003)
	Replaces Fe		(Ke et al., 2005)
Arsenite	Down-regulates the expression of FIH-1 and ARD1		(Salnikow et al., 2004)
	Depletes intracellular ascorbate		(Gao et al., 2004)
	PI3K	Increased HIF-1 α	(Duyndam et al., 2001)
Chromium	ROS		(Duyndam et al., 2003)
	p38 MAPK	Increased HIF-1 α	(Gao et al., 2004)
Vanadate	p38 MAPK, ROS	Increased HIF-1 α	(Gao et al., 2002)
	PI3K/Akt, ROS	Increased HIF-1 α	(Hwang et al., 2004)
Desferrioxamine (DFO)	AMP-activated protein kinase (AMPK)		(Wang and Semenza, 1993)
Insulin	Fe chelator	Increased HIF-1 α	(Stiehl et al., 2002)
Interleukin-1 (IL-1)	PI3K	Increased HIF-1 α	(Feldser et al., 1999)
insulin-like growth factor (IGF)-1, IGF-2			(Richard et al., 2000)
Fetal calf serum	ROS	Increased HIF-1 α	(Richard et al., 2000)
Angiotensin II (Ang II)			(Gorlach et al., 2001)
Thrombin, Platelet-derived growth factor (PDGF)			
Nitric oxide (NO)	MAPK, PI3K	Increased HIF-1 α	(Kasuno et al., 2004)
	Inhibits PHDs		(Metzen et al., 2003b)

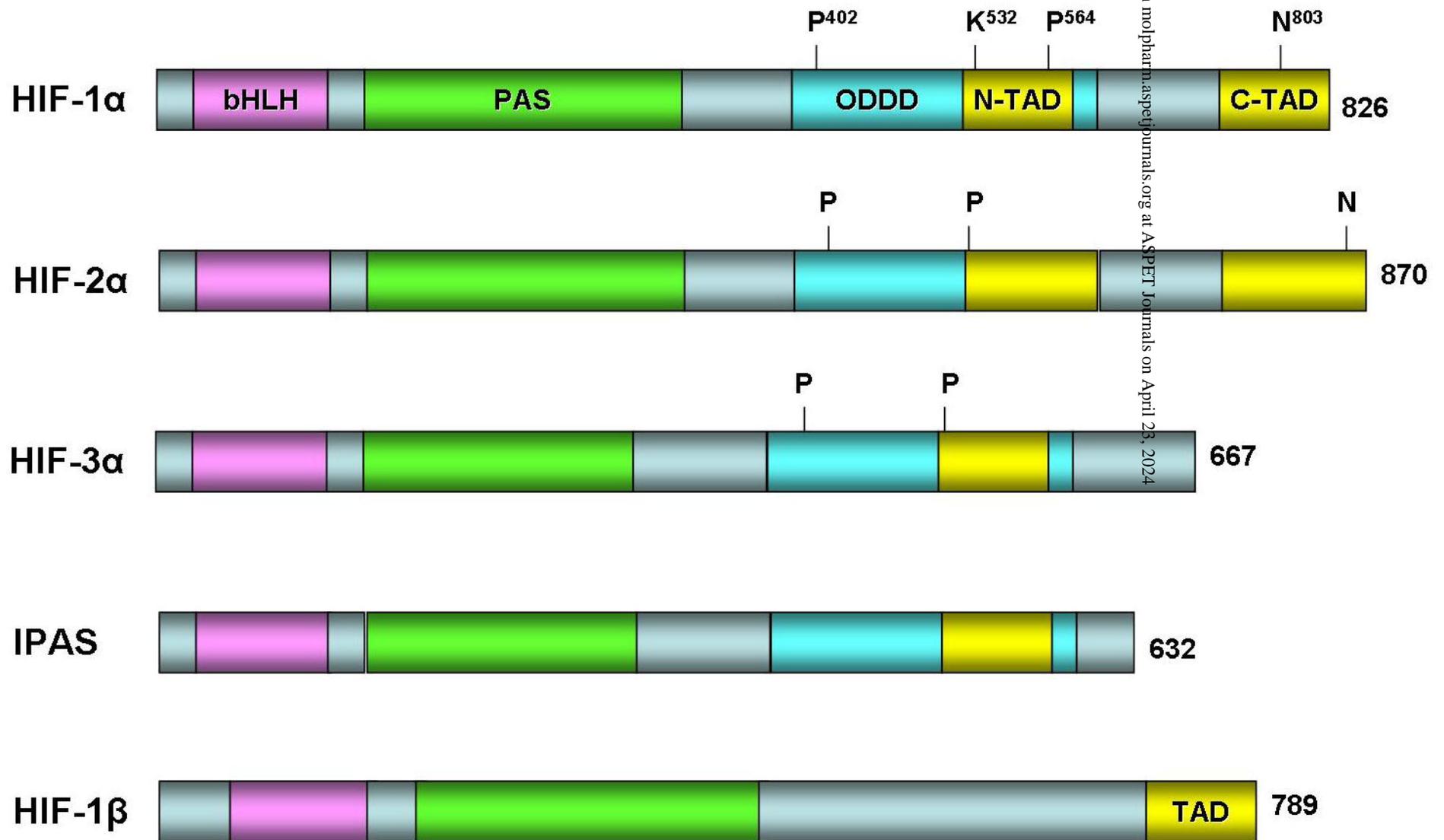
MOL #27029

Nitric oxide (NO) under hypoxia	Inhibits mitochondrial O ₂ consumption	Decreased HIF-1 α	(Hagen et al., 2003)
	Increases intracellular Fe and PHDs activity		(Callapina et al., 2005)
TGF- α	ROS	Increased HIF-1 α translocation and activity	(Haddad and Land, 2001)
		Increased DNA binding	(Hellwig-Burgel et al., 1999)

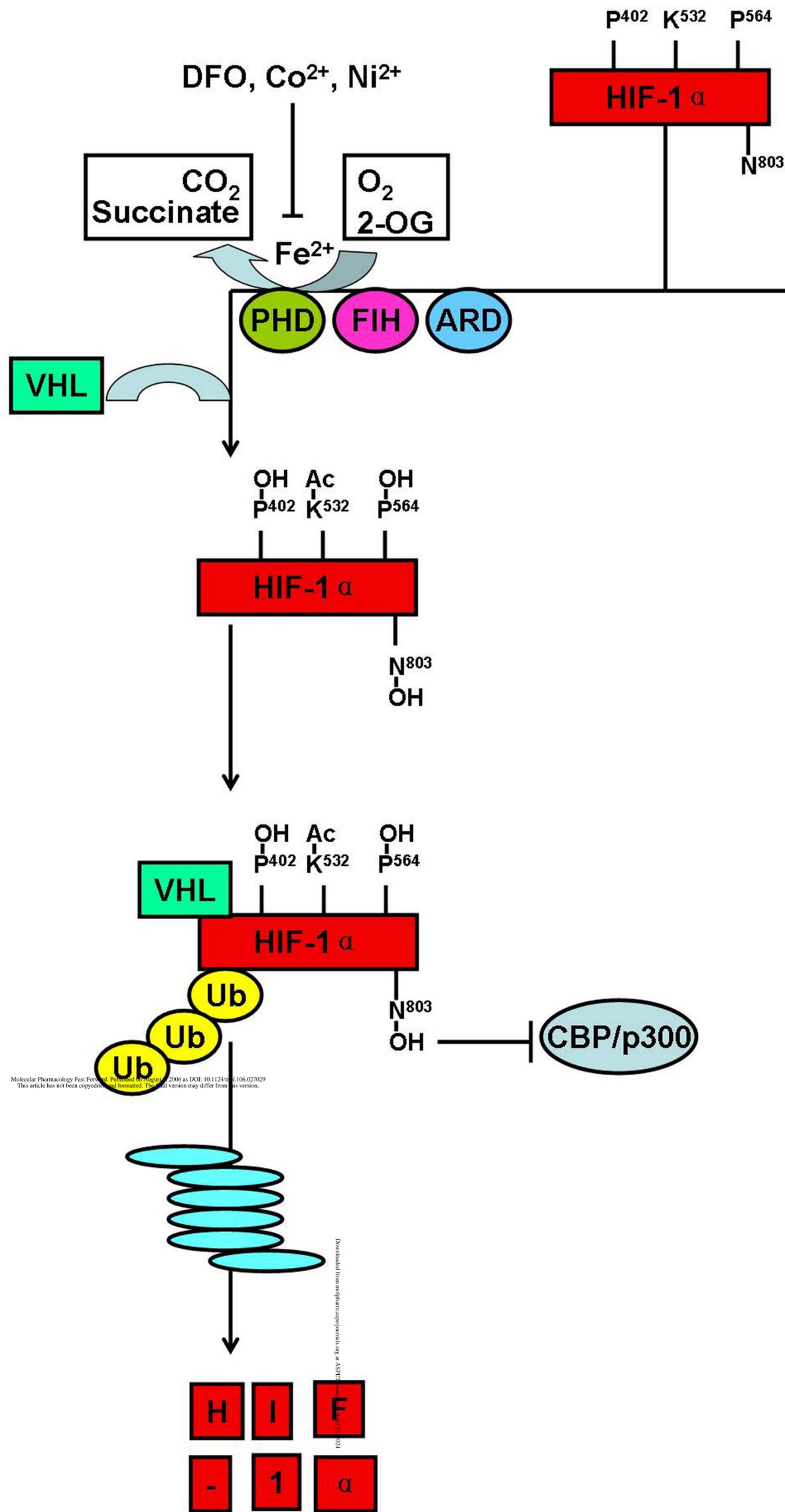
MOL #27029

Table 2. HIF-1 target genes.

Function	Gene (abbreviation)	Reference
Erythropoiesis/ iron metabolism	Erythropoietin (EPO) Transferrin (Tf) Transferrin receptor (Tfr) Ceruloplasmin	(Semenza et al., 1991) (Rolfs et al., 1997) (Bianchi et al., 1999) (Lok and Ponka, 1999)
Angiogenesis	Vascular endothelial growth factor (VEGF) Endocrine-gland-derived VEGF (EG-VEGF) Leptin (LEP) Transforming growth factor-beta3 (TGF- β 3)	(Levy et al., 1995) (LeCouter et al., 2001) (Grosfeld et al., 2002) (Scheid et al., 2002)
Vascular tone	Nitric oxide synthase (NOS2) Heme oxygenase 1 Endothelin 1 (ET1) Adrenomedulin (ADM) α 1B-adrenergic receptor	(Melillo et al., 1995) (Lee et al., 1997) (Hu et al., 1998) (Nguyen and Claycomb, 1999) (Eckhart et al., 1997)
Matrix metabolism	Matrix metalloproteinases (MMPs) Plasminogen activator receptors and inhibitors (PAIs) Collagen prolyl hydroxylase	(Ben-Yosef et al., 2002) (Kietzmann et al., 1999) (Takahashi et al., 2000)
Glucose metabolism	Adenylate kinase-3 Aldolase-A,C (ALDA,C) Carbonic anhydrase-9 Enolase-1 (ENO1) Glucose transporter-1,3 (GLU1,3) Glyceraldehyde phosphate dehydrogenase (GAPDH) Hexokinase 1,2 (HK1,2) Lactate dehydrogenase-A (LDHA) Pyruvate kinase M (PKM) Phosphofructokinase L (PFKL) Phosphoglycerate kinase 1 (PGK1) 6-phosphofructo-2-kinase/gructose-2,6-bisphosphate-3 (PFKFB3)	(O'Rourke et al., 1996) (Semenza et al., 1996) (Wykoff et al., 2000) (Semenza et al., 1996) (Chen et al., 2001) (Graven et al., 1999) (Mathupala et al., 2001) (Semenza et al., 1996) (Semenza et al., 1994) (Semenza et al., 1994) (Semenza et al., 1994) (Semenza et al., 1994) (Minchenko et al., 2002)
Cell proliferation/ survival	Insulin-like growth factor-2 (IGF2) Transforming growth factor- α (TGF- α) Adrenomedullin (ADM)	(Feldser et al., 1999) (Krishnamachary et al., 2003) (Cormier-Regard et al., 1998)
Apoptosis	Bcl-2/adenovirus E1B 19kD-interacting protein 3 (BNip3) Nip3-like protein X (NIX)	(Carrero et al., 2000) (Bruick, 2000)



Normoxia



Hypoxia

