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Hypoxia-inducible factor (HIF-1)α: its protein stability and biological functions

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Abbreviations: 17-AAG, 17-allyl-aminogel-danamycin; ARNT, aryl hydrocarbon receptor nuclear translocator; EGFR, epidermal growth factor receptor; EPAS1, endothelial PAS domain protein 1; FIH-1, factor inhibiting HIF-1; HDAC, histone deacetylase; HIF-1, hypoxia-inducible factor; HRE, hypoxia response element; HSP 90, molecular chaperon heat shock protein 90; IGF2, insulin like growth factor-2; IGFIR, insulin-like growth factor 1 receptor; IPAS, inhibitory PAS domain protein; MAPK, mitogen-activated protein kinase; MEK, MAP/ERK kinase; NAT, N-acetyltransferase; NO, Nitric oxide; ODD domain, oxygen-dependent degradation domain; PHD, prolyl hydroxylase; PI3K, phosphatidylinositol 3-kinase; PMA, phorbol-12-myristate-13-acetate; TAD-C, C-terminal transactivation domains; TAD-N, N-terminal transactivation domains; TGF-α, transforming growth factor-α; VEGF, vascular endothelial cell growth factor

Abstract

Hypoxia-inducible factor (HIF-1) is an oxygen-dependent transcriptional activator, which plays crucial roles in the angiogenesis of tumors and mammalian development. HIF-1 consists of a constitutively expressed HIF-1β subunit and one of three subunits (HIF-1α, HIF-2α or HIF-3α). The stability and activity of HIF-1α are regulated by various post-translational modifications, hydroxylation, acetylation, and phosphorylation. Therefore, HIF-1α interacts with several protein factors including PHD, pVHL, ARD-1, and p300/CBP. Under normoxia, the HIF-1α subunit is rapidly degraded *via* the von Hippel-Lindau tumor suppressor gene product (pVHL)-mediated ubiquitin-proteasome pathway. The association of pVHL and HIF-1α under normoxic conditions is triggered by the hydroxylation of prolines and the acetylation of lysine within a polypeptide segment known as the oxygen-dependent degradation (ODD) domain. On the contrary, in the hypoxia condition, HIF-1α subunit becomes stable and interacts with coactivators such as p300/CBP to modulate its transcriptional activity. Eventually, HIF-1 acts as a master regulator of numerous hypoxia-inducible genes under hypoxic conditions. The target genes of HIF-1 are especially related to angiogenesis, cell proliferation/survival, and glucose/iron metabolism. Moreover, it was reported that the activation of HIF-1α is closely associated with a variety of tumors and oncogenic pathways. Hence, the blocking of HIF-1α itself or HIF-1α interacting proteins inhibit tumor growth. Based on these findings, HIF-1 can be a prime target for anticancer therapies. This review summarizes the molecular mechanism of HIF-1α stability, the biological functions of HIF-1 and its potential applications of cancer therapies.

Keywords: ARD1; angiogenesis; anticancer therapy; cell proliferation/survival; HIF-1; glucose metabolismi; PHD; pVHL; p300/CBP; iron metabolism; transcription factor

Introduction

To maintain oxygen homeostasis, higher eukaryotes have adopted specialized mechanisms to enhance O_2 uptake and distribution. Hence, conserved oxygen-dependent responsive pathways are expressed in almost every mammalian cell (Bruick, 2003). In mammalian cells, the transcriptional complex HIF-1 plays an essential role in cellular and systemic oxygen homeostasis (Iyer *et al*., 1998; Semenza, 1999; Semenza, 2000). HIF-1 induces the transcription of more than 60 proteins, including VEGF and erythropoietin. These protein products increase oxygen availability by promoting erythropoiesis and angiogenesis, which activates genes involved in glucose transport and metabolism (Semenza, 2002; Semenza, 2003). HIF-1 functions as a master regulator of oxygen and undergoes conformational changes in reponse to oxygen concentrations (Bruick and McKnight, 2001; Epstein *et al.,* 2001; Ivan *et al.,* 2001; Jaakkola *et al.,* 2001; Masson *et al.,* 2001). It binds a core sequence of the HRE in the promoters of hypoxia-responsive genes and induces their expressions. HIF-1 consists of α and β subunits, both are basic helix-loop-helix factors. The expression of the α subunit is remarkably high during

hypoxia and is maintained at low levels in most cells under normoxic conditions. Unlike the α subunit, β subunit is constitutively expressed and its activity is controlled in an oxygen-independent manner (Wang and Semenza, 1993). In this review, we will summerize the oxygen dependent regulation of HIF-1 and functions of HIF-1 in the field of anticancer therapies.

HIF-1α and β subunits

HIF is a heterodimeric transcription factor composed of an -α subunit and a -β subunit also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) (Wang *et al*., 1995). Each subunit belongs to the bHLH-PAS (Per/Arnt/Sim) family. Oxygen levels can affect the protein stability, subcellular localization and transcriptional potency of the HIF-α subunits, whereas the ARNT subunit is constitutively expressed in the nucleus and its activity is not affected by hypoxia (Li *et al*., 1996). HIF-1α dimerizes with ARNT to play a role as a transcription factor. Each subunit contains two PAS domains, designated PAS-A and PAS-B. The bHLH and PAS domains are required for heterodimer formation between the α and β subunit and for DNA binding (Jiang *et al*,. 1996). The HIF-1α subunit contains TAD-N and TAD-C (N- and C-terminal transactivation domains, respectively) bridged by an inhibitory domain (Figure 1) (Ruas *et al*., 2002). The TAD-N overlaps with the oxygen-dependent degradation (ODD) domain, and it is continuous with protein stability. The TAD-C interacts with coactivator such as p300/CBP, independent of protein stability and is required for full HIF activity (Lando *et al*., 2002).

HIF-α isoforms

Besides the HIF-1α, there are two more members of the bHLH-PAS superfamily: HIF-2α, referred as endothelial PAS domain protein 1 (EPAS1) and HIF-3 (Tian *et al*., 1997). These isoforms have structural similarity and are classified as bHLH-PAS family. The structure and functions of HIF-1α were more extensively studied than that of HIF-2α or -3α. Five isoforms of HIF-1α are reported. HIF-1α^{FL} is similar to wild type HIF-1α except for additional three base pairs, TAG between exon1 and exon2. Next, HIF-1α^{736} loses exon14, therefore it lacks C-terminal transactivation domain. HIF-1α^{FL} and HIF-1α^{736} activate the VEGF promoter upon hypoxia (Gothie *et al*., 2000). In contrast to HIF-1α^{FL} and HIF-1α^{736}, HIF-1α^{557} (HIF-1αZ) and HIF-1α^{516} function as a dominant negative isoforms of HIF-1α. HIF-1αZ loses exon 12, which is induced by zinc ion, and HIF-1α^{516} lacks exon11, 12 (Chun *et al*., 2001; Chun *et al*., 2002). The recently known HIF-1α^{785} contains all functional domains, therefore, HIF-1α^{785} acts as a transcriptional activator, but it is deprived of exon11 in the ODD domain (Chun *et al*., 2003). The roles of 2α and 3α class subunits are not known in much detail. Like HIF-1α, HIF-2α is also regulated by enzymatic hydroxylation of conserved proline residue that causes the degradation under the normoxia *via* the ubiquitin E3 ligase complex (Maxwell *et al.,* 1999; Tanimoto *et al.,* 2000; Ivan *et al.,* 2001; Jaakkola *et al.,* 2001; Masson *et al.,* 2001). Recently, it was reported that HIF-3α also undergoes the degradation through polyubiquitination-proteasome pathway (Maynard *et al.,* 2003). Human HIF-3α has multiple spliced variants: hHIF-3α1-6 (Figure 2). hHIF-3α1, 2 and 3 share a common ODD

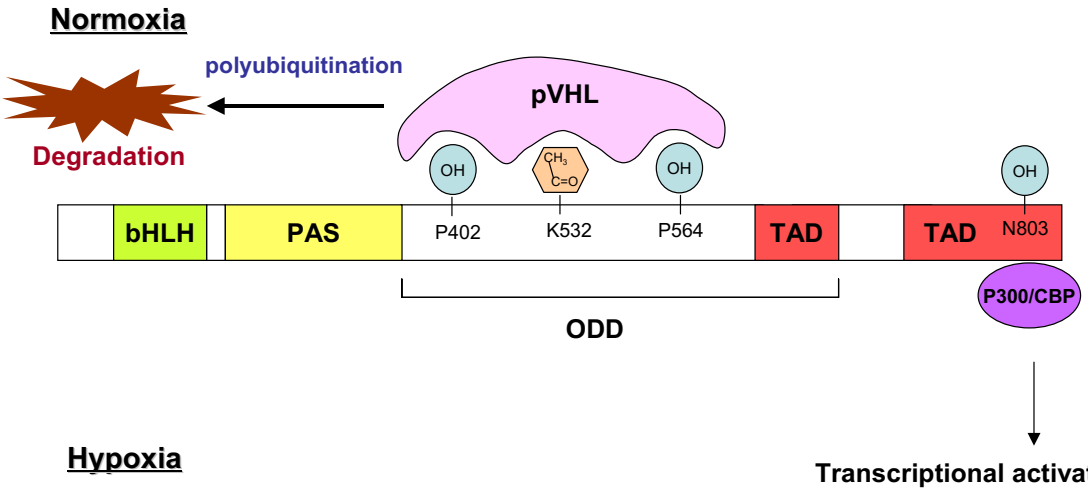

Figure 1. Molecular mechanism of HIF-1α stability. Oxygen level regulates the degradation *via* hydroxylation or acetylation-mediated VHL binding and also transcriptional activity of HIF-1α. P402, P564 and K532 is target amino acid residues of PHD and ARD1, respectively. FIH hydroxylates N803 under normoxia and inhibits the association of p300/CBP to HIF-1α leading to the downregulation of transcriptional activity of HIF-1α.

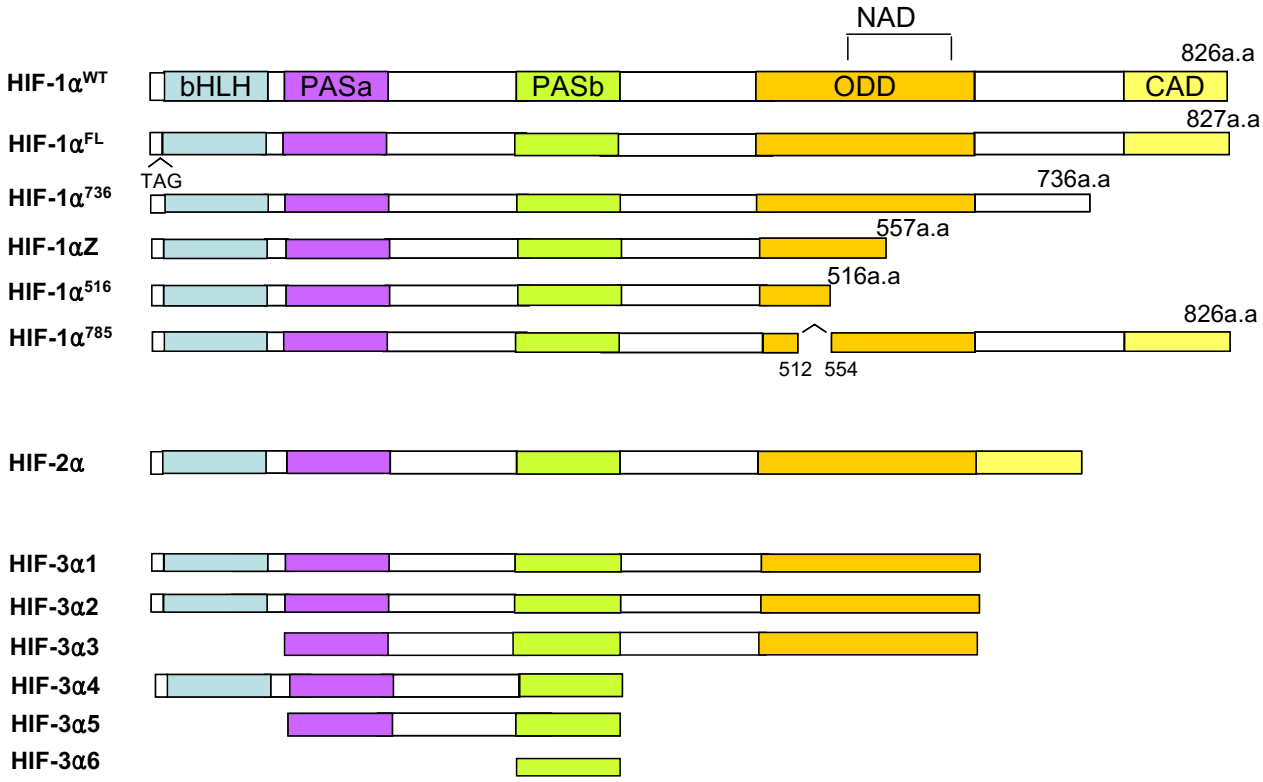

Figure 2. Splice variants of the α subunit of HIF. bHLH; basic helix-loop-helix, PAS, Per/Arnt/Sim domain; ODD, oxygen dependent degradation domain; NAD, N-terminal transactivation domain; CAD, C-terminal transactivation domain.

that includes the consensus motif of proline hydroxylase and binds VHL E3 ligase complex under normoxic condition. However, HIF-3α lacks a trans activation domain. A dominant negative regulator of HIF-1 called IPAS (inhibitory PAS domain protein), which lacks transactivation domain, was identified as an alternatively spliced variants of HIF-3α (Makino *et al*., 2002). IPAS prevents the interaction of HIF-1α to HIF-1β because IPAS dimerizes HIF-1α and IPAS/HIF-1α complex does not bind to the hypoxia- response element. Therefore, IPAS fails to the HIF mediated target gene expression and inhibits transcriptional activation of HIF-1α. Therefore, HIF-3α can act as an antagonist of HIF system. In contrast to HIF-3α, HIF-2α activates transcription and induces hypoxia-mediated gene expression such as VEGF.

It was reported that hypoxia did not affect mRNA levels of HIF-1α, HIF-1β and HIF-2α, but HIF-3α mRNA increased after 2 h hypoxia (Heidbreder *et al*., 2003). However, how hypoxia increases mRNA level of HIF-3α is not known. Therefore, a further study about the expression and stability of HIF-3α is required.

The effect of HIF family on gene expression may be different according to cell types. For example, HIF-1α and -2α are abundantly expressed in the kidney. Nevertheless, the overexpression of HIF-2α, but not HIF-1α promotes growth of renal carcinoma cell (Kondo *et al*., 2002; Maranchie *et al*., 2002; Rosenberger *et al*., 2002). However, in the breast cancer cell line, HIF-1α is the major isoform required for induction of hypoxic genes (Blancher *et al*., 2000).

Molecular mechanism of HIF-1α stability

Regulation of HIF-1α protein by oxygen level

HIF-1α protein is subject to rapid degradation at normoxia by process of pVHL-mediated ubiquitin-proteasome pathway, whereas hypoxia blocks degradation leading to accumulation (Huang *et al*., 1996; Kallio *et al*., 1997). The association of HIF-1α with pVHL is triggered by the post-translational hydroxylation of proline residue that is mediated by prolyl hydroxylase (PHD) or HIF prolyl hydroxylase (HPH). The HIF-1α contains two sites for hydroxylation, Pro 402 and Pro 564 within its ODD domain and each site contains a conserved LXXLAP motif (Masson *et al*., 2001). The hydroxyproline residue becomes buried within the hydrophobic core of pVHL, the von Hippel-Lindau tumor suppressor protein, a part of the ubiquitin ligase protein complex (Hon *et al*., 2002; Min *et al*., 2002).

All three paralogs of the HPH/PHD family have been discovered and named as follows; HPH-1/PHD-3, HPH-2/PHD-2 and HPH-3/PHD-1. When overexpressed as tagged proteins, HPH-2/PHD-2 resides in the cytoplasm, whereas HPH-3/PHD-1 is predominantly in the nuclear (Huang *et al*., 2002; Metzen *et al*., 2003). Berra *et al.* (2003) proposed that HPH-2/PHD-2 is primarily responsible for HIF hydroxylation in the normoxic condition.

The prolyl hydroxylase is dioxygenase requiring oxygen and 2-oxoglutarate as substrate. This enzyme transfers one oxygen atoms to the proline residue, and the second oxygen atom reacts with 2-oxoglutarate generating succinate. The activity of PHD to HIF-1α is known to depend on the O_2 concentration. Therefore, PHD was suggested as an oxygen sensor (Epstein *et al*., 2001; Jewell *et al*., 2001).

Another protein interacting with the ODD domain of HIF-1α is ARD1 acetyltransferase (Jeong *et al*., 2002). The yeast homolog of ARD-1 is required for the expression of protein N-acetyltransferase (NAT) in lower eukaryotes and bacteria, but whose function is not defined in mammalian cells (Tribioli *et al*. 1994; Ingram *et al*., 2000). Jeong *et al.* (2002) reported that ARD1 acetylates the Lys-532 residue in the ODD domain of HIF-1α by transferring an acetyl group from Ac-CoA. The acetylation level of HIF-1α gradually decreases as the length of hypoxic exposure time increases, which is due to the reduced expression of ARD1. The acetylation of Lys532 by ARD1 is critical to the proteasomal degradation of HIF-1α. A K532R mutant not acetylated by ARD1 was stabilized and showed decreased interaction with pVHL (Jeong *et al*., 2002). Interestingly, it was previously reported that Lys 532 is critical for the degradation of HIF-1α under normoxic condition (Tanimoto *et al*., 2000). Although it is unclear how the acetylation of HIF-1α leads to its decreased stability, a conformational change of HIF-1α may effectively increase its interaction with pVHL and enhance the following proteasomal degradation.

Mechanisms stabilizing HIF-1α under normoxic conditions

Many growth factors and cytokines are known to stabilize HIF-1α under normoxic conditions, including insulin, insulin-like growth factors, transforming growth factor, platelet-derived growth factor, epidermal growth factor, interleukin-1β (Zelzer *et al*., 1998; Feldser *et al*., 1999; Hellwig-Burgel *et al*., 1999; Richard *et al*., 2000; Gorlach *et al*., 2001; Haddad *et al*., 2001; Stiehl *et al*., 2002). Although it is not known how these factors stabilize HIF-1α, a common cellular kinase pathway may be included.

Nitric oxide (NO) has been reported to stabilize HIF-1α under normoxia (Palmer *et al*., 2000). Adverse reports have been published showing that NO treatment interferes with HIF-1 activity (Sogawa *et al*., 1998). This contradiction may be due to the cell culture model diversity and the physiological situation.

To date, four splice variants of human HIF-1α mRNA have been reported (Figure 2). Recently, the fifth splice variant of HIF-1α, HIF-1α^{785} was identified (Chun *et al*., 2003). HIF-1α^{785} contains all of the essential domains of HIF-1α except for some part of the ODD domain (amino acid 512-554) and functions as an active transcription activator. HIF-1α^{785} was markedly induced by PMA (phorbol-12-myristate-13-acetate) and hyperthermia under normoxia, whereas HIF-1α was induced by hypoxia (Chun *et al*., 2003). Since HIF-1α^{785} is deprived of Lys532 residue, the acetylation at Lys532 may play an important role in the HIF-1α protein stability under normoxic condition (Jeong *et al*., 2002).

HIF-1α protein synthesis

Growth factors induce HIF-1α protein translation irrespectively of hypoxia whereas hypoxia is associated with decreased degradation of HIF-1α. Thus it can overcome the oxygen sensor mediated HIF-1α degradation under normoxic conditions. Probably, growth factor binding to receptor tyrosine kinase activates the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (Zhong *et al*., 2000; Zundel *et al*., 2000; Laughner *et al*., 2001; Fukuda *et al*., 2002; Hellwig-Burgel *et al*., 2003). Then, PI3K activates Akt also known as protein kinase B (PKB). In the MAPK pathway, growth factor activates RAS and its downstream, MAP/ERK kinase (MEK) (Figure 3). In addition to the role of HIF-1α synthesis, MAPK pathway enhances the transcriptional activity of HIF. This effect is due to phosphorylation of the p300 by ERK.

Transcriptional activity

A hydroxylation-dependent switch also regulates the ability of the HIF-1α TAD-C to interact with coactivators such as CBP/p300 only under hypoxic condition (Lando *et al*., 2002; Sang *et al*., 2002). Under normoxic conditions, factor inhibiting HIF-1 (FIH-1) hydroxylates asparagine-803 of HIF-1α within the TAD-C, which prevents the interaction of HIF-1α with coactivators (Hewitson *et al*., 2002; Lando *et al*., 2002). Like the prolyl hydroxylase, FIH-1 is a Fe(II)- and 2-oxoglutarate dependent dioxygenase enzyme and also requires vitamin C to retain the ferrous state of the iron (Hewiston *et al*., 2002; Lando *et al*., 2002). FIH-1 can serve as a second oxygen sensor because of the utilization of oxygen as a substrate. Further-

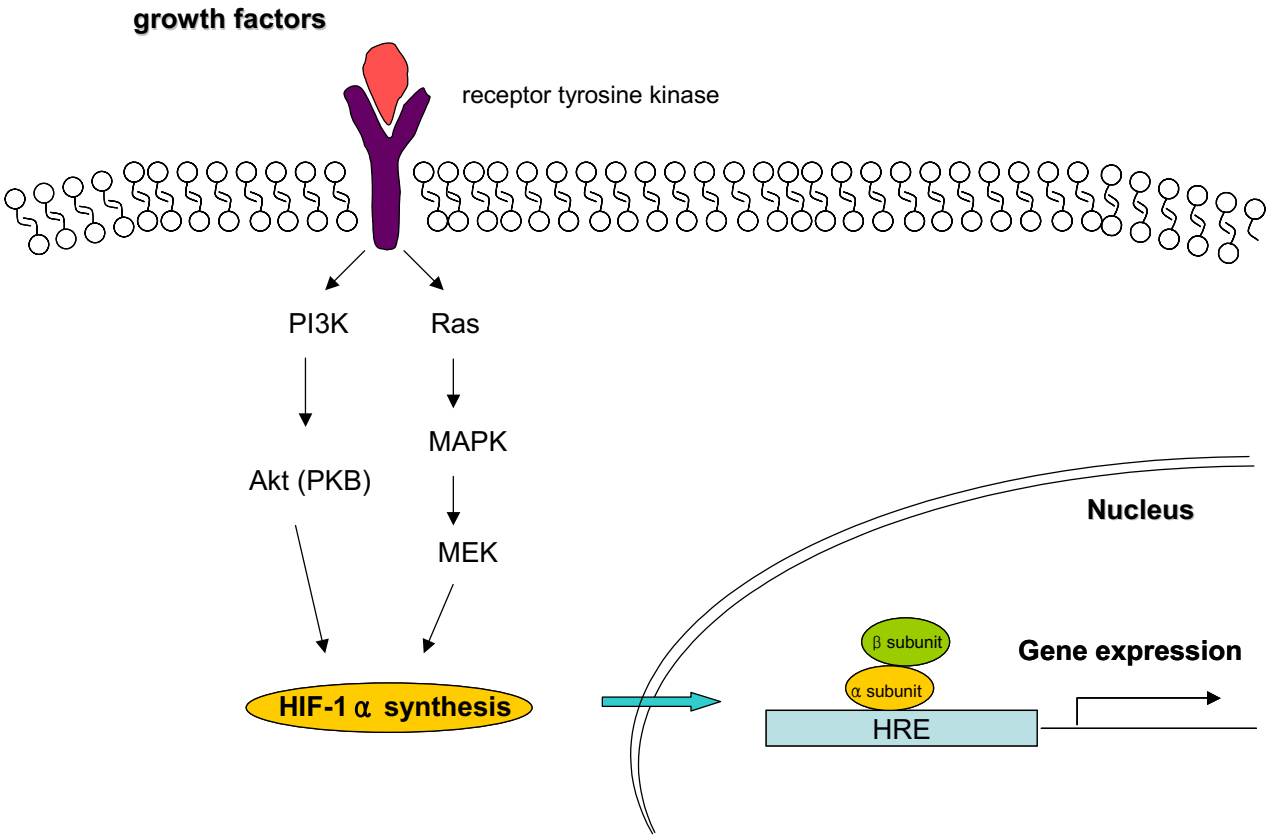

Figure 3. Growth factors such as IGF and TGF synthesize HIF-1α independent of oxygen level *via* PI3K or MAPK pathways.

more, FIH-1 binds pVHL independent of its hydroxylase activity and pVHL recruits histone deacetylase (HDAC) that interferes with the transactivation domain function (Mahon *et al.*, 2001). Although HIF activity may be regulated by additional mechanisms, the oxygen dependent hydroxylases seem to be primarily responsible for regulating HIF in response to oxygen level.

HIF-1 target genes

HIF-1 acts as a master regulator of oxygen-regulated gene expression. More than 60 putative HIF-1 target genes have been identified (Table 1). HIF-1 target genes are particularly relevant to cancer encoding angiogenic factors, proliferation/survival factors, glucose transporters and glycolytic enzymes (Semenza, 2003).

Angiogenesis

The avascular tumor or stroma cells in low-oxygen tension (hypoxia) pathologically or physiologically triggers angiogenesis as a consequence of an oxygen-sensing mechanism and subsequent induction of a variety of pro-angiogenic genes (Bunn and Poyton, 1996; Giordano and Johnson, 2001; Semenza, 2002). Vascular endothelial cell growth factor (VEGF) is one of the major target genes, specifically recruits endothelial cells into hypoxic and avascular area and stimulates their proliferation. VEGF is the most potent endothelial-specific mitogen and is known to directly participate in angiogenesis (An *et al.*, 2000; Berra *et al.*, 2000; Harris, 2000; Josko *et al.*, 2000; Conway *et al.*, 2001). This growth factor interacts with its receptor, VEGFR, which is specifically expressed in endothelial cells, and stimulates endothelial cell proliferation (Neufeld *et al.*, 1999; Berra *et al.*, 2000; Harris, 2000; Josko *et al.*, 2000). It was also shown that hypoxia induces the expression of VEGF mRNA and protein, suggesting that hypoxia is a stimulus of angiogenesis through the up-regulation of the VEGF expression (Neufeld *et al.*, 1999; Ahnmed *et al.*, 2000; Berra *et al.*, 2000; Harris, 2000; Josko *et al.*, 2000). The induction of angiogenesis leads to an increase in the vascular density and hence a decrease in the oxygen diffusion distance. However, local blood flow under pathophygiological conditions is controlled by modulation of the vascular tone through production of NO (inducible nitric oxide synthase), CO (heme oxygenease 1), endothelin 1, adrenomedulin, or activa-

Table 1. Transcriptionally activated genes by HIF-1.

Function	Genes
Cell proliferation	Cyclin G2, IGF2, IGF-BP1, IGF-BP-2, IGF-BP-3, WAF-1, TGF-α, TGF-β3
Cell survival	ADM, EPO, IGF2, IGF-BP1, IGF-BP-2, IGF-BP-3, NOS2, TGF-α, VEGF
Apoptosis	NIP3, NIX, RTP801
Motility	ANF/GPI, c-MET, LRP1, TGF-α
Cytoskeletal structure	KRT14, KRT18, KRT19, VIM
Cell adhesion	MIC2
Erythropoiesis	EPO
Angiogenesis	EG-VEGF, ENG, LEP, LRP1, TGF-β3, VEGF
Vascular tone	α_{1B}-adrenergic receptor, ADM, ET1, Haem oxygenase-1, NOS2
Transcriptional regulation	DEC1, DEC2, ETS-1, NUR77
pH regulation	Carbonic anhydrase 9
Regulation of HIF-1 activity	P35srj
Epithelial homeostasis	Intestinal trefoil factor
Drug resistance	MDR1
Nucleotide metabolism	Adenylate kinase 3, Ecto-5'-nucleotidase
Iron metabolism	Ceruloplasmin, Transferrin, Transeferrin receptor
Glucose metabolism	HK1, HK2, AMF/GPI, ENO1, GLUT1, GAPDH, LDHA, PFKBF3, PFKL, PGK1, PKM, TPI, ALDA, ALDC
Extracellular-matrix metabolism	CATHD, Collagen type V (α1), FN1, MMP2, PAI1, Prolyl-4-hydroxylase α (1), UPAR
Energy metabolism	LEP
Amino-Acid metabolism	Transglutaminase 2

ADM, adrenomedullin; ALDA, aldolase A; ALDC, aldolase C; AMF, autocrine motility factor; CATHD, cathepsin D; EG-VEGF, endocrine-gland-derived VEGF; ENG, endoglin; ET1, endothelin-1; ENO1, enolase 1; EPO, erythropoietin; FN1, fibronectin 1; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; GAPDH, glyceraldehyde-3-P-dehydrogenase; HK1, hexokinase 1; HK2, hexokinase 2; IGF2, insulin-like growth -factor 2; IGF-BP1, IGF-factor-binding-protein 1; IGF-BP2, IGF-factor-binding-protein 2; IGF-BP3, IGF-factor-binding-protein 3; KRT14, keratin 14; KRT18, keratin 18; KRT19, keratin 19; LDHA, lactate dehydrogenase A; LEP, leptin; LRP1, LDL-receptor-related protein 1; MDR1, multidrug resistance 1; MMP2, matrix metalloproteinase 2; NOS2, nitric oxide synthase 2; PFKBF3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3; PFKL, phosphofructokinase L; PGK 1, phosphoglycerate kinase 1; PAI1, plasminogen-activator inhibitor 1; PKM, pyruvate kinase M; TGF-α, transforming growth factor-α; TGF-β3, transforming growth factor-β3; TPI, triosephosphate isomerase; VEGF, vascular endothelial growth factor; UPAR, urokinase plasminogen activator receptor; VEGFR2, VEGF receptor-2; VIM, vimentin.

tion of the α_{1B}-adrenergic receptor, all of which involve HIF-1 target genes (Melillo *et al*., 1995; Eckhart *et al*., 1997; Lee *et al*., 1997; Hu *et al*.,1998; Palmer *et al*., 1998; Nguyen and Claycomb, 1999). HIF-1 therefore contributes to angiogenesis by mechanisms far more complex than simple VEGF induction, probably by recruiting additional target genes involved in vessel maturation (Wenger, 2002).

Cell proliferation/survival

Hypoxia-induced growth factors are known to promote cell proliferation and survival. Several growth factors, most notably insulin-like growth factor-2 (IGF2) and transforming growth factor-α (TGF-α), are also HIF-1 target genes (Feldser *et al*., 1999; Krishnamachary *et al*., 2003). Binding of these factors to their cognate receptors, the insulin-like growth factor 1 receptor (IGFIR) and epidermal growth factor receptor (EGFR), respectively activates signal transduction pathways that lead both to HIF-1α expression and to cell proliferation/survival (Semenza, 2003). The p42/p44 mitogen-activated protein kinases, which regulate cell proliferation in response to extra-cellular growth factors, have been shown to phosphorylate HIF-1α and activate transcription of HIF-1 target genes (Berra *et al*., 2000). Phosphatidylinositol 3-OH kinase (PI3K) activity is also increased in some cell types under hypoxic conditions (Chen *et al*., 2001). PI3K is one of the key downstream mediators of many tyrosine kinase sig-

naling pathways and is involved in regulating cell proliferation and suppression of apoptosis. The PI3K pathway is inhibited by the phosphoinositide phosphatase PTEN, and mutations in PTEN enhance HIF-1 activated responses (Zundel *et al*., 2000). PTEN regulates cell growth and proliferation, and is deleted or mutated in several human cancers, including glioblastoma, endometrial tumors and prostate cancer (Harris, 2001). Therefore, HIF-1 contributes to autocrine-signalling pathways that are crucial for cancer progression.

Glucose metabolism

Under hypoxic conditions, cells switch to the oxygen-independent metabolic pathway, and they use glycolysis as a primary mechanism of ATP production (Dang and Semenza, 1999; Seagroves *et al*., 2001). Glycolysis provides only two ATP molecules for each glucose molecule, in contrast to the TCA cycle, which provides 38 ATP molecules. Many genes involved in glucose uptake and glycolysis were identified as HIF-1 target genes (Wenger, 2000). HIF-1 regulate expressions of all enzymes in the glycolytic pathway, as well as expression of the glucose transporters GLUT1 and GLUT3, which mediate cellular glucose uptake (Chen *et al*., 2001). Enhanced lactate production and hence a decrease in pH results from the increase in anaerobic glycolysis, potentially limiting this source of ATP despite sufficient glucose supply. Transmembrane carbonic anhydrases were reported to be HIF-1 target genes (Wykoff *et al*., 2000). In fact, the increased glycolysis is a normal response to proliferation, and that migrating cells also use this pathway as an energy source (Mazurek *et al*., 1997). The intermediary metabolites of the glycolytic pathway provide the precursors for synthesis of glycine, serine, purine, pyrimidine and phospholipids, all of which are essential for cell growth and maintenance of cells under stress (Harris, 2001).

Iron metabolism

Hypoxia was found to increase the expression of transferrin, probably to enhance the iron transport to erythroid tissues (Rolfs *et al*., 1997). The transferring receptor is a hypoxia-inducible HIF-1 target gene, enabling cellular transferrin uptake (Tacchini *et al*., 1999). And ceruloplasmin was reported to be a HIF-1 target gene (Lok and Ponka, 1999; Mukhopadhyay *et al*., 2000). Ceruloplasmin, also known as a ferroxidase, is required to oxidize ferrous to ferric iron. The only ferric iron can be bound by transferrin, hypoxic ceruloplasmin induction is likely to support iron supply to erythroid tissue (Wenger, 2002).

HIF-1 targeted therapies

HIF-1α is overexpressed in many human cancer (Zhong *et al*., 1999; Talks *et al*., 2000). Significant associations between HIF-1α overexpression and patient mortality have been shown in cancers of the brain, breast, cervix, oropharynx, ovary and uterus. In contrast, associations between HIF-1α overexpression and the decreased mortality were reported for patients with head and neck cancer and non-small cell lung cancer (Volm and Koomagi, 2000; Beasley *et al*., 2002). So, the effect of HIF-1α overexpression is dependent on the cancer type. Blocking of HIF-1α activity may be advantageous in inhibiting cancer progression as this would help starve growing tumors of oxygen and nutrient supply (Lando *et al*., 2003). Recent studies have provided evidence indicating that HIF-1α mediates resistance to chemotherapy and radiation (Aebersold *et al*., 2001; Unruh *et al*., 2003). Inhibition of HIF-1α activity could therefore represent an important component of combinatory anti-angiogenic therapies, which are under development to block HIF-1α itself or HIF-1α interacting proteins. HIF-α antisense therapy might act synergistically with immunotherapy. *In vivo* delivery of antisense to HIF-α alone by direct intratumor injection inhibited tumor growth, but combination of the two treatments caused marked tumor regression and a sustained antitumor immune response (Sun *et al*., 2001). A gene-therapy strategy to block the interaction between HIF-1α and its transcriptional co-activator CBP/p300 led to attenuation of hypoxia-inducible gene expression and inhibition of tumor growth in a mouse xenograft model (Kung *et al*., 2000). And HIF-1α interacts with the chaperone HSP90, and the HSP90 inhibitor 17-allylminogel-danamycin (17-AAG) induces HIF-1α degradation in a VHL-independent manner (Isaacs *et al*., 2002; Mabjeesh *et al*., 2002; Zagzag *et al*., 2003). The small molecule YC-1 [3-(-5'-hydroxy-methyl-2'-uryl)-1-benzylindazole] was also shown to reduce HIF-1α levels and xenograft growth (Yeo *et al*., 2003). The mechanism by which YC-1 reduces HIF-1α levels has not been established (Semenza, 2003), although YC-1 is known to stimulate soluble guanylate-cyclase activity, but this effect is not required for inhibition of HIF-1α levels.

Hypoxia response elements (HREs) linked to marker genes or prodrug activation systems can be used for selective therapeutics in hypoxic regions (Dachs *et al*., 1997; Lemmon *et al*., 1997). Gene-therapy vectors that carry pro-apoptotic or anti-proliferation genes driven by HREs can be selectively targeted to cancer cells in hypoxic regions of the tumor (Harris, 2000). For example, *in vivo*, HRE-mediated trans gene expression was localized adjacent to areas of pyknotic cells and necrosis (Dachs *et al*., 1997). In addition to anti-angiognesis agents, it is clear that

many novel therapeutic agents that target signal-transduction pathways have anti-angiogenic effects. This effect seems to be due in part to the fact that inhibition of signal-transduction pathways result in decreased levels of HIF-1α (Semenza, 2003).

Conclusion

Hydroxylation and acetylation is essential to the regulation of HIF-1α protein stability. Under normoxic conditions, the HIF-1α ODD domain encompasses sequences that mediate O_2-dependent ubiquitination of HIF-1α protein through the interaction with pVHL, which is an E3 ubiquitin-protein ligase that targets HIF-1α for proteasomal degradation (Salceda and Caro, 1997; Cockman *et al*., 2000; Kamura *et al*., 2000; Sutter *et al*., 2000; Jeong *et al*., 2002). Furthermore, ubiquitination of HIF-1α is mediated by interaction with p53 that promotes Mdm-2-mediated ubiquitination and proteasomal degradation of the HIF-1α through direct interaction with HIF-1α in hypoxia (An *et al*., 1998; Ravi *et al*., 2000). Under hypoxia conditions, HIF-1α is stabilized, which is determined by balance between negative regulators such as p53 and positive unknown factors, and accumulated in nucleus (Ema *et al*., 1999). Stabilized HIF-1α exerts its transcriptional activity by binding to the p300/CBP, SRC (steroid receptor coactivator-1) family coactivators, nuclear redox regulator Ref-1, and molecular chaperon heat shock protein 90 (HSP 90) (Arany *et al*., 1996; Ema *et al*., 1999; Minet *et al*., 1999; Carrero *et al*., 2000). They synergistically enhance HIF-1α-mediated transcriptional regulation under hypoxic conditions. The modulation of HIF-1α stability and activation requires interaction of these multiproteins with HIF-1α (Bae *et al*., 2002).

In addition to angiogenesis, HIF-1α is also a master transcription factor related to cell proliferation/survival, glucose metabolism, and iron metabolism. Hence HIF-1α plays in many diseases, such as cancer, stroke, and heart disease that generate a hypoxic microenvironment (Semenza, 2000). Especially, hypoxic tumor conditions might activate expression of genes that promote tumor growth leading to a more aggressive phenotype (Harris, 2001). Thus, the activation of HIF-1α has been associated with a variety of tumors and oncogenic pathways. On the contrary, the blocking of HIF-1α itself or HIF-1α interacting proteins inhibit tumor growth (Semenza, 2003). Particularly, HIF-1α antisense therapy, gene-therapy strategy to block the interaction between HIF-1α and its transcriptional co-activator CBP/p300 and small molecules such as 17AAG, YC-1 showed a possibility for cancer therapy (Kung *et al*., 2000; Sun *et al*., 2001). Based on these findings, HIF-1 can be a prime target for anticancer therapies (Harris, 2001; Semenza, 2003), therefore, the increased understanding of HIF-1α regulation will provide a key solution for novel therapeutical approaches.

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