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Review

Hypoxia-induced Angiogenesis during Carcinogenesis

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The formation of new blood vessels, angiogenesis, is an essential process during development and disease. Angiogenesis is well known as a crucial step in tumor growth and progression. Angiogenesis is induced by hypoxic conditions and regulated by the hypoxia-inducible factor 1 (HIF-1). The expression of HIF-1 correlates with hypoxia-induced angiogenesis as a result of the induction of the major HIF-1 target gene, vascular endothelial cell growth factor (VEGF). In this review, a brief overview of the mechanism of angiogenesis is discussed, focusing on the regulatory processes of the HIF-1 transcription factor. HIF-1 consists of a constitutively expressed HIF-1 beta (HIF-1β) subunit and an oxygen-regulated HIF-1 alpha (HIF-1α) subunit. The stability and activity of HIF-1α are regulated by the interaction with various proteins, such as pVHL, p53, and p300/CBP as well as by post-translational modifications, hydroxylation, acetylation, and phosphorylation. It was recently reported that HIF-1α binds a co-activator of the AP-1 transcription factor, Jab-1, which inhibits the p53-dependent degradation of HIF-1 and enhances the transcriptional activity of HIF-1 and the subsequent VEGF expression under hypoxic conditions. ARD1 acetylates HIF-1α and stimulates pVHL-mediated ubiquitination of HIF-1α. With a growing knowledge of the molecular mechanisms in this field, novel strategies to prevent tumor angiogenesis can be developed, and from these, new anticancer therapies may arise.

Keywords: Angiogenesis, HIF-1α, Hypoxia

Introduction

Carcinogenesis is a multistep process, which results from the loss of control in cell cycle regulation and, in the case of malignant disease, is followed by the metastatic spread of cancerous cells from the primary tissue site to other, distal tissue sites. The formation of new blood vessels at both the primary and metastatic sites is a critical step in the growth and spread of the primary and secondary tumors. Blood vessels are formed by the various processes of vasculogenesis, arteriogenesis, and angiogenesis. Vasculogenesis describes the earliest vascular development from the angioblasts or hemangioblasts in the embryo. Arteriogenesis represents the formation of collateral arteries from pre-existing arterioles as a process that is adaptive to arterial occlusion. Angiogenesis is defined as the formation of capillaries by the sprouting of pre-existing vessels, as in tumor angiogenesis (Schaper and Buschmann, 1999; Buschmann and Schaper, 2000; Cho *et al.*, 2001; Scholz *et al.*, 2001).

The critical role of angiogenesis in tumorigenesis was first suggested by Judah Folkman (Folkman *et al.*, 1971; Hanahan *et al.*, 1996). It is now accepted that a tumor mass cannot exceed ~1 mm^3 in an avascular state because of the limited nutrients and oxygen. 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). Perhaps the most important of these target genes, vascular endothelial cell growth factor (VEGF), specifically recruits endothelial cells into hypoxic and avascular area and stimulates their proliferation. Oxygen homeostasis is primarily operated by a cellular oxygen-sensing transcription factor, hypoxia-inducible factor 1 (HIF-1), which induces the transcription of more than 40 proteins, including VEGF (Table 1) (Semenza, 2002).

HIF-1 functions as a master regulator of oxygen and undergoes conformational changes in response to oxygen concentrations (Bruick and McKnight, 2001; Epstein *et al.*, 2001; Ivan *et al.*, 2001; Jaakkola *et al.*, 2001; Masson *et al.*, 2001). HIF-1 occupies the center of the hypoxia-signaling pathway. It binds a core sequence of the hypoxia response element (HRE) in the promoters of hypoxia-responsive genes and induces their expression. HIF-1 consists of α and β subunits, both of which are basic helix-loop-helix transcription factors. The expression of the α subunit is

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Table 1. Genes regulated by HIF-1

Genes	References
Growth factor-related	
Endocrine gland-derived VEGF	(LeCouter *et al.*, 2001)
Erythropoietin	(Jiang *et al.*, 1996)
IGF-II	(Feldser *et al.*, 1999)
IGF-binding protein I	(Tazuke *et al.*, 1998)
IGF-binding protein II	(Feldser *et al.*, 1999)
IGF-binding protein III	(Feldser *et al.*, 1999)
$TGF\beta_3$	(Caniggia *et al.*, 2000)
VEGF	(Carmeliet *et al.*, 1998; Iyer *et al.*, 1998)
Receptors	
FLT-1	(Gerber *et al.*, 1997)
α1B-adrenergic receptor	(Eckhart *et al.*, 1997)
Transferrin receptor	(Lok and Ponka, 1999; Tacchini *et al.*, 1999)
Glucose metabolism	
Aldolase A (ALDA)	(Iyer *et al.*, 1998; Ryan *et al.*, 1998)
Aldolase C (ALDC)	(Iyer *et al.*, 1998; Ryan *et al.*, 1998)
Enolase 1	(Iyer *et al.*, 1998)
Glucose transporter 1	(Ryan *et al.*, 1998; Wood *et al.*, 1998)
Glucose transporter 3	(Iyer *et al.*, 1998)
Glyceraldehyde-3-P-dehydrogenase	(Iyer *et al.*, 1998; Ryan *et al.*, 1998)
Hexokinase 1	(Iyer *et al.*, 1998)
Hexokinase 2	(Iyer *et al.*, 1998)
Phosphoglycerate kinase 1	(Carmeliet *et al.*, 1998; Iyer *et al.*, 1998)
Lactate dehydrogenase A (LDHA)	(Iyer *et al.*, 1998; Ryan *et al.*, 1998)
Pyruvate kinase M (PKM)	(Iyer *et al.*, 1998)
Others related in vascular growth and metabloism	
Adenylate kinase 3	(Wood *et al.*, 1998)
Adrenomedulin	(Cormier-Regard *et al.*, 1998)
Ceruloplasmin	(Mukhopadhyay *et al.*, 2000)
Collagen type V, α1	(Wykoff *et al.*, 2000)
Endothelin-1	(Hu *et al.*, 1998)
ETS-1	(Oikawa *et al.*, 2001)
Heme oxygenase-1	(Lee *et al.*, 1997)
LDL receptor-related protein 1	(Wykoff *et al.*, 2000)
Notric oxide synthase 2	(Melillo *et al.*, 1995; Palmer *et al.*, 1998)
p21	(Carmeliet *et al.*, 1998)
p35srj	(Bhattacharya *et al.*, 1999)
Plasminogen activator inhibitor 1 (PAI-1)	(Kietzmann *et al.*, 1999)
Prolyl-4-hydroxylase αI	(Takahashi *et al.*, 2000)
Transferrin	(Rolfs *et al.*, 1997)
Transglutaminase II	(Wykoff *et al.*, 2000)

remarkably high during hypoxia and is maintained at low levels in most cells under normoxic conditions. Unlike the α subunit, the β subunit is constitutively expressed and its activity is controlled in an oxygen-independent manner (Wang and Semenza, 1993). This review provides a general introduction to the molecular events in angiogenesis and hypoxia-induced angiogenesis in cancer.

Angiogenic Factors Involved in Cancer

Angiogenesis is stimulated by a number of angiogenic factors,

including a variety of growth factors and cytokines. The action of these factors includes proliferation and migration of endothelial cells, ECM degradation, and tube formation. A discussion of the growth factors and cytokines that are known to play a role in angiogenesis follows.

VEGF is the most potent endothelial-specific mitogen and is known to directly participate in the 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. PlGF, a VEGF family member, binds to VEGFR-1, which is selectively expressed in placenta and induces angiogenesis. In addition to VEGF, the VEGFR expression level is also important in endothelial cell proliferation. The three high-affinity receptors that bind VEGF are VEGFR-1 (Flt-1, *fms*-like tyrosine kinase-1), VEGFR-2 (KDR/Flk-1, kinase insert domain-containing receptor/fetal liver kinase-1), and VEGFR-3 (FLT4). It was reported that the expression of VEGF receptors is closely related in tumor angiogenesis (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; Ahmed *et al.*, 2000; Berra *et al.*, 2000; Harris, 2000; Josko *et al.*, 2000).

The up-regulation of the VEGF expression leads to an increased formation of the placenta growth factor (PlGF)/VEGF heterodimers that modulates VEGF activity when they are co-expressed (Cao *et al.*, 1996). The platelet-derived growth factor (PDGF) is found as an angiogenic factor for microvascular sprouting endothelial cells. PDGF-BB and receptors especially recruit the pericytes and smooth muscle cells around nascent vessel sprouts (Carmeliet and Jain, 2000). The expression of the PDGF-A and B chains are also regulated by oxygen. The PDGF-B expression is remarkably stimulated by hypoxia 13-fold (Gleadle *et al.*, 1995).

Our lab (Kim *et al.*, 1998) demonstrated that IGF-II functions in the angiogenic process of hepatocellular carcinoma, both directly and indirectly, by increasing the secretion of VEGF. IGF-II substantially increased the VEGF mRNA and protein levels in a time-dependent manner in HepG2 cells. Moreover, IGF-II increases the stability of HIF-1α protein, which is a key factor in the induction of the VEGF expression (Feldser *et al.*, 1999). It was also shown that hypoxia stimulated the insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells, resulting from an interaction of HIF-1 with a hypoxia response element (HRE) that is located in the first intron of the human IGFBP-1 gene (Tazuke *et al.*, 1998; Park *et al.*, 2001). HGF is also known to increase the DNA binding activity of HIF-1 in the HepG2 cells as HGF triggers a signal transduction cascade that involves the PI3K-AKT pathway to induce HIF-1 (Tacchini *et al.*, 2001).

The expression of chemokine IL-8 is also increased by hypoxia in tumor cells, and it induces the angiogenesis (Xie, 2001). In addition, cytokine IL-6 is an angiogenic stimulator in Huh7 hepatoma cells (Tsukamoto *et al.*, 1999).

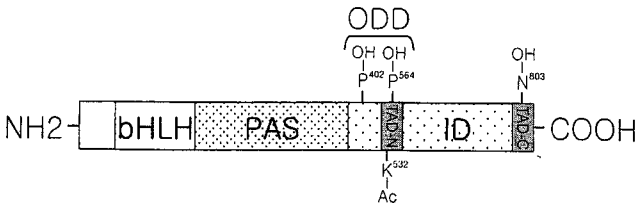

Fig. 1. Structure of HIF-1α.

Biology of HIF-1

The two subunits, HIF-1α and HIF-1β, aryl hydrocarbon receptor nuclear translocator (ARNT) belong to the bHLH-PAS (Per/Arnt/Sim) family (Fig. 1). HIF-1α dimerizes with ARNT to play a role as a transcription factor. The constitutively-expressed ARNT resides in the nucleus and acts as a common subunit of multiple bHLH-PAS transcription factors; whereas, HIF-1α constantly varies in its protein level, according to changes in the microenvironmental oxygen concentration. Therefore, the HIF-1α transcriptional activity is determined by the hypoxic induction and modifications of the HIF-1α protein level (Semenza, 1999; Minet *et al.*, 2001).

Beginning at the N-terminus, HIF-1α contains the basic helix-loop-helix (bHLH) domain, PAS domain, ID (inhibitory domain), TAD-N, and TAD-C (N- and C-terminal transactivation domains, respectively). These domains are involved in the regulatory machinery of HIF-1 biological activation. The bHLH and PAS domains are required for dimerization with HIF-1β and DNA binding (Salceda *et al.*, 1997; Huang *et al.*, 1998; Maxwell *et al.*, 1999; Berra *et al.*, 2001). The transcriptional activity of HIF-1 is regulated through TAD-N, TAD-C, and ID (Ruas *et al.*, 2002). Under normoxia, the hydroxylation of Pro-402 or Pro-564 in ID or TAD-N of HIF-1α, respectively, by HIF-1 prolyl hydroxylase is required for the binding of VHL, which recruits an E3 ubiquitin-protein ligase that targets HIF-1α for degradation (An *et al.*, 1998; Maxwell *et al.*, 1999; Kallio *et al.*, 1999; Ohh *et al.*, 2000; Ravi *et al.*, 2000). In the absence of oxygen, the prolyl hydroxylase is not active, consequently the unhydroxylated prolyl-HIF-1α cannot interact with pVHL (Bruick and McKnight, 2001; Epstein *et al.*, 2001; Ivan *et al.*, 2001; Jaakkola *et al.*, 2001; Masson *et al.*, 2001). For this reason, these domains are also called oxygen-dependent degradation (ODD) domains. VHL also recruits histone deacetylases (HDAC) that interfere with the transactivation domain function. An asparaginyl hydroxylase, FIH-1 (Factor inhibiting HIF-1), which binds to both VHL and HIF-1α, also inhibits the transcriptional activity of HIF-1 (Mahon *et al.*, 2001; Hewitson *et al.*, 2002).

The protein level of HIF-1α is regulated at the level of synthesis and stability. Activation of tyrosine kinases, such as SRC, $HER2^{neu}$, and the IGF and EGF receptors, stimulates the PI3K-AKT-FRAP and ERK/MAPK signal transduction pathways, which lead to the increased translation of HIF-1α mRNA into protein (Zhong *et al.*, 2000; Laughner *et al.*, 2001).

Regulation of HIF-1α Stability under Hypoxia

As previously mentioned, HIF-1α is rapidly degraded by a pVHL-mediated ubiquitin-proteosome pathway under normal oxygen conditions (An *et al.*, 1998; Kallio *et al.*, 1999; Maxwell *et al.*, 1999; Ohh *et al.*, 2000; Ravi *et al.*, 2000). In addition, p53 promotes the Mdm-2 mediated ubiquitination and proteosomal degradation of the HIF-1α through direct interaction with HIF-1α in hypoxia (Ravi *et al.*, 2000). The inhibitory PAS domain (IPAS) protein is also reported to target HIF-1 and plays a role as a negative regulator of the hypoxia-induced gene expression. The expression of IPAS in hepatoma cells impairs the induction of genes under hypoxic conditions, notably the VEGF gene, and results in retarded tumor growth and tumor vascular density *in vivo* (Makino *et al.*, 2001). HIF-1α stability is determined by a balance between the negative regulator and positive factor (Ema *et al.*, 1999). Under hypoxic conditions, HIF-1α is stabilized and exerts its transcriptional activity by binding to the p300/CBP (Arany *et al.*, 1996), SRC-1 (steroid receptor coactivator-1) family co-activators, nuclear redox regulator Ref-1 (Carrero *et al.*, 2000), and molecular chaperone heat shock protein 90 (HSP90) (Minet *et al.*, 1999). The HIF-1α-mediated transcriptional regulation is synergistically enhanced by p300/CBP, SRC-1, and Ref-1. The control of HIF-1α stability by post-translational modification or protein-protein interaction appears to be essential for the transcriptional activity of HIF-1α. Although HIF-1α is much more stable in hypoxia than normoxia, the mechanism of the hypoxia-dependent stabilization of HIF-1 is still unclear.

Using the yeast two-hybrid system, we recently identified the HIF-1α-binding protein, Jab-1, which was originally described as a transcriptional co-activator of c-Jun and Jun D (Bae *et al.*, 2002). Jab-1 plays a role in the variety of signaling pathways. These include integrin signaling (Rossetti *et al.*, 2002), cell cycle control (Tomoda *et al.*, 1999), and steroid hormone signaling (Chauchereau *et al.*, 2000). This protein is also known as a subunit of the mammalian CSN complex (CSN5), which phosphorylates transcriptional regulators such as c-Jun, IκB, p105, and p53 (Seeger *et al.*, 1998; Bech-Otschir *et al.*, 2001). Jab-1 interacts with the ODD domain of HIF-1α and is shown to control HIF-1α stability and activity by competition with p53. The interaction of Jab-1 with HIF-1α contributes to the up-regulation of the HIF-1α protein level under hypoxic conditions. However, under normoxia, Jab-1 has no effect on the up-regulation of the HIF-1α protein level. This is due to the strong affinity of pVHL for HIF-1α, which leads to the rapid degradation of HIF-1α. The p53 directly interacts with the ODD domain of HIF-1α and down-regulates the hypoxia-induced expression of HIF-1α by promoting the ubiquitination of HIF-1α under hypoxic conditions (Maxwell *et al.*, 1999; Ravi *et al.*, 2000), but not under normal oxygen concentrations. Therefore, Jab-1 directly interferes with the HIF-1α-p53 interaction and leads to the stabilization of the HIF-1α protein under hypoxia, but it probably exhibits a negligible effect on HIF-1α protein stability in response to normoxia. The Jab-1 mediated stabilization of HIF-1α plays a central role in the HIF-1 regulation under hypoxic conditions.

Regulation of HIF-1α Stability under Normoxia

During normoxia, the most predominant regulation process of HIF-1α is the pVHL-mediated ubiquitin-proteasome pathway (Salceda and Caro, 1997; Huang *et al.*, 1998; Maxwell *et al.*, 1999; Berra *et al.*, 2001). As previously mentioned, the association of HIF-1α with pVHL is triggered by the post-translational hydroxylation of proline residues (Pro-402, Pro-564) within its ODD domain that is mediated by HIF-1α prolyl-hydroxylase (HIF-PH) (Bruick and McKnight, 2001; Epstein *et al.*, 2001; Ivan *et al.*, 2001; Jaakkola *et al.*, 2001; Masson *et al.*, 2001). Recently, the Epstein group (Epstein *et al.*, 2001) identified *C. elegans* EGL-9 as a dioxygenase that regulates HIF-1 activity by prolyl hydroxylation and the consequent modulation of the pVHL binding to HIF-1α (Masson *et al.*, 2001). The activity of HIF-PH to HIF-1α is known to depend on the O_2 concentration, suggesting that this enzyme acts as an oxygen sensor (Epstein *et al.*, 2001). Another hydroxylation at Asn-803 in the C-terminal transactivation domain (CAD) of HIF-1α under normal oxygen concentrations inhibits the interaction of CAD with the p300/CBP co-activator and down-regulates the transcriptional activity of HIF-1 (Lando *et al.*, 2002b). The hydroxylated Asn-803 of HIF-1α recruits FIH-1 and inhibits the transactivation function of CAD. FIH-1 binds to pVHL and the FIH-1-pVHL complex associates with histone deacetylases (HDACs), which results in the inhibition of the HIF-1α transactivation function (Mahon *et al.*, 2001; Lando *et al.*, 2002a).

The regulation of HIF-1α stability and its activity occur at multiple levels. In addition to Jab-1, which regulates the protein level of HIF-1α by a protein-protein interaction manner, HIF-1α is also regulated by its post-translational modification. Studies on the post-translational modification of HIF-1 have presently been restricted to hydroxylation, ubiquitination, and phosphorylation.

Another protein that interacts with HIF-1α in the yeast two-hybrid system is ARD1 acetyltransferase (Jeong *et al.*, 2002), which is required for the expression of protein N-acetyltransferase (NAT) activity in lower eukaryotes and

bacteria, but whose function is not defined in mammalian cells (Park and Szostak, 1992; Tribioli *et al.*, 1994; Ingram *et al.*, 2000). Acetylation is found in various proteins, including histone, E2F1, MyoD, GATA-1, and p53 (Boyes *et al.*, 1998; Ogryzko *et al.*, 1998; Kouzarides, 1999; Kouzarides, 2000). ARD1 binds HIF-1α more strongly under normoxic conditions than under hypoxic conditions. The over-expression of ARD1 significantly decreases HIF-1α protein stability; whereas, the down-regulation of the ARD1 level with the antisense ARD1 transfection increases the stability of HIF-1α. This suggests that ARD1 functions as a negative regulator of HIF-1α (Jeong *et al.* 2002).

ARD1 acetylates the Lys-532 residue in the ODD domain of HIF-1α by transferring an acetyl group from Ac-CoA. The mutation of Lys-532 to arginine prevents acetylation by ARD1, and subsequently inhibits the pVHL-dependent ubiquitination of HIF-1α under normoxic conditions (Jeong *et al.*, 2002). Interestingly, it was previously reported that Lys-532 is critical for the degradation of HIF-1α under normoxia (Tanimoto *et al.*, 2000). The ODD domain contains sequences that mediate the O_2-dependent pVHL-E3 ubiquitination of the HIF-1α protein (Cockman *et al.*, 2000; Kamura *et al.*, 2000; Sutter *et al.*, 2000). Although it is unclear how the ARD1-mediated acetylation of HIF-1α leads to its decreased stability, a conformational change of HIF-1α by the acetylation may effectively facilitate its interaction with pVHL and enhance the subsequent proteosomal ubiquitination of HIF-1α. Therefore, the ARD1-mediated HIF-1α acetylation appears to be involved in a major regulation mechanism of HIF-1α under normoxic conditions.

Other Hypoxia-induced Transcriptional Factors

Egr-1, a zinc-finger-containing transcription factor is expressed under hypoxic conditions in mononuclear phagocytes and has been shown to mediate tissue factor production by endothelial cells that lead to the VEGF expression (Semenza, 2000). We previously reported that Egr-1 increases IGF-II transcription by direct activation of the P3 promoter of IGF-II under hypoxic conditions in HepG2 cells (Bae *et al.*, 1999). The stability of Egr-1 appears to be dependent on the oxygen tension via its ubiquitination and proteosome-dependent degradation (unpublished observation). Therefore, hypoxia enhances the expression and stability of Egr-1, resulting in an increase of the VEGF expression through IGF-II (Bae *et al.*, 1999).

The transcription factor, Ets-1, is expressed during angiogenesis in the normal and pathological development in endothelial cells (Lelievre *et al.*, 2001). Hypoxia is known to increase the expression of Ets-1 through the activation of its promoter by HIF-1 in the human bladder cancer cell line, T24 (Oikawa *et al.*, 2001). Therefore, the increased expression of Ets-1 may contribute to angiogenesis under hypoxic conditions.

Concluding Remarks

Oxygen homeostasis is involved in a variety of diseases, which generate a hypoxic microenvironment. The nucleus of an avascular tumor mass is a typical example of the hypoxic condition, and the hypoxia-induced angiogenesis of this area is a major turning point for the advancement of the tumor. Defining the mechanisms of angiogenesis will provide novel therapeutical approaches in anti-angiogenesis.

References

Ahmed, A., Dunk, C., Ahmad, S. and Khaliq, A. (2000) Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen-a review. *Placenta* **21 Suppl. A**, S16-S24.

An, F. Q., Matsuda, M., Fujii, H. and Matsumoto, Y. (2000) Expression of vascular endothelial growth factor in surgical specimens of hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* **126**, 153-160.

An, W. G., Kanekal, M., Simon, M. C., Maltepe, E., Blagosklonny, M. V. and Neckers, L. M. (1998) Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. *Nature* **392**, 405-408.

Arany, Z., Huang, L. E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M. A., Bunn, H. F. and Livingston, D. M. (1996) An essential role for p300/CBP in the cellular response to hypoxia. *Proc. Natl. Acad. Sci. USA* **93**, 12969-12973.

Bae, M. K., Ahn, M. Y., Jeong, J. W., Bae, M. H., Lee, Y. M., Bae, S. K., Park, J. W., Kim, K. R. and Kim, K. W. (2002) Jab1 interacts directly with HIF-1alpha and regulates its stability. *J. Biol. Chem.* **277**, 9-12.

Bae, S. K., Bae, M. H., Ahn, M. Y., Son, M. J., Lee, Y. M., Bae, M. K., Lee, O. H., Park, B. C. and Kim, K. W. (1999) Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Res.* **59**, 5989-5994.

Bech-Otschir, D., Kraft, R., Huang, X., Henklein, P., Kapelari, B., Pollmann, C. and Dubiel, W. (2001) COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. *EMBO J.* **20**, 1630-1639.

Berra, E., Pages, G. and Pouyssegur, J. (2000) MAP kinases and hypoxia in the control of VEGF expression. *Cancer Metastasis Rev.* **19**, 139-145.

Berra, E., Roux, D., Richard, D. E. 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**, 615-620.

Bhattacharya, S., Michels, C. L., Leung, M. K., Arany, Z. P., Kung, A. L. and Livingston, D. M. (1999) Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. *Genes Dev.* **13**, 64-75.

Boyes, J., Byfield, P., Nakatani, Y. and Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* **396**, 594-598.

Bruick, R. K. and McKnight, S. L. (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **294**, 1337-1340.

Bunn, H. F. and Poyton, R. O. (1996) Oxygen sensing and

molecular adaptation to hypoxia. *Physiol Rev.* **76**, 839-885.

Buschmann, I. and Schaper, W. (2000) The pathophysiology of the collateral circulation (arteriogenesis). *J. Pathol.* **190**, 338-342.

Caniggia, I., Mostachfi, H., Winter, J., Gassmann, M., Lye, S. J., Kuliszewski, M. and Post, M. (2000) Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta (3). *J. Clin. Invest.* **105**, 577-587.

Cao, Y., Linden, P., Shima, D., Browne, F. and Folkman, J. (1996) *In vivo* angiogenic activity and hypoxia induction of heterodimers of placenta growth factor/vascular endothelial growth factor. *J. Clin. Invest.* **98**, 2507-2511.

Carmeliet, P., Dor, Y., Herbert, J. M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F., Abramovitch, R., Maxwell, P., Koch, C. J., Ratcliffe, P., Moons, L., Jain, R. K., Collen, D., Keshert, E. and Keshet, E. (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumor angiogenesis. *Nature* **394**, 485-490.

Carmeliet, P. and Jain, R. K. (2000) Angiogenesis in cancer and other diseases. *Nature* **407**, 249-257.

Carrero, P., Okamoto, K., Coumailleau, P., O'Brien, S., Tanaka, H. and Poellinger, L. (2000) Redox-regulated recruitment of the transcriptional co-activators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. *Mol. Cell Biol.* **20**, 402-415.

Chauchereau, A., Georgiakaki, M., Perrin-Wolff, M., Milgrom, E. and Loosfelt, H. (2000) JAB1 interacts with both the progesterone receptor and SRC-1. *J. Biol. Chem.* **275**, 8540-8548.

Cho, H., Kim, W. J., Lee, S. W., Kim, Y. M., Choi, E. Y., Park, S., Kwon, Y. G. and Kim, K. W. (2001) Anti-angiogenic activity of mouse N-/C-terminal deleted endostation. *J. Biochem. Mol. Biol.* **34**, 206-211.

Cockman, M. E., Masson, N., Mole, D. R., Jaakkola, P., Chang, G. W., Clifford, S. C., Maher, E. R., Pugh, C. W., Ratcliffe, P. J. and Maxwell, P. H. (2000) Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J. Biol. Chem.* **275**, 25733-25741.

Conway, E. M., Collen, D. and Carmeliet, P. (2001) Molecular mechanisms of blood vessel growth. *Cardiovasc. Res.* **49**, 507-521.

Cormier-Regard, S., Nguyen, S. V. and Claycomb, W. C. (1998) Adrenomedullin gene expression is developmentally regulated and induced by hypoxia in rat ventricular cardiac myocytes. *J. Biol. Chem.* **273**, 17787-17792.

Eckhart, A. D., Yang, N., Xin, X. and Faber, J. E. (1997) Characterization of the alpha1B-adrenergic receptor gene promoter region and hypoxia regulatory elements in vascular smooth muscle. *Proc. Natl. Acad. Sci. USA* **94**, 9487-9492.

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**, 1905-1914.

Epstein, A. C., Gleadle, J. M., McNeill, L. A., Hewitson, K. S., O'Rourke, J., Mole, D. R., Mukherji, M., Metzen, E., Wilson, M. I., Dhanda, A., Tian, Y. M., Masson, N., Hamilton, D. L., Jaakkola, P., Barstead, R., Hodgkin, J., Maxwell, P. H., Pugh, C. W., Schofield, C. J. and Ratcliffe, P. J. (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**, 43-54.

Feldser, D., Agani, F., Iyer, N. V., Pak, B., Ferreira, G. and Semenza, G. L. (1999) Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res.* **59**, 3915-3918.

Folkman, J., Merler, E., Abernathy, C. and Williams, G. (1971) Isolation of a tumor factor responsible or angiogenesis. *J. Exp. Med.* **133**, 275-288.

Gerber, H. P., Condorelli, F., Park, J. and Ferrara, N. (1997) Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J. Biol. Chem.* **272**, 23659-23667.

Giordano, F. J. and Johnson, R. S. (2001) Angiogenesis: the role of the microenvironment in flipping the switch. *Curr. Opin. Genet. Dev.* **11**, 35-40.

Gleadle, J. M., Ebert, B. L., Firth, J. D. and Ratcliffe, P. J. (1995) Regulation of angiogenic growth factor expression by hypoxia, transition metals, and chelating agents. *Am. J. Physiol* **268**, C1362-C1368.

Hanahan, D., Christofori, G., Naik, P. and Arbeit, J. (1996) Transgenic mouse models of tumor angiogenesis: the angiogenic switch, its molecular controls, and prospects for preclinical therapeutic models. *Eur. J. Cancer* **32A**, 2386-2393.

Harris, A. L. (2000) von Hippel-Lindau syndrome: target for anti-vascular endothelial growth factor (VEGF) receptor therapy. *Oncologist* **5 Suppl. 1**, 32-36.

Hewitson, K. S., McNeill, L. A., Riordan, M. V., Tian, Y. M., Bullock, A. N., Welford, R. W., Elkins, J. M., Oldham, N. J., Bhattacharya, S., Gleadle, J. M., Ratcliffe, P. J., Pugh, C. W. and Schofield, C. J. (2002) Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. *J. Biol. Chem.* **277**, 26351-26355.

Hu, J., Discher, D. J., Bishopric, N. H. and Webster, K. A. (1998) Hypoxia regulates expression of the endothelin-1 gene through a proximal hypoxia-inducible factor-1 binding site on the antisense strand. *Biochem. Biophys. Res. Commun.* **245**, 894-899.

Huang, L. E., Gu, J., Schau, M. and Bunn, H. F. (1998) Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci. USA* **95**, 7987-7992.

Ingram, A. K., Cross, G. A. and Horn, D. (2000) Genetic manipulation indicates that ARD1 is an essential N(infinity)-acetyltransferase in Trypanosoma brucei. *Mol. Biochem. Parasitol.* **111**, 309-317.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S. and Kaelin, W. G., Jr. (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* **292**, 464-468.

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y. and Semenza, G. L. (1998) Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.* **12**, 149-162.

Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., Kriegsheim, A., Hebestreit, H. F., Mukherji, M.,

Schofield, C. J., Maxwell, P. H., Pugh, C. W. and Ratcliffe, P. J. (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* **292**, 468-472.

Jeong, J. -W., Bae, M. -K., Ahn, M. -Y., Kim, S. -H., Shon, T. -K., Bae, M. -H., Yoo, H. -A., Song, E. J., Lee, K. -J. and Kim, K. -W. (2002) Regulation and destabilization of HIF-1 alpha by ARD1-mediated acetylation. *Cell* **111**, 709-720..

Jiang, B. H., Rue, E., Wang, G. L., Roe, R. and Semenza, G. L. (1996) Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J. Biol. Chem.* **271**, 17771-17778.

Josko, J., Gwozdz, B., Jedrzejowska-Szypulka, H. and Hendryk, S. (2000) Vascular endothelial growth factor (VEGF) and its effect on angiogenesis. *Med. Sci. Monit.* **6**, 1047-1052.

Kallio, P. J., Wilson, W. J., O'Brien, S., Makino, Y. and Poellinger, L. (1999) Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. *J. Biol. Chem.* **274**, 6519-6525.

Kamura, T., Sato, S., Iwai, K., Czyzyk-Krzeska, M., Conaway, R.C. and Conaway, J.W. (2000) Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc. Natl. Acad. Sci. USA* **97**, 10430-10435.

Kietzmann, T., Roth, U. and Jungermann, K. (1999) Induction of the plasminogen activator inhibitor-1 gene expression by mild hypoxia via a hypoxia response element binding the hypoxia-inducible factor-1 in rat hepatocytes. *Blood* **94**, 4177-4185.

Kim, K. W., Bae, S. K., Lee, O. H., Bae, M. H., Lee, M. J. and Park, B. C. (1998) Insulin-like growth factor II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. *Cancer Res.* **58**, 348-351.

Kouzarides, T. (1999) Histone acetylases and deacetylases in cell proliferation. *Curr. Opin. Genet. Dev.* **9**, 40-48.

Kouzarides, T. (2000) Acetylation: a regulatory modification to rival phosphorylation? *EMBO J.* **19**, 1176-1179.

Lando, D., Peet, D. J., Gorman, J. J., Whelan, D. A., Whitelaw, M. L. and Bruick, R. K. (2002a) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev.* **16**, 1466-1471.

Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J. and Whitelaw, M. L. (2002b) Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* **295**, 858-861.

Laughner, E., Taghavi, P., Chiles, K., Mahon, P. C. and Semenza, G. L. (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol. Cell Biol.* **21**, 3995-4004.

LeCouter, J., Kowalski, J., Foster, J., Hass, P., Zhang, Z., Dillard-Telm, L., Frantz, G., Rangell, L., DeGuzman, L., Keller, G. A., Peale, F., Gurney, A., Hillan, K. J. and Ferrara, N. (2001) Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature* **412**, 877-884.

Lee, P. J., Jiang, B. H., Chin, B. Y., Iyer, N. V., Alam, J., Semenza, G. L. and Choi, A. M. (1997) Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. *J. Biol. Chem.* **272**, 5375-5381.

Lelievre, E., Lionneton, F., Soncin, F. and Vandenbunder, B. (2001) The Ets family contains transcriptional activators and repressors involved in angiogenesis. *Int. J. Biochem. Cell Biol.* **33**, 391-407.

Lok, C. N. and Ponka, P. (1999) Identification of a hypoxia response element in the transferrin receptor gene. *J. Biol. Chem.* **274**, 24147-24152.

Mahon, P. C., Hirota, K. and Semenza, G. L. (2001) FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* **15**, 2675-2686.

Makino, Y., Cao, R., Svensson, K., Bertilsson, G., Asman, M., Tanaka, H., Cao, Y., Berkenstam, A. and Poellinger, L. (2001) Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* **414**, 550-554.

Masson, N., Willam, C., Maxwell, P. H., Pugh, C. W. and Ratcliffe, P. J. (2001) Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. *EMBO J.* **20**, 5197-5206.

Maxwell, P. H., Wiesener, M. S., Chang, G. W., Clifford, S. C., Vaux, E. C., Cockman, M. E., Wykoff, C. C., Pugh, C. W., Maher, E. R. and Ratcliffe, P. J. (1999) The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271-275.

Melillo, G., Musso, T., Sica, A., Taylor, L. S., Cox, G. W. and Varesio, L. (1995) A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. *J. Exp. Med.* **182**, 1683-1693.

Minet, E., Michel, G., Mottet, D., Raes, M. and Michiels, C. (2001) Transduction pathways involved in Hypoxia-Inducible Factor-1 phosphorylation and activation. *Free Radic. Biol. Med.* **31**, 847-855.

Minet, E., Mottet, D., Michel, G., Roland, I., Raes, M., Remacle, J. and Michiels, C. (1999) Hypoxia-induced activation of HIF-1: role of HIF-1alpha-Hsp90 interaction. *FEBS Lett.* **460**, 251-256.

Mukhopadhyay, C. K., Mazumder, B. and Fox, P. L. (2000) Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. *J. Biol. Chem.* **275**, 21048-21054.

Neufeld, G., Cohen, T., Gengrinovitch, S. and Poltorak, Z. (1999) Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* **13**, 9-22.

Ogryzko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J. and Nakatani, Y. (1998) Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94**, 35-44.

Ohh, M., Park, C. W., Ivan, M., Hoffman, M. A., Kim, T. Y., Huang, L. E., Pavletich, N., Chau, V. and Kaelin, W. G. (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nature Cell Biol.* **2**, 423-427.

Oikawa, M., Abe, M., Kurosawa, H., Hida, W., Shirato, K. and Sato, Y. (2001) Hypoxia induces transcription factor ETS-1 via the activity of hypoxia-inducible factor-1. *Biochem. Biophys. Res. Commun.* **289**, 39-43.

Palmer, L. A., Semenza, G. L., Stoler, M. H. and Johns, R. A. (1998) Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. *Am. J. Physiol* **274**, L212-L219.

Park, J. H., Park, T., Kim, H. Y. and Yang, Y. M. (2001) The IGFBP-1 mRNA experession in HepG2 cells is affected by

inhibition of heme biosynthesis. *J. Biochem. Mol. Biol.* **34**, 385-389.

Park, E. C. and Szostak, J. W. (1992) ARD1 and NAT1 proteins form a complex that has N-terminal acetyltransferase activity. *EMBO J.* **11**, 2087-2093.

Ravi, R., Mookerjee, B., Bhujwalla, Z. M., Sutter, C. H., Artemov, D., Zeng, Q., Dillehay, L. E., Madan, A., Semenza, G. L. and Bedi, A. (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev.* **14**, 34-44.

Rolfs, A., Kvietikova, I., Gassmann, M. and Wenger, R. H. (1997) Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. *J. Biol. Chem.* **272**, 20055-20062.

Rossetti, G., Collinge, M., Bender, J. R., Molteni, R. and Pardi, R. (2002) Integrin-dependent regulation of gene expression in leukocytes. *Immunol. Rev.* **186**, 189-207.

Ruas, J. L., Poellinger, L. and Pereira, T. (2002) Functional Analysis of Hypoxia-inducible Factor-1alpha-mediated Transactivation. *J. Biol. Chem.* **277**, 38723-38730.

Ryan, H. E., Lo, J. and Johnson, R. S. (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J.* **17**, 3005-3015.

Salceda, S., Beck, I., Srinivas, V. and Caro, J. (1997) Complex role of protein phosphorylation in gene activation by hypoxia. *Kidney Int.* **51**, 556-559.

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**, 22642-22647.

Schaper, W. and Buschmann, I. (1999) VEGF and therapeutic opportunities in cardiovascular diseases. *Curr. Opin. Biotechnol.* **10**, 541-543.

Scholz, D., Cai, W.J. and Schaper, W. (2001) Arteriogenesis, a new concept of vascular adaptation in occlusive disease. *Angiogenesis.* **4**, 247-257.

Seeger, M., Kraft, R., Ferrell, K., Bech-Otschir, D., Dumdey, R., Schade, R., Gordon, C., Naumann, M. and Dubiel, W. (1998) A novel protein complex involved in signal transduction possessing similarities to 26S proteasome subunits. *FASEB J.* **12**, 469-478.

Semenza, G. L. (1999) Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annu. Rev. Cell Dev. Biol.* **15**, 551-578.

Semenza, G. L. (2000) Oxygen-regulated transcription factors and their role in pulmonary disease. *Respir. Res.* **1**, 159-162.

Semenza, G. L. (2002) HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol. Med.* **8**, S62-S67.

Sutter, C. H., Laughner, E. and Semenza, G. L. (2000) Hypoxia-inducible factor 1alpha protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. *Proc. Natl. Acad. Sci. USA* **97**, 4748-4753.

Tacchini, L., Bianchi, L., Bernelli-Zazzera, A. and Cairo, G. (1999) Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. *J. Biol. Chem.* **274**, 24142-24146.

Tacchini, L., Dansi, P., Matteucci, E. and Desiderio, M. A. (2001) Hepatocyte growth factor signaling stimulates hypoxia inducible factor-1 (HIF-1) activity in HepG2 hepatoma cells. *Carcinogenesis* **22**, 1363-1371.

Takahashi, Y., Takahashi, S., Shiga, Y., Yoshimi, T. and Miura, T. (2000) Hypoxic induction of prolyl 4-hydroxylase alpha (I) in cultured cells. *J. Biol. Chem.* **275**, 14139-14146.

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**, 4298-4309.

Tazuke, S. I., Mazure, N. M., Sugawara, J., Carland, G., Faessen, G. H., Suen, L. F., Irwin, J.C., Powell, D. R., Giaccia, A. J. and Giudice, L. C. (1998) Hypoxia stimulates insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: a possible model for IGFBP-1 expression in fetal hypoxia. *Proc. Natl. Acad. Sci. USA* **95**, 10188-10193.

Tomoda, K., Kubota, Y. and Kato, J. (1999) Degradation of the cyclin-dependent-kinase inhibitor p27Kip1 is instigated by Jab1. *Nature* **398**, 160-165.

Tribioli, C., Mancini, M., Plassart, E., Bione, S., Rivella, S., Sala, C., Torri, G. and Toniolo, D. (1994) Isolation of new genes in distal Xq28: transcriptional map and identification of a human homologue of the ARD1 N-acetyl transferase of Saccharomyces cerevisiae. *Human Mol. Genet.* **3**, 1061-1067.

Tsukamoto, A., Kaneko, Y., Yoshida, T., Ichinose, M. and Kimura, S. (1999) Regulation of angiogenesis in human hepatomas: possible involvement of p53-inducible inhibitor of vascular endothelial cell proliferation. *Cancer Lett.* **141**, 79-84.

Wang, G. L. and Semenza, G. L. (1993) General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc. Natl. Acad. Sci. USA* **90**, 4304-4308.

Wood, S. M., Wiesener, M. S., Yeates, K. M., Okada, N., Pugh, C. W., Maxwell, P. H. and Ratcliffe, P. J. (1998) Selection and analysis of a mutant cell line defective in the hypoxia-inducible factor-1 alpha-subunit (HIF-1alpha). Characterization of hif-1alpha-dependent and -independent hypoxia-inducible gene expression. *J. Biol. Chem.* **273**, 8360-8368.

Wykoff, C. C., Pugh, C. W., Maxwell, P. H., Harris, A. L. and Ratcliffe, P. J. (2000) Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumor suppressor by mRNA differential expression profiling. *Oncogene* **19**, 6297-6305.

Xie, K. (2001) Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev.* **12**, 375-391.

Zhong, H., Chiles, K., Feldser, D., Laughner, E., Hanrahan, C., Georgescu, M. M., Simons, J. W. and Semenza, G. L. (2000) Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res.* **60**, 1541-1545.