Cell. Mol. Life Sci. 64 (2007) 2170 – 2180
1420-682X/07/162170-11
DOI 10.1007/s00018-007-7082-2

Cellular and Molecular Life Sciences

Review

The role of hypoxia-inducible factors in cancer

M. A. Maynard and M. Ohh*

Department of Laboratory Medicine and Pathobiology, University of Toronto, 1 King's College Circle, Toronto, Ontario (Canada), M5S 1A8, e-mail: michael.ohh@utoronto.ca, Fax: +416 978 5959

Received 14 February 2007; accepted 26 April 2007
Online First 18 May 2007

Abstract. Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor that mediates the adaptive responses to hypoxia by effecting the transcription of numerous hypoxia-inducible genes. HIF is frequently overexpressed in solid tumors, and the transactivation of HIF targets in transformed cells provides a distinct survival advantage. Accordingly, the upregulation of HIF correlates with increased progression or aggressiveness of the cancer and poor prognosis. In addition to the induction of HIF by hypoxia, its expression is induced by the loss of tumor suppressors VHL, PTEN, TSC1/2, PML, and SDH, as well as by the increased activity of PI3K and/or MAPK signaling pathways, underscoring the significance of HIF in oncogenesis.

Keywords. HIF, VHL, PI3K, MAPK, PHD.

Expression of HIFs

Hypoxia-inducible factors (HIFs) are heterodimeric transcription factors of the basic helix-loop-helix PAS (Per/ARNT/Sim) family of DNA-binding proteins that induce the transcription of a diverse array of genes to effect the hypoxic response. HIFs are composed of an α subunit and a β subunit of which the most ubiquitously expressed is the aryl hydrocarbon receptor nuclear translocator (ARNT) [1, 2]. HIF activity is regulated at the level of the α subunit, with ARNT being constitutively expressed and stable [3]. There are three HIF-α genes in humans: HIF-1α, HIF-2α, and HIF-3α. There are multiple splice variants of HIF-1α and HIF-3α, with dominant-negative protein products being produced in some tissues [4 – 9]. The role of the different HIF-α subunits has been investigated in development and in disease processes such as cancer where they are frequently overexpressed. In mice, knockout of HIF-1α or HIF-2α is embryonic lethal, while the mouse knockout of HIF-3α has not yet been addressed. HIF-1α–/– embryos die by day E10.5 of cardiac and vascular malformations [10, 11] and HIF-2α–/– embryos die by day E16.5 of failed cardiac function due to decreased release of catecholamines [12]. In human tissues, HIF-1α messenger RNA (mRNA) expression is generally ubiquitous with high expression in the kidneys, while HIF-2α mRNA is predominantly expressed in the heart, placenta, and lungs [13, 14]. HIF-2α may play a role in development of the tubular system and vascular remodelling as it is also expressed in endothelial cells [15 – 18]. Human HIF-3α mRNA expression has been found to be highest in the heart, placenta, lung, and skeletal muscle [6].

* Corresponding author.

HIF expression in cancer

HIFs are frequently upregulated in cancer and their metastases because transcription of their downstream target genes can promote growth and survival. Increased expression and activity of HIF-α in cancer may occur by loss of tumor suppressors such as VHL, activation of oncogenes, and increased activity of the PI3K and MAPK signalling pathways discussed below. High levels of HIF-1α have been positively correlated with tumor progression and poor prognosis in patients with brain, non-small cell lung carcinoma (NSCLC), breast, oesophageal, stomach, fibrosarcoma, colorectal carcinoma (CRC), prostate, ovarian, uterine, and cervical tumors [19–34]. Overexpression of HIF-2α has been correlated with tumor progression and poor prognosis in patients with NSCLC, head and neck squamous cell carcinoma (HN-SCC), CRC, and VHL–/– clear cell-renal cell carcinoma (CC-RCC) [31, 32, 34–38]. HIF-3α expression is detectable in several human cancer cell lines [6, 39], and while expression of the dominant-negative HIF-3α inhibits endogenous HIF-1-mediated transcription [6, 8], the significance of the full-length HIF-3α for tumor progression is unknown. Generally, HIF overexpression promotes tumorigenesis, but there are several examples to the contrary. Knock-down of HIF-2α in rat glioma tumors reduced apoptosis, and overexpression of HIF-2α reduced growth of these tumors [40], while in VHL–/– CC-RCC xenograft assays, overexpression of HIF-1α inhibited tumor growth and overexpression of HIF-2α promoted tumor progression [37].

Regulation of HIF-α by PHDs and VHL

HIF activity is regulated at the level of the α subunit by mechanisms affecting its protein expression, stability, and transcriptional activity. The most well characterized regulatory mechanism for HIF-α is its degradation in the presence of oxygen [1]. Oxygen-dependent hydroxylation by a family of prolyl-hydroxylase domain-containing proteins, or PHDs 1–3 [41, 42], at a conserved residue in the oxygen-dependent degradation (ODD) domain mediates binding to the von Hippel Lindau (VHL) tumor suppressor protein, pVHL [6,43–48]. pVHL is the substrate-docking interface for an E3 ubiquitin ligase complex that polyubiquitylates HIF-α, targeting it for degradation by the common 26S proteasome [46–48]. Under conditions of reduced oxygen availability PHDs do not hydroxylate HIF-α [41,42], the VHL E3 ligase does not recognize the α subunit, and it is no longer targeted for ubiquitin-mediated destruction by the 26S proteasome [46–48]. Patients with VHL disease have inherited an inactivating mutation in one allele of VHL and have a subsequent somatic mutation of the remaining allele [49]. CC-RCC-causing mutations in VHL patients that prevent VHL from recognizing HIF-α or those that disrupt the formation of a functional E3 ligase complex result in the failure to target HIF-α for efficient oxygen-dependent degradation and a loss of tumor suppressor function for VHL in the kidney [50, 51]. In these VHL patients or individuals with somatic mutations of VHL in the kidney epithelium, patients develop CC-RCC and haemangioblastomas [52–56]. As mentioned, overexpression of HIF-α, particularly HIF-2α, has been implicated in the tumorigenesis of VHL–/– CC-RCC. Interestingly, mutations in tumor suppressors other than VHL may also disregulate HIF-α stability by affecting pVHL's ability to bind HIF-α. Loss of the succinate dehydrogenase (SDH) or fumarate hydratase (FH) tumor suppressors result in increased levels of succinate and fumarate, respectively, which inhibit PHD activity preventing hydroxylation of HIF-α [57].

Regulation of HIF-α protein levels by phosphorylation signalling cascades

Both phosphoinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling cascades can regulate HIF-1α protein levels in an oxygen-independent manner. Increased expression of HIF-1α via the PI3K signalling pathway may occur by gain-of-function mutations in upstream positive regulators such as receptor tyrosine kinases and Ras, or loss-of-function mutations in tumor suppressors such as tuberous sclerosis (TSC) 1 or 2 or phosphatase and tensin homolog (PTEN) [58–64]. The PI3K pathway can increase translation of HIF-1α mRNA by both mammalian target of rapamycin (mTOR)-dependent or mTOR-independent mechanisms [58, 61, 65, 66]. As part of a signalling complex, mTOR kinase regulates protein synthesis via S6 kinase-mediated phosphorylation of S6 ribosomal binding protein to increase expression of translational mediators and via activation of eukaryotic initiation factors to increase both cap-dependent and cap-independent translation [66]. The mTOR-dependent mechanism of increased HIF-1α translation is most active under low serum conditions and has been observed in TSC2–/– mouse embryonic fibroblast (MEF) cells and osteosarcoma U2OS cells with TSC2 knock-down [61], in neuroblastoma cells downstream of brain-derived growth factor (BDGF)-mediated activation of the TrkB receptor via PI3K [30], in IGF-2-overexpressing rhabdomyosarcoma cell lines [67], in the MCF7 breast

cancer cell line via activation of the human epidermal growth factor receptor 2 (HER2) [58], and in PML–/– MEFs via loss of Ras homolog enriched in brain (Rheb) regulation [68]. PI3K can also upregulate translation of HIF-1α mRNA by a mechanism that is mTOR-independent and may be dependent on internal ribosomal entry sequence (IRES)-mediated translation instead of the cap-dependent translation potentiated by mTOR [66, 69].

Regulation of HIF-α expression by other mechanisms

There is also some evidence that reactive oxygen species can increase HIF-driven gene expression by modulating both HIF-α levels and transcriptional activity [70]. Recently it was shown that nitric oxide synthase expression in oral squamous cell carcinoma is responsible for accumulation of HIF-1α [71] and that NADPH oxidase 4 (NOX4) expression in CC-RCC is required for endogenous HIF-2α mRNA and protein expression [72].

Regulation of HIF transactivation

HIFs bind to hypoxia-responsive elements (HREs) in the enhancers of target genes, which requires the basic helix-loop-helix, PAS, and PAS-associated C-terminal (PAC) domains at the amino-termini of the α subunits [73]. Two transactivation domains mediate gene transcription by HIF-1α and HIF-2α: the N-terminal transactivation domain (N-TAD), which partially overlaps with the ODD domain, and the C-terminal transactivation domain (C-TAD) [74, 75]. HIF-3α has an N-TAD but does not possess the C-TAD of HIF-1α and HIF-2α [6, 39, 76]. Although HIF-3α may have weaker transactivation activity at hypoxia-responsive elements than HIF-1 and HIF-2 [39, 76], its novel C-terminus may have additional uncharacterized transactivation properties [6, 39]. The C-TAD of HIF-1α and HIF-2α bind to p300/CREB binding protein (CBP), steroid receptor co-activator-1 (SRC-1), and transcription intermediary factor-2 (TIF-2) as co-activators to effect transcription [75, 77–80]. For HIF-2α, the C-TAD may not be necessary for transcription of all HIF-mediated genes such that the N-TAD alone appears to sometimes suffice [81]. The mechanism of transactivation by the N-TAD is not well characterized, and although p300/CBP can potentiate its activity, there is no evidence for a direct interaction between the N-TAD and p300/CBP as there is for the C-TAD [77–79, 82, 83]. The binding of coactivators to the C-TAD is regulated by post-translational hydroxylation and cysteine reduction events. Hydroxylation of a conserved asparagine residue in the C-TAD in the presence of oxygen by factor inhibiting HIF-1 (FIH-1) prevents binding of p300/CBP to the C-TAD [84]. Under hypoxia, the C-TAD is no longer hydroxylated, and p300/CBP may be recruited by HIF-1α [84]. The HIF-2α C-TAD appears to be resistant to FIH1-mediated inhibition under normoxia [81]. To promote C-TAD-mediated transactivation, reduction of a conserved cysteine residue in the C-TAD of HIF-1α and HIF-2α by REF-1 in conjunction with transferrin potentiates binding of p300/CBP and SRC-1 to this domain [77]. Interestingly, knockout of the C-TAD-interacting CH1 domain of p300 and CBP in mouse has revealed that less than 50% of HIF-1-inducible genes in MEFs are dependent on this interaction [85]. Most HIF-1 regulated/CH1-dependent genes were sensitive to histone deacetylase (HDAC) inhibitors, and some required both HDAC activity and p300/CBP [85]. HIF-1α has been shown to interact with HDACs 4, 6, and 7, and these interactions have also been shown to stabilize and potentiate transcription by HIF-1α [86, 87]. The role of direct acetylation of HIF-1α in regulating it's stability and transcription activity is controversial and will not be discussed [88–92].

Phosphorylation of HIF-1α and p300 also regulates transactivation. P300 phosphorylation by the MAPK pathway improves the recruitment of p300/CBP to the C-TAD of HIF-1α [93]. Direct phosphorylation of HIF-1α by the MAPK pathway has been shown to potentiate transactivation by HIF-1 of target genes [94, 95], and may involve protecting the subunit for exportin-dependent nuclear export [96].

A differential role for the N-TAD and C-TAD in HIF transcription

It has been suggested that as oxygen concentration decreases during hypoxia, inhibition of PHD-mediated hydroxylation of the ODD domain may occur before inhibition of FIH-1-mediated hydroxylation of the C-TAD [97]. This would lead to activation of the N-TAD before the C-TAD, and perhaps selective regulation of some genes in a positive manner by the N-TAD, but negatively once cofactors can be recruited to the C-TAD [97].

HIF transcriptional targets in the development and progression of cancer

Many of the genes regulated by HIF-1 and HIF-2 to allow a cell and tissue to adapt to hypoxic conditions also promote the survival of a tumorigenic phenotype

in cancer. HIF-1 can upregulate more than 60 targets, including those involved in angiogenesis, anaerobic glucose metabolism, metastasis, cell motility, iron metabolism, growth and survival, apoptosis, telomere maintenance, and drug-export mechanisms. Several of these processes and the relevant HIF targets will now be discussed.

The most notable protein induced by HIF-1 and HIF-2 involved in angiogenesis is vascular endothelial growth factor (VEGF)-A [16, 98–100], the potent endothelial cell mitogen highly expressed in many solid tumor types. Angiogenesis is required for a tumor to grow beyond the size accommodated by diffusion from the existing vasculature alone [101]. Interestingly, increased angiogenesis in solid tumors does not result in the tumor losing the induction of the hypoxic response if HIF can be targeted for degradation because the tumor vasculature is highly irregular allowing for regions of hypoxia to persist [102–104]. HIF-1 has also been shown to increase the expression of transforming growth factor (TGF)-β3, leptin, and endoglin to promote angiogenesis [10, 98, 99, 105–107]. In addition, HIF-1 can increase the permeability and dilation of the vasculature by inducing the expression of VEGF-A, nitric oxide synthase 2, haem oxygenase-1, endothelin-1, and the $\alpha_{1\beta}$-adrenergic receptor [98, 99, 108–113]. HIF-2 has also been shown to transactivate VEGF receptor-2 (VEGFR2) and the endothelial receptor tyrosine kinase Tie-2 [16, 18, 114].

To adapt to reduced oxygen availability and subsequent decreased oxidative phosphorylation, HIF-1 upregulates the enzymes of glycolysis and glucose transporters (GLUTs) 1 and 3 to maintain cellular ATP pools [115, 116]. This switch to anaerobic metabolism is also another trademark of solid tumor biology. Interestingly, the glucose metabolites pyruvate and oxaloacetate appear to regulate normoxic HIF-1α expression in cancer cell lines by inhibiting PHD-mediated hydroxylation of HIF-1α [117, 118]. Therefore, there may be a role for glycolysis in a positive feedback loop of sustained HIF-1α activation in cancer [117, 118].

Increasing the invasive potential of a cell and changing the extracellular environment to promote migration is required for tumor spreading and metastasis. HIF-1 regulates autocrine motility factor (AMF), TGF-α, urokinase-type plasminogen activator receptor (uPAR), matrix metalloproteinase-2 (MMP-2), E-cadherin (negatively), lysyl oxidase, and chemokine (C-X-C motif) receptor 4 (CXCR4) to potentiate migration and invasion of cancer cells [34, 112, 119–128]. E-cadherin, a homophilic adhesion molecule of epithelial cells, was recently identified as being positively regulated by VHL in a HIF-dependent manner (HIF induces the expression of inhibitory regulators of transcription) [124–126]. Loss of E-cadherin expression occurs early in pre-neoplastic lesions and potentiates migration and invasion of VHL–/– CC-RCC cells [124, 129]. Lysyl oxidase promotes metastasis of breast cancer tumors by promoting focal adhesion kinase activity and cell-to-matrix adhesion [123]. Induction of CXCR-4 and its ligand stromal cell-derived factor-1alpha (SDF-1α) by HIF-1 and HIF-2 promotes invasiveness of VHL–/– CC-RCC cells locally and metastasis distally to organs expressing SDF-1α ligand [34, 127, 128]. Deposition of vimentin, which is also regulated by HIF-1, an extracellular matrix (ECM) component, leads to a de-differentiated ECM (instead of the more differentiated keratin-rich ECM) that promotes increased motility of cancer cells [122].

HIF-1 and HIF-2 regulate the expression of many growth and survival factors for tumor maintenance and progression, including VEGF-A, transforming growth factor (TGF)-α, survivin, cyclooxygenase (COX)-2, platelet-derived growth factor (PDGF)-β, insulin-like growth factor (IGF-2), and insulin-like growth factor binding proteins (IGFBPs) 1–3 [16, 98–100, 119, 130–135]. TGF-α is a powerful mitogen for renal proximal tubule epithelial cells (the suggested origin of CC-RCC) and VHL –/– CC-RCC cell lines, and signalling downstream of its receptor epithelial growth factor receptor (EGFR) may result in an autocrine signalling loop with PI3K further activating expression of HIF-1 and HIF-2 [119]. Expression of survivin, an anti-apoptotic factor, has been partly attributed to downstream signalling from the EGFR in breast cancer via HIF-1 [135]. Similarly, COX-2 expression by HIF-1 in CRC cells leads to production of the survival factor prostaglandin E2 (PGE2) and positive feedback on HIF-1 activity by activating the MAPK pathway [134].

HIF-1 also has the potential to induce several pro-apoptotic genes such as BCL2/adenovirus E1B 19-kDa interacting protein 3 (BniP3), BCL2/adenovirus E1B 19-kDa interacting protein 3-like (BNip3L), and DNA-damage-inducible transcript 4 (DDIT4) [136–138]. Mutation of p53 and epigenetic silencing, such as for the BNip3 promoter in CRC cancer cell lines [139], may explain the apparent contradiction of increased HIF-1 expression generally correlating with poor prognosis.

HIF-1 can contribute to radiotherapy and chemotherapy treatment resistance. It increases the expression of the P-gp drug efflux pump that is commonly overexpressed in cancer which confers resistance to chemotherapeutics [140]. It has also been implicated in double-strand break repair and transcriptionally upregulating expression of the catalytic subunit of

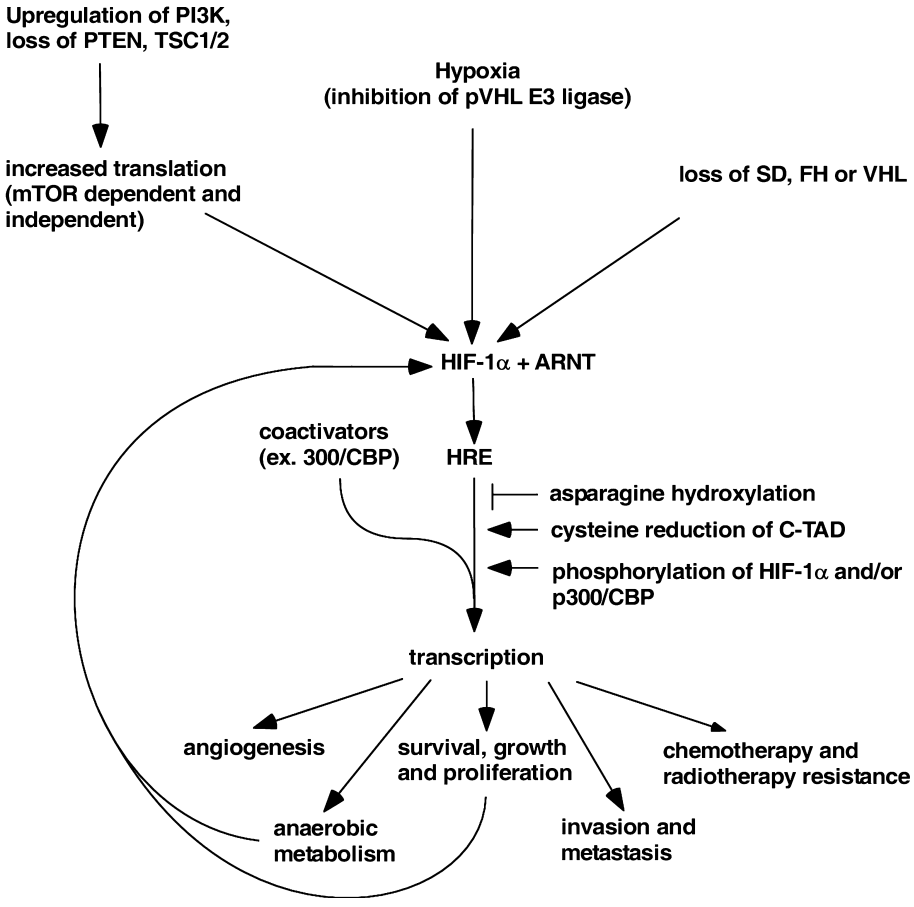

Figure 1. Deregulation of hypoxia-inducible factor (HIF) in cancer by oxygen-dependent and independent mechanisms. Loss of tumor suppressor proteins, hypoxia, and increased activity of phosphorylation signalling cascades increase the expression and/or stability of HIF in cancers. As well, positive feedback by the glycolytic metabolites and growth factor signalling downstream of HIF-1 increase HIF-1α expression. HIF transactivation is tightly regulated by phosphorylation and hydroxylation events, and the upregulation of HIF-target genes promotes the growth and survival of tumor by multiple mechanisms.

telomerase, human telomerase reverse transcriptase (hTERT) [141–143]. Increased expression of hTERT is also a trademark of cancer allowing the number of cell divisions to be unhinged to telomere length of the chromosomes [144].

Tissue specificity of HIF-1 and HIF-2 dependent transcription

An emerging area of research in hypoxia-mediated gene expression is the tissue-specific gene transcription profile of HIF-1 versus HIF-2. As mentioned, HIF-2α plays an important role in the development and progression of VHL–/– CC-RCC. Support for the preferential expression of HIF-2α in VHL–/– CC-RCC is from data demonstrating that HIF-2 appears to be responsible for activating transcription of the pro-tumorigenic genes such as TGF-α, VEGF, and cyclin D1, while HIF-1 upregulates the pro-apoptotic factor BNip-3 [37]. Another group has also reported similar results demonstrating HIF-2α was predominantly responsible for VEGF, uPAR, and PAI-1 expression in VHL –/– CC-RCC [145]. However, this group found that in MCF-7 breast cancer cells, it was HIF-1α that primarily regulates VEGF and IGF-1 [145]. As well, HIF-2 and HIF-1 seem to negatively affect the expression of one another in both VHL–/– CC-RCC cell lines and MCF-7 [37, 145].

HIF-2 has unique targets additional to those of HIF-1. The VEGFR2 (Flk-1) is a HIF-2 regulated gene whose transcription is dependent on the interaction of the N-TAD of HIF-2α with the E26 transformation-specific (ETS) transcription factors [114]. Several other genes have been reported to be HIF-2 driven in an ETS-dependent manner as well, including CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2) and WNT1 inducible signaling pathway protein 1 (WISP1) in the MCF-7 cell line [146]. In the 11 genes identified as being HIF-2 regulated, almost all had an ETS-binding site adjacent to the HRE, and knock-down of ETS family member ELK-1 significantly reduced the expression of these genes [146]. The mechanisms of transcription for the HIF-2-specific targets octomer binding transcription factor-4 (Oct-4) and erythropoietin are not as well understood and may involve interaction of HIF-2α with as of yet uncharacterized co-factors. Oct-4 is a transcription factor essential for maintaining stem cell pluripotency [147]. Significantly, knock-in expression of HIF-2α in

Oct-4–/– homozygous mouse embryos caused large embryonic-stem cell-derived tumors [147]. Erythropoietin (EPO) was first reported to be a HIF-1 regulated gene; it has now been shown that knockdown of HIF-2α but not HIF-1α by siRNA (small-interfering RNA) transfection significantly decreased the expression of EPO in Kelly neuroblastoma and Hep3B cell lines (the cell line that was used to identify EPO as a HIF-1 target) [148]. While the EPO HRE in isolation was responsive to both HIF-1α and HIF-2α knockdown and showed no cell-type specific expression, when the entire EPO enhancer was used, there was a significant difference in luciferase activation, suggesting an explanation for the earlier findings that EPO was a HIF-1-regulated gene [148].

Conclusion

Hypoxia-inducible gene expression is regulated by a family of HIF transcription factors of which HIF-1 is the most well characterized, HIF-2 has emerged as a non-redundant player, and the role of full-length HIF-3 is as of yet unknown. Oxygen-dependent regulation of the α subunit of these factors is well established, as is the role for both PI3K and MAPK signalling cascades (see Fig. 1). Deregulation of these pathways by loss of any one of several tumor suppressors leads to increased expression and/or activity of HIFs during tumorigenesis, providing a growth and survival advantage to the cells. Interestingly, drugs that have been shown to inhibit HIF have demonstrated significant activity in clinical trials, and in the case of kidney cancer, represent major advances in the treatment of these diseases. However, when treatments involve non-specific targeting of several HIF-α subunits, consideration should be given to the fact that the role of a given subunit is tissue-specific, and in some instances they can play an inhibitory role in tumorigenesis.

1 Wang, G. L., Jiang, B. H., Rue, E. A. and Semenza, G. L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc. Natl. Acad. Sci. USA 92, 5510 – 5514.

2 Carver, L. A., Hogenesch, J. B., and Bradfield, C. A. (1994) Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. Nucleic Acids Res. 22, 3038 – 3044.

3 Kallio, P. J., Pongratz, I., Gradin, K., McGuire, J., and Poellinger, L. (1997) Activation of hypoxia-inducible factor 1alpha: posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. Proc. Natl. Acad. Sci. USA 94, 5667 – 5672.

4 Chun, Y. S., Choi, E., Kim, T. Y., Kim, M. S., and Park, J. W. (2002) A dominant-negative isoform lacking exons 11 and 12 of the human hypoxia-inducible factor-1alpha gene. Biochem. J. 362, 71 – 79.

5 Gothie, E., Richard, D. E., Berra, E., Pages, G., and Pouyssegur, J. (2000) Identification of alternative spliced variants of human hypoxia-inducible factor-1alpha. J. Biol. Chem. 275, 6922 – 6927.

6 Maynard, M. A., Qi, H., Chung, J., Lee, E. H., Kondo, Y., Hara, S., Conaway, R. C., Conaway, J. W., and Ohh, M. (2003) Multiple splice variants of the human HIF-3alpha locus are targets of the VHL E3 ubiquitin ligase complex. J. Biol. Chem. 278, 11032 – 11040.

7 Maynard, M. A., Evans, A. J., Hosomi, T., Hara, S., Jewett, M. A., and Ohh, M. (2005) Human HIF-3alpha4 is a dominant-negative regulator of HIF-1 and is down-regulated in renal cell carcinoma. FASEB J. 19, 1396 – 1406.

8 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.

9 Makino, Y., Kanopka, A., Wilson, W. J., Tanaka, H., and Poellinger, L. (2002) IPAS is an hypoxia-inducible splicing variant of the HIF-3α locus. J. Biol. Chem. 277, 32405 – 32408.

10 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. et al. (1998) Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 12, 149 – 162.

11 Ryan, H., Lo, J., and Johnson, R. (1998) HIF-1α is required for solid tumor formation and embryonic vascularization. EMBO J. 17, 3005 – 3015.

12 Tian, H., Hammer, R., Matsumoto, A., Russell, D., and McKnight, S. (1998) The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. Genes Dev. 12, 3320 – 3324.

13 Hogenesch, J. B., Chan, W. K., Jackiw, V. H., Brown, R. C., Gu, Y. Z., Pray-Grant, M., Perdew, G. H., and Bradfield, C. A. (1997) Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J. Biol. Chem. 272, 8581 – 8593.

14 Wiener, C. M., Booth, G., and Semenza, G. L. (1996) In vivo expression of mRNAs encoding hypoxia-inducible factor 1. Biochem. Biophys. Res. Commun. 225, 485 – 488.

15 Flamme, I., Frohlich, T., von Reutern, M., Kappel, A., Damert, A., and Risau, W. (1997) HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. Mech. Dev. 63, 51 – 60.

16 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. USA 94, 4273 – 4278.

17 Peng, J., Zhang, L., Drysdale, L., and Fong, G. H. (2000) The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. Proc. Natl. Acad. Sci. USA 97, 8386 – 8391.

18 Tian, H., McKnight, S. L., and Russell, D. W. (1997) Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev. 11, 72 – 82.

19 Koukourakis, M. I., Giatromanolaki, A., Skarlatos, J., Corti, L., Blandamura, S., Piazza, M., Gatter, K. C., and Harris, A. L. (2001) Hypoxia inducible factor (HIF-1a and HIF-2a) expression in early esophageal cancer and response to photodynamic therapy and radiotherapy. Cancer Res. 61, 1830 – 1832.

20 Zhong, H., De Marzo, A., Laughner, E., Lim, M., Hilton, D., Zagzag, D., Buechler, P., Isaacs, W., Semenza, G., and Simons, J. (1999) Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res. 59, 5830 – 5835.

21 Talks, K. L., Turley, H., Gatter, K. C., Maxwell, P. H., Pugh, C. W., Ratcliffe, P. J., and Harris, A. L. (2000) The expression and distribution of the hypoxia-inducible factors HIF-1alpha and

HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am. J. Pathol. 157, 411 – 421.
22 Schindl, M., Schoppmann, S. F., Samonigg, H., Hausmaninger, H., Kwasny, W., Gnant, M., Jakesz, R., Kubista, E., Birner, P., and Oberhuber, G. (2002) Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin. Cancer Res. 8, 1831 – 1837.
23 Birner, P., Schindl, M., Obermair, A., Breitenecker, G., and Oberhuber, G. (2001) Expression of hypoxia-inducible factor 1alpha in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. Clin. Cancer Res. 7, 1661 – 1668.
24 Birner, P., Gatterbauer, B., Oberhuber, G., Schindl, M., Rossler, K., Prodinger, A., Budka, H., and Hainfellner, J. A. (2001) Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer 92, 165 – 171.
25 Birner, P., Schindl, M., Obermair, A., Plank, C., Breitenecker, G., and Oberhuber, G. (2000) Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res. 60, 4693 – 4696.
26 Bos, R., van der Groep, P., Greijer, A. E., Shvarts, A., Meijer, S., Pinedo, H. M., Semenza, G. L., van Diest, P. J., and van der Wall, E. (2003) Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 97, 1573 – 1581.
27 Sivridis, E., Giatromanolaki, A., Gatter, K. C., Harris, A. L., and Koukourakis, M. I. (2002) Association of hypoxia-inducible factors 1alpha and 2alpha with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. Cancer 95, 1055 – 1063.
28 Takahashi, R., Tanaka, S., Hiyama, T., Ito, M., Kitadai, Y., Sumii, M., Haruma, K., and Chayama, K. (2003) Hypoxia-inducible factor-1alpha expression and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncol. Rep. 10, 797 – 802.
29 Detwiller, K. Y., Fernando, N. T., Segal, N. H., Ryeom, S. W., D'Amore, P. A., and Yoon, S. S. (2005) Analysis of hypoxia-related gene expression in sarcomas and effect of hypoxia on RNA interference of vascular endothelial cell growth factor A. Cancer Res. 65, 5881 – 5889.
30 Nakamura, K., Martin, K. C., Jackson, J. K., Beppu, K., Woo, C. W., and Thiele, C. J. (2006) Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha in neuroblastoma cells. Cancer Res. 66, 4249 – 4255.
31 Winter, S. C., Shah, K. A., Han, C., Campo, L., Turley, H., Leek, R., Corbridge, R. J., Cox, G. J., and Harris, A. L. (2006) The relation between hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression with anemia and outcome in surgically treated head and neck cancer. Cancer 107, 757 – 766.
32 Yoshimura, H., Dhar, D. K., Kohno, H., Kubota, H., Fujii, T., Ueda, S., Kinugasa, S., Tachibana, M., and Nagasue, N. (2004) Prognostic impact of hypoxia-inducible factors 1alpha and 2alpha in colorectal cancer patients: correlation with tumor angiogenesis and cyclooxygenase-2 expression. Clin. Cancer Res. 10, 8554 – 8560.
33 Generali, D., Berruti, A., Brizzi, M. P., Campo, L., Bonardi, S., Wigfield, S., Bersiga, A., Allevi, G., Milani, M., Aguggini, S. et al. (2006) Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin. Cancer Res. 12, 4562 – 4568.
34 Liu, Y. L., Yu, J. M., Song, X. R., Wang, X. W., Xing, L. G., and Gao, B. B. (2006) Regulation of the chemokine receptor CXCR4 and metastasis by hypoxia-inducible factor in non small cell lung cancer cell lines. Cancer Biol. Ther. 5, 1320 – 1326.
35 Giatromanolaki, A., Koukourakis, M. I., Sivridis, E., Turley, H., Talks, K., Pezzella, F., Gatter, K. C., and Harris, A. L. (2001) Relation of hypoxia inducible factor 1 alpha and 2 alpha in operable non-small cell lung cancer to angiogenic/molecular profile of tumours and survival. Br. J. Cancer 85, 881 – 890.
36 Koukourakis, M. I., Giatromanolaki, A., Sivridis, E., Simopoulos, C., Turley, H., Talks, K., Gatter, K. C., and Harris, A. L. (2002) Hypoxia-inducible factor (HIF1A and HIF2A), angiogenesis, and chemoradiotherapy outcome of squamous cell head-and-neck cancer. Int. J. Radiat. Oncol. Biol. Phys. 53, 1192 – 1202.
37 Raval, R. R., Lau, K. W., Tran, M. G., Sowter, H. M., Mandriota, S. J., Li, J. L., Pugh, C. W., Maxwell, P. H., Harris, A. L., and Ratcliffe, P. J. (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol. Cell. Biol. 25, 5675 – 5686.
38 Chen, H. H., Su, W. C., Lin, P. W., Guo, H. R., and Lee, W. Y. (2006) Hypoxia-inducible factor-1alpha correlates with MET and metastasis in node-negative breast cancer. Breast Cancer Res. Treat.[Epub ahead of print].
39 Gu, Y. Z., Moran, S. M., Hogenesch, J. B., Wartman, L., and Bradfield, C. A. (1998) Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. Gene Expression 7, 205 – 213.
40 Acker, T., Diez-Juan, A., Aragones, J., Tjwa, M., Brusselmans, K., Moons, L., Fukumura, D., Moreno-Murciano, M. P., Herbert, J. M., Burger, A., et al. (2005) Genetic evidence for a tumor suppressor role of HIF-2alpha. Cancer Cell 8, 131 – 141.
41 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. et al. (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. [see comments]. Cell 107, 43 – 54.
42 Bruick, R. K., and McKnight, S. L. (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. Science 294, 1337 – 1340.
43 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.
44 Srinivas, V., Zhang, L., Zhu, X., and Caro, J. (1999) Characterization of an oxygen/redox-dependent degradation domain of hypoxia-inducible factor alpha (HIF-alpha) proteins. Biochem. Biophys. Res. Commun. 260, 557 – 561.
45 O'Rourke, J., Tian, Y. M., Ratcliffe, P. J., and Pugh, C. W. (1999) Oxygen-regulated and transactivation domains in endothelial PAS protein 1: comparison with hypoxia-inducible factor-1a. J. Biol. Chem. 274, 2060 – 2071.
46 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 O2 sensing. Science 292, 464 – 468.
47 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. et al. (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292, 468 – 472.
48 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.
49 Stolle, C., Glenn, G., Zbar, B., Humphrey, J., Choyke, P., Walther, M., Pack, S., Hurley, K., Andrey, C., Klausner, R. et al. (1998) Improved detection of germline mutations in the

von Hippel-Lindau disease tumor suppressor gene. Hum. Mutat. 12, 417 – 423.
50 Clifford, S. C., Cockman, M. E., Smallwood, A. C., Mole, D. R., Woodward, E. R., Maxwell, P. H., Ratcliffe, P. J., and Maher, E. R. (2001) Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease. Hum. Mol. Genet. 10, 1029 – 1038.
51 Sutter, C., Laughner, E., and Semenza, G. (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.
52 Shuin, T., Kondo, K., Ashida, S., Okuda, H., Yoshida, M., Kanno, H., and Yao, M. (1999) Germline and somatic mutations in von Hippel-Lindau disease gene and its significance in the development of kidney cancer. Contrib. Nephrol. 128, 1 – 10.
53 Shuin, T., Kondo, K., Torigoe, S., Kishida, T., Kubota, Y., Hosaka, M., Nagashima, Y., Kitamura, H., Latif, F., Zbar, B. et al. (1994) Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. Cancer Res. 54, 2852 – 2855.
54 Foster, K., Prowse, A., van den Berg, A., Fleming, S., Hulsbeek, M. M. F., Crossey, P. A., Richards, F. M., Cairns, P., Affara, N. A., Ferguson-Smith, M. A. et al. (1994) Somatic mutations of the von Hippel-Lindau disease tumor suppressor gene in non-familial clear cell renal carcinoma. Hum. Mol. Gen. 3, 2169 – 2173.
55 Kanno, H., Kondo, K., Ito, S., Yamamoto, I., Fujii, S., Torigoe, S., Sakai, N., Hosaka, M., Shuin, T., and Yao, M. (1994) Somatic mutations of the von Hippel-Lindau Tumor supressor gene in sporadic central nervous systems hemangioblastomas. Cancer Res. 54, 4845 – 4847.
56 Lee, J.-Y., Dong, S.-M., Park, W.-S., Yoo, N.-J., Kim, C.-S., Jang, J.-J., Chi, J.-G., Zbar, B., Lubensky, I., Linehan, W. et al. (1998) Loss of heterozygosity and somatic mutations of the VHL tumor suppressor gene in sporadic cerebellar hemangioblastomas. Cancer Res. 58, 504 – 508.
57 Esteban, M. A., and Maxwell, P. H. (2005) HIF, a missing link between metabolism and cancer. Nat. Med. 11, 1047 – 1048.
58 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.
59 Zhong, H., Agani, F., Baccala, A. A., Laughner, E., Rioseco-Camacho, N., Isaacs, W. B., Simons, J. W., and Semenza, G. L. (1998) Increased expression of hypoxia inducible factor-1alpha in rat and human prostate cancer. Cancer Res. 58, 5280 – 5284.
60 Zundel, W., Schindler, C., Haas-Kogan, D., Koong, A., Kaper, F., Chen, E., Gottschalk, A., Ryan, H., Johnson, R., Jefferson, A. et al. (2000) Loss of *PTEN* facilitates HIF-1-mediated gene expression. Genes Dev. 14, 391 – 396.
61 Brugarolas, J. B., Vazquez, F., Reddy, A., Sellers, W. R., and Kaelin Jr, W. G. (2003) TSC2 reguates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 4, 147 – 158.
62 Blancher, C., Moore, J. W., Robertson, N., and Harris, A. L. (2001) Effects of ras and von Hippel-Lindau (VHL) gene mutations on hypoxia-inducible factor (HIF)-1alpha, HIF-2alpha, and vascular endothelial growth factor expression and their regulation by the phosphatidylinositol 3′-kinase/Akt signaling pathway. Cancer Res. 61, 7349 – 7355.
63 Jiang, B. H., Jiang, G., Zheng, J. Z., Lu, Z., Hunter, T., and Vogt, P. K. (2001) Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ. 12, 363 – 369.
64 Fukuda, R., Hirota, K., Fan, F., Jung, Y. D., Ellis, L. M., and Semenza, G. L. (2002) Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J. Biol. Chem. 277, 38205 – 38211.
65 Hudson, C. C., Liu, M., Chiang, G. G., Otterness, D. M., Loomis, D. C., Kaper, F., Giaccia, A. J., and Abraham, R. T. (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol. Cell. Biol. 22, 7004 – 7014.
66 Pore, N., Jiang, Z., Shu, H. K., Bernhard, E., Kao, G. D., and Maity, A. (2006) Akt1 activation can augment hypoxia-inducible factor-1alpha expression by increasing protein translation through a mammalian target of rapamycin-independent pathway. Mol. Cancer Res. 4, 471 – 479.
67 Wan, X., Shen, N., Mendoza, A., Khanna, C., and Helman, L. J. (2006) CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1alpha/VEGF signaling. Neoplasia 8, 394 – 401.
68 Bernardi, R., Guernah, I., Jin, D., Grisendi, S., Alimonti, A., Teruya-Feldstein, J., Cordon-Cardo, C., Simon, M. C., Rafii, S., and Pandolfi, P. P. (2006) PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature 442, 779 – 785.
69 Brugarolas, J. B., Vazquez, F., Reddy, A., Sellers, W. R., and Kaelin, W. G., Jr. (2003) TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 4, 147 – 158.
70 Kietzmann, T., and Gorlach, A. (2005) Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. Semin. Cell Dev. Biol. 16, 474 – 486.
71 Quintero, M., Brennan, P. A., Thomas, G. J., and Moncada, S. (2006) Nitric oxide is a factor in the stabilization of hypoxia-inducible factor-1alpha in cancer: role of free radical formation. Cancer Res. 66, 770 – 774.
72 Maranchie, J. K., and Zhan, Y. (2005) Nox4 is critical for hypoxia-inducible factor 2-alpha transcriptional activity in von Hippel-Lindau-deficient renal cell carcinoma. Cancer Res. 65, 9190 – 9193.
73 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.
74 Jiang, B. H., Zheng, J. Z., Leung, S. W., Roe, R., and Semenza, G. L. (1997) Transactivation and inhibitory domains of hypoxia-inducible factor 1a. J. Biol. Chem. 272, 19253 – 19260.
75 Pugh, C., O'Rourke, J., Nagao, M., Gleadle, J., and Ratcliffe, P. (1997) Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. J. Biol. Chem. 272, 11205 – 11214.
76 Hara, S., Hamada, J., Kobayashi, C., Kondo, Y., and Imura, N. (2001) Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha. Biochem. Biophys. Res. Commun. 287, 808 – 813.
77 Ema, M., Hirota, K., Mimura, J., Abe, H., Yodoi, J., Sogawa, K., Poellinger, L. and Fukii-Kuriyama, Y. (1999) Molecular mechanisms of transcription activation by HLF and HIF1a in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. EMBO J. 18, 1905 – 1914.
78 Kallio, P. J., 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-1alpha. EMBO J. 17, 6573 – 6586.
79 Carrero, P., Okamoto, K., Coumailleau, P., O'Brien, S., Tanaka, H., and Poellinger, L. (2000) Redox-regulated recruitment of the transcriptional coactivators CREB-bind-

ing protein and SRC-1 to hypoxia-inducible factor 1alpha. Mol. Cell. Biol. 20, 402 – 415.
80 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. Nat. Acad. Sci. of the USA 93, 12969 – 12973.
81 Yan, Q., Bartz, S., Mao, M., Li, L., and Kaelin, W. G., Jr. (2007) The HIF2{alpha} N-terminal and C-terminal transactivation domains cooperate to promote renal tumorigenesis in vivo. Mol. Cell. Biol. 27, 2092 – 2102.
82 Pereira, T., Zheng, X., Ruas, J. L., Tanimoto, K., and Poellinger, L. (2003) Identification of residues critical for regulation of protein stability and the transactivation function of the hypoxia-inducible factor-1alpha by the von Hippel-Lindau tumor suppressor gene product. J. Biol. Chem. 278, 6816 – 6823.
83 Freedman, S. J., Sun, Z. Y., Poy, F., Kung, A. L., Livingston, D. M., Wagner, G., and Eck, M. J. (2002) Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha. Proc. Natl. Acad. Sci. USA 99, 5367 – 5372.
84 Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J., and Whitelaw, M. L. (2002) Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 295, 858 – 861.
85 Kasper, L. H., Boussouar, F., Boyd, K., Xu, W., Biesen, M., Rehg, J., Baudino, T. A., Cleveland, J. L., and Brindle, P. K. (2005) Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. EMBO J. 24, 3846 – 3858.
86 Kato, H., Tamamizu-Kato, S., and Shibasaki, F. (2004) Histone deacetylase 7 associates with hypoxia-inducible factor 1alpha and increases transcriptional activity. J. Biol. Chem. 279, 41966 – 41974.
87 Qian, D. Z., Kachhap, S. K., Collis, S. J., Verheul, H. M., Carducci, M. A., Atadja, P., and Pili, R. (2006) Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1{alpha}. Cancer Res. 66, 8814 – 8821.
88 Bilton, R., Mazure, N., Trottier, E., Hattab, M., Dery, M. A., Richard, D. E., Pouyssegur, J., and Brahimi-Horn, M. C. (2005) Arrest-defective-1 protein, an acetyltransferase, does not alter stability of hypoxia-inducible factor (HIF)-1alpha and is not induced by hypoxia or HIF. J. Biol. Chem. 280, 31132 – 31140.
89 Fisher, T. S., Etages, S. D., Hayes, L., Crimin, K., and Li, B. (2005) Analysis of ARD1 function in hypoxia response using retroviral RNA interference. J. Biol. Chem. 280, 17749 – 17757.
90 Arnesen, T., Kong, X., Evjenth, R., Gromyko, D., Varhaug, J. E., Lin, Z., Sang, N., Caro, J., and Lillehaug, J. R. (2005) Interaction between HIF-1 alpha (ODD) and hARD1 does not induce acetylation and destabilization of HIF-1 alpha. FEBS Lett. 579, 6428 – 6432.
91 Kim, S. H., Park, J. A., Kim, J. H., Lee, J. W., Seo, J. H., Jung, B. K., Chun, K. H., Jeong, J. W., Bae, M. K., and Kim, K. W. (2006) Characterization of ARD1 variants in mammalian cells. Biochem. Biophys. Res. Commun. 340, 422 – 427.
92 Jeong, J. W., Bae, M. K., Ahn, M. Y., Kim, S. H., Sohn, T. K., Bae, M. H., Yoo, M. A., Song, E. J., Lee, K. J., and Kim, K. W. (2002) Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 111, 709 – 720.
93 Sang, N., Stiehl, D. P., Bohensky, J., Leshchinsky, I., Srinivas, V., and Caro, J. (2003) MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300. J. Biol. Chem. 278, 14013 – 14019.
94 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, 53 – 58.
95 Das, B., Yeger, H., Tsuchida, R., Torkin, R., Gee, M. F., Thorner, P. S., Shibuya, M., Malkin, D., and Baruchel, S. (2005) A hypoxia-driven vascular endothelial growth factor/Flt1 autocrine loop interacts with hypoxia-inducible factor-1alpha through mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 pathway in neuroblastoma. Cancer Res. 65, 7267 – 7275.
96 Mylonis, I., Chachami, G., Samiotaki, M., Panayotou, G., Paraskeva, E., Kalousi, A., Georgatsou, E., Bonanou, S., and Simos, G. (2006) Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. J. Biol. Chem. 281, 33095 – 33106.
97 Dayan, F., Roux, D., Brahimi-Horn, M. C., Pouyssegur, J., and Mazure, N. M. (2006) The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res. 66, 3688 – 3698.
98 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.
99 Carmeliet, P., Dor, Y., Herbert, J. M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F., Abramovitch, R., Maxwell, P. et al. (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394, 485 – 490.
100 Wiesener, M. S., Turley, H., Allen, W. E., Willam, C., Eckardt, K. U., Talks, K. L., Wood, S. M., Gatter, K. C., Harris, A. L., Pugh, C. W. et al. (1998) Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood 92, 2260 – 2268.
101 Folkman, J. (1992) The role of angiogenesis in tumor growth. Semin. Cancer Biol. 3, 65 – 71.
102 Chang, Y. S., di Tomaso, E., McDonald, D. M., Jones, R., Jain, R. K., and Munn, L. L. (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc. Natl. Acad. Sci. USA 97, 14608 – 14613.
103 Vaupel, P., Thews, O., and Hoeckel, M. (2001) Treatment resistance of solid tumors: role of hypoxia and anemia. Med. Oncol. 18, 243 – 259.
104 Vaupel, P., Kelleher, D. K., and Hockel, M. (2001) Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin. Oncol. 28, 29 – 35.
105 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.
106 Grosfeld, A., Andre, J., Hauguel-De Mouzon, S., Berra, E., Pouyssegur, J., and Guerre-Millo, M. (2002) Hypoxia-inducible factor 1 transactivates the human leptin gene promoter. J. Biol. Chem. 277, 42953 – 42957.
107 Sanchez-Elsner, T., Botella, L. M., Velasco, B., Langa, C., and Bernabeu, C. (2002) Endoglin expression is regulated by transcriptional cooperation between the hypoxia and transforming growth factor-beta pathways. J. Biol. Chem. 277, 43799 – 43808.
108 Bodi, I., Bishopric, N. H., Discher, D. J., Wu, X., and Webster, K. A. (1995) Cell-specificity and signaling pathway of endothelin-1 gene regulation by hypoxia. Cardiovasc. Res. 30, 975 – 984.
109 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.
110 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.
111 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.

112 Melillo, G., Musso, T., Sica, A., Taylor, L., Cox, G., 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.

113 Palmer, L., Semenza, G., Stoler, M., and Johns, R. (1998) Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. Am. J. Physiol. 274, L212 – 219.

114 Elvert, G., Kappel, A., Heidenreich, R., Englmeier, U., Lanz, S., Acker, T., Rauter, M., Plate, K., Sieweke, M., Breier, G. etal. (2003) Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). J. Biol. Chem. 278, 7520 – 7530.

115 Chen, J., Zhao, S., Nakada, K., Kuge, Y., Tamaki, N., Okada, F., Wang, J., Shindo, M., Higashino, F., Takeda, K. et al. (2003) Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. Am. J. Pathol. 162, 1283 – 1291.

116 Akakura, N., Kobayashi, M., Horiuchi, I., Suzuki, A., Wang, J., Chen, J., Niizeki, H., Kawamura, K., Hosokawa, M., and Asaka, M. (2001) Constitutive expression of hypoxia-inducible factor-1alpha renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. Cancer Res. 61, 6548 – 6554.

117 Lu, H., Forbes, R. A., and Verma, A. (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J. Biol. Chem. 277, 23111 – 23115.

118 Lu, H., Dalgard, C. L., Mohyeldin, A., McFate, T., Tait, A. S., and Verma, A. (2005) Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. J. Biol. Chem. 280, 41928 – 41939.

119 Gunaratnam, L., Morley, M., Franovic, A., de Paulsen, N., Mekhail, K., Parolin, D. A., Nakamura, E., Lorimer, I. A., and Lee, S. (2003) Hypoxia inducible factor activates the transforming growth factor-alpha/epidermal growth factor receptor growth stimulatory pathway in VHL(−/−) renal cell carcinoma cells. J. Biol. Chem. 278, 44966 – 44974.

120 Yoon, D. Y., Buchler, P., Saarikoski, S. T., Hines, O. J., Reber, H. A., and Hankinson, O. (2001) Identification of genes differentially induced by hypoxia in pancreatic cancer cells. Biochem. Biophys. Res. Commun. 288, 882 – 886.

121 Graham, C. H., Forsdike, J., Fitzgerald, C. J., and Macdonald-Goodfellow, S. (1999) Hypoxia-mediated stimulation of carcinoma cell invasiveness via upregulation of urokinase receptor expression. Int. J. Cancer 80, 617 – 623.

122 Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., Ferreira, G., Iyer, N., LaRusch, J., Pak, B., Taghavi, P. et al. (2003) Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res. 63, 1138 – 1143.

123 Erler, J. T., Bennewith, K. L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q. T., Chi, J. T., Jeffrey, S. S., and Giaccia, A. J. (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440, 1222 – 1226.

124 Evans, A. J., Russell, R. C., Roche, O., Burry, T. N., Fish, J. E., Chow, V. W., Kim, W. Y., Saravanan, A., Maynard, M. A., Gervais, M. L. et al. (2007) VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and snail. Mol. Cell. Biol. 27, 157 – 169.

125 Esteban, M. A., Tran, M. G., Harten, S. K., Hill, P., Castellanos, M. C., Chandra, A., Raval, R., O'Brien T, S., and Maxwell, P. H. (2006) Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. Cancer Res. 66, 3567 – 3575.

126 Krishnamachary, B., Zagzag, D., Nagasawa, H., Rainey, K., Okuyama, H., Baek, J. H., and Semenza, G. L. (2006) Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. Cancer Res. 66, 2725 – 2731.

127 Schioppa, T., Uranchimeg, B., Saccani, A., Biswas, S. K., Doni, A., Rapisarda, A., Bernasconi, S., Saccani, S., Nebuloni, M., Vago, L. et al. (2003) Regulation of the chemokine receptor CXCR4 by hypoxia. J. Exp. Med. 198, 1391 – 1402.

128 Melillo, G. (2006) Inhibiting hypoxia-inducible factor 1 for cancer therapy. Mol. Cancer Res. 4, 601 – 605.

129 Nelson, W. J., and Nusse, R. (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. Science 303, 1483 – 1487.

130 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.

131 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.

132 Zhang, S. X., Gozal, D., Sachleben, L. R., Jr., Rane, M., Klein, J. B., and Gozal, E. (2003) Hypoxia induces an autocrine-paracrine survival pathway via platelet-derived growth factor (PDGF)-B/PDGF-beta receptor/phosphatidylinositol 3-kinase/Akt signaling in RN46A neuronal cells. FASEB J. 17, 1709 – 1711.

133 Pause, A., Lee, S., Lonergan, K. M., and Klausner, R. D. (1998) The von Hippel-Lindau tumor suppressor gene is required for cell cycle exit upon serum withdrawal. Proc. Natl. Acad. Sci. (USA) 95, 993 – 998.

134 Kaidi, A., Qualtrough, D., Williams, A. C., and Paraskeva, C. (2006) Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. Cancer Res. 66, 6683 – 6691.

135 Peng, X. H., Karna, P., Cao, Z., Jiang, B. H., Zhou, M., and Yang, L. (2006) Cross-talk between epidermal growth factor receptor and hypoxia-inducible factor-1alpha signal pathways increases resistance to apoptosis by up-regulating survivin gene expression. J. Biol. Chem. 281, 25903 – 25914.

136 Bruick, R. K. (2000) Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc. Natl. Acad. Sci. USA 97, 9082 – 9087.

137 Sowter, H. M., Ratcliffe, P. J., Watson, P., Greenberg, A. H., and Harris, A. L. (2001) HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res. 61, 6669 – 6673.

138 Shoshani, T., Faerman, A., Mett, I., Zelin, E., Tenne, T., Gorodin, S., Moshel, Y., Elbaz, S., Budanov, A., Chajut, A. et al. (2002) Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. Mol. Cell. Biol. 22, 2283 – 2293.

139 Bacon, A. L., Fox, S., Turley, H., and Harris, A. L. (2007) Selective silencing of the hypoxia-inducible factor 1 target gene BNIP3 by histone deacetylation and methylation in colorectal cancer. Oncogene 26, 132 – 141.

140 Wartenberg, M., Ling, F. C., Muschen, M., Klein, F., Acker, H., Gassmann, M., Petrat, K., Putz, V., Hescheler, J., and Sauer, H. (2003) Regulation of the multidrug resistance transporter P-glycoprotein in multicellular tumor spheroids by hypoxia-inducible factor (HIF-1) and reactive oxygen species. FASEB J. 17, 503 – 505.

141 Unruh, A., Ressel, A., Mohamed, H. G., Johnson, R. S., Nadrowitz, R., Richter, E., Katschinski, D. M., and Wenger, R. H. (2003) The hypoxia-inducible factor-1 alpha is a negative factor for tumor therapy. Oncogene 22, 3213 – 3220.

142 Nishi, H., Nakada, T., Kyo, S., Inoue, M., Shay, J. W., and Isaka, K. (2004) Hypoxia-inducible factor 1 mediates upregulation of telomerase (hTERT). Mol. Cell. Biol. 24, 6076 – 6083.

143 Yatabe, N., Kyo, S., Maida, Y., Nishi, H., Nakamura, M., Kanaya, T., Tanaka, M., Isaka, K., Ogawa, S., and Inoue, M. (2004) HIF-1-mediated activation of telomerase in cervical cancer cells. Oncogene 23, 3708 – 3715.
144 Stewart, S. A., and Weinberg, R. A. (2006) Telomeres: cancer to human aging. Annu. Rev. Cell Dev. Biol. 22, 531 – 557.
145 Carroll, V. A., and Ashcroft, M. (2006) Role of hypoxia-inducible factor (HIF)-1alpha versus HIF-2alpha in the regulation of HIF target genes in response to hypoxia, insulin-like growth factor-I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. Cancer Res. 66, 6264 – 6270.
146 Aprelikova, O., Wood, M., Tackett, S., Chandramouli, G. V., and Barrett, J. C. (2006) Role of ETS transcription factors in the hypoxia-inducible factor-2 target gene selection. Cancer Res. 66, 5641 – 5647.
147 Covello, K. L., Kehler, J., Yu, H., Gordan, J. D., Arsham, A. M., Hu, C. J., Labosky, P. A., Simon, M. C., and Keith, B. (2006) HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev. 20, 557 – 570.
148 Warnecke, C., Zaborowska, Z., Kurreck, J., Erdmann, V. A., Frei, U., Wiesener, M., and Eckardt, K. U. (2004) Differentiating the functional role of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2alpha target gene in Hep3B and Kelly cells. FASEB J. 18, 1462 – 1464.