ANTIOXIDANTS & REDOX SIGNALING
Volume 5, Number 4, 2003

Forum Review

HIF-1 in Cell Cycle Regulation, Apoptosis, and Tumor Progression

NOBUHITO GODA, SARA J. DOZIER, and RANDALL S. JOHNSON

ABSTRACT

Cells in a low oxygen, or hypoxic, microenvironment must have the ability to sense oxygen levels in the nucleus in order to maintain oxygen homeostasis by gene regulation. Hypoxia inducible factor-1 (HIF-1) serves as a molecular bridge between the sensation and utilization of oxygen, and thus functions as a key player in oxygen homeostasis. HIF-1 is a heterodimeric transcription factor and is composed of two subunits, the oxygen-sensitive HIF-1α and constitutively expressed HIF-1β. HIF-1 regulates the expression of a broad range of genes that facilitate acclimation to low oxygen conditions by changes in protein levels in circulation, metabolism, and proliferation. Appropriate temporal and spatial activation of HIF-1 is crucial not only in developmental and physiological processes, characterized by programmed cellular proliferation, but also in pathophysiological conditions such as tumorigenesis, which exhibit unregulated cellular proliferation. However, many contradictory reports as to the role of HIF-1 in the regulation of cellular proliferation have been put forward in recent years. In this review, our first aim is to summarize the current knowledge of oxygen-dependent HIF-1 activation mechanisms based on its structure. Then we will describe the proposed mechanisms through which HIF-1 regulates cellular proliferation of different cell types, including tumor cells as well as non-transformed, nonimmortalized cells under normoxic and hypoxic conditions. *Antioxid. Redox Signal.* 5, 467–473.

INTRODUCTION

THE AVAILABILITY OF MOLECULAR OXYGEN allows complex multicellular organisms to exist in many different environments. Sophisticated systems have evolved to utilize oxygen and prevent reactive oxygen cell damage during adaptation to oxygen-rich environments. Over 90% of oxygen intake is consumed as an electron sink through oxidative phosphorylation in the mitochondria to produce ATP, the ubiquitous biological energy source. Organisms use the residual fraction of oxygen molecules to carry out other important biochemical reactions, such as oxygenation and hydroxylation, which generate a group of biologically active substances that maintain important cellular functions. Metabolic shifts in the evolution from anaerobic toward aerobic organisms conferred the ability to sense a decrease in environmental oxygen concentrations through a well conserved hypoxic response pathway (36, 46, 56). This pathway facilitates acclimation to hypoxia-evoked physiological stress by modulating the expression of specific genes, and plays a central role not only in various physiological events during ontogenetic processes, including neovascularization and angiogenesis (26, 33, 41), but also in pathophysiological events such as tumorigenesis (6, 8, 28, 42). Despite considerable efforts devoted to understanding this system, the molecular mechanisms used by cells in higher eukaryocytes to sense and respond to changes in oxygen availability were unclear until recently.

In the early 1990s, a novel hypoxia-inducible transcription factor, hypoxia-inducible factor-1 (HIF-1), was identified by its binding at the 3′ region of the hypoxia response element

Molecular Biology Section, Division of Biology, University of California, San Diego, La Jolla, CA 92093.

(HRE) of the erythropoietin gene after hypoxic exposure (48, 52). This information increased our understanding of molecular acclimation to a wide range of alterations in environmental oxygen concentrations (36, 46, 55). At present, many processes involved in oxygen homeostasis are believed to be mediated by a family of HIFs, which transcriptionally regulate the expression of several dozen target genes (46, 55). These genes encode proteins involved in angiogenesis, erythropoiesis, glucose metabolism, and cellular proliferation. In the last few years, a surprisingly simple and conceptually attractive model of oxygen sensing has emerged. Oxygen-dependent hydroxylation of HIF is a reversible molecular switch activated by low oxygen concentrations in the surrounding environment (5, 11, 22, 23). This mechanism allows HIFs to function as a molecular bridge between external oxygen stimuli and intracellular effectors.

STRUCTURE OF HIF-1, ESSENTIAL FOR ACCLIMATION TO HYPOXIA

HIF-1 is a heterodimeric transcription factor consisting of two distinct components, the oxygen-sensitive α subunit (HIF-1α) and constitutively expressed β subunit [HIF-1β, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT)], both of which are members of the PAS (Per/ARNT/Sim) protein family (53, 54). Semenza *et al.* initially identified this factor by its DNA binding activity to the erythropoietin 3′ HRE in hypoxia-treated Hep3B cells and extracted the dimer from nuclear fractions (48). This protein–DNA interaction was later observed in various cell types under hypoxia and lies at the heart of oxygen sensing and signal transduction mechanisms in a variety of organisms (46, 55). In addition to this ubiquitously expressed protein, at least two other HIFs, designated as HIF-2α (10, 58) and HIF-3α (17, 37), have been cloned. These HIF family members show expression patterns that are more cell type-specific than HIF-1α, although the patterns do partially overlap (51, 58). HIF-2α has many biochemical properties similar to HIF-1α, most significantly the ability to bind HIF-1β and induce hypoxic gene expression (57, 58). The HIF-3α locus has recently been shown to produce several splice variants (37), one of which encodes an inhibitory PAS domain protein that may act as a negative regulator of hypoxia-inducible gene expression through competitive inhibition of HIF-1α by binding to HIF-1β (31, 32).

Analysis of the full-length cDNA clone of HIF-1α allows us to predict the structural features of this protein and, thus, its biological functions. Its N-terminal domain contains a basic helix–loop–helix domain and a PAS domain that are essential for dimerization with HIF-1β and binding to the conserved HRE DNA sequences (5′-RCGTG-3′) (54). The C-terminal portion of HIF-1α contains several regulatory domains responsible for oxygen-dependent gene expression. These include two separate transactivation domains, TAD-N (N-terminal transactivaton domain; residues 531–575) and TAD-C (C-terminal transactivation domain; residues 786–826), which bind general transcriptional coactivators such as CBP/p300, SRC-1 and TIF-2 (2, 7). The two transactivation domains flank an inhibitory domain (residues 576–785), which, when deleted, increases the function of the transactivation domains under normoxic conditions (39, 46). Nuclear localization signals, identified at both N- and C-termini (residues 17–74 and residues 718–721, respectively), translocate HIF-1α into the nucleus, independent of dimerization with HIF-1β (25). The oxygen-dependent degradation domain (ODD, residues 401–603) is a critical component of HIF-1α and is involved in HIF-1α protein stability. Under normoxic conditions, the ODD causes rapid degradation of HIF-1α protein through the ubiquitin-proteasome pathway (22, 23). This ODD contains two PEST-like motifs at residues 499–518 and 581–600, which may also regulate HIF-1α protein degradation (54).

OXYGEN-DEPENDENT REGULATION OF HIF-1α SUBUNIT

Until recently, mechanisms by which organisms sense alterations in oxygen concentration and subsequently induce HIF-1 activity remained obscure. The first key finding was evidence that von Hippel–Lindau tumor suppressor gene product, pVHL, binds directly with the ODD of HIF-1α under normoxic conditions, which leads to ubiquitin-dependent proteolysis of HIF-1α (35, 36). In contrast, hypoxia prevents the binding of HIF-1α by pVHL protein, causing rapid accumulation of HIF-1α in the nucleus within a few minutes. pVHL forms an E3 ubiquitin ligase complex with elongin B, elongin C, Cul2, and Rbx1 (also called as ROC1 or Hrt1) that is similar to SCF (Skp1/Cdc53 or Cullin/F-box protein) complexes. This complex specifically recognizes HIF-1α as a substrate (38). Cells lacking functional pVHL express a high level of HIF-1α even under normoxia, which induces expression of many HIF-1-dependent genes (36, 38). Moreover, hereditary VHL disease is well known to be associated with highly vascularized tumors in retinal and central nervous system hemangioblastomas, in part driven by inhibition of HIF-1α degradation (24).

The next important step toward understanding how pVHL is able to distinguish normoxic from hypoxic HIF-1α protein was the identification of two specific prolyl residues (Pro402 and Pro564) embedded within the LXXLAP motif of the ODD in HIF-1α that are hydroxylated as a function of increased oxygen availability (22, 23). This oxygen-dependent modification is enzymatic and requires a prolyl-4-hydroxylase, a member of a subfamily of novel protein hydroxylases distinct from those involved in collagen stabilization (5, 11). In mammals, three homologues [termed as HIF-prolyl hydroxylase (HPH) 3, 2, and 1] of *C. elegans* Egl-9 have been cloned based on sequence similarity to collagen-modifying prolyl hydroxylases. They show specific activities for ODD in both HIF-1α and HIF-2α (5, 20). The HPHs belong to the extended superfamily of dioxygenases, and require molecular oxygen and 2-oxoglutarate as cosubstrates (5, 11). In the active sites of the HPHs, the carboxylate of an aspartate residue and two histidine residues bind iron. The HPH must be in its ferrous state in order to capture oxygen through a vitamin C-dependent reduction. Under normoxic conditions, hydroxylation serves as a recognition element for pVHL, resulting in

rapid degradation of HIF-1α subunits. Hypoxia diminishes the hydroxylase activity of the HPHs and allows HIF-1α protein to escape from pVHL-associated proteolytic destruction, accumulate rapidly in the nucleus, and subsequently activate target gene expression (5, 19).

Modulation of HIF-1α protein stability by pVHL demonstrates one pathway for HIF-1 activation in response to hypoxia. However, oxygen tension has been reported to affect not only HIF-1α protein degradation, but also the subcellular localization, DNA-binding capability, and transactivation functions of HIF-1. This indicates that multiple steps regulated by HIF-1 are necessary for transmission of extracellular hypoxic signals into the nucleus (25, 36, 46, 56). Indeed, previous reports show that the C-terminal transactivation domain (TAD-C) of HIF-1α, when fused to a heterologous DNA-binding domain, is stable and shows hypoxic induction of downstream gene expression. This suggests a distinct oxygen-regulated pathway of HIF-1 activation through TAD-C (43). Recent evidence indicated that hydroxylation of a critical asparagine residue (Asn803and Asn851 in HIF-1α and HIF-2α, respectively) within the TAD-C occurs in an oxygen-dependent manner, and in turn renders TAD-C unable to associate with the CH1 domain of the CBP/p300 transcriptional coactivators (29). This reinforces the importance of TAD-C in the oxygen-dependent modulation of HIF-1 activity. Subsequently, Lando and colleagues determined that the asparagine hydroxylase involved in the catalytic reaction is identical to factor-inhibiting hypoxia-inducible factor-1 (FIH-1), initially identified by Semenza and coworkers as a novel HIF-1 binding protein and later shown to repress TAD-C functions (29, 30). FIH-1 is a 2-oxoglutarate-dependent dioxygenase that uses molecular oxygen to modify its substrate. This is similar to the aforementioned prolyl hydroxylase, and thus appears to serve as another oxygen-dependent system controlling HIF-1 activity. In addition, structural analysis of FIH-1 reveals that it forms a homodimer, which is essential for substrate recognition and enzymatic activity. This provides new clues toward understanding oxygen-dependent HIF-1 regulation and the kinetic differences among related hydroxylase enzymes including prolyl and lysyl hydroxylase (9, 21). Two distinct types of oxygen-dependent enzymatic reactions involved in HIF-1 activation may lead to a more complete understanding of the evolutionary processes wherein aerobic organisms acquired the ability to sense and adapt to subtle alterations in oxygen concentrations. Further investigation will determine if other oxygen-dependent, enzymatically mediated post-translational modifications of HIF-1 are involved.

THE ROLE OF HYPOXIA AND HIF IN APOPTOSIS AND CELL CYCLE REGULATION

Mammalian cells can respond differentially to wide ranges of oxygen concentration through alterations in both metabolic states and growth rates. A growing body of evidence indicates that hypoxia alters cellular proliferation in two distinct ways by programmed cell death and cell cycle arrest (44, 49). In transformed and/or immortalized cells, apoptosis is the predominant response to hypoxia, occurring via activation of a p53 tumor suppressor gene product-dependent pathway (44, 49). These alterations are believed to take place through the direct action of p53 in apoptotic pathways. However, accumulation and transcriptional activation of p53 protein are observed only under near-anoxic conditions, not under hypoxia (15, 27). Recent studies have suggested a role as well for HIF-1 in hypoxia-induced apoptosis (6, 26). Biochemical analyses have demonstrated that the tumor suppressor p53 has the potential to associate directly with HIF-1α subunits, leading to stabilization and activation of p53 under hypoxia (4, 50). Supporting data for this interaction have emerged within the last year, when Hansson and colleagues determined that the DNA-binding domain of p53 binds two specific motifs adjacent to and within the ODD domain of HIF-1α and that this interaction occurs irrespective of HIF-1α hydroxylation status (18). Moreover, another report showed that HIF-1α null cells accumulate more p53 protein than wild-type cells at low oxygen concentrations. This suggests that the expression of HIF-1α may cause p53 protein to accumulate in hypoxic cells, and thus induce apoptosis through p53 activation (1). In addition to the possible effects of HIF-1α on p53 activity, HIF may be a player in another aspect of p53-dependent apoptosis through transcriptional activation of a HIF-1 target gene, Nip3. The proapoptotic protein Nip3 can bind with antiapoptotic Bcl-2 family members such as Bcl-2 and Bcl-X_L and may promote apoptosis by sequestering these factors (40). These data, in conjunction with a recent report showing that the phosphorylated form of HIF-1α binds to HIF-1β, whereas the dephosphorylated form associates with p53 (50), indicate that HIF-1 may play a role in hypoxia-induced apoptosis. Further investigation will determine the critical oxygen concentration for increased expression and transcriptional activation of p53, and in what manner HIF-1 affects p53-dependent apoptotic pathways.

It is important to know whether p53 is involved in regulatory mechanisms of hypoxia-induced cell cycle arrest in non-transformed, nonimmortalized cells, and what role HIF-1 plays in this process. Recent studies from our laboratory provide new insight into the effects of hypoxic stress on cells and, specifically, cell cycle progression (14). Although several lines of contradicting data as to the effects of p53 on G1 arrest under hypoxia have been reported, p53-null cells show an accumulation of cells at the G1 phase under hypoxia (12, 15, 16). Our detailed cell cycle analyses have revealed that p53 is not an essential factor in hypoxia-induced cell cycle arrest in two different primary cell types. We found that, under hypoxic conditions, the numbers of cells in G1 phase in p53 null cultures are increased relative to wild-type cultures. This change may be attributable to enhanced HIF-1 activity by inactivation of p53, rather than the direct action of p53, because expression and transcriptional activity of p53 change little under hypoxia.

We observed that HIF-1α null, p53 wild-type cells do not show any hypoxia-induced G1 arrest. Rather, S-phase entry is accelerated, indicating that HIF-1, but not p53, plays an essential role in the regulation of cell cycle progression under hypoxia. Surprisingly, cells lacking functional copies of both p53 and HIF-1α did not display any change in the proportion of cells entering S-phase, as was seen in HIF-1α null cells.

These cells appear to lose the ability to sense and respond to hypoxia. Collectively, these data strongly suggest that two transcription factors, HIF-1 and p53, cooperate to regulate the cell cycle progression through distinct mechanisms, but that HIF-1 serves as the primary determinant for cell cycle regulation under hypoxia (Fig. 1).

Previous studies have indicated that hypoxia-induced cell cycle arrest is accompanied by a decreased activity of certain cyclin–cyclin-dependent kinase (CDK) complexes and hypophosphorylation of retinoblastoma (Rb) protein, leading to inhibition of cell cycle progression (12). However, little information has been available as to the role of HIF-1 in the regulation of cell cycle machinery. An exception is one report that showed cyclin G2, a negative regulator of cell cycle progression via binding with protein phosphatase 2A in certain cell types, is induced by hypoxia through HIF-1 activation (3, 59). Our recent studies elucidated the critical role of HIF-1 in the regulation of cell cycle progression under hypoxia, by showing that the induction of HIF-1 activation prevents G1/S transition through at least two distinct mechanisms (14). First, the expression of two cyclin-dependent kinase inhibitors (CKIs), $p21^{Cip1}$ and $p27^{Kip1}$, is increased transcriptionally in a HIF-1-dependent manner. Sustained expression of these CKIs is observed in wild-type cells, but not in HIF-1α null cells. $p21^{Cip1}$ and $p27^{Kip}$ suppress cyclin/CDK2 activity, and thus can reduce the ratio of phosphorylated to dephosphorylated Rb protein, resulting in cell cycle arrest at the G1/S interface.

Collectively, these data clearly indicate that CKIs function with HIF-1 to regulate the cell cycle in response to hypoxia. Secondarily, HIF-1 may regulate cyclin E, but not cyclin A protein levels; both of these bind CDK2 and modulate its kinase activity dependent upon cell cycle phase. In our recent studies, hypoxic cells lacking HIF-1α displayed enhanced and sustained accumulation of cyclin E, but not cyclin A, without any effect on CDK2 protein expression, relative to wild-type cells (14). In accordance with changes in cyclin E expression, cyclin E/CDK2 kinase activity in HIF-1α-deficient cells was also increased, resulting in somewhat retarded, but still substantial, cell growth, even under hypoxia. Thus, HIF-1 could serve as a primary gatekeeper at the G1/S transition in two distinct ways: through the action of CKIs and the regulation of cyclin E expression. More detailed investigation will reveal whether other HIF-1-dependent regulatory mechanisms of the cell cycle, including cyclin G2, are involved.

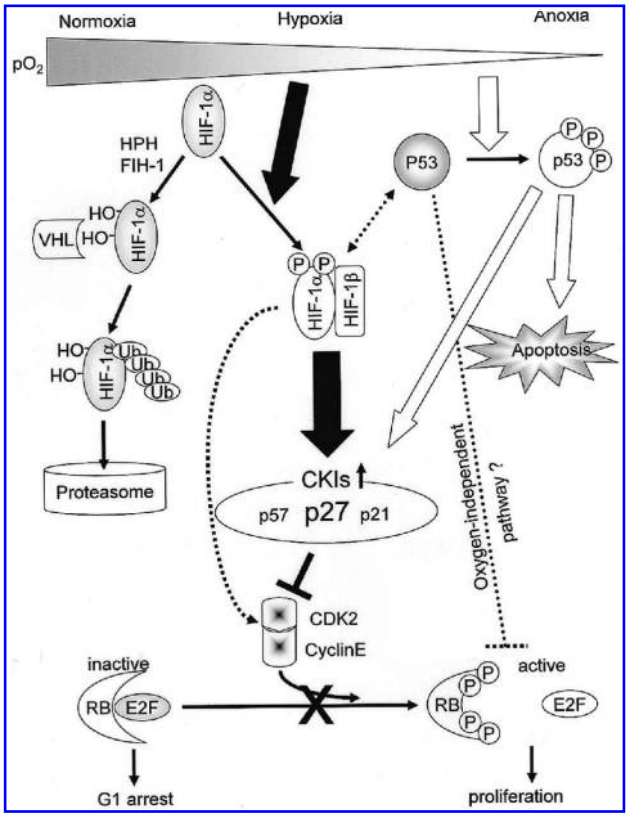

FIG. 1. A proposed model of oxygen-dependent regulation of cell cycle progression at the G1/S interface. HIF-1 and p53 are activated by a decrease in oxygen concentration with different optimal threshold. Under moderate hypoxia, HIF-1 acts as a central regulator of cell cycle, and evokes G1 arrest through induction of CKI transcripts. Severe hypoxia, on the other hand, leads to activation of p53 and subsequent apoptosis and/or cell cycle arrest in the cell type-specific manners.

ACTIVATION OF HIF-1 IN TUMOR PROGRESSION

Microenvironmental hypoxia is a hallmark of tumor progression. This is attributable to a decrease in capillary density and functional disorganization of the vasculature surrounding proliferating tumor cells. Although hypoxia alone has negative effects on cellular proliferation (15, 44), the acclimation to decreased oxygen concentration transcriptionally activates many genes involved in angiogenesis, anaerobic metabolism and the cell cycle, through the activation of HIF-1 (33, 46, 55). Recent immunohistochemical analyses using an antibody specific for the HIF-1α subunit revealed high-level expression of the protein in various types of human cancers, including mammary gland, prostate, lung, colon, and brain, suggesting critical roles of HIF-1 in tumor formation (51). In addition, clinical studies have shown that HIF-1α immunostaining is pronounced in aggressive tumors (13).

In addition to the effects of intratumoral hypoxia, genetic alterations in a number of tumor suppressor genes and oncogenes have also been implicated in the normoxic activation of HIF-1 pathways in the multistep process of tumor development. Effected molecules include pVHL, PTEN (phosphatase and tensin homologue deleted on chromosome 10), Ha-*ras*, v-*Src*, and p53, and comprise a diverse and complex array of pathways that involve not only protein stabilization, but also phosphorylation, nuclear translocation, and transcriptional cofactor recruitment of HIF-1α (4, 24, 47, 55).

Regardless of the stimulus for HIF-1α activation, tumor progression appears to favor sustained HIF-1α expression, inasmuch as HIF-1 acts as a major regulator of angiogenesis and anaerobic energy metabolism under certain conditions. Among over two dozen HIF-1 target genes, vascular endothelial growth factor (VEGF) is the most prominent and plays

important roles in tumor neovascularization, which supplies sufficient amounts of oxygen and nutrients to tumor cells to allow tumor propagation and metastasis (34). Furthermore, induction of anaerobic glycolytic enzymes and genes involved in glucose uptake allows the tumor to maintain energy production independent of oxidative phosphorylation and survive with a limited oxygen supply. Indeed, tumor cells, along with other highly proliferative cells, have been shown to use glycolytic pathways, rather than oxidative phosphorylation for ATP production. This might be due to constitutive HIF-1 expression even under normoxia (56). Further support for the view that HIF-1 is a positive regulator of tumor progression comes from studies of experimental tumors using mouse hepatoma cells either wild-type or null for HIF-1β (34). Tumors lacking HIF-1β grow more slowly and form a defective vascular network relative to wild-type HIF-1β cells when injected into mice, although both cell types are able to proliferate on tissue culture dishes under either normoxia or hypoxia. These results indicate that loss of HIF-1 activity suppresses tumor angiogenesis, but not tumor proliferation, due to reduced production of angiogenic factors such as VEGF. Another recent study used peptides to block the interaction between HIF-1α and p300. These cells displayed tumor growth retardation, as well as decreased angiogenesis (28). Our previous results are consistent with the concept of HIF-1 as a positive modulator of tumorigenesis in which tumors derived from HIF-1α null mouse embryonic stem cells showed a significant reduction in size. However, alterations in vascular density within tumors were not observed in HIF-1α null tumors (41, 42, 45). Collectively, these data suggest HIF-1 activation has different effects on tumor progression with a variable net outcome under different conditions.

CONCLUSION

Hypoxia plays an important role in various types of pathophysiological conditions, such as cancer, as well as physiological and developmental processes. HIF-1 is a central regulator of cellular hypoxic response and controls expression of critical hypoxia-responsive genes, allowing cells to acclimate to different oxygen concentrations. Recent studies of HIF-1 regulatory systems provide new clues as to how organisms sense alterations in oxygen concentration and transmit this information to downstream effectors. It remains to be determined how HIF-1 functions in the maintenance of cell and organ integrity, how loss of HIF-1 function alters cellular mechanisms, and how other transcription factors, including other HIFs, cooperate to control gene expression. As new information is collected, we will gain a more complete picture of the effect of oxygen on biological processes.

ACKNOWLEDGEMENTS

We thank the members of the Johnson Laboratory for helpful discussions and insight. This work was supported by a grant for the Promotion of the Advancement of Education and Research in Graduate Schools.

ABBREVIATIONS

ARNT, aryl hydrocarbon nuclear translocator; CDK, cyclin-dependent kinase; CKI, cyclin-dependent kinase inhibitor; FIH-1, factor-inhibiting hypoxia-inducible factor-1; HIF-1, hypoxia-inducible factor-1; HPH, HIF-prolyl hydroxylase; HRE, hypoxia response element; ODD, oxygen-dependent degradation domain; PAS, Per/ARNT/Sim; pVHL, Von Hippel–Lindau; Rb, retinoblastoma; TAD-C, C-terminal transactivation domain; VEGF, vascular endothelial growth factor.

REFERENCES

1. An WG, Kanekal M, Simon MC, Maltepe E, Blagosklonny MV, and Neckers LM. Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. *Nature* 392: 405–408, 1998.
2. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, and Livingston DM. An essential role for p300/CBP in the cellular response to hypoxia. *Proc Natl Acad Sci U S A* 93: 12969–12973, 1996.
3. Bennin DA, Don AS, Brake T, McKenzie JL, Rosenbaum H, Ortiz L, DePaoli-Roach AA, and Horne MC. Cyclin G2 associates with protein phosphatase 2A catalytic and regulatory B′ subunits in active complexes and induces nuclear aberrations and a G1/S phase cell cycle arrest. *J Biol Chem* 277: 27449–27467, 2002.
4. Blagosklonny MV, An WG, Romanova LY, Trepel J, Fojo T, and Neckers L. p53 inhibits hypoxia-inducible factor-stimulated transcription. *J Biol Chem* 273: 11995–11998, 1998.
5. Bruick RK and McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294: 1337–1340, 2001.
6. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E, and Keshet E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394: 485–490, 1998.
7. Carrero P, Okamoto K, Coumailleau P, O'Brien S, Tanaka H, and Poellinger L. Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. *Mol Cell Biol* 20: 402–415, 2000.
8. Dachs GU, Patterson AV, Firth JD, Ratcliffe PJ, Townsend KM, Stratford IJ, and Harris AL. Targeting gene expression to hypoxic tumor cells. *Nat Med* 3: 515–520, 1997.
9. Dann CE 3rd, Bruick RK, and Deisenhofer J. Structure of factor-inhibiting hypoxia-inducible factor 1: an asparaginyl hydroxylase involved in the hypoxic response pathway. *Proc Natl Acad Sci U S A* 99: 15351–15356, 2002.
10. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, and Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved

in lung and vascular development. *Proc Natl Acad Sci U S A* 94: 4273–4278, 1997.
11. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, and Ratcliffe PJ. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107: 43–54, 2001.
12. Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, and Dang CV. Hypoxia inhibits G1/S transition through regulation of p27 expression. *J Biol Chem* 276: 7919–7926, 2001.
13. Giatromanolaki A and Harris AL. Tumour hypoxia, hypoxia signaling pathways and hypoxia inducible factor expression in human cancer. *Anticancer Res* 21: 4317–4324, 2001.
14. Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC, and Johnson RS. Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. *Mol Cell Biol* 23: 359–369, 2003.
15. Graeber TG, Peterson JF, Tsai M, Monica K, Fornace AJ Jr, and Giaccia AJ. Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol* 14: 6264–6277, 1994.
16. Green SL, Freiberg RA, and Giaccia AJ. p21(Cip1) and p27(Kip1) regulate cell cycle reentry after hypoxic stress but are not necessary for hypoxia-induced arrest. *Mol Cell Biol* 21: 1196–1206, 2001.
17. Gu YZ, Moran SM, Hogenesch JB, Wartman L, and Bradfield CA. Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. *Gene Expr* 7: 205–213, 1998.
18. Hansson LO, Friedler A, Freund S, Rudiger S, and Fersht AR. Two sequence motifs from HIF-1alpha bind to the DNA-binding site of p53. *Proc Natl Acad Sci U S A* 99: 10305–10309, 2002.
19. Hon WC, Wilson MI, Harlos K, Claridge TD, Schofield CJ, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DI, and Jones EY. Structural basis for the recognition of hydroxyproline in HIF-1 alpha by pVHL. *Nature* 417: 975–978, 2002.
20. Huang J, Zhao Q, Mooney SM, and Lee FS. Sequence determinants in hypoxia-inducible factor-1alpha for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. *J Biol Chem* 277: 39792–39800, 2002.
21. Huang LE, Pete EA, Schau M, Milligan J, and Gu J. Leu-574 of HIF-1alpha is essential for the von Hippel–Lindau (VHL)-mediated degradation pathway. *J Biol Chem* 277: 41750–41755, 2002.
22. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, and Kaelin WG Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* 292: 464–468, 2001.
23. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, and Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel–Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001.
24. Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer* 2: 673–682, 2002.
25. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, and Poellinger L. 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, 1998.
26. Kotch LE, Iyer NV, Laughner E, and Semenza GL. Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol* 209: 254–267, 1999.
27. Koumenis C, Alarcon R, Hammond E, Sutphin P, Hoffman W, Murphy M, Derr J, Taya Y, Lowe SW, Kastan M, and Giaccia A. Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Mol Cell Biol* 21: 1297–1310, 2001.
28. Kung AL, Wang S, Klco JM, Kaelin WG, and Livingston DM. Suppression of tumor growth through disruption of hypoxia-inducible transcription. *Nat Med* 6: 1335–1340, 2000.
29. Lando D, Peet DJ, Whelan DA, Gorman JJ, and Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* 295: 858–861, 2002.
30. Mahon PC, Hirota K, and Semenza GL. 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, 2001.
31. Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, Cao Y, Berkenstam A, and Poellinger L. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* 414: 550–554, 2001.
32. Makino Y, Kanopka A, Wilson WJ, Tanaka H, and Poellinger L. Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus. *J Biol Chem* 277: 32405–32408, 2002.
33. Maxwell PH and Ratcliffe PJ. Oxygen sensors and angiogenesis. *Semin Cell Dev Biol* 13: 29–37, 2002.
34. Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, Hankinson O, Pugh CW, and Ratcliffe PJ. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 94: 8104–8109, 1997.
35. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, and Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399: 271–275, 1999.
36. Maxwell PH, Pugh CW, and Ratcliffe PJ. The pVHL-hIF-1 system. A key mediator of oxygen homeostasis. *Adv Exp Med Biol* 502: 365–376, 2001.
37. Maynard MA, Qi H, Chung J, Lee EH, Kondo Y, Hara S, Conaway RC, Conaway JW, and Ohh M. Multiple splice variants of the human HIF-3 alpha locus are targets of the von Hippel–Lindau E3 ubiquitin ligase complex. *J Biol Chem* 278: 11032–11040, 2003.

38. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, and Kaelin WG. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel–Lindau protein. *Nat Cell Biol* 2: 423–427, 2000.
39. Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, and Ratcliffe PJ. Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit. *J Biol Chem* 272: 11205–11214, 1997.
40. Ray R, Chen G, Vande Velde C, Cizeau J, Park JH, Reed JC, Gietz RD, and Greenberg AH. BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. *J Biol Chem* 275: 1439–1448, 2000.
41. Ryan HE, Lo J, and Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J* 17: 3005–3015, 1998.
42. Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, and Johnson RS. Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res* 60: 4010–4015, 2000.
43. Sang N, Fang J, Srinivas V, Leshchinsky I, and Caro J. Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel–Lindau protein-independent, hydroxylation-regulated association with p300/CBP. *Mol Cell Biol* 22: 2984–2992, 2002.
44. Schmaltz C, Hardenbergh PH, Wells A, and Fisher DE. Regulation of proliferation-survival decisions during tumor cell hypoxia. *Mol Cell Biol* 18: 2845–2854, 1998.
45. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, and Johnson RS. Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. *Mol Cell Biol* 21: 3436–3444, 2001.
46. Semenza GL. Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* 15: 551–578, 1999.
47. Semenza GL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. *Pediatr Res* 49: 614–617, 2001.
48. Semenza GL, Nejfelt MK, Chi SM, and Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. *Proc Natl Acad Sci U S A* 88: 5680–5684, 1991.
49. Shimizu S, Eguchi Y, Kosaka H, Kamiike W, Matsuda H, and Tsujimoto Y. Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-xL. *Nature* 374: 811–813, 1995.
50. Suzuki H, Tomida A, and Tsuruo T. Dephosphorylated hypoxia-inducible factor 1alpha as a mediator of p53-dependent apoptosis during hypoxia. *Oncogene* 20: 5779–5788, 2001.
51. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, and Harris AL. 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, 2000.
52. Wang GL and Semenza GL. Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia. *J Biol Chem* 268: 21513–21518, 1993.
53. Wang GL and Semenza GL. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270: 1230–1237, 1995.
54. Wang GL, Jiang BH, Rue EA, and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci U S A* 92: 5510–5514, 1995.
55. Wenger RH. Mammalian oxygen sensing, signalling and gene regulation. *J Exp Biol* 203 Pt 8: 1253–1263, 2000.
56. Wenger RH. Cellular adaptation to hypoxia: O_2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2-regulated gene expression. *FASEB J* 16: 1151–1162, 2002.
57. Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, Wood SM, Gatter KC, Harris AL, Pugh CW, Ratcliffe PJ, and Maxwell PH. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. *Blood* 92: 2260–2268, 1998.
58. Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH, and Eckardt KU. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *FASEB J* 17: 271–273, 2003.
59. Wykoff CC, Pugh CW, Maxwell PH, Harris AL, and Ratcliffe PJ. Identification of novel hypoxia dependent and independent target genes of the von Hippel–Lindau (VHL) tumour suppressor by mRNA differential expression profiling. *Oncogene* 19: 6297–6305, 2000.

Address reprint requests to:
Nobuhito Goda, M.D., Ph.D.
Department of Biochemistry and Integrative Medical Biology
School of Medicine, Keio University
35 Shinanomachi, Shinjyuku-ku
Tokyo 160–8582, Japan

E-mail: goda@sc.itc.keio.ac.jp

Received for publication December 27, 2002; accepted March 19, 2003.

This article has been cited by:

1. Michael Murphy, A. Phelps, H. Swede, K. Claffey. 2012. Hypoxia-induced response of cell cycle and apoptosis regulators in melanoma. *International Journal of Dermatology* **51**:10, 1263-1267. [CrossRef]
2. Gibin G. Powathil, Kirsty E. Gordon, Lydia A. Hill, Mark A.J. Chaplain. 2012. Modelling the effects of cell-cycle heterogeneity on the response of a solid tumour to chemotherapy: Biological insights from a hybrid multiscale cellular automaton model. *Journal of Theoretical Biology* **308**, 1-19. [CrossRef]
3. Omolara O. Ogunshola , Abraham Al-Ahmad . 2012. HIF-1 at the Blood-Brain Barrier: A Mediator of Permeability?. *High Altitude Medicine Biology* **13**:3, 153-161. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Hong Kang, Zhen Sheng, Ruixin Zhu, Qi Huang, Qi Liu, Zhiwei Cao. 2012. Virtual Drug Screen Schema Based on Multiview Similarity Integration and Ranking Aggregation. *Journal of Chemical Information and Modeling* 120229101425008. [CrossRef]
5. Angelique A. M. van Oorschot, Anke M. Smits, Evangelia Pardali, Pieter A. Doevendans, Marie-José Goumans. 2011. Low oxygen tension positively influences cardiomyocyte progenitor cell function. *Journal of Cellular and Molecular Medicine* **15**:12, 2723-2734. [CrossRef]
6. Assefa Wondimu, Lynda Weir, Douglas Robertson, Alexandre Mezentsev, Sergey Kalachikov, Andrey A Panteleyev. 2011. Loss of Arnt (Hif1#) in mouse epidermis triggers dermal angiogenesis, blood vessel dilation and clotting defects. *Laboratory Investigation* . [CrossRef]
7. G Xenaki, T Ontikatze, R Rajendran, I J Stratford, C Dive, M Krstic-Demonacos, C Demonacos. 2008. PCAF is an HIF-1# cofactor that regulates p53 transcriptional activity in hypoxia. *Oncogene* **27**:44, 5785-5796. [CrossRef]
8. Song-yi Yao, Mohammed Soutto, Subramaniam Sriram. 2008. Bacterial cell wall products increases stabilization of HIF-1 alpha in an oligodendrocyte cell line preconditioned by cobalt chloride or desferrioxamine. *Journal of Neuroimmunology* **200**:1-2, 17-26. [CrossRef]
9. Song-yi Yao, Mohammed Soutto, Subramaniam Sriram. 2008. Preconditioning with cobalt chloride or desferrioxamine protects oligodendrocyte cell line (MO3.13) from tumor necrosis factor-#-mediated cell death. *Journal of Neuroscience Research* **86**:11, 2403-2413. [CrossRef]
10. Z. Gunnur Dikmen, Ginelle C. Gellert, Pakize Dogan, Heejeong Yoon, Young Bok Lee, Chang Ho Ahn, Jerry W. Shay. 2008. In vivo and in vitro effects of a HIF-1# inhibitor, RX-0047. *Journal of Cellular Biochemistry* **104**:3, 985-994. [CrossRef]
11. Tong Zhao, Cui-ping Zhang, Zhao-hui Liu, Li-ying Wu, Xin Huang, Hai-tong Wu, Lei Xiong, Xuan Wang, Xiao-min Wang, Ling-ling Zhu, Ming Fan. 2008. Hypoxia-driven proliferation of embryonic neural stem/progenitor cells – role of hypoxia-inducible transcription factor-1#. *FEBS Journal* **275**:8, 1824-1834. [CrossRef]
12. A.J. Harvey, A. Navarrete Santos, M. Kirstein, K.L. Kind, B. Fischer, J.G. Thompson. 2007. Differential expression of oxygen-regulated genes in bovine blastocysts. *Molecular Reproduction and Development* **74**:3, 290-299. [CrossRef]
13. Jared W. Allen , Salman R. Khetani , Randall S. Johnson , Sangeeta N. Bhatia . 2006. In Vitro Liver Tissue Model Established from Transgenic Mice: Role of HIF-1alpha on Hypoxic Gene Expression. *Tissue Engineering* **12**:11, 3135-3147. [Abstract] [Full Text PDF] [Full Text PDF with Links]
14. Jared W. Allen, Salman R. Khetani, Randall S. Johnson, Sangeeta N. Bhatia. 2006. In Vitro Liver Tissue Model Established from Transgenic Mice: Role of HIF1alpha on Hypoxic Gene Expression. *Tissue Engineering*, ahead of print061004065151004. [CrossRef]
15. Daizo Yoshida, Kyonsong Kim, Masahiro Noha, Akira Teramoto. 2006. Anti-Apoptotic Action by Hypoxia Inducible Factor 1-Alpha in Human Pituitary Adenoma Cell Line, HP-75 in Hypoxic Condition. *Journal of Neuro-Oncology* **78**:3, 217-225. [CrossRef]

16. Derek John Atkins, Christian Gingert, Christina Justenhoven, Gerd Emil Schmahl, Marcellus Stephanus Bonato, Hiltrud Brauch, Stephan Störkel. 2005. Concomitant deregulation of HIF1# and cell cycle proteins in VHL-mutated renal cell carcinomas. *Virchows Archiv* **447**:3, 634-642. [CrossRef]
17. Peter Johnson, Robert Elsner, Tania Zenteno-Savín. 2005. Hypoxia-inducible factor 1 proteomics and diving adaptations in ringed seal. *Free Radical Biology and Medicine* **39**:2, 205-212. [CrossRef]
18. B H Shen, T W Hermiston. 2005. Effect of hypoxia on Ad5 infection, transgene expression and replication. *Gene Therapy* **12**:11, 902-910. [CrossRef]
19. R BINDRA, P GLAZER. 2005. Genetic instability and the tumor microenvironment: towards the concept of microenvironment-induced mutagenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **569**:1-2, 75-85. [CrossRef]
20. Yun Li, Changman Zhou, John W. Calvert, Austin R.T. Colohan, John H. Zhang. 2005. Multiple effects of hyperbaric oxygen on the expression of HIF-1# and apoptotic genes in a global ischemia–hypotension rat model. *Experimental Neurology* **191**:1, 198-210. [CrossRef]
21. Tobias Schmid, Jie Zhou, Bernhard Brüne. 2004. HIF-1 and p53: communication of transcription factors under hypoxia. *Journal of Cellular and Molecular Medicine* **8**:4, 423-431. [CrossRef]
22. C Liu. 2003. Suppression of the dual-specificity phosphatase MKP-1 enhances HIF-1 trans-activation and increases expression of EPO. *Biochemical and Biophysical Research Communications* **312**:3, 780-786. [CrossRef]
23. Makoto Suematsu . 2003. Quartet Signal Transducers in Gas Biology. *Antioxidants & Redox Signaling* **5**:4, 435-437. [Citation] [Full Text PDF] [Full Text PDF with Links]