

Review

Biology of hypoxia-inducible factor-2 α in development and disease

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The transcriptional response to hypoxia is primarily mediated by two hypoxia-inducible factors – HIF-1 α and HIF-2 α . While these proteins are highly homologous, increasing evidence suggests they have unique transcriptional targets and differential impact on tumor growth. Furthermore, non-transcriptional effects of the HIF- α subunits, including effects on the Notch and c-Myc pathways, contribute to their distinct functions. HIF-2 α transcriptional targets include genes involved in erythropoiesis, angiogenesis, metastasis, and proliferation. Therefore, HIF-2 α contributes significantly to both normal physiology as well as tumorigenesis. Here, we summarize the function of HIF-2 α during development as well as its contribution to pathologic conditions, such as tumors and vascular disease.

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Cells, tissues, and organisms experience reduced oxygen (O₂) tension, or ‘hypoxia,’ under both physiologic and pathologic conditions.¹ During the rapid cell proliferation associated with early embryonic development, physiologic hypoxia stimulates the development of the hematopoietic and circulatory systems.² Rapidly growing tumors also experience hypoxia due to insufficient and defective vascularization.³ The hypoxia-inducible factors (HIFs) mediate the adaptive response to decreased O₂ availability at the cellular and organismal level. HIFs are heterodimeric proteins belonging to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim (PAS) domain family of transcription factors.⁴ Under normoxic conditions, the HIF- α subunits are hydroxylated on key proline residues, which allows for recognition by the von Hippel-Lindau (pVHL) tumor suppressor, the substrate recognition component of an E3 ubiquitin ligase complex that targets HIF- α for proteosomal degradation.^{5–9} In hypoxic conditions, these hydroxylation events are inhibited allowing the α subunits to enter the nucleus and bind the stable subunit or aryl hydrocarbon receptor nuclear translocator (ARNT), forming a functional transcriptional complex. Among HIF transcriptional targets are genes involved in glycolysis, angiogenesis, erythropoiesis, cell death, and differentiation.¹⁰ HIF-1 α and HIF-2 α are the most extensively studied of the three HIF- α isoforms. While these proteins share significant sequence homology, they have unique tissue distributions, embryonic deletion phenotypes and effects on tumorigenesis.

Tissue Distribution

HIF-2 α is also known as endothelial PAS domain protein 1 (EPAS1), HIF-like factor, HIF-related factor or member of PAS superfamily 2 (MOP2).^{11–14} While it was initially detected in endothelial cells, HIF-2 α is expressed in the parenchyma and interstitial cells of multiple organs.^{11,15,16} HIF-2 α stabilization can be observed by immunohistochemistry in renal interstitial cells, hepatocytes, epithelial cells of the duodenum, cardiomyocytes, and astrocytes in rodents exposed to systemic hypoxia.¹⁶ While HIF-2 α is stabilized at higher O₂ tensions than HIF-1 α *in vitro*, HIF-2 α was not detected under normoxic conditions in the organs examined.^{16–18} Accumulation of HIF-2 α is predominantly due to post-translational regulation as mRNA levels are not significantly induced under hypoxia.¹⁶ Interestingly, HIF-2 α mRNA levels are particularly high in tissues that are important for the systemic delivery of O₂, for example the lung, heart, and endothelium.^{11,16} In the lung, HIF-2 α protein is stabilized in type II pneumocytes and pulmonary endothelial cells in response to hypoxia, while HIF-1 α is not detectable.^{16,19} HIF-2 α transcript levels are also developmentally regulated in the lung, with an induction in mRNA levels occurring during the late stages of fetal development.¹⁹ Neural crest derivatives, which synthesize hormones important in modulating vascular tone and cardiac output, also express HIF-2 α .¹⁵ In particular, HIF-2 α is highly expressed in the organ of Zuckerkandl, an important site for catecholamine synthesis during embryonic development.

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Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; Epo, erythropoietin; HIFs, hypoxia-inducible factors; HRE, hypoxia response element; IRE, iron response element; IRP, iron regulatory protein; LOX, lysyl oxidase; PAS, Per-ARNT-Sim; pVHL, von Hippel-Lindau; RCC, renal clear cell carcinoma; TGF- α , transforming growth factor- α ; TAD, transcriptional activation domain; UTR, untranslated region; VEGF, vascular endothelial growth factor

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Analysis of HIF- α -staining patterns in ischemic myocardium showed that acute induction of both α subunits occurs primarily in the region surrounding the infarcted tissue, including cardiomyocytes, endothelial cells, and macrophages.²⁰ With time HIF-2 α also becomes detectable in more distant areas of myocardium. These distinct spatio-temporal patterns of HIF expression suggest that the two α subunits may have unique and complementary roles in the adaptive response to tissue hypoxia.

Transcriptional Targets

Both HIF- α subunits dimerize with ARNT and recognize identical DNA sequences (5'-CGTG-3') found within the promoters or enhancers of target genes, termed hypoxia response elements (HREs). Despite these similarities, expression profiling and functional studies have revealed that the HIF- α subunits regulate both shared and unique target genes.^{21–25} For instance, HIF-1 α uniquely stimulates the expression of glycolytic enzymes, such as phosphoglycerate kinase and lactate dehydrogenase-A, carbonic anhydrase-9, and the pro-apoptotic gene *BNIP-3*.^{21,23,24,26} In contrast, the embryonic transcription factor *Oct-4* (*Pou5f1*, *Oct-3/4*), *CYCLIN D1*, *TWIST1*, transforming growth factor- α (*TGF- α*), and erythropoietin (*EPO*) are upregulated under hypoxia in a HIF-2 α -dependent manner.^{22,24,27–31} A third group of genes, including vascular endothelial growth factor (*VEGF*), adrenomedullin (*ADM*), and glucose transporter 1 (*GLUT-1*) are regulated by both α subunits.^{18,21}

The HIF- α subunits display the highest degree of sequence homology in the bHLH (85%), PAS-A (68%), and PAS-B (73%) domains (Figure 1).¹¹ HIF- α subunits also contain two transcriptional activation domains (TADs) termed the N-terminal (N-TAD) and C-terminal (C-TAD).³² The C-TAD recruits the transcriptional co-activators Creb-binding protein CBP/p300 in an O₂-dependent fashion. During normoxic conditions, a conserved asparagine residue in the C-TAD is hydroxylated by the factor-inhibiting HIF-1 α (FIH-1), preventing interaction with CBP/p300.^{33,34} Domain-swapping and chromatin immunoprecipitation studies have shown that

selective HIF target gene activation is not based on the unique DNA-binding abilities of these factors. Both HIF- α subunits can bind to the endogenous HREs of hypoxia-responsive genes; however, binding is not sufficient for target gene activation.^{35,36} In contrast, C-terminal regions of each protein are required for specificity. For select targets, the N-TAD is sufficient to confer target gene specificity and replacement of the HIF-2 α N-TAD with the analogous region of HIF-1 α switches the specificity of these proteins.³⁶ In contrast, targets such as prolyl hydroxylase-3 (PHD3) require additional regions of the HIF-2 α protein to be induced.³⁵

Cooperation with other transcription factors may also be required for maximal and cell-type-specific upregulation of HIF target genes (Figure 2). Multiple Ets family transcription factors can cooperate with HIF-2 α for hypoxic gene induction.^{36–38} For example, Ets-1 and HIF-2 α synergistically activate the expression of VEGF receptor-2 (VEGFR-2 or Flk-1).³⁷ Interestingly, physical interaction between HIF-2 α and Ets-1 is mediated by the C terminus of HIF-2 α . Another Ets family transcription factor, Elk-1, cooperates with HIF-2 α to activate the target genes *CITED-2*, *EPO*, insulin-like growth factor-binding protein-3 (*IGFBP3*), and *PAI-1*.^{36,38} Several other genes preferentially regulated by HIF-2 α in MCF7 breast cancer cells exhibit Ets-binding sites adjacent to HREs, suggesting this may be a common mechanism for regulating target gene selectivity by HIF-2 α .³⁸ The NF- κ B essential modulator (NEMO) was also identified as a HIF-2 α -interacting protein through yeast two-hybrid screening. NEMO physically interacts with HIF-2 α specifically, but not HIF-1 α , and enhances HIF-2 α transcriptional activity in reporter assays. However, the target gene selectivity and requirement for NEMO at endogenous HIF-2 α responsive genes have not been explored.

Loss-of-function Phenotypes

Targeted disruption of HIF-2 α leads to embryonic lethality between E9.5 and E13.5, with divergent phenotypes depending on the genetic background of the mice (Table 1). Tian *et al.*¹⁵ found that mice lacking HIF-2 α died at mid-gestation with bradycardia and reduced levels of noradrenaline, suggesting that HIF-2 α regulates embryonic catecholamine synthesis in response to physiologic hypoxia. Indeed, HIF-2 α is expressed in neural crest derivatives that synthesize catecholamines and tyrosine hydroxylase, a critical enzyme in catecholamine synthesis that is hypoxia inducible.⁴¹ A fraction of these embryos could be rescued to birth by exogenous administration of D,L-threo-3,4-dihydroxyphenylserine, a metabolic intermediate that can be directly converted to noradrenaline.¹⁵ Nevertheless, homozygous pups died soon after birth. This is due to impaired fetal lung maturation, leading to perinatal lethality and respiratory distress syndrome.¹⁹ HIF-2 α -mediated upregulation of VEGF appears to be required for stimulating surfactant production by type II pneumocytes of the lung. Inhibition of VEGF receptor signaling using neutralizing anti-Flk-1 antibodies recapitulated the lung phenotype of HIF-2 α -deficient mice and intra-amniotic VEGF administration improved lung function and survival of preterm mice. In an independent HIF-2 α knockout model generated by Peng *et al.*³⁹ vascular defects in the yolk

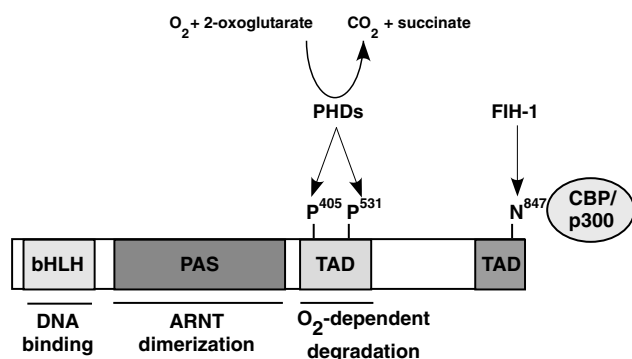


Figure 1 Structure of HIF-2 α , including the bHLH DNA-binding domain, PAS domain, and transactivation domain (TAD). Prolyl hydroxylases (PHDs) hydroxylate proline residues 405 and 531 in the oxygen-dependent degradation (ODD) of HIF-2 α under normoxic conditions, targeting it for degradation by the proteasome. In addition, hydroxylation of asparagine 847 in the C-terminal TAD by factor-inhibiting HIF (FIH-1) inhibits interaction with the co-activators CBP/p300

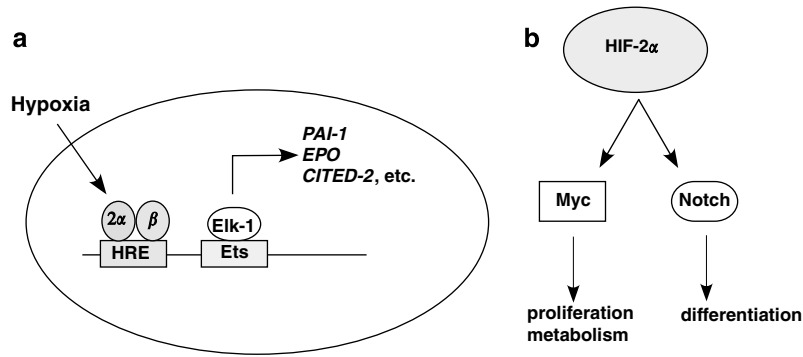


Figure 2 (a). Transcription-dependent functions. Ets family transcription factors have been shown to cooperate specifically with HIF-2 α to activate select target genes (e.g. PAI-1, EPO, CITED-2). (b). Transcription-independent activities. HIF-2 α regulates the activity of transcription factors such as Notch and c-Myc, thereby influencing cell proliferation, metabolism, and differentiation

Table 1 Summary of HIF-2 α knockout phenotypes

Study	Phenotype
Tian <i>et al.</i> ¹⁵	Bradycardia, reduced noradrenaline, partial rescue with metabolic intermediate
Peng <i>et al.</i> ³⁹	Vascular defects in yolk sac and embryo
Compernelle <i>et al.</i> ¹⁹	Reduced surfactant production, respiratory distress syndrome
Scortegagna <i>et al.</i> ⁴⁰	Pancytopenia, hepatic steatosis, cardiac hypertrophy, retinopathy, increased oxidative stress
Gruber <i>et al.</i> ³¹	Global postnatal deletion, anemia rescued by exogenous Epo

sac and embryos of mutant mice were observed. HIF-2 α also appears to play a role in regulating blood vessel dynamics during pathologic conditions. Heterozygosity for HIF-2 α can be protective against pulmonary hypertension and right ventricular hypertrophy induced by chronic hypoxia. This effect may be mediated by hypoxic upregulation of endothelin-1 (ET-1), which causes pulmonary vasoconstriction and remodeling of pulmonary vasculature. Mice heterozygous for HIF-2 α did not upregulate ET-1 or increase circulating levels of catecholamines in response to hypoxia.⁴²

By crossing mice in different genetic backgrounds, Scortegagna *et al.*⁴⁰ were able to obtain a small fraction of viable *Hif-2 α* -null adult mice. These mice exhibit multiorgan dysfunction, including pancytopenia, hepatic steatosis, cardiac hypertrophy, and retinopathy, associated with increased oxidative stress. This study suggested a novel role for HIF-2 α in regulating the expression of antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase. Bone marrow transplant experiments demonstrated that the hematopoietic defect in these mice is non-cell autonomous.⁴³ Reconstitution of lethally irradiated wild-type recipients with hematopoietic stem cells from *Hif-2 α* ^{-/-} mice corrected the pancytopenia, suggesting that the phenotype was mediated by the microenvironment of the cells. *Hif-2 α* ^{-/-} mice had inappropriately low levels of renal *Epo* mRNA and failed to upregulate *Epo* in response to intermittent hypoxia, suggesting that decreased *Epo* production was responsible for defective hematopoiesis. Notably, administration of exogenous *Epo* corrected the pancytopenic phenotype.⁴⁴

Given these disparate phenotypes for global HIF-2 α deletion, the differential requirements for the α subunits during development remained unclear. As a genetic test of the ability of HIF-2 α to compensate for HIF-1 α function, we 'knocked-in' the HIF-2 α coding sequence into the HIF-1 α locus, thereby expanding HIF-2 α expression to all tissues in which HIF-1 α is normally expressed.⁴⁵ In addition to demonstrating that the HIFs are not redundant proteins, as expanded HIF-2 α expression was unable to rescue lack of HIF-1 α , this study revealed a gain-of-function phenotype for HIF-2 α knock-in, due to its unique ability to regulate *Oct-4* and *Tgf- α* .²⁹ Increased *Oct-4* expression in mice homozygous for the HIF-2 α knock-in allele led to early embryonic lethality. Tumors derived from homozygous HIF-2 α knock-in embryonic stem (ES) cells displayed increased growth and angiogenesis in xenograft assays.^{29,45} These tumors were also characterized by an increased fraction of undifferentiated cells, which could be reversed by knock down of *Oct-4*.

Using a conditional deletion system, we studied the function of HIF-2 α postnatally independent of the confounding requirements for HIF-2 α during development. Acute deletion of HIF-2 α using a tamoxifen-inducible ubiquitously expressed Cre leads to anemia associated with decreased levels of circulating *Epo*, demonstrating that HIF-2 α is the physiologic regulator of *Epo* production in adult mice.³¹ Deletion of HIF-2 α also impaired stress-induced *Epo* synthesis and erythropoiesis. Prior to the onset of renal *Epo* production, the liver is the major source of systemic *Epo* in neonatal mice. Consistent with the global deletion of HIF-2 α , hepatocyte-specific deletion using an albumin-Cre reveals that in neonatal mice hepatic *Epo* production is dependent on HIF-2 α activity. Moreover, HIF-2 α binds the endogenous *EPO* 3' enhancer HRE in hepatocytes.^{31,46} Local synthesis of *Epo* by astrocytes in the central nervous system also appears to be regulated by HIF-2 α and may be important for survival of neurons during periods of ischemia.⁴⁷

O₂ transport by red blood cells is mediated by reversible binding to iron present in the hemoglobin molecule. Therefore, organisms must coordinate erythropoiesis with iron availability. HIFs regulate the expression of several genes involved in iron homeostasis, such as transferrin.⁴⁸ Iron regulatory proteins (IRPs), on the other hand, control iron metabolism through post-transcriptional regulation of select mRNAs. IRPs

regulate translation of these mRNAs by binding to iron response elements (IREs) present in untranslated regions (UTRs) of the transcript.⁴⁹ A conserved IRE was recently identified in the 5' UTR of HIF-2 α .⁵⁰ This element promotes HIF-2 α protein translation under conditions of increased iron availability, thereby coupling erythropoiesis with iron homeostasis.

Role in Tumorigenesis

HIF-2 α is frequently expressed in solid tumors. In particular, the role of HIF-2 α in the pathogenesis of renal clear cell carcinoma (RCC) has been most extensively studied. Loss of *VHL* is associated with the autosomal-dominant hereditary cancer syndrome VHL disease, in which patients inherit one mutant allele of *VHL* through the germline. Somatic mutation, deletion, or epigenetic silencing of the remaining allele leads to the formation of highly vascular tumors in specific tissues, including hemangioblastomas of the central nervous system, RCCs, and pheochromocytomas.⁵¹ Loss of pVHL function is also associated with the majority (80–90%) of sporadic RCC.⁵² In the absence of pVHL, the HIF- α subunits accumulate, leading to a constitutive hypoxia-like pattern of gene expression. Examination of kidneys from patients with VHL renal disease suggested that HIF activation in renal epithelium precedes the development of overt malignancy.^{24,53} HIF-2 α staining was absent in early lesions, but frequently observed in overt carcinomas and cell lines derived from renal tumors.

Restoration of pVHL protein in *VHL*-null renal carcinoma cell lines inhibits tumor growth in xenograft models.^{54,55} Given the highly vascular lesions associated with VHL disease, it was hypothesized that dysregulated HIF activity stimulated the growth of *VHL*-deficient tumors. Surprisingly, expression of a normoxically stable HIF-1 α protein further inhibited xenograft growth in pVHL-rescued tumors while expression of stabilized HIF-2 α promoted it, demonstrating that suppression of HIF-2 α activity is critical for tumor suppression by pVHL.^{54,55} Elimination of HIF-2 α by shRNA-mediated knock-down similarly inhibited the growth of *VHL*-null renal cell lines in xenograft experiments.⁵⁶ These studies were among the first to suggest differential abilities of the HIF- α subunits to promote tumor growth and prompted investigation into the differences between the α subunits.

von Hippel–Lindau disease is characterized by striking genotype–phenotype correlations, with select point mutations conferring increased risk of renal carcinoma in patients.⁵² Introduction of pVHL mutations (Y98N, Y112N, W117R, and R167Q) that are associated with a high risk of renal carcinoma (type 2B) into *VHL*-deficient cell lines leads to increased normoxic HIF-1 α and HIF-2 α stabilization compared to wild-type VHL.^{57,58} Cells harboring these mutations also display increased proliferation in serum-depleted media and growth in orthotopic xenograft assays, which can be reversed by shRNA-mediated knock down of HIF-2 α .⁵⁷ The R167Q mutation, in particular, uniquely disrupts regulation of HIF-2 α in ES cells.⁵⁸ In contrast, type 2A mutations (Y98H, Y112H, A149T, and T157I), which have a lower risk for renal tumors, display reduced levels of normoxic HIF- α accumulation and growth in xenograft assays compared to type 2B mutants.⁵⁷

In contrast to tumor-associated VHL mutations, the R200W mutation in pVHL leads to Chuvash polycythemia, a heritable disease characterized by elevated levels of serum Epo and VEGF. Importantly, mice homozygous for the R200W mutation recapitulate the human disease and display upregulation of HIF-2 α -preferential target genes in multiple tissues.⁵⁹

HIF-2 α may also play a role in renal carcinomas caused by mutations in the tumor suppressor tuberous sclerosis-2 (*Tsc-2*). Cell lines and primary tumors derived from the Eker rat, a model of spontaneous renal carcinoma caused by heterozygous mutation in *Tsc-2*, display increased levels of HIF-2 α protein. High levels of VEGF expression and increased vascularization are features of these tumors as well.⁶⁰

CYCLIN D1 and *TGF- α* represent two HIF-2 α -regulated genes that could mediate these pro-tumorigenic properties in renal tumors.^{24,27,28,61} Activation of an autocrine signaling loop through TGF- α -mediated stimulation of the epidermal growth factor receptor (EGFR) has been proposed to drive serum-independent growth of renal carcinoma cells.^{28,62} Indeed, silencing of EGFR in VHL-deficient RCC cell lines impairs proliferation in the absence of serum and growth in xenograft assays.⁶¹ Given the challenge of developing drugs that target transcription factors, identifying signaling pathways activated downstream of HIF-2 α will be critical for developing therapeutics.

An apparent paradox of constitutive HIF activation in *VHL*-deficient renal tumors is the ability of FIH-1 to inhibit recruitment of the co-activators CBP/p300 by hydroxylating the C-TAD. Dissection of the relative contributions of the C-TAD and N-TAD of HIF-2 α to target gene activation in RCC lines indicates that the C-TAD is largely dispensable for transcriptional activation by HIF-2 α .⁶³ Nevertheless, deletion of either the C-TAD or N-TAD impairs tumor formation in nude mice, suggesting that subtle changes in gene expression and transcription-independent functions of HIF-2 α mediated by these domains could contribute to tumor growth. The HIF-2 α C-TAD also appears to be resistant to the effects of FIH-1 in transcriptional activation.

Transcription-independent functions of the HIF- α subunits also contribute to their differential activity (Figure 2b). HIF-1 α functionally antagonizes c-Myc transcriptional activity under hypoxia, leading to upregulation of the cyclin-dependent kinase inhibitor p21 and cell cycle arrest.⁶⁴ In contrast, HIF-2 α promotes cell cycle progression by enhancing c-Myc-mediated expression of cyclin D2 and E2F1 in multiple cell types.⁶⁵ HIF-2 α also enhances c-Myc- and Ras-induced transformation of primary fibroblasts. Increased expression of c-Myc target genes can also be observed in human tumor samples that solely express HIF-2 α (J Gordon, submitted). HIF-2 α -only expressing tumors also exhibit increased proliferation compared to tumors expressing both α subunits. Both HIFs have also been shown to regulate the expression of *Mxi1*, which inhibits c-Myc activity by competing for Max.^{66,67} Furthermore, it has been shown that HIF-1 α targets c-Myc for proteosomal degradation independently of *Mxi1*.⁶⁶ Decreased c-Myc activity in *VHL*-deficient RCC lines was correlated with reduced mitochondrial DNA content and O₂ consumption.

The HIFs can also enhance stabilization of the Notch intracellular domain and expression of Notch target genes,

such as *Hes1* and *Hey2*.⁶⁸ Enhanced Notch signaling under hypoxia blocks myogenic and neuronal differentiation. It will be interesting to compare the relative contributions of the two α subunits in enhancing Notch activity and determine whether this function contributes to tumorigenesis.

In addition to cell intrinsic responses to hypoxia, changes in the tumor microenvironment can significantly impact tumor growth and metastasis. Infiltrating immune cells influence angiogenesis and metastasis by secreting chemokines and matrix-remodeling enzymes.⁶⁹ HIF-2 α is highly expressed in tumor-associated macrophages of human breast carcinomas and correlates with increased microvessel density and high tumor grade.^{70,71} HIF-2 α also upregulates several genes that are key mediators of metastasis, including *CXCR4*, *LOX*, and *TWIST1*.^{30,72,73} The chemokine receptor *CXCR4* is a HIF-2 α -inducible gene in RCC, and high levels of expression correlate with reduced patient survival. Lysyl oxidase (LOX) is critical for metastasis of breast cancer xenografts. High levels of LOX expression are correlated with poor metastasis-free and overall survival rates in human breast and head/neck cancers. Recently, an RNAi screen in *Caenorhabditis elegans* to identify genes in the HIF pathway isolated *hlh-8*, a homolog of human *TWIST1*. *TWIST1* is a bHLH transcription factor that promotes epithelial–mesenchymal transition and is required for metastasis in murine breast cancer models.⁷⁴ Subsequent analysis in human tumor cell lines showed that *TWIST1* is uniquely regulated by HIF-2 α , although direct binding of HIF-2 α to *TWIST1* regulatory elements is yet to be demonstrated.

A pro-tumorigenic function for HIF-2 α has also been demonstrated in neuroblastoma, a childhood malignancy derived from sympathetic neural precursors. Hypoxia alters the phenotypic properties of these tumors, promoting de-differentiation toward a neural crest-like state.⁷⁵ HIF-2 α stabilization can be detected in well-vascularized regions of neuroblastoma patient samples and at higher O₂ tensions (5%) in neuroblastoma cell lines than HIF-1 α .¹⁷ The temporal pattern of HIF- α subunit accumulation is also varied in these cells. HIF-1 α is stabilized acutely, while HIF-2 α protein remains stabilized over longer periods of hypoxia. Transient knock down of HIF-2 α inhibits growth of neuroblastoma xenografts in nude mice. In addition, high levels of HIF-2 α expression in patient samples correlate with advanced clinical stage and poor prognosis.

In contrast to human neuroblastomas, overexpression of Hif-2 α in rodent glioma cells has been shown to reduce tumor growth through increased apoptosis, despite enhanced vascularization.⁷⁶ Teratomas derived from *Hif-2 α ^{-/-}* ES cells were also observed to grow faster than control teratomas. To date, this is the only study suggesting that HIF-2 α can function as a tumor suppressor and there is a conflicting report on the role of HIF-2 α in apoptosis. A previous study of *Hif-2 α ^{-/-}* ES cells suggested that hypoxia-induced accumulation of p53 and apoptosis is HIF-1 α dependent and that unlike HIF-1 α , deletion of *Hif-2 α* did not protect against hypoxia-induced apoptosis.⁷⁷ Furthermore, the pro-apoptotic gene *BNIP-3* is positively regulated by HIF-1 α and can be repressed by HIF-2 α in certain cell types.²³ Collectively, these studies suggest that HIF-2 α has cell-type-dependent effects on tumor growth and its role in apoptosis is unresolved.

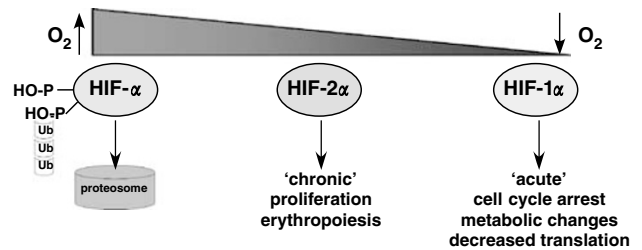


Figure 3 The HIF- α subunits may mediate different adaptive responses to hypoxia. HIF-2 α appears to be stabilized at higher O₂ levels and displays fewer growth-inhibitory properties than HIF-1 α . Moreover, prolonged accumulation of HIF-2 α suggests it could be important for adaptation to chronic hypoxia

Renal clear cell carcinomas are unique tumors in that the majority of cases have mutations in *VHL*, resulting in sustained HIF- α accumulation, independent of O₂ tension. HIF-1 α is ubiquitously expressed and mediates multiple adaptive responses to hypoxia that may inhibit tumor growth when activated constitutively. For instance, HIF-1 α inhibits proliferation by antagonizing c-Myc transcriptional activity and reprograms the metabolism of cells to reduce anabolic processes through the upregulation of pyruvate dehydrogenase kinase-1 (PDK1).⁷⁸ Reduced growth of *VHL*-deficient fibrosarcomas and teratocarcinomas also suggests that HIF-1 α can inhibit tumor growth.^{79,80} HIF-2 α , on the other hand, promotes c-Myc activity and expression of cyclin D1, both of which can promote tumor growth (Figure 3). HIF-2 α is also stabilized at higher O₂ levels (5%) and over prolonged time periods in neuroblastomas. Thus, tissues in which HIF-2 α is preferentially stabilized at higher O₂ levels may show stronger dependence of this α subunit during tumorigenesis. However, to clearly discern the functional significance of the individual α subunits on tumorigenesis it will be important to use conditional alleles to individually knockout these genes in mouse models of disease, particularly RCCs and neuroblastomas. An important prerequisite for these studies will be the development of appropriate mouse models of renal tumorigenesis. Conditional deletion of *VHL* in the kidney leads to cyst formation, but not frank carcinoma.⁸¹ Moreover, the two subunits may also behave differently depending upon whether they are deleted in the tumors themselves or in cells within the tumor microenvironment, such as macrophages or endothelial cells.

Conclusions

While HIF-1 α was the first HIF- α subunit to be identified and has been most extensively studied, recent examination of HIF-2 α in the context of both development and tumorigenesis demonstrates significant differences in the biological function of these two proteins. Loss-of-function studies suggest that the contribution of HIF-2 α activity to diseases such as pulmonary hypertension, polycythemia, and anemia will be interesting to investigate further. In addition, both HIF- α subunits regulate genes involved in tumor angiogenesis, metastasis, and differentiation, but appear to have cell-type-specific effects on tumor progression. Given the importance of hypoxia in solid tumors, identification of small molecule inhibitors of the HIF pathway has been a growing area

of interest and several promising compounds have been identified.^{82–84} Understanding the differences between the α subunits and their relative contributions to different tumor types will be important for appropriately testing these compounds for use in cancer therapy.

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