Published in final edited form as:

Trends Biochem Sci. 2012 September ; 37(9): 364–372. doi:10.1016/j.tibs.2012.06.004.

Passing the baton: The HIF switch

Mei Yee Koh¹ and Garth Powis

Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, Texas 77030 USA

Abstract

Hypoxia is an inadequate oxygen supply to tissues and cells, which can restrict their function. The hypoxic response is primarily mediated by the hypoxia-inducible transcription factors, HIF-1 and HIF-2, which have both overlapping and unique target genes. HIF target gene activation is highly context specific and is not a reliable indicator of which HIF- α isoform is active. For example in some cell lines, the individual HIFs have specific temporal and functional roles: HIF-1 drives the initial response to hypoxia (<24 hours) and HIF-2 drives the chronic response (>24 hours). Here, we review the significance of the HIF switch and the relationship between HIF-1 and HIF-2 under both physiological and pathophysiological conditions.

Key terms

Hypoxia; HIF; development; angiogenesis; cancer; stem cells

Hypoxia and the HIFs in human physiology and disease

Physiological tissue oxygen tensions are significantly lower than ambient oxygen tensions as a result of the dramatic decrease in blood oxygen content as it travels from the lungs throughout the body (Table 1) [1]. Oxygen gradients play an important and beneficial role in mammalian physiology; low oxygen or hypoxia provides the required extracellular stimulus for proper embryogenesis and wound healing, and maintains the pluripotency of stem cells. Hypoxia that involves oxygen tensions below the normal physiological range can restrict the function of organs, tissues, or cells (Table 2). Pathological hypoxia can be caused by a reduction in oxygen supply such as caused by high altitude or localized ischemia due to the disruption of blood flow to a given area. Additionally, most solid tumors contain hypoxic regions because of the severe structural abnormality of tumor microvessels [2]. Crucial mediators of the hypoxic response are the HIF transcription factors, which transactivate a large number of genes including those promoting angiogenesis, anaerobic metabolism and resistance to apoptosis. HIFs are heterodimers comprising one of three major oxygen labile HIF- α subunits (HIF-1 α , HIF-2 α or HIF-3 α), and a constitutive HIF-1 β subunit (also known as aryl hydrocarbon receptor nuclear translocator or ARNT), which together form the HIF-1, HIF-2 and HIF-3 transcriptional complexes respectively [3]. Of the three α -subunits, HIF-1 α and HIF-2 α are the best studied. HIF-3 α has high similarity to HIF-1 α and HIF-2 α in the basic helix-loop-helix (bHLH) and Per-Arnt-SIM (PAS) domains (Figure 1), but lacks

© 2012 Elsevier Ltd. All rights reserved.

¹Corresponding author: Mei Yee Koh, PhD. (mykoh@mdanderson.org).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the C-terminal transactivation domain (TAD-C). HIF-3 α has multiple splice variants, the most studied being the inhibitory PAS domain protein (IPAS), which is a truncated protein that acts as a dominant negative inhibitor of HIF-1 α [4].

HIF-1 α and HIF-2 α have 48% amino acid sequence identity and similar protein structures, but are non-redundant and have distinct target genes and mechanisms of regulation. Intriguingly, it appears that in some cell lines, HIF-1 α is most active during short periods (2–24 hours) of intense hypoxia or anoxia (< 0.1% O₂), whereas HIF-2 α is active under mild or physiological hypoxia (<5% O₂), and continues to be active even after 48–72 hours of hypoxia [5]. Hence, in certain contexts HIF-1 drives the initial response to hypoxia, but during chronic hypoxic exposure it is HIF-2 that plays the major role in driving the hypoxic response [5, 6]. This HIF ‘switch’ results in HIF-1 and HIF-2 playing divergent but complementary roles during the hypoxic response of tissues under both physiological and pathophysiological conditions. This review provides an update on HIF-1 and HIF-2 regulation and describes the mechanism and function of the HIF ‘switch’ under both normal and diseased conditions.

HIF regulation

Under aerobic conditions, HIF-1/2 α is hydroxylated by specific prolyl hydroxylases (PHDs) at two conserved proline residues (P402/P564 and P405/P531 for human HIF-1 α and HIF-2 α , respectively) situated within the oxygen-dependent degradation domain (ODD) in a reaction requiring oxygen, 2-oxoglutarate, ascorbate, and iron (Fe²⁺) as a cofactor. HIF-1/2 α hydroxylation facilitates binding of von Hippel-Lindau protein (pVHL) to the HIF-1/2 α ODD [7]. pVHL forms the substrate recognition module of an E3 ubiquitin ligase complex comprising elongin C, elongin B, cullin-2, and ring-box 1, which directs HIF-1/2 α poly-ubiquitylation and proteasomal degradation [8]. Under hypoxic conditions, PHD activity is inhibited, pVHL binding is abrogated, and HIF-1 α and HIF-2 α are stabilized. HIF-1/2 α enter the nucleus, where they heterodimerize with HIF-1 β and bind to a conserved DNA sequence known as the hypoxia responsive element (HRE), to transactivate a variety of hypoxia-responsive genes [9]. Under normoxic conditions, the ability of HIF-1/2 α to activate transcription is prevented by another oxygen regulated enzyme, factor inhibiting HIF-1 (FIH-1). FIH-1 hydroxylates Asn 803 within the TAD-C of human HIF-1 α , disrupting its interaction with the transcription co-activators p300/CBP [10]. FIH-1 can also hydroxylate the corresponding Asn within HIF-2 α , albeit at a much lower efficiency compared to HIF-1 α [11]. As with the PHDs, Asn hydroxylation is inhibited under hypoxic conditions, allowing the p300/CBP complex to bind to HIF-1/2 α , thus allowing HIF transactivation.

Although pVHL has been well-established as the key player in the oxygen-dependent degradation of HIF- α , a number of alternative HIF regulatory pathways have been described. These pathways can be either oxygen-dependent or -independent, and either HIF- α isoform-selective or affect both HIF-1 α and HIF-2 α (Figure 1). Oxygen independent regulators include receptor of activated protein kinase C (RACK1), which competes with heat shock protein 90 (Hsp90) for binding to HIF-1 α and promotes HIF-1 α degradation; and human double minute 2 (Hdm2), which induces HIF-1 α proteasomal degradation via a p53-HIF-1 α interaction [12, 13]. It is unclear whether either modulator affects HIF-2 α stability. Modulators known to be HIF- α selective include the hypoxia-associated factor (HAF), which causes HIF-1 α ubiquitylation and degradation, but promotes HIF-2 α transactivation under prolonged hypoxia; and the Hsp70/CHIP complex, which degrades HIF-1 α but not HIF-2 α under prolonged hypoxia or under high glucose conditions [14–16].

Outcomes of HIF-1 versus HIF-2 activation

Since its identification over a decade ago, HIF-1 α has been described as the master regulator of the hypoxic response and the crucial node in ensuring survival during hypoxic stress [3]. HIF-2 α was initially identified as the endothelial PAS domain protein (EPAS1), an endothelial specific HIF- α isoform, and was therefore considered to have a more specialized function than HIF-1 α [17]. However, HIF-2 α is also expressed in many other tissues including brain, heart, lung, kidney, liver, pancreas, and intestine, suggesting that it also has a widespread role in the hypoxia response [18]. Recent data now show that both HIF-1 and HIF-2 participate in hypoxia-dependent gene regulation through complex and sometimes antagonistic interactions, such as that observed in kidney cancer cells [19]. Intriguingly, although both HIF-1 and HIF-2 bind to the same HRE consensus sequence in the regulatory regions of target genes, DNA binding does not necessarily correspond to increased transcriptional activity, suggesting that post-DNA binding mechanisms might be required for transactivation [20]. Hence, although HIF-1 α was originally proposed to promote the hypoxic induction of erythropoietin (*EPO*) by binding to the HRE in the *EPO* enhancer, it is now clear that in intact cells and in mice, endogenous HIF-2 α is the main driver of *EPO* production [21–23]. A list of HIF-1- and HIF-2-specific target genes in various contexts is available elsewhere [24]. In general, HIF-1 preferentially induces genes that encode glycolytic enzymes, such as phosphofructokinase (*PFK*) and lactate dehydrogenase A (*LDHA*); that are involved in pH regulation, such as monocarboxylate transporter 4 (*MCT4*) and carbonic anhydrase 9 (*CA-IX*); and that promote apoptosis, such as BCL2/adenovirus E1B 19kDa interacting protein 3 (*BNIP3*) and BCL2/adenovirus E1B 19kDa interacting protein 3-like (*BNIP3L/NIX*). By contrast, HIF-2 induces genes that are involved in invasion, including the matrix metalloproteinases (*MMP*) 2, and 13, and the stem cell factor *OCT-3/4* [24]. However, HIF-2 has also been shown to regulate enzymes in the glycolytic pathway in the absence of HIF-1, and HIF-1 is capable of activating some MMPs, suggesting that under some circumstances, HIF-1 α and -2 α can each substitute for the other's isoform-specific functions [22, 25]. HIF-1 and HIF-2 also have common target genes, such as the vascular endothelial growth factor A (*VEGFA*) and glucose transporter 1 (*GLUT1*). Thus, the ability of HIF-1 and HIF-2 to activate specific target genes appears to be context dependent and therefore each target gene must be carefully defined in terms of HIF-1 and HIF-2 responsiveness when examined within a specific context. In addition their intrinsic specificities for downstream target genes, HIF-1 and HIF-2 also show variations in their regulation by major upstream or downstream modulators that contribute to distinct temporal and functional responses to hypoxia, and these will be discussed next.

Different temporal and functional roles of HIF-1 versus HIF-2

The temporal regulation of HIF-1/2 is largely mediated by the oxygen dependent hydroxylases that regulate HIF-1/2 α stability and activity. The actions of the PHDs on different HIF isoforms are generally not equivalent; PHD2 has relatively more influence on HIF-1 α than HIF-2 α , and PHD3 has relatively more influence on HIF-2 α than HIF-1 α [26]. HIF-2 α is also hydroxylated at a much lower efficiency than HIF-1 α by both the PHDs and FIH-1, which can result in the stabilization and activation of HIF-2 α at higher oxygen tensions than HIF-1 α [11]. In addition to post-translational mechanisms, HIF-1 α (but not HIF-2 α) mRNA stability is inhibited after prolonged hypoxia, due to HIF-1/2 α -dependent expression of HIF-1 α antisense RNA [27]. Furthermore, HIF-2 α (but not HIF-1 α) translation is linked to iron metabolism due to an iron-responsive element (IRE) in the 5'UTR of HIF-2 α ; HIF-2 α translation is inhibited when iron is depleted, thus inhibiting erythropoiesis [28]. Context-specific regulation also comes into play by controlling the availability of isoform-specific cofactors and transactivators, such as the HIF-2 α -specific coactivator, avian erythroblastosis virus E26 oncogene homolog 1 (Ets-1) (discussed in the

next section). Differential activation of downstream target genes has also been linked to differences between the N-terminal transactivation domains of HIF-1 α versus HIF-2 α , suggesting that inherent differences in the coding sequences of the HIF- α isoforms might also contribute to isoform-specific function [29]. Hence, multiple mechanisms converge to dictate context-dependent, HIF- α isoform specific activation in response to variations in hypoxic intensities and duration. This exquisite balance between HIF-1 and HIF-2 activation enables the coordinate regulation of the complex hypoxia-dependent processes that occur in normal physiology, such as during formation of the vascular and skeletal system, which will be outlined in the following sections.

HIFs in vascular development

During early embryonic development, the rapid cellular proliferation in gastrulating embryos results in physiological hypoxia that is necessary for the patterning of the embryonic vascular system [30]. The HIFs are activated by this hypoxic microenvironment, and also by non-hypoxic stimuli such as the renin-angiotensin system, growth factors, and immunogenic cytokines, all of which play important roles in the regulation of placental development and maturation. Embryonic blood vessels form through vasculogenesis, a process by which undifferentiated precursors differentiate into endothelial cells that assemble into a primary vascular plexus [31]. Additional blood vessels are generated by both sprouting and non-sprouting angiogenesis, and are progressively pruned and remodeled into a functional adult circulatory system. The most important signaling molecules for the regulation of vasculature development are VEGF(A–D), the VEGF receptors (VEGFR1–3), and the angiopoietin growth factors (Ang1, Ang2, Ang3/4), and their receptors (Tie1, Tie2) [32]. The initial assembly of the vasculature requires VEGF and its receptors, whereas the Ang-Tie system is essential for later stages of vascular development, when the vessels remodel and acquire their pericyte and/or smooth muscle cell coating (Figure 2a) [33]. VEGFA is one of the most potent pro-angiogenic growth factors and its cognate receptor, VEGFR-2, is considered the major signaling VEGFR in endothelial cells. VEGFR-1 is also important for normal vascular development, but it acts as a decoy receptor for VEGFA, thus suppressing VEGFR-2 signaling [34]. The main mediator of HIF-1 driven angiogenesis is VEGFA [35]. However, angiogenesis mediated by VEGFA alone results in highly permeable, abnormal vasculature that soon regresses once VEGFA is withdrawn, because of low pericyte recruitment and incomplete arterialization. Thus, complete vascular development requires the appropriate spatiotemporal expression of a variety of ligands and receptors, a large number of which are either HIF-1 or HIF-2 regulated.

The Ets-1 proto-oncogene is expressed in endothelial, vascular smooth muscle and epithelial cells, and regulates the expression of several angiogenic and extracellular matrix remodeling factors, thus promoting an invasive phenotype [36]. 90% of HIF-2-specific genes contain Ets family transcription factor binding sites within 60bp of the HRE, including Tie-2, VEGFR-1, VEGFR-2 and VE-cadherin [37]. Intriguingly, HIF-2 (but not HIF-1) stimulates expression of the aforementioned genes either co-operatively or synergistically with Ets-1 [17, 38–40]. Notably, although Tie-2 and VE-cadherin contain HRE-like motifs, they do not appear to be hypoxia inducible. This suggests that HIF-2 can also promote the transcription of specific target genes independently of hypoxia.

The differential requirement for HIF-1 versus HIF-2 activation during vessel formation and maturation has been demonstrated using knockout mouse studies. HIF-1 α ^{-/-} knockout mice display impaired erythropoiesis and defects in neural fold formation, cephalic vascularization, and the cardiovascular system, resulting in embryonic lethality around E10.5 [41–44]. By contrast, depending on genetic background, HIF-2 α ^{-/-} mice can die either by E12.5 with vascular defects, deficient catecholamine metabolism and bradycardia;

as neonates, due to impaired lung maturation; or several months after birth, due to multiorgan pathology and metabolic abnormalities related to impaired homeostasis of reactive oxygen species [45–48]. Hence, the HIFs play essential roles but cannot fully compensate for each other during embryonic development. Intriguingly, selective loss of either HIF-1 α or HIF-2 α in endothelial cells is not embryonic lethal and does not profoundly affect vascular development, but it inhibits tumor angiogenesis in the adult mouse [49, 50]. This suggests that HIF-1 plays a crucial role in driving vasculogenesis and the early stages of angiogenesis, which become rate limiting around E8.5, whereas HIF-2 is required for the remodeling and maturation of the primitive vascular network, which occurs between E9.5 and E12.5 and involves the recruitment of peri-endothelial support cells that contribute to the vessel wall [33, 50]. Although the mechanism regulating the transition from HIF-1- to HIF-2-dependent transcription during embryonic vascular development is not yet known, it might be linked to the increase in oxygen tension as the vasculature develops. Formation of the primitive vascular network, for which HIF-1 plays a crucial role, occurs under intensely hypoxic conditions. As the vasculature matures, oxygen concentrations increase as a result of increased perfusion, promoting greater dependence on HIF-2, which is less hypoxia-dependent, to complete the remodeling and stabilization of the newly formed vasculature (Figure 2a). Thus the formation of complete vasculature requires the seamless transition from largely HIF-1-dependent transcription during early vasculogenesis, through a phase in which both HIF-1 and HIF-2 drive overlapping functions, to gene transcription that is mainly dependent upon HIF-2 during the final stages of vascular maturation. A similar process can be observed during the development of skeletal bone, described in the next section.

HIFs in bone development

Bone formation occurs via two different mechanisms: intramembranous and endochondral ossification [51]. Intramembranous ossification occurs during the formation of the flat skull bones and involves the differentiation of mesenchymal cells directly into osteoblasts. Endochondral ossification occurs during the development of most other bones, and involves a two-stage mechanism, whereby mesenchymal cells become chondrocytes, the primary cell type of cartilage, which form an avascular and highly hypoxic matrix template or growth plate. As a permanent stress, hypoxia influences general chondrocyte metabolism, and most importantly tissue-specific production of cartilage matrix proteins such as type II collagen. This is followed by the replacement of the cartilaginous matrix with highly vascularized bone tissue via degradation of the matrix and blood vessel invasion (Figure 2b). The process of endochondral ossification requires both the hypertrophic differentiation of chondrocytes, which is characterized by secretion of type X collagen, and the conversion of avascular cartilage tissue into highly vascularized bone tissue via degradation of the cartilage matrix, and vascular invasion, mainly via the activation of VEGF [51].

Recent studies demonstrate that the HIF pathway is involved in membranous ossification and in both stages of endochondral ossification by coupling angiogenesis to osteogenesis, and regulating the spatio-temporal onset of angiogenesis in the growth plate [52]. Although both HIF-1 α and HIF-2 α are expressed in growth plate chondrocytes, HIF-1 α is expressed at similar levels during all stages of chondrocyte differentiation, and its activity is enhanced by hypoxia, whereas HIF-2 α levels increase with chondrocyte differentiation, and its function is independent of oxygen-dependent hydroxylation [53]. In this respect, HIF-1 acts as a survival factor in hypoxic chondrocytes by enhancing anaerobic glycolysis and inhibiting apoptosis [54]. HIF-1 also promotes autophagy, which can extend the lifespan of chondrocytes [55]. Additionally HIF-1 is important for extracellular matrix (ECM) synthesis, by inducing the expression of important components required by proliferating chondrocytes in the proliferating zone, such as Type II collagen and aggrecan; and also by

inducing SOX9, a key transcription factor that is important for all phases of chondrocytic development and differentiation [54]. By contrast, HIF-2 regulates the extent of HIF-1-induced autophagy, acting as a brake to the accelerator function of HIF-1, and does not appear to play an important role in ECM synthesis within the proliferating zone [56]. Instead, HIF-2 is a potent transactivator several genes, including type X collagen (*COL10A1*), which is secreted by hypertrophic chondrocytes within the hypertrophic zone; a variety of MMPs (*MMP1, 3, 9, 12, 13*) and cartilage proteinases (*ADAMTS4,5*), which are important for regulating catabolic cartilage destruction; and *VEGF*, the crucial switch for vascular invasion [53, 57]. Consistent with its role as an important regulator of cartilage destruction, elevated HIF-2 α levels have been associated with the development of osteoarthritis [53, 57]. Mice bearing osteoblast-targeted deletions of HIF-1 α but not of HIF-2 α show decreased bone volume, although deletion of either isoform results in comparable decreases in skeletal angiogenesis [58]. Taken together, this suggests that HIF-1 plays a central role in hypoxia-dependent cartilage formation and maintenance, whereas HIF-2 α is involved in endochondral ossification and cartilage destruction, which might be less hypoxia-dependent [53]. However, both HIF-1 and HIF-2 are required for developing skeletal vascularity. The temporal regulation of HIF-1 α and HIF-2 α that occurs during bone development resembles the switch that occurs during vascular development, whereby HIF-1 is crucial for the early stages during severely hypoxic conditions, and HIF-2 becomes more important during later stages in a hypoxia-independent manner (Figure 2b). The roles of the HIFs during normal physiological processes, such as bone and vascular development, can be usurped and misregulated to drive disease processes, the clearest example of which is cancer initiation and progression.

HIFs in stem cells and cancer

Tumor hypoxia is of major clinical significance because it promotes both tumor progression and resistance to therapy [59]. In addition to promoting tumor cell survival by shifting cells towards anaerobic metabolism, neovascularization and resistance to apoptosis, hypoxia drives other responses that contribute to tumor aggressiveness, such as increased genetic instability, invasion, metastasis and de-differentiation, largely through activation of the HIFs (Figure 3a) [60]. Elevated levels of tumor HIF-1 α are associated with poor patient prognosis in multiple tumor types [61]. Elevated HIF-2 α is also associated with poor prognosis in specific tumor types such as renal clear cell carcinoma (RCC), which is the most common type of kidney cancer, neuroblastoma, glioblastoma (GBM) and non-small cell lung cancer [5, 62].

Consistent with the differential activation of HIF-1 versus HIF-2 in response to variations in oxygen tension, tumor HIF-1 provides a swift response to acute or transient hypoxia due to its rapid induction and negative feedback regulation, whereas chronic hypoxia appears to favor activation of HIF-2 (Figure 3b). However, whether HIF-1, HIF-2, or both HIFs drive tumor growth is very much dependent upon the mutational landscape of the tumor cells; this includes the availability of required co-factors, and the functionality of HIF- α isoform-specific pathways, such as apoptosis (primarily HIF-1 driven) or cell cycle progression (primarily HIF-2 driven). The HIF switch is particularly evident during the development of RCC, where there is a gradual shift from HIF-1 α to HIF-2 α expression with increasing tumor grade [19, 63]. RCC is most typically initiated by loss of pVHL, resulting in the pseudo-hypoxic activation of both HIF-1 and HIF-2. However, HIF-2 α drives tumor progression in RCC, whereas HIF-1 α , whose expression is frequently lost, inhibits growth and predicts for better patient prognosis [64]. This antagonistic effect might be explained by the unique ability of HIF-2 α to co-operate with and potentiate c-Myc transcriptional activity, thus stimulating cellular proliferation in clear cell RCC; by contrast, c-Myc activity and RCC cell proliferation are inhibited by HIF-1 α [65].

Stem cells are a rare population of cells with the capacity for self-renewal, multi-lineage differentiation potential, and long term viability. Embryonic stem (ES) cells can be isolated from the inner cell mass of blastocysts, whereas adult stem cells are found in various tissues such as blood, bone marrow and adipose tissue [66]. Multipotent neoplastic cancer stem or progenitor cells (CSCs) have been identified in a variety of tumor types including GBM, leukemia and breast cancer, and share important properties with normal tissue stem cells, including self-renewal and differentiation capacity [67]. CSCs have been associated with increased radio- and/or chemoresistance, and promote tumor maintenance and recurrence [68]. Both normal and malignant stem cells reside in specialized niches where specific micro-environmental factors such as low oxygen play a crucial role in maintaining pluripotency and viability [69]. Indeed, tumor cells have been shown to undergo de-differentiation when grown under hypoxic conditions [70]. In this context, HIF-1 and HIF-2 both promote the hypoxia-induced undifferentiated phenotype by activating the Notch pathway, and inducing the transcription of other stem cell-specific factors [71]. In non-neoplastic ES cells, HIF-1 is the main driver for hypoxia-inducible transcription; by contrast, HIF-2 appears to be non-functional, possibly due to the presence of an endogenous, titrateable repressor [72]. In cancer, although HIF-1 α is required for the proliferation of both stem and non-stem GBM cells, HIF-2 α is preferentially expressed by the GBM stem cell population, and is specifically required for their survival [73]. Intriguingly, HIF-1 is also required for maintenance of the undifferentiated phenotype in GBM stem cells, but only under hypoxic conditions [74]. HIF-1/2 α can also have hypoxia-independent functions in CSC maintenance. For instance, HIF-2 α levels are elevated in a hypoxia-independent manner in GBM and neuroblastoma stem cells, and is required for maintenance of the undifferentiated phenotype [75]. Similarly, HIF-1 α is required, in a hypoxia-independent manner, for the maintenance of stem cells in hematologic malignancies; although in this case, it functions via down-regulation of pVHL [76]. Hence, although further studies are needed to clarify the involvement of HIF-1 and HIF-2 in stem cell survival under both normal and malignant settings, it appears that HIF-1 is the central regulator of both normal and neoplastic stem cells during either hypoxia or when pVHL is downregulated. Thus, in this context the function of HIF-2 appears to generally overlap that of HIF-1. However, HIF-2 plays a unique role in stem cell maintenance under physiological oxygen tension, independently of hypoxia, such as the peri-vascular stem cell niche, which might experience higher oxygen tension due to its proximity to the vasculature [77–79].

Mediators of the HIF switch

Recent studies have revealed the existence of HIF switches: mechanisms capable of directly changing HIF- α isoform dependency (Table 3). For example, the Hsp70/CHIP axis promotes the specific degradation of HIF-1 α during diabetes-associated hypoxia and hyperglycemia, resulting in diabetic complications that are associated with an impaired hypoxic response and cell death [16]. Another HIF switch is the histone deacetylase SIRT1, which deacetylates HIF-2 α . HIF-2 α is acetylated during hypoxia, and HIF-2 α deacetylation by SIRT1 increases HIF-2 activity [80, 81]. However, the evidence for SIRT1 in regulating HIF-1 is less clear as independent groups propose a neutral, activating, or repressing role for SIRT1 on HIF-1 α [80–82]. Another crucial HIF- α isoform-specific regulator, HAF, selectively binds and degrades HIF-1 α in an oxygen-independent manner, but promotes HIF-2 α transactivation and stability [6]. HAF itself is decreased following acute hypoxia but increases with prolonged hypoxic exposure, thus providing a mechanism for the switch from HIF-1 α towards HIF-2 α -dependent transcription after prolonged hypoxic exposure (Figure 3b).

Concluding remarks

Much progress has been made towards understanding the complex regulation of the HIFs in both physiological and pathophysiological processes. It is evident that hypoxia, as a complex micro-environmental stimulus that can vary both in intensity and duration, requires a sliding scale response that is largely provided by the intricate interplay between HIF-1 and HIF-2. Developmentally, HIF-1 plays a central role in early vascular and bone development; a role that is later assumed by HIF-2 as oxygen tension increase. Hence, despite some overlap between the HIFs, differences in their temporal regulation and downstream target genes explain why the HIFs are non-redundant, but instead play complementary roles. The HIF switch is also evident in solid tumors where, although HIF-1 drives the initial response to hypoxia, it is HIF-2 that plays the major role in driving the hypoxic response during chronic hypoxia exposure. Hence, there is a necessity for cells to switch from HIF-1 to HIF-2 dependency and vice versa in a tightly regulated and cellular context-specific manner. Even though HIF activation can form part of a developmental or recovery process (such as in wound healing and recovery from stroke/ischemia), HIF activation can also drive disease progression (such as in arthritis and cancer). Understanding the molecular mechanisms that determine HIF- α dependency in these contexts will facilitate the clinical application and targeting of HIF- α -driven maladies.

Acknowledgments

The authors would like to acknowledge NIH grants CA095060 and CA098920 to GP.

References

1. Carreau A, et al. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med.* 2011; 15:1239–1253. [PubMed: 21251211]
2. Pouyssegur J, et al. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature.* 2006; 441:437–443. [PubMed: 16724055]
3. Wang GL, et al. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A.* 1995; 92:5510–5514. [PubMed: 7539918]
4. Makino Y, et al. Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3 α locus. *J Biol Chem.* 2002; 277:32405–32408. [PubMed: 12119283]
5. Holmquist-Mengelbier L, et al. Recruitment of HIF-1 α and HIF-2 α to common target genes is differentially regulated in neuroblastoma: HIF-2 α promotes an aggressive phenotype. *Cancer Cell.* 2006; 10:413–423. [PubMed: 17097563]
6. Koh MY, et al. The Hypoxia-Associated Factor Switches Cells from HIF-1{ α }- to HIF-2{ α }-Dependent Signaling Promoting Stem Cell Characteristics, Aggressive Tumor Growth and Invasion. *Cancer Res.* 2011; 71:4015–4027. [PubMed: 21512133]
7. Jaakkola P, et al. Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science.* 2001; 292:468–472. [PubMed: 11292861]
8. Ohh M, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the β -domain of the von Hippel-Lindau protein. *Nat Cell Biol.* 2000; 2:423–427. [PubMed: 10878807]
9. Maxwell PH, et al. Activation of the HIF pathway in cancer. *Curr Opin Genet Dev.* 2001; 11:293–299. [PubMed: 11377966]
10. Mahon PC, et al. FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* 2001; 15:2675–2686. [PubMed: 11641274]
11. Koivunen P, et al. Catalytic Properties of the Asparaginyl Hydroxylase (FIH) in the Oxygen Sensing Pathway Are Distinct from Those of Its Prolyl 4-Hydroxylases. *Journal of Biological Chemistry.* 2004; 279:9899–9904. [PubMed: 14701857]

12. Liu YV, Semenza GL. RACK1 vs. HSP90: competition for HIF-1 alpha degradation vs. stabilization. *Cell Cycle*. 2007; 6:656–659. [PubMed: 17361105]
13. Ravi R, et al. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev*. 2000; 14:34–44. [PubMed: 10640274]
14. Koh MY, et al. Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1alpha, leading to its oxygen-independent degradation. *Mol Cell Biol*. 2008; 28:7081–7095. [PubMed: 18838541]
15. Luo W, et al. Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1alpha but Not HIF-2alpha. *J Biol Chem*. 2010; 285:3651–3663. [PubMed: 19940151]
16. Bento CF, et al. The chaperone-dependent ubiquitin ligase CHIP targets HIF-1alpha for degradation in the presence of methylglyoxal. *PLoS One*. 2010; 5:e15062. [PubMed: 21124777]
17. Tian H, et al. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev*. 1997; 11:72–82. [PubMed: 9000051]
18. Wiesener MS, et al. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *Faseb J*. 2003; 17:271–273. [PubMed: 12490539]
19. Raval RR, et al. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol*. 2005; 25:5675–5686. [PubMed: 15964822]
20. Mole DR, et al. Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem*. 2009; 284:16767–16775. [PubMed: 19386601]
21. Kapitsinou PP, et al. Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. *Blood*. 2010; 116:3039–3048. [PubMed: 20628150]
22. Warnecke C, et al. 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*. 2004; 18:1462–1464. [PubMed: 15240563]
23. Scortegagna M, et al. HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood*. 2005; 105:3133–3140. [PubMed: 15626745]
24. Keith B, et al. HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer*. 2012; 12:9–22. [PubMed: 22169972]
25. Fujiwara S, et al. Silencing hypoxia-inducible factor-1alpha inhibits cell migration and invasion under hypoxic environment in malignant gliomas. *Int J Oncol*. 2007; 30:793–802. [PubMed: 17332917]
26. Appelhoff RJ, et al. Differential Function of the Prolyl Hydroxylases PHD1, PHD2, and PHD3 in the Regulation of Hypoxia-inducible Factor. *Journal of Biological Chemistry*. 2004; 279:38458–38465. [PubMed: 15247232]
27. Uchida T, et al. Prolonged hypoxia differentially regulates hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression in lung epithelial cells: implication of natural antisense HIF-1alpha. *J Biol Chem*. 2004; 279:14871–14878. [PubMed: 14744852]
28. Sanchez M, et al. Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. *Nat Struct Mol Biol*. 2007; 14:420–426. [PubMed: 17417656]
29. Hu CJ, et al. The N-terminal transactivation domain confers target gene specificity of hypoxia-inducible factors HIF-1alpha and HIF-2alpha. *Mol Biol Cell*. 2007; 18:4528–4542. [PubMed: 17804822]
30. Pringle KG, et al. Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. *Hum Reprod Update*. 2010; 16:415–431. [PubMed: 19926662]
31. Flamme I, et al. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *J Cell Physiol*. 1997; 173:206–210. [PubMed: 9365523]
32. Augustin HG, et al. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. *Nat Rev Mol Cell Biol*. 2009; 10:165–177. [PubMed: 19234476]
33. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol*. 2007; 8:464–478. [PubMed: 17522591]

34. Fong GH, et al. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature*. 1995; 376:66–70. [PubMed: 7596436]
35. Oladipupo S, et al. VEGF is essential for hypoxia-inducible factor-mediated neovascularization but dispensable for endothelial sprouting. *Proc Natl Acad Sci U S A*. 2011; 108:13264–13269. [PubMed: 21784979]
36. Oda N, et al. ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin beta3. *J Cell Physiol*. 1999; 178:121–132. [PubMed: 10048576]
37. Aprelikova O, et al. Role of ETS transcription factors in the hypoxia-inducible factor-2 target gene selection. *Cancer Res*. 2006; 66:5641–5647. [PubMed: 16740701]
38. Takeda N, et al. Endothelial PAS domain protein 1 gene promotes angiogenesis through the transactivation of both vascular endothelial growth factor and its receptor, Flt-1. *Circ Res*. 2004; 95:146–153. [PubMed: 15192019]
39. Elvert G, et al. 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*. 2003; 278:7520–7530. [PubMed: 12464608]
40. Le Bras A, et al. HIF-2alpha specifically activates the VE-cadherin promoter independently of hypoxia and in synergy with Ets-1 through two essential ETS-binding sites. *Oncogene*. 2007; 26:7480–7489. [PubMed: 17563748]
41. Iyer NV, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev*. 1998; 12:149–162. [PubMed: 9436976]
42. Kotch LE, et al. Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol*. 1999; 209:254–267. [PubMed: 10328919]
43. Ryan HE, et al. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *Embo J*. 1998; 17:3005–3015. [PubMed: 9606183]
44. Yoon D, et al. Hypoxia-inducible Factor-1 Deficiency Results in Dysregulated Erythropoiesis Signaling and Iron Homeostasis in Mouse Development. *Journal of Biological Chemistry*. 2006; 281:25703–25711. [PubMed: 16787915]
45. Peng J, et al. The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. *Proc Natl Acad Sci U S A*. 2000; 97:8386–8391. [PubMed: 10880563]
46. Tian H, et al. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev*. 1998; 12:3320–3324. [PubMed: 9808618]
47. Compemolle V, et al. Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med*. 2002; 8:702–710. [PubMed: 12053176]
48. Scortegagna M, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in *Epas1*^{-/-} mice. *Nat Genet*. 2003; 35:331–340. [PubMed: 14608355]
49. Tang N, et al. Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. *Cancer Cell*. 2004; 6:485–495. [PubMed: 15542432]
50. Skuli N, et al. Endothelial deletion of hypoxia-inducible factor-2alpha (HIF-2alpha) alters vascular function and tumor angiogenesis. *Blood*. 2009; 114:469–477. [PubMed: 19439736]
51. Kronenberg HM. Developmental regulation of the growth plate. *Nature*. 2003; 423:332–336. [PubMed: 12748651]
52. Rankin EB, et al. A central role for hypoxic signaling in cartilage, bone, and hematopoiesis. *Curr Osteoporos Rep*. 2011; 9:46–52. [PubMed: 21360287]
53. Saito T, et al. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. *Nat Med*. 2010; 16:678–686. [PubMed: 20495570]
54. Duval E, et al. Hypoxia-inducible factor 1alpha inhibits the fibroblast-like markers type I and type III collagen during hypoxia-induced chondrocyte redifferentiation: hypoxia not only induces type II collagen and aggrecan, but it also inhibits type I and type III collagen in the hypoxia-inducible

- factor 1alpha-dependent redifferentiation of chondrocytes. *Arthritis Rheum.* 2009; 60:3038–3048. [PubMed: 19790048]
55. Bohensky J, et al. HIF-1 regulation of chondrocyte apoptosis: induction of the autophagic pathway. *Autophagy.* 2007; 3:207–214. [PubMed: 17224629]
 56. Bohensky J, et al. Regulation of autophagy in human and murine cartilage: hypoxia-inducible factor 2 suppresses chondrocyte autophagy. *Arthritis Rheum.* 2009; 60:1406–1415. [PubMed: 19404942]
 57. Yang S, et al. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med.* 2010
 58. Shomento SH, et al. Hypoxia-inducible factors 1alpha and 2alpha exert both distinct and overlapping functions in long bone development. *J Cell Biochem.* 2010; 109:196–204. [PubMed: 19899108]
 59. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev.* 2007; 26:225–239. [PubMed: 17440684]
 60. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer.* 2011; 11:393–410. [PubMed: 21606941]
 61. Zhong H, et al. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res.* 1999; 59:5830–5835. [PubMed: 10582706]
 62. Franovic A, et al. Human cancers converge at the HIF-2alpha oncogenic axis. *Proc Natl Acad Sci U S A.* 2009; 106:21306–21311. [PubMed: 19955413]
 63. Mandriota SJ, et al. HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell.* 2002; 1:459–468. [PubMed: 12124175]
 64. Shen C, et al. Genetic and Functional Studies Implicate HIF1 α as a 14q Kidney Cancer Suppressor Gene. *Cancer Discovery.* 2011
 65. Gordan JD, et al. HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. *Cancer Cell.* 2008; 14:435–446. [PubMed: 19061835]
 66. Wagers AJ, Weissman IL. Plasticity of Adult Stem Cells. *Cell.* 2004; 116:639–648. [PubMed: 15006347]
 67. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer.* 2008; 8:755–768. [PubMed: 18784658]
 68. Eyler CE, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. *J Clin Oncol.* 2008; 26:2839–2845. [PubMed: 18539962]
 69. Scadden DT. The stem-cell niche as an entity of action. *Nature.* 2006; 441:1075–1079. [PubMed: 16810242]
 70. Jögi A, et al. Hypoxia alters gene expression in human neuroblastoma cells toward an immature and neural crest-like phenotype. *Proceedings of the National Academy of Sciences.* 2002; 99:7021–7026.
 71. Mukherjee T, et al. Interaction Between Notch and Hif- α in Development and Survival of Drosophila Blood Cells. *Science.* 2011; 332:1210–1213. [PubMed: 21636775]
 72. Hu CJ, et al. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol.* 2003; 23:9361–9374. [PubMed: 14645546]
 73. Li Z, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell.* 2009; 15:501–513. [PubMed: 19477429]
 74. Soeda A, et al. Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1[alpha]. *Oncogene.* 2009; 28:3949–3959. [PubMed: 19718046]
 75. Pietras A, et al. The HIF-2alpha-driven pseudo-hypoxic phenotype in tumor aggressiveness, differentiation, and vascularization. *Curr Top Microbiol Immunol.* 2010; 345:1–20. [PubMed: 20517717]
 76. Wang Y, et al. Targeting HIF1 α Eliminates Cancer Stem Cells in Hematological Malignancies. *Cell Stem Cell.* 2011; 8:399–411. [PubMed: 21474104]
 77. Tavazoie M, et al. A Specialized Vascular Niche for Adult Neural Stem Cells. *Cell Stem Cell.* 2008; 3:279–288. [PubMed: 18786415]

78. Gilbertson RJ, Rich JN. Making a tumour's bed: glioblastoma stem cells and the vascular niche. *Nat Rev Cancer*. 2007; 7:733–736. [PubMed: 17882276]
79. Pietras A, et al. HIF-2alpha maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells. *Proc Natl Acad Sci U S A*. 2009; 106:16805–16810. [PubMed: 19805377]
80. Lim JH, et al. Sirtuin 1 Modulates Cellular Responses to Hypoxia by Deacetylating Hypoxia-Inducible Factor 1α. *Molecular Cell*. 2010; 38:864–878. [PubMed: 20620956]
81. Chen R, et al. Hypoxia Increases Sirtuin 1 Expression in a Hypoxia-inducible Factor-dependent Manner. *Journal of Biological Chemistry*. 2011; 286:13869–13878. [PubMed: 21345792]
82. Laemmle A, et al. Inhibition of SIRT1 Impairs the Accumulation and Transcriptional Activity of HIF-1alpha Protein under Hypoxic Conditions. *PLoS One*. 2012; 7:e33433. [PubMed: 22479397]

Box 1**Outstanding questions**

- Is there a role for the HIFs in non-hypoxic tissue in the adult?
- What is the extent of the non-transcriptional role of the HIFs, for example in protein-protein interactions?
- Why do HIF-1 and HIF-2 have distinct tumor suppressor and tumor-promoting roles only in clear cell renal carcinoma?
- Does HIF drive different responses in tumors compared to the surrounding stroma, and if so, what is the source of the disparity?
- Is it possible to selectively target HIF (instead of targeting upstream or downstream of HIF)?
- Would HIF- α isoform-specific inhibitors be more beneficial than pan-HIF- α inhibitors?

Figure 1.

The structural domains of hypoxia inducible factor (HIF)-1/2/3 α and their transcriptional binding partner, HIF-1 β /ARNT (aryl hydrocarbon nuclear translocator) that together, form the HIF-1, HIF-2 and HIF-3 transcriptional complexes, respectively. The basic helix-loop-helix (bHLH) and per-Arnt-SIM (PAS) domains are involved in DNA binding and heterodimerization; the oxygen -dependent degradation (ODD) domain is required for oxygen-dependent hydroxylation and degradation; and the N -terminal and C-terminal transactivation domains (TAD-N and TAD-C, respectively) are required for transcriptional activation. The binding domains of known modulators of HIF- α are depicted, along with the effects of these interactions on activity of the HIF transcriptional complex (red font indicates inhibitory interactions, green font indicates activating interactions). The von Hippel Lindau protein (pVHL) E3 ligase complex regulates the oxygen-dependent degradation of all three major HIF- α subunits. Factor inhibiting HIF-1 (FIH-1) hydroxylates HIF -2 α at a lower efficiency (broken oval) than HIF-1 α (unbroken oval). Receptor for activated protein kinase C 1 (RACK1) promotes the degradation of HIF-1 α when heat shock protein 90 (Hsp90) is inhibited, such as by Hsp90 inhibitors. The hypoxia-associated factor (HAF) selectively binds to HIF-1 α and HIF-2 α , mediating degradation and transactivation, respectively. Hsp70 promotes the binding of carboxyl terminus of Hsp70-interacting protein (CHIP) to HIF-1 α , resulting in HIF-1 α degradation. Sirtuin 1 (SIRT1) deacetylates HIF -2 α , resulting in inactivation.

Figure 2.

The transition from HIF-1- to HIF-2-dependent transcription is dependent on oxygen tension during vascular and bone development. **(a)** HIF-1 plays a dominant role during vasculogenesis (i), which occurs under intense hypoxia. Although both HIF-1 and HIF-2 play complementary roles during angiogenesis (ii), the final stages of vascular remodeling and stabilization (iii, iv), such as pericyte (pct) and smooth muscle cell (smc) recruitment, occur under less hypoxic conditions and are mainly HIF-2 driven. HIF target genes involved in the various stages of vascular development are indicated in *italics*. **(b)** During the initial stage of bone development, HIF-1 is required for mesenchymal cell condensation, chondrocyte proliferation, and synthesis of the cartilaginous extracellular matrix (ECM) (i). Next, proliferation continues to be promoted by HIF-1, while cells within the hypertrophic zone require HIF-2 to undergo hypertrophic differentiation (ii). This is followed by vascular invasion of the hypertrophic zone, which requires both HIF-1 and HIF-2 (iii). Finally, degradation of cartilage and its eventual replacement with bone is promoted by HIF-2 (iv). Major HIF target genes are indicated in *italics*.

Figure 3.

The switch from HIF-1- to HIF-2-dependent transcription during chronic hypoxia in solid tumors. **(a)** The diffusion limitation of oxygen results in oxygen gradients within solid tumors (i). Acute hypoxia caused by vessel occlusion or rapid tumor growth promotes induction of both HIF-1 α and -2 α (ii); however, HIF-1 is the major driver of the acute response. This causes activation of the HIF-1 transcriptional program, which promotes alleviation of hypoxia through angiogenesis and/or reperfusion, or (iii) cell death (broken circles), depending on the mutational landscape of the tumor cells. Alternatively, chronic hypoxia (iv) can increase HAF and HIF-2 α levels, mediating a switch to HIF-2-dependent transcription that promotes tumor adaptation, proliferation and progression. **(b)** The temporal regulation of HIF-1 α (blue line), HIF-2 α (green line) and HAF (red line) in response to prolonged hypoxic exposure. The window in which the HIF-1 α to HIF-2 α switch occurs is indicated by broken vertical lines.

Table 1

Physiological oxygen tensions in a selection of normal human tissue types [1].

Organ	Normal pO ₂ (mmHg)	%O ₂
Trachea	150	19.7
Alveoli	110	14.5
Arterial blood	100	13.2
Pulmonary arterial blood	40	5.3
Brain	35	4.4
Intestinal tissue	58	7.6
Liver	41	5.4
Kidney	72	9.5
Muscle	29	3.8
Bone marrow	49	6.4

Table 2

Frequently used definitions describing low oxygen.

Condition	pO ₂ (mmHg)	% O ₂
Normoxia (ambient)	159	21%
Physiological hypoxia	15–68	2% – 9%
Mild hypoxia	8–38	1% – 5%
Hypoxia	< 8	<1%
Anoxia	<0.08	< 0.1%

Table 3

Major post-translational modifiers of HIF-1 α and HIF-2 α . (ND: not determined).

Modulator	Function	Affect on HIF-1 α	Affect on HIF-2 α	Mechanism	Stimuli
PHD1-3	Prolyl hydroxylase	Inhibit	Inhibit	Degradation	Oxygen
pVHL	Part of E3 ligase complex	Inhibit	Inhibit	Degradation	Oxygen
Hsp90	Protein chaperone	Promote	Promote	Stabilization	Endogenous
RACK1	Hsp90 competitor	Inhibit	ND	Degradation	HSP90 inhibition
SIRT1	Histone deacetylase	Inhibit	Activate	Inhibits p300/CBP binding (HIF-1 α); Promotes transactivation (HIF-2 α)	Decreased NAD+
HAF	E3 ligase/coactivator	Inhibit	Activate	Degradation (HIF-1 α); Promotes transactivation (HIF-2 α)	Prolonged hypoxia
Hsp70/CHIP	Protein chaperone/E3 ligase complex	Inhibit	ND	Degradation	High glucose
Ets-1	Transcription factor	Unchanged	Activate	Binds DNA and promotes transactivation	ND