Published in final edited form as:

Cell Cycle. 2009 May 1; 8(9): 1359–1366.

HAF: the new player in oxygen-independent HIF-1 α degradation

Mei Yee Koh¹ and Garth Powis

Department of Experimental Therapeutics M.D. Anderson Cancer Center, Houston, Texas, 77030

Abstract

Adaptation to hypoxia is primarily mediated by the hypoxia-inducible transcription factor, HIF. The regulation of HIF activity by the oxygen-dependent degradation of the HIF-1 α and HIF-2 α subunits by the pVHL E3 ligase complex has been well characterized. We have recently described the hypoxia-associated factor, HAF, as an E3 ligase for HIF-1 α that does not degrade HIF-2 α . Here we summarize the mechanism of HAF-mediated HIF-1 α degradation and the importance of oxygen-independent HIF-1 α regulation in cancer. We also discuss the implications of the new HAF: HIF-1 α degradation pathway with respect to other novel mediators of oxygen-independent HIF- α degradation. Finally, we review the significance of HAF as an isoform-specific E3 ligase in light of new information on the non-overlapping functions of HIF-1 α and HIF-2 α in cancer.

Introduction

Hypoxia is a state of reduced oxygen availability that restricts the function of organs, tissues, or cells in the adult mammal¹. However, hypoxia plays an important and beneficial role in mammalian physiology; and is critical for proper embryogenesis. Many solid tumors also contain hypoxic regions, due to the inability of the local vasculature to supply sufficient oxygen to the rapidly growing tumor and because of the severe structural abnormality of tumor microvessels². Tumor hypoxia is of major clinical significance since it can promote both tumor progression, and resistance to radiation and chemotherapy³.

The cellular adaptation to hypoxia includes the induction of angiogenesis, a switch from aerobic metabolism to anaerobic glycolysis, and the expression of a variety of stress proteins regulating cell death or survival. The hypoxia-inducible factor (HIF) is recognized as the master regulator of the hypoxia response, activating the transcription of a large number of genes critical for the adaptation to hypoxia⁴. HIF also contributes to tumor growth and its increased expression has been correlated with poor patient prognosis⁵. The involvement of HIF in pathophysiological conditions such as ischemia and cancer and its value as a therapeutic target have generated considerable interest in the understanding of HIF regulation⁶.

The HIF transcription factor is a heterodimer of the oxygen-regulated HIF- α subunit, and the constitutively expressed HIF-1 β subunit (also known as the aryl hydrocarbon receptor nuclear translocator (ARNT)⁷. Under aerobic conditions, HIF- α is degraded by the von Hippel Lindau protein (pVHL) through the ubiquitin-proteasomal pathway. Under hypoxic conditions, HIF- α is stabilized, enters the nucleus and heterodimerizes with HIF-1 β and binds to a conserved DNA sequence known as the hypoxia responsive element (HRE) to transactivate a variety of hypoxia-responsive genes⁸. Recruitment of the coactivator p300/CBP to the C-terminal transactivation (CTAD) domain of HIF- α , is required for transcription of HIF downstream targets. Under normoxic conditions, the ability of HIF- α to activate transcription is further

¹Requests for reprints should be addressed to: Mei Yee Koh Ph.D. Department of Experimental Therapeutics M.D. Anderson Cancer Center 1515 Holcombe Blvd. Houston, Texas 77030 Email: E-mail: mykoh@mdanderson.org.

prevented by factor inhibiting HIF (FIH), which hydroxylates an asparagine residue within the C-TAD of HIF- α , hence disrupting its interaction with p300/CBP⁹.

To date, three HIF- α isoforms (HIF-1/2/3 α) have been described (Figure 1), of which HIF-1 α and HIF-2 α are the best characterized. HIF-1 α is expressed ubiquitously whereas HIF-2 α displays more tissue-specific expression¹⁰. Although HIF-1 α and HIF-2 α display 48% amino acid identity and have similar protein structure and mechanism of regulation, they regulate both common and unique target genes^{11, 12}, and may be differentially regulated depending on the duration and severity of hypoxia exposure¹³. The existence of distinct HIF-1 α /HIF-2 α downstream targets can be at least partly explained by their different affinities for specific regulators of HIF-1/2 α activity and stability such as the prolyl hydroxylases (described in greater detail below) and FIH, and differential recruitment of additional isoform-specific transcriptional co-activators or the repressors^{12, 14, 15}. *HIF-1 α ^{-/-}* mice exhibit midgestation lethality with severe developmental defects such as in the neural tubes and cardiovascular malformations^{16, 17}. *HIF-2 α ^{-/-}* mice manifest a variety of defects depending on the study, possibly due to the different genetic backgrounds of the mice studied, including embryonic lethality due to defective vascular remodeling during development¹⁸, embryonic lethality due to impaired catecholamine synthesis¹⁹, and death either midgestation or soon after birth from severe respiratory failure due to insufficient surfactant production²⁰. In cancer, while both HIF- α isoforms have been associated with poor patient prognosis, differences in their target genes has led to a proposed association of HIF-1 α with the regulation of glycolysis, and HIF-2 α with increased proliferation and tumor metastasis²¹⁻²³.

In addition to altering cellular energy metabolism and angiogenesis, hypoxia also influences the proliferation and differentiation of various stem/progenitor cell populations. An emerging theme relating to HIF and cancer has been the implication of HIF in the maintenance of cancer stem cells (or tumor initiating/progenitor cells)²⁴. Cancer stem cells are defined as a specific minority of tumor cells which are, like their normal counterparts, endowed with extensive potential for self-renewal and for generating the diverse tumor cell population^{25, 26}. Quiescent cancer stem cells are believed to be more resistant to chemotherapy and to be the main source of tumor recurrence²⁷. Furthermore, low stages of differentiation in certain tumor types that can be induced by hypoxia, are associated with poor outcome and disseminated disease^{28, 29}. In this respect, HIF-1 α has been shown to interact with and stabilize the intracellular domain (ICD) of Notch, a factor important for the maintenance of the stem/progenitor cell state, and to activate transcription of Notch downstream targets such as Hey-2 and Hes-1³⁰. The hypoxia-induced activation of the Notch pathway was also observed in A-498 renal carcinoma cells that exclusively express HIF-2 α ³¹, suggesting that HIF-2 α may play a similar role as HIF-1 α in Notch activation³⁰. On the other hand, HIF-2 α but not HIF-1 α , selectively induces the expression of OCT-4, an important transcription factor for the maintenance of the stem cell phenotype and embryonic pluripotency³². The targeted replacement of HIF-1 α by a HIF-2 α knock-in allele in murine embryonic stem cells generated teratomas with increased growth and an increased population of undifferentiated cells compared to their controls through a mechanism dependent upon the HIF-2 α -induced activation of OCT-4^{32, 33}.

Oxygen-dependent HIF- α degradation

Under aerobic conditions, HIF-1/2 α is hydroxylated by specific prolyl hydroxylases (PHD1, PHD2, PHD3 and, recently, PHD4) at conserved proline residues within its oxygen-dependent degradation (ODD) domain in a reaction requiring molecular oxygen, 2-oxoglutarate, and ascorbate^{34, 35}. While PHD2 is a primary hydroxylase for both HIF-1 α and HIF-2 α ³⁶, genetic studies suggest that PHD3 is mainly responsible for HIF-2 α hydroxylation³⁷. Hydroxylated HIF- α is recognized by pVHL, which forms the substrate recognition module of an E3 ubiquitin ligase complex comprising elongin C, elongin B, cullin-2, and ring-box 1 (Rbx1), which directs

HIF- α poly-ubiquitination and proteasomal degradation^{38, 39}. The central role of pVHL in HIF- α regulation is manifested in VHL disease where the inactivation of the *VHL* gene results in inappropriate overexpression of HIF-target genes including those involved in angiogenesis and invasion, and the development of highly vascularized tumors in the kidney, retina and central nervous system⁴⁰.

The oxygen-dependent degradation of HIF-1 α by pVHL can also be mediated by hypoxia-induced HIF-1 α small ubiquitin-like modifier (SUMO)ylation. In this regard, hypoxia induces the SUMOylation of HIF-1 α through an unknown mechanism, which allows the binding of the pVHL E3 ligase complex to HIF-1 α without requiring HIF-1 α prolyl hydroxylation, leading to HIF-1 α degradation⁴¹. The pVHL-mediated degradation of SUMOylated HIF-1 α in hypoxia is prevented by SENP1 (SUMO1/sentrin specific peptidase 1), a nuclear SUMO protease that deconjugates SUMOylated HIF-1 α , allowing it to escape pVHL-mediated degradation. Further work will be necessary to demonstrate whether HIF-2 α is subject to the same SUMOylation/deSUMOylation regulation as HIF-1 α .

Oxygen-independent HIF- α degradation

While pVHL has been well established as the key player in the regulation of the oxygen-dependent degradation of HIF-1 α , there has been increasing evidence of pVHL-independent pathways of HIF-1 α degradation⁴². Indeed, although primarily regulated by changes in cellular oxygen tension, it has now become clear that HIF-1 α may be also activated by a wide range of growth-promoting stimuli and oncogenic pathways such as insulin, insulin-like growth factor-1, epidermal growth factor (EGF) and mutant Ras, Src and/or PI-3K pathways⁴³⁻⁴⁶. This is particularly important in cancer where deregulation of growth factors and/or their cognate receptors can result in constitutive activation of HIF-1 α . The main mechanism implicated for HIF-1 α activation by the PI-3K/mTOR pathway is an increase in HIF-1 α protein translation, without a change in HIF-1 α degradation^{9, 47, 48}. The increase in HIF-1 α translation appears sufficient to shift the balance between synthesis and degradation towards an accumulation of HIF-1 α in normoxia. Additionally, oncogene activation such as by Ras mutation or by Src activation results in increased HIF-1 α activity through p42/p44 MAPK phosphorylation^{49, 50}.

Although HIF-1 α promotes the cellular adaptation to hypoxia, continuous HIF-1 α activation has, under certain settings, been reported to inhibit cell-cycle progression^{51, 52} and to promote apoptosis⁵³. It is thus likely that other mechanisms contribute to the maintenance of HIF activity at a level conducive for continued cell growth and survival. We propose that HIF activity requires tight regulation by both oxygen-dependent and oxygen-independent mechanisms to cope with diverse growth conditions and cellular stimuli.

Compared to the pVHL pathway, the regulation of these new oxygen-independent pathways may be less affected by oxygen availability but more dependent upon specific cellular conditions such as calcium or the presence of growth factors. pVHL- and oxygen-independent degradation of HIF-1 α has been observed in overexpression studies, such as by the overexpression of GSK3 or FOXO4; or in drug studies, such as by treatment with histone deacetylase (HDAC) inhibitors or heat shock protein 90 (Hsp90) inhibitors⁵⁴⁻⁵⁷. Additionally, HIF-1 α has been shown to be subject to transcriptional-dependent proteasomal degradation, independent of the PHDs and pVHL, which may limit the accumulation of HIF-1 α under severe hypoxia⁵⁸. Furthermore, constitutive, ubiquitin-independent degradation of HIF-1 α and HIF-2 α via the proteasomal pathway has also been proposed⁵⁹. We have recently identified a new oxygen-independent modulator of HIF-1 α degradation termed the Hypoxia-Associated Factor (HAF)⁶⁰. Here we will discuss the role of HAF as an important new mediator of HIF-1 α degradation and the potential ramifications of oxygen-independent HIF- α regulation and of modulation of the balance between HIF-1 α and HIF-2 α activity.

HAF mediates HIF-1 α -specific oxygen-independent degradation

HAF, also known as SART1₈₀₀ (squamous cell carcinoma antigen recognized by T-cells), was originally identified as a nuclear protein expressed in proliferating cells⁶¹. HAF is a component of the U4/U6•U5 tri-snRNP (small nuclear ribonucleoprotein) complex and is essential for the assembly of mature spliceosomes⁶². The murine homolog of SART1₈₀₀, HAF, was given its name due to its ability to bind to the promoter of the erythropoietin (*EPO*) gene and to contribute to its hypoxia inducibility⁶³. In our study, we showed that HAF promotes HIF-1 α degradation independently of cellular oxygen tension⁶⁴. HAF overexpression decreased HIF-1 α levels, whereas HAF knockdown increased HIF-1 α levels independently of pVHL or oxygen. Intriguingly, neither HAF knockdown nor overexpression caused any change in the levels of HIF-2 α . This suggests that HAF is a novel HIF-1 α isoform specific E3 ligase. Further characterization showed that HAF bound to HIF-1 α causing HIF-1 α ubiquitination and proteasomal degradation in an oxygen- and pVHL-independent manner. In this respect, we found that recombinant HAF ubiquitinated non-hydroxylated recombinant HIF-1 α in *in vitro* ubiquitination assays in an E2-dependent manner. These studies demonstrated that HAF is a bona fide E3 ligase and not a member of a larger multiprotein E3 ligase complex like pVHL. Hence, our study identified HAF as a novel E3 ligase for HIF-1 α and the mediator of a new mechanism of HIF-1 α degradation that is independent of pVHL and oxygen.

Consistent with HAF's ability to degrade HIF-1 α irrespective of cellular oxygen tension, we found that HAF knockdown using siRNA increased endogenous levels of HIF-1 α in normoxia, hypoxia and under EGF stimulation in a panel of cell lines. Furthermore, HAF siRNA increased the half life of HIF-1 α in pVHL-deficient RCC cells, whereas HAF overexpression decreased the half life of hypoxia-induced HIF-1 α in HT29 cells expressing wild-type pVHL. In contrast, knockdown of pVHL using siRNA increased the levels of HIF-1 α only under normoxic conditions but did not affect HIF-1 α levels induced by hypoxia or EGF. The effect of HAF under a variety of conditions is an important demonstration of a critical role for HAF in the regulation of HIF-1 α turnover/degradation beyond that of pVHL, and that it is functional under conditions in which pVHL is inactive or insufficient, such as during hypoxia or in cells with elevated HIF-1 α levels due to growth factor activation. Hence, HAF mediates an oxygen-independent HIF-1 α degradation pathway that is complementary to that of the oxygen-dependent pathway mediated by pVHL, providing an additional layer of control that allows the precise regulation of HIF-1 α levels under diverse conditions. Additionally, the unique ability of HAF to discriminate between HIF-1 α and HIF-2 α for degradation provides a novel mechanism for a switch from HIF-1 α to HIF-2 α signaling. This is significant in view of recent findings on the non-redundancy of HIF-1 α and HIF-2 α target genes and may facilitate tumor progression to a more aggressive, undifferentiated phenotype.

The role of HAF as a multifunctional protein

The N-terminus of HAF has previously been shown to have DNA binding activity and to induce the transcription of *EPO* and *VEGF*⁶³. This indicates that HAF may be a modular protein with DNA binding activity within its N-terminus and E3 ligase activity within its C-terminus, in addition to its previously described role in pre-mRNA splicing. The dual-functionality of DNA binding and E3 ligase activity is not unique to HAF but has also been demonstrated by other key regulatory proteins such as BRCA1, which can bind DNA independently of its E3 ubiquitin ligase domain⁶⁵, and p300, which acts as both a transcriptional coactivator and ubiquitin ligase for p53⁶⁶. The multi-functionality of these proteins may be indicative of their significance in specific cellular processes where mutation or loss may be highly detrimental and potentially oncogenic. Indeed, HAF has been identified in a genomic RNAi screen as a gene essential for cell division, the silencing of which results in mitotic arrest⁶⁷. However, the dual functionality

of HAF appears contradictory in that it inhibits HIF-1 α signaling by degradation, and yet promotes the transcription of some of its key downstream targets. In this regard, HAF may provide a measure of selectivity by promoting the activation of only a subset of HIF downstream targets while degrading HIF-1 α . Additionally, HAF transcriptional activity may contribute to HIF-2 α mediated transcription. Hence, the dual-functionality of HAF may provide avenues for regulation by varied inputs or opportunities for therapeutic modulation.

Our mapping studies have shown that the C-terminus of HAF is responsible for both the HAF E3 ligase activity and its binding to HIF-1 α . Specifically, we found that the C-terminus of HAF (amino acids 654-800) bound to HIF-1 α within amino acids 296-400, a region flanking the HIF-1 α ODD domain. Intriguingly, HAF 654-800 did not contain a proto-typical E3 ligase motif (HECT, RING or U-Box), whereas mutational analyses showed that HAF did not require catalytic cysteines for its ubiquitin ligase activity. This suggests that HAF is one of a growing list of atypical E3 ligases^{68, 69}. Moreover, we observed that overexpressed HAF is mono-ubiquitinated which, unlike poly-ubiquitination, does not serve as a proteasome targeting signal. Mono-ubiquitination has also been reported in tumor suppressors such as p53, Forkhead Box (FOXO) and phosphatase and tensin homolog (PTEN) and can be induced by certain cellular stimuli (such as oxidative stress in the case of FOXO), and can affect substrate protein activity, stability, cellular localization or interaction with other proteins⁷⁰. Additionally, HAF is also a target for SUMOylation⁷¹. Further work will reveal whether the post-translational modification of HAF by mono-ubiquitination and/or SUMOylation by certain physiological stimuli plays a role in regulating HAF-mediated HIF-1 α degradation.

The role of HAF in cancer

HAF has been detected in all cell lines (both normal and tumor-derived) and in proliferating tissues but is undetectable in normal, non-proliferating tissue⁶¹. Additionally, HAF has been detected at higher levels in some tumors compared with the surrounding, non-proliferating normal tissue^{72, 73}. As tumors are almost invariably highly proliferative, it is difficult to determine whether HAF expression plays a significant role in tumorigenesis, or is simply a reflection of the increased growth rate of tumor cells. In our study, we found that HAF overexpression did not affect cell proliferation *in vitro*. However, HAF overexpression was associated with marked inhibition of xenograft tumor growth *in vivo*, decreased HIF-1 α levels and transactivation, and reduced tumor angiogenesis⁶⁴. This is consistent with a HIF-1 α dependent effect on proliferation as much of the effect of HIF-1 α on tumor growth can be attributed to VEGF inhibition, which is not required for cell growth *in vitro*. This suggests that under certain conditions, HAF, by degrading HIF-1 α , may act as a tumor suppressor, but this appears to conflict with the observations of increased HAF expression in cancer. Intriguingly, although HIF-1 α has been associated with aggressive tumors and poor patient prognosis, high levels of HIF-1 α in some cases such as in renal cell carcinoma (RCC), has been related to increased patient survival⁷⁴. Additionally, overexpression of the HIF-2 α subunit has been shown to enhance the growth of pVHL-deficient RCC xenografts, whereas overexpression of the HIF-1 α subunit has been shown to retard xenograft growth⁷⁵. This suggests that at least in some settings, HIF-1 α and HIF-2 α have opposing roles in tumorigenesis whereby HIF-1 α inhibits, whereas HIF-2 α promotes tumor growth. In support of this notion, it has been reported that HIF-1 α expression gradually decreases, whereas HIF-2 α expression increases as RCCs develop⁷⁶. This trend has also been observed in colorectal cancer where tumor aggressiveness has been significantly correlated with overexpression of HIF-2 α but not with HIF-1 α ⁷⁷, and in neuroblastoma where high levels of HIF-2 α have been proposed to promote an aggressive tumor phenotype¹³. Nevertheless, although the precise pro/anti-tumorigenic properties of HIF-1 α in comparison to HIF-2 α are still under debate⁷⁸, they do highlight the complex interaction between HIF-1 α and HIF-2 α and their different roles in tumorigenesis. Hence, we propose that HAF promotes tumor progression in cells expressing both HIF-1 α and HIF-2 α .

isoforms by degrading HIF-1 α whilst allowing HIF-2 α to activate downstream transcription (Figure 2). This may facilitate the propensity of HIF-2 α to activate specific pro-tumorigenic factors such as OCT-4 and c-Myc that are not activated, or even antagonized (in the case of c-Myc) by HIF-1 α ^{22, 23}. Similarly, the selection of the HIF-2 α in favor of HIF-1 α by HAF may allow the hypoxic adaptation of tumor cells without the activation of the HIF-1 α specific downstream targets such as BNIP3 and BNIP3L that may promote apoptosis. On the other hand, although HIF-2 α has received increased attention as a potent pro-tumorigenic factor, the case for HIF-1 α as an indicator of morbidity has also been well established⁷⁹⁻⁸¹. Indeed, the pro-apoptotic activity of HIF-1 α through BNIP3 and BNIP3L may only be manifest under conditions of severe hypoxia, necrosis and/or acidosis and these may be counteracted by a number of other anti-apoptotic factors induced by HIF-1 α ⁸². Hence, the differential outcomes of HIF-1 α versus HIF-2 α activation in tumors are certainly not clear cut. Consequently, in addition to promoting tumor growth in cells expressing both HIF- α isoforms by facilitating HIF-2 α signaling, we propose that HAF may play more of a tumor suppressor role in tumors in which HIF-1 α is the driving factor.

Other oxygen-independent HIF- α regulators

In addition to HAF, other mediators of oxygen-independent HIF- α degradation have been reported. For the most part, although oxygen-dependent HIF-1 α degradation has been observed, investigators have been unable to identify the E3 ligase or precise mechanism involved^{55, 56, 58}. Additionally, the situations under which the constitutive, proteasomal dependent, ubiquitin-independent degradation of HIF-1 α occurs remain unclear^{59, 83}. However, two new mediators of HIF- α degradation — RACK1 and Int6 have been characterized in greater detail and are summarized below.

RACK1

HIF-1 α degradation mediated by Hsp90 inhibitors has been attributed to receptor of activated protein kinase C (RACK1)⁸⁴. RACK1 competes with Hsp90 for binding to the PAS-A domain of HIF-1 α . Consequently, inhibition of Hsp90 by Hsp90 inhibitors such as 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), promote the oxygen-independent degradation of HIF-1 α through increased binding of RACK1⁸⁴. RACK1 binds HIF-1 α and recruits elongin C and other components of the E3 ligase complex to HIF-1 α , in a manner mechanistically similar to pVHL, leading to HIF-1 α ubiquitination and proteasomal degradation. Intriguingly, a RACK1-dependent mechanism has also been implicated in HIF-1 α degradation mediated by HDAC inhibitors as treatment with trichostatin A increases Hsp90 acetylation, which destabilizes the HIF-1 α -Hsp90 interaction and promotes HIF-1 α degradation⁸⁵. However, the involvement of RACK1 in HDAC inhibitor mediated HIF-1 α degradation remains to be demonstrated.

RACK1-mediated HIF-1 α degradation can be inhibited by calcium through the activity of calcineurin, a calcium/calmodulin dependent and serine/threonine-specific protein phosphatase⁸⁶. Hence, increasing intracellular calcium concentration by treatment with ionomycin protects HIF-1 α from RACK1-mediated degradation. Paradoxically, the chelation of intracellular calcium in normoxia may cause the accumulation of HIF-1 α protein through inhibition of HIF PHDs, hence preventing pVHL-induced HIF-1 α degradation⁸⁷. Thus, modulation of intracellular calcium concentration can affect the stability of HIF-1 α protein through both the pVHL and RACK1 pathways and the final outcome may well be context dependent. Certainly the fact that both pVHL and RACK require the E3 ligase complex (Elongin B/C, Cul 2, Rbx1) for HIF-1 α ubiquitination and subsequent degradation lends the possibility of co-regulation of both pVHL and RACK pathways by fluctuations in the availability or activities of the various E3 ligase components. In contrast, the HAF C-terminus displays E3 ligase activity without requiring any ancillary factors and so degrades HIF-1 α even

in the absence of Elongin B/C and hence may be regulated in a manner entirely independently of the pVHL pathway⁶⁴.

Int6

The murine mammary tumor integration site 6 gene (*Int6*), also known as EIF3E, has been described as a HIF-2 α -specific E3 ligase⁸⁸. The *Int6* gene was first identified from a screen in which the mouse mammary tumor virus (MMTV) was employed as an insertional mutagen to identify genes whose functions are critical for breast tumor formation⁸⁹. The MMTV insertion into *Int6* generates a C-terminal truncated protein (Int6 Δ C) that is a dominant negative, transforms cells, which, when injected into nude mice, induces tumor formation⁹⁰. Int6 interacts specifically with HIF-2 α (but not with HIF-1 α or HIF-3 α) and mediates the proteasomal dependent degradation of HIF-2 α in an oxygen- and pVHL-independent manner⁸⁸. Overexpression of Int6 Δ C induces HIF-2 α expression even during normoxia. While the mechanism of Int6-induced HIF-2 α degradation remains unclear, overexpression of Int6 induces the degradation of endogenous and overexpressed HIF-2 α , whereas Int6 knockdown increased the expression of endogenous HIF-2 α in even under normoxia. In this regard, although Int6 has no clear homology with the ubiquitin ligase family members, it has been known to associate with the 26S proteasome⁹¹, and it has thus been suggested that this association between Int6, HIF-2 α , the proteasome, and potentially an unknown E3 ligase, promotes HIF-2 α degradation⁸⁸.

The ability of Int6 to selectively degrade HIF-2 α resembles the HIF-1 α -selective activity of HAF. These isoform-specific mediators of HIF- α degradation may provide a mechanism for the selective recruitment of either HIF-1 α or HIF-2 α to HIF-1 β to form the HIF heterodimer in order to fulfill precise cellular requirements.

Summary

Since the identification of the HIF transcription factor in 1992, the understanding of the regulation of the hypoxic response by HIF has dramatically increased. From the identification and characterization of HIF-1 α /HIF2 α /HIF-3 α and HIF-1 β subunits, to the elucidation of pVHL-mediated oxygen dependent regulation of HIF, and the involvement of HIF in normal development and in cancer morbidity, the field of HIF regulation has come a long way. Our recent identification of HAF, a ubiquitin ligase specific for HIF-1 α that promotes its oxygen-independent degradation is yet another piece to fit into the complex HIF puzzle. HAF and other recent oxygen-independent modulators of HIF- α degradation highlight an until recently, unexpected role for HIF in non-hypoxic gene regulation and the need for HIF- α isoform-specific degradation. However, the work with HAF raises further questions as to how HAF activity is regulated and in what context it is beneficial to the cell to degrade HIF-1 α in favor of HIF-2 α . Certainly, it raises the question of the role of HAF in cancer, whether HAF is present due to the high proliferative activity of cancer cells or whether it plays a more active role in tumorigenesis/cancer progression. Further work should provide new insights into the HAF: HIF connection that may be utilized for therapeutic benefit.

Aknowledgements

Supported by NIH grants CA 098920, CA 109552, CA 017094

References

1. Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 2001;93:266–76. [PubMed: 11181773]
2. Bilton R, Trotter E, Pouyssegur J, Brahimi-Horn MC. ARDent about acetylation and deacetylation in hypoxia signalling. *Trends in Cell Biology* 2006;16:616–621. [PubMed: 17070052]

3. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 2007;26:225–39. [PubMed: 17440684]
4. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 1992;12:5447–54. [PubMed: 1448077]
5. Semenza GL. Intratumoral hypoxia, radiation resistance, and HIF-1. *Cancer Cell* 2004;5:405–6. [PubMed: 15144945]
6. Welsh SJ, Koh MY, Powis G. The hypoxic inducible stress response as a target for cancer drug discovery. *Semin Oncol* 2006;33:486–97. [PubMed: 16890803]
7. Wang GL, Jiang BH, Rue EA, Semenza GL. 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;92:5510–4. [PubMed: 7539918]
8. Maxwell PH, Pugh CW, Ratcliffe PJ. Activation of the HIF pathway in cancer. *Curr Opin Genet Dev* 2001;11:293–9. [PubMed: 11377966]
9. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 2001;21:3995–4004. [PubMed: 11359907]
10. 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, Eckardt KU. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *Faseb J* 2003;17:271–3. [PubMed: 12490539]
11. Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol* 2003;23:9361–74. [PubMed: 14645546]
12. Hu CJ, Iyer S, Sataur A, Covelto KL, Chodosh LA, Simon MC. Differential regulation of the transcriptional activities of hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha in stem cells. *Mol Cell Biol* 2006;26:3514–26. [PubMed: 16611993]
13. Holmquist-Mengelbier L, Fredlund E, Lofstedt T, Noguera R, Navarro S, Nilsson H, Pietras A, Vallon-Christersson J, Borg A, Gradin K, Poellinger L, Pahlman S. Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. *Cancer Cell* 2006;10:413–23. [PubMed: 17097563]
14. Bracken CP, Fedele AO, Linke S, Balrak W, Lisy K, Whitelaw ML, Peet DJ. Cell-specific regulation of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha stabilization and transactivation in a graded oxygen environment. *J Biol Chem* 2006;281:22575–85. [PubMed: 16760477]
15. Hu CJ, Sataur A, Wang L, Chen H, Simon MC. The N-terminal transactivation domain confers target gene specificity of hypoxia-inducible factors HIF-1alpha and HIF-2alpha. *Mol Biol Cell* 2007;18:4528–42. [PubMed: 17804822]
16. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 1998;12:149–62. [PubMed: 9436976]
17. Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *Embo J* 1998;17:3005–15. [PubMed: 9606183]
18. Peng J, Zhang L, Drysdale L, Fong GH. The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. *Proc Natl Acad Sci U S A* 2000;97:8386–91. [PubMed: 10880563]
19. Tian H, Hammer RE, Matsumoto AM, Russell DW, McKnight SL. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev* 1998;12:3320–4. [PubMed: 9808618]
20. Compennolle V, Brusselmans K, Acker T, Hoet P, Tjwa M, Beck H, Plaisance S, Dor Y, Keshet E, Lupu F, Nemery B, Dewerchin M, Van Veldhoven P, Plate K, Moons L, Collen D, Carmeliet P. 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;8:702–10. [PubMed: 12053176]

21. Gordan JD, Simon MC. Hypoxia-inducible factors: central regulators of the tumor phenotype. *Curr Opin Genet Dev* 2007;17:71–7. [PubMed: 17208433]
22. Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC. HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. *Cancer Cell* 2007;11:335–47. [PubMed: 17418410]
23. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL. HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. *Cancer Cell* 2008;14:435–46. [PubMed: 19061835]
24. Barnhart BC, Simon MC. Metastasis and stem cell pathways. *Cancer Metastasis Rev* 2007;26:261–71. [PubMed: 17647111]
25. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008;8:755–68. [PubMed: 18784658]
26. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. *Cell* 2006;124:1111–5. [PubMed: 16564000]
27. Eyler CE, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. *J Clin Oncol* 2008;26:2839–45. [PubMed: 18539962]
28. Axelson H, Fredlund E, Ovenberger M, Landberg G, Pahlman S. Hypoxia-induced dedifferentiation of tumor cells—a mechanism behind heterogeneity and aggressiveness of solid tumors. *Semin Cell Dev Biol* 2005;16:554–63. [PubMed: 16144692]
29. Jogi A, Ora I, Nilsson H, Lindeheim A, Makino Y, Poellinger L, Axelson H, Pahlman S. Hypoxia alters gene expression in human neuroblastoma cells toward an immature and neural crest-like phenotype. *Proc Natl Acad Sci U S A* 2002;99:7021–6. [PubMed: 12011461]
30. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U, Bondesson M. Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell* 2005;9:617–28. [PubMed: 16256737]
31. Lou F, Chen X, Jalink M, Zhu Q, Ge N, Zhao S, Fang X, Fan Y, Bjorkholm M, Liu Z, Xu D. The opposing effect of hypoxia-inducible factor-2alpha on expression of telomerase reverse transcriptase. *Mol Cancer Res* 2007;5:793–800. [PubMed: 17699105]
32. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith B. HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev* 2006;20:557–70. [PubMed: 16510872]
33. Covello KL, Simon MC, Keith B. Targeted replacement of hypoxia-inducible factor-1alpha by a hypoxia-inducible factor-2alpha knock-in allele promotes tumor growth. *Cancer Res* 2005;65:2277–86. [PubMed: 15781641]
34. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* 2001;292:468–72. [PubMed: 11292861]
35. Koivunen P, Tiainen P, Hyvarinen J, Williams KE, Sormunen R, Klaus SJ, Kivirikko KI, Myllyharju J. An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. *J Biol Chem* 2007;282:30544–52. [PubMed: 17726031]
36. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolylhydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *Embo J* 2003;22:4082–90. [PubMed: 12912907]
37. Bishop T, Gallagher D, Pascual A, Lygate CA, de Bono JP, Nicholls LG, Ortega-Saenz P, Oster H, Wijeyekoon B, Sutherland AI, Grosfeld A, Aragones J, Schneider M, van Geyte K, Teixeira D, Diez-Juan A, Lopez-Barneo J, Channon KM, Maxwell PH, Pugh CW, Davies AM, Carmeliet P, Ratcliffe PJ. Abnormal sympathoadrenal development and systemic hypotension in PHD3^{-/-} mice. *Mol Cell Biol* 2008;28:3386–400. [PubMed: 18332118]
38. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999;399:271–5. [PubMed: 10353251]

39. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* 2000;2:423–7. [PubMed: 10878807]
40. Kaelin WG. Von hippel-lindau disease. *Annu Rev Pathol* 2007;2:145–73. [PubMed: 18039096]
41. Cheng J, Kang X, Zhang S, Yeh ET. SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. *Cell* 2007;131:584–95. [PubMed: 17981124]
42. Koh MY, Spivak-Kroizman TR, Powis G. HIF-1 regulation: not so easy come, easy go. *Trends Biochem Sci* 2008;33:526–34. [PubMed: 18809331]
43. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG Jr. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* 2004;18:2893–904. [PubMed: 15545625]
44. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol* 2002;22:7004–14. [PubMed: 12242281]
45. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D, Giaccia AJ. Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* 2000;14:391–6. [PubMed: 10691731]
46. Gray MJ, Zhang J, Ellis LM, Semenza GL, Evans DB, Watowich SS, Gallick GE. HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. *Oncogene* 2005;24:3110–20. [PubMed: 15735682]
47. Zhou J, Brune B. Cytokines and hormones in the regulation of hypoxia inducible factor-1alpha (HIF-1alpha). *Cardiovasc Hematol Agents Med Chem* 2006;4:189–97. [PubMed: 16842205]
48. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 2000;60:1541–5. [PubMed: 10749120]
49. Berra E, Milanini J, Richard DE, Le Gall M, Vinals F, Gothie E, Roux D, Pages G, Pouyssegur J. Signaling angiogenesis via p42/p44 MAP kinase and hypoxia. *Biochem Pharmacol* 2000;60:1171–8. [PubMed: 11007955]
50. Richard DE, Berra E, Gothie E, Roux D, Pouyssegur J. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. *J Biol Chem* 1999;274:32631–7. [PubMed: 10551817]
51. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. *Embo J* 2004;23:1949–56. [PubMed: 15071503]
52. Mack FA, Patel JH, Biju MP, Haase VH, Simon MC. Decreased growth of Vhl-/- fibrosarcomas is associated with elevated levels of cyclin kinase inhibitors p21 and p27. *Mol Cell Biol* 2005;25:4565–78. [PubMed: 15899860]
53. 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, Keshet E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 1998;394:485–90. [PubMed: 9697772]
54. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem* 2002;277:29936–44. [PubMed: 12052835]
55. Flugel D, Gorlach A, Michiels C, Kietzmann T. Glycogen synthase kinase-3 phosphorylates hypoxia-inducible factor-1 alpha and mediates its destabilization in a VHL-independent manner. *Mol Cell Biol*. 2007
56. Tang TT, Lasky LA. The forkhead transcription factor FOXO4 induces the down-regulation of hypoxia-inducible factor 1 alpha by a von Hippel-Lindau protein-independent mechanism. *J Biol Chem* 2003;278:30125–35. [PubMed: 12761217]

57. Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, Atadja P, Pili R. Class II Histone Deacetylases Are Associated with VHL-Independent Regulation of Hypoxia-Inducible Factor 1 {alpha}. *Cancer Res* 2006;66:8814–21. [PubMed: 16951198]
58. Demidenko ZN, Rapisarda A, Garayoa M, Giannakakou P, Melillo G, Blagosklonny MV. Accumulation of hypoxia-inducible factor-1alpha is limited by transcription-dependent depletion. *Oncogene* 2005;24:4829–38. [PubMed: 15897903]
59. Kong X, Alvarez-Castelao B, Lin Z, Castano JG, Caro J. Constitutive/hypoxic degradation of HIF-alpha proteins by the proteasome is independent of von Hippel Lindau protein ubiquitylation and the transactivation activity of the protein. *J Biol Chem* 2007;282:15498–505. [PubMed: 17403672]
60. Koh, MY.; Darnay, BG.; Powis, G. HAF, a novel E3-ubiquitin ligase binds and ubiquitinates HIF-1 α leading to its pVHL-and oxygen-independent degradation. 2008. Under review
61. Shichijo S, Nakao M, Imai Y, Takasu H, Kawamoto M, Niiya F, Yang D, Toh Y, Yamana H, Itoh K. A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. *J Exp Med* 1998;187:277–88. [PubMed: 9449708]
62. Makarova OV, Makarov EM, Luhrmann R. The 65 and 110 kDa SR-related proteins of the U4/U6.U5 tri-snRNP are essential for the assembly of mature spliceosomes. *EMBO J* 2001;20:2553–63. [PubMed: 11350945]
63. Gupta M, Mungai PT, Goldwasser E. A new transacting factor that modulates hypoxia-induced expression of the erythropoietin gene. *Blood* 2000;96:491–7. [PubMed: 10887110]
64. Koh MY, Darnay BG, Powis G. 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;28:7081–95. [PubMed: 18838541]
65. Paull TT, Cortez D, Bowers B, Elledge SJ, Gellert M. Direct DNA binding by Brca1. *Proc Natl Acad Sci U S A* 2001;98:6086–91. [PubMed: 11353843]
66. Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. *Science* 2003;300:342–4. [PubMed: 12690203]
67. Kittler R, Putz G, Pelletier L, Poser I, Heninger AK, Drechsel D, Fischer S, Konstantinova I, Habermann B, Grabner H, Yaspo ML, Himmelbauer H, Korn B, Neugebauer K, Pisabarro MT, Buchholz F. An endoribonuclease-prepared siRNA screen in human cells identifies genes essential for cell division. *Nature* 2004;432:1036–40. [PubMed: 15616564]
68. Le Cam L, Linares LK, Paul C, Julien E, Lacroix M, Hatchi E, Triboulet R, Bossis G, Shmueli A, Rodriguez MS, Coux O, Sardet C. E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation. *Cell* 2006;127:775–88. [PubMed: 17110336]
69. Linares LK, Kiernan R, Triboulet R, Chable-Bessia C, Latreille D, Cuvier O, Lacroix M, Le Cam L, Coux O, Benkirane M. Intrinsic ubiquitination activity of PCAF controls the stability of the oncoprotein Hdm2. *Nat Cell Biol* 2007;9:331–8. [PubMed: 17293853]
70. Salmena L, Pandolfi PP. Changing venues for tumour suppression: balancing destruction and localization by monoubiquitylation. *Nat Rev Cancer* 2007;7:409–13. [PubMed: 17508027]
71. Vertegaal AC, Ogg SC, Jaffray E, Rodriguez MS, Hay RT, Andersen JS, Mann M, Lamond AI. A proteomic study of SUMO-2 target proteins. *J Biol Chem* 2004;279:33791–8. [PubMed: 15175327]
72. Sasatomi T, Yamana H, Shichijo S, Tanaka S, Okamura T, Ogata Y, Itoh K, Shirouzu K. Expression of the SART1 tumor-rejection antigens in colorectal cancers. *Dis Colon Rectum* 2000;43:1754–8. [PubMed: 11156463]
73. Imaizumi T, Kuramoto T, Matsunaga K, Shichijo S, Yutani S, Shigemori M, Oizumi K, Itoh K. Expression of the tumor-rejection antigen SART1 in brain tumors. *Int J Cancer* 1999;83:760–4. [PubMed: 10597192]
74. Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Vasko J, Ljungberg B. The expression of hypoxia-inducible factor 1alpha is a favorable independent prognostic factor in renal cell carcinoma. *Clin Cancer Res* 2005;11:1129–35. [PubMed: 15709180]
75. Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 2005;25:5675–86. [PubMed: 15964822]

76. Mandriota SJ, Turner KJ, Davies DR, Murray PG, Morgan NV, Sowter HM, Wykoff CC, Maher ER, Harris AL, Ratcliffe PJ, Maxwell PH. HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* 2002;1:459–68. [PubMed: 12124175]
77. Yoshimura H, Dhar DK, Kohno H, Kubota H, Fujii T, Ueda S, Kinugasa S, Tachibana M, Nagasue N. Prognostic impact of hypoxia-inducible factors 1 α and 2 α in colorectal cancer patients: correlation with tumor angiogenesis and cyclooxygenase-2 expression. *Clin Cancer Res* 2004;10:8554–60. [PubMed: 15623639]
78. Imamura T, Kikuchi H, Herraiz MT, Park DY, Mizukami Y, Mino-Kenduson M, Lynch MP, Rueda BR, Benita Y, Xavier RJ, Chung DC. HIF-1 α and HIF-2 α have divergent roles in colon cancer. *Int J Cancer* 2009;124:763–71. [PubMed: 19030186]
79. Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, Semenza GL. Expression of hypoxia-inducible factor-1 α : a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Res* 2001;61:2911–6. [PubMed: 11306467]
80. Birner P, Schindl M, Obermair A, Plank C, Breitenacker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1 α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. *Cancer Res* 2000;60:4693–6. [PubMed: 10987269]
81. Birner P, Schindl M, Obermair A, Breitenacker G, Oberhuber G. Expression of hypoxia-inducible factor 1 α in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. *Clin Cancer Res* 2001;7:1661–8. [PubMed: 11410504]
82. Mellor HR, Harris AL. The role of the hypoxia-inducible BH3-only proteins BNIP3 and BNIP3L in cancer. *Cancer Metastasis Rev* 2007;26:553–66. [PubMed: 17805942]
83. Jariel-Encontre I, Bossis G, Piechaczyk M. Ubiquitin-independent degradation of proteins by the proteasome. *Biochim Biophys Acta* 2008;1786:153–77. [PubMed: 18558098]
84. Liu YV, Baek JH, Zhang H, Diez R, Cole RN, Semenza GL. RACK1 competes with HSP90 for binding to HIF-1 α and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1 α . *Mol Cell* 2007;25:207–17. [PubMed: 17244529]
85. Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J. Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1 α . *Mol Cell Biol* 2006;26:2019–28. [PubMed: 16507982]
86. Liu YV, Hubbi ME, Pan F, McDonald KR, Mansharamani M, Cole RN, Liu JO, Semenza GL. Calcineurin promotes hypoxia-inducible factor 1 α expression by dephosphorylating RACK1 and blocking RACK1 dimerization. *J Biol Chem* 2007;282:37064–73. [PubMed: 17965024]
87. Berchner-Pfannschmidt U, Petrat F, Doege K, Trinidad B, Freitag P, Metzen E, de Groot H, Fandrey J. Chelation of cellular calcium modulates hypoxia-inducible gene expression through activation of hypoxia-inducible factor-1 α . *J Biol Chem* 2004;279:44976–86. [PubMed: 15322093]
88. Chen L, Uchida K, Endler A, Shibasaki F. Mammalian tumor suppressor Int6 specifically targets HIF-2 α to degradation by hypoxia- and pVHL-independent regulation. *J Biol Chem*. 2007
89. Marchetti A, Buttitta F, Miyazaki S, Gallahan D, Smith GH, Callahan R. Int-6, a highly conserved, widely expressed gene, is mutated by mouse mammary tumor virus in mammary preneoplasia. *J Virol* 1995;69:1932–8. [PubMed: 7853537]
90. Mayeur GL, Hershey JW. Malignant transformation by the eukaryotic translation initiation factor 3 subunit p48 (eIF3e). *FEBS Lett* 2002;514:49–54. [PubMed: 11904180]
91. Alves, K Hoareau; Bochar, V.; Rety, S.; Jalinot, P. Association of the mammalian proto-oncoprotein Int-6 with the three protein complexes eIF3, COP9 signalosome and 26S proteasome. *FEBS Lett* 2002;527:15–21. [PubMed: 12220626]

Figure 1. Domain structures of HIF-1α, HIF-2α, HIF-3α and HIF-1β/ARNT

The HIF-α and HIF-1β/ARNT subunits are basic helix-loop-helix/Per-ARNT-Sim homology (bHLH/PAS) transcription factors. HIF-1α and HIF-2α contain two transactivation (TAD) domains, one at the amino terminal and the other at the carboxy terminal. Six splice versions of HIF-3α have been identified (only two are depicted here) and those that have been mapped contain only an N-TAD. HIF-1β/ARNT, the constitutive member of the HIF heterodimer contains a C-TAD, which is not required for HIF transcriptional activity.

Figure 2. HAF, the new player in HIF-1α degradation

Under aerobic conditions, the hydroxylation of both HIF-1α and HIF-2α by prolyl hydroxylases facilitates the binding of the pVHL E3 ligase complex (pVHL E3L) to HIF-1α/HIF-2α. This results in the poly-ubiquitination of HIF-1α/HIF-2α and their proteasomal degradation. Under hypoxic conditions, HIF-1α/HIF-2α are stabilized and enter the nucleus where they heterodimerize with HIF-1β and initiate downstream transcription. The presence of growth factors or specific extracellular stimuli may promote the binding of HAF to HIF-1α by a yet to be identified oxygen-independent mechanism. This promotes the poly-ubiquitination and degradation of HIF-1α while HIF-2α is allowed remain to activate downstream transcription.