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HIF1 α and HIF2 α : sibling rivalry in hypoxic tumor growth and progression

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Preface

Hypoxia inducible factors (HIFs) are broadly expressed in human cancers, and HIF1 α and HIF2 α were previously suspected of promoting tumor progression through largely overlapping functions. However, this relatively simple model has now been challenged in light of recent data from genome-wide analyses of human tumors, genetically engineered mouse models of cancer, and systems biology approaches that reveal unique and sometimes opposing HIF α activities in both normal physiology and disease. These effects are mediated in part through regulation of unique target genes, as well as direct and indirect interactions with important oncoproteins and tumor suppressors, including MYC and p53. As HIF inhibitors are currently under clinical evaluation as cancer therapeutics, a more thorough understanding of unique roles performed by HIF1 α and HIF2 α in human neoplasia is warranted. This Review summarizes our rapidly changing understanding of shared and independent HIF1 α and HIF2 α activities in tumor growth and progression, and the implications for using selective HIF inhibitors as cancer therapeutics.

Introduction

Oxygen (O₂) levels are known to vary widely across sub-domains of solid tumors, due to rapid cell division and aberrant tumor angiogenesis and blood flow. Although extended exposure to complete O₂ deprivation (anoxia) can result in necrosis, viable hypoxic cancer cells often surround necrotic zones. Tumor hypoxia has long been associated with increased malignancy, poor prognosis and resistance to radiotherapy and chemotherapy (reviewed in^{1,2}), prompting intensive research into cellular responses to O₂ deprivation. Particular interest has been focused on the mechanisms by which hypoxic tumor cells alter their transcriptional profiles to modulate glycolysis, proliferation, survival and invasion to persist under conditions of hypoxic stress³.

The Hypoxia Inducible Factor (HIF) transcription factors mediate the primary transcriptional responses to hypoxic stress in normal and transformed cells. HIFs are

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heterodimeric complexes composed of bHLH-PAS proteins including an O₂-labile alpha subunit (HIF1 α , HIF2 α , or HIF3 α) and a stable beta subunit (HIF1 β , also known as ARNT), which together bind hypoxia responsive elements (HREs) containing a conserved RCGTG core sequence (see Box 1). Hypoxic HIF activity is controlled primarily through post-translational modification and stabilization of HIF1 α and HIF2 α subunits, so that HIF α protein levels and overall HIF transcriptional activity increase as cells become more hypoxic. The central molecular mechanisms underlying the O₂-lability of HIF α subunits were first elaborated in 2001 by multiple groups, and are the subject of several recent reviews^{4,5} (Box 1). Briefly, HIF α subunits are modified by HIF-specific prolyl-hydroxylases (PHDs) in the presence of O₂, leading to normoxic proteasomal degradation mediated in part by the Von Hippel Lindau tumor suppressor protein (pVHL) (Box 1). It is also important to note that elevated oncogenic signaling in cancer cells can induce HIF α expression through O₂-independent mechanisms including increased transcription and/or translation of HIF α mRNA⁶.

HIF1 α was first described by Semenza and colleagues in 1995, and was shown to play a central role in mediating O₂-dependent transcriptional responses⁷. The identification of HIF2 α by independent groups in 1997 (initially called endothelial PAS protein 1 (EPAS1)⁸, HIF-related factor (HRF)⁹, HIF1 α -like factor (HLF)¹⁰, and member of PAS family 2 (MOP2)¹¹) indicated that HIF regulation was more complex. Whereas HIF1 α appears to be expressed in nearly all cell types, RNA in situ hybridization on mouse embryos revealed that HIF2 α expression is more restricted, and particularly abundant in blood vessels. This observation led to the hypothesis that the primary role of HIF2 α is to modulate vascular endothelial cell (Ee) function, an idea supported in part by the close correlation of HIF2 α and VEGF mRNA expression patterns⁸. A more complex view emerged as HIF2 α protein expression was identified in multiple cell types in hypoxic rat kidney, lung, and colonic epithelia, as well as hepatocytes, macrophages, muscle cells and astrocytes¹², indicating that both HIF1 α and HIF2 α are co-expressed in a large number of cell types.

The majority of HIF transcriptional responses have been attributed to HIF1 α and HIF2 α ; however, a third HIF α subunit (HIF3 α) has also been described¹³. HIF3 α mRNA is differentially spliced to produce multiple isoforms that either promote or inhibit the activity of other HIF complexes, although little is yet known about the impact of HIF3 α on hypoxic tumor progression¹⁴⁻¹⁷. Similarly, a second ARNT protein (ARNT2) has been identified¹⁸ and shown to regulate neuronal development¹⁹ and exhibit overlapping activity with ARNT₂₀; however, its activity in human cancer cells has not been studied in depth²¹. Although it will be important to determine whether (and how) HIF3 α and ARNT2 affect HIF-mediated responses in cancers, the available evidence suggests that HIF1 α and HIF2 α account for the vast majority of HIF-dependent effects on tumor growth and progression described to date.

Elevated expression of HIF1 α and HIF2 α protein has been observed in a broad array of human cancer cell types, and associated with poor prognosis in many cases (Table 1). Particular attention has been focused on renal clear cell carcinomas (RCCs), approximately 90% of which lose function of the Von Hippel-Landau tumor suppressor protein (pVHL), which binds prolyl-hydroxylated HIF α subunits and targets them for ubiquitin-mediated proteolysis²² (Box 1). pVHL-deficient RCC cell lines consequently cannot degrade HIF α subunits in an O₂-dependent manner, and have been used extensively to investigate the roles of HIF1 α and HIF2 α in tumor growth.

The observations summarized in Table 1 have led to the general view that elevated HIF α protein expression in tumor cells, whether induced by hypoxia or aberrant oncogenic signaling, actively drives tumor growth and progression by regulating the expression of

critical target genes. Disparate correlations have been observed in some tumor types; for example, HIF1 α expression has been associated with both better and worse prognosis in separate analyses of renal and non-small cell lung cancers (see Table 1). The basis of these apparent discrepancies is not understood, but may reflect the consequences of HIF activity in different cancer subtypes, or at different stages of tumor progression. In some tumors, including gastric cancers and glioma, only one HIF α subunit is correlated with prognosis, suggesting it plays a particularly important role or predominant role in these tumor cell types. Interestingly, multiple recent studies have also revealed unexpected tumor suppressive activities of HIF1 α and HIF2 α in specific contexts²³⁻²⁶. Although initially viewed as having largely overlapping functions, there is now mounting evidence that HIF1 α and HIF2 α can promote highly divergent – even opposing – outcomes when expressed in the same cell type. It appears that HIF1 α and HIF2 α mediate these disparate responses partly through independent regulation of distinct target genes, but also through direct and indirect interactions with complexes containing important oncoproteins and tumor suppressors.

Direct regulation of gene expression by HIF1 α and HIF2 α

Numerous early studies revealed that either HIF1 α or HIF2 α could regulate the expression of many hypoxically induced genes, but that each HIF α isoform also had unique targets (Table 2)^{27,28}. By swapping protein domains between HIF1 α and HIF2 α , several groups demonstrated that this transcriptional specificity resided in the N-terminal activation domain (N-TAO), suggesting that differential interactions with transcriptional co-factors likely determine differential gene activation^{29,30}. Recently, multiple groups have used chromatin immunoprecipitation coupled to tiled microarrays (ChIP-chip) to assess HIF α binding across the genome³¹⁻³⁵. These analyses confirmed the RCGTG core binding sequence, and revealed no additional sequences absolutely required for HIF binding^{32,35}.

Direct comparison of HIF1 α and HIF2 α binding in MCF7 breast cancer cells demonstrated that although some sites bind HIF1 α exclusively, many others bind both HIF α subunits with equal affinity, despite the fact that HIF2 α contributes to the hypoxic expression of relatively few genes in these cells³³. Subsequent analysis using high-resolution ChIP-seq techniques revealed that HIFs bind to approximately 500 high-affinity sites across the genome, many of which are located at great distances (>100 kbp) from the genes they regulate³⁵. Perhaps not surprisingly, HIF1 α and HIF2 α were shown to bind preferentially to specific genes each is known to preferentially regulate (Table 2): for example, a significantly higher level of HIF1 α binding was associated with glycolytic pathway genes, whereas relatively greater HIF2 α binding was observed at the *Oct4* locus. Strikingly, however, significant levels of both proteins were detected at essentially all HIF binding sites, further implicating differential interactions with specific co-factors, perhaps mediated by distinct posttranslational modifications, in controlling target gene specificity³⁵. Interestingly, it appears that HIFs are recruited to genes already expressed in normoxic cells (as revealed by DNaseI hypersensitivity), and are therefore unlikely to direct hypoxic changes in chromatin structure of target genes^{32,35}. The spectrum of HIF target genes may therefore be determined largely by underlying cell type-specific patterns of chromatin structure, a speculation supported by the limited concordance (40-60%) of HIF binding sites detected in MCF7 and RCC cells³⁵. Intriguingly, several reports indicate that HIF1 α binds and regulates the expression of multiple Jumonji-domain containing histone demethylases (JMJDs), which may contribute directly to changes in hypoxic target gene expression^{31,34}.

Assessing HIF1 α and HIF2 α function in tumor models

Multiple xenograft tumor models (Table 3) support the contention that HIF1 α and HIF2 α promote tumor progression by regulating both shared and unique target genes. As global deletion of the mouse *Hif1a* gene results in lethality at E9.5^{36,37}, and HIF2 α deficiency causes embryonic and perinatal lethality³⁸⁻⁴⁰, or severe developmental abnormalities⁴¹ conditional alleles^{37, 42} were required to investigate the specific effects of HIF α deficiency in autochthonous mouse tumor models. Only a small number of studies have been reported to date (Table 3), but these have demonstrated independent roles for HIF1 α and HIF2 α in different cancers, as well as stromal cell types, at various stages of tumor growth and progression. For example, HIF1 α deletion in a mouse mammary tumor virus (MMTV) promoter-driven Polyoma middle T cancer model reduced overall tumor burden and pulmonary metastasis, even when equivalent tumor burdens were allowed to occur in control mice⁴³ (Table 3).

In a direct comparison of HIF1 α and HIF2 α function in a KRAS-driven lung tumor model, HIF1 α deletion had surprisingly little effect on tumor burden and progression, whereas loss of HIF2 α actually increased tumor growth and progression²⁶. This latter effect correlates to HIF2 α -driven expression of the *Scgb3a1* gene, which encodes the putative tumor suppressor secretoglobin 3a1 protein⁴⁴. Surprisingly, overexpression of a stabilized HIF2 α protein in the identical KRAS lung tumor model also promoted tumor angiogenesis and invasion by increasing expression of vascular endothelial growth factor (VEGF) and SNAIL⁴⁵, respectively. The observation that either HIF2 α overexpression or deletion can promote tumor growth in the same tumor context, albeit by different mechanisms, suggests that effective targeting of HIF α subunits in cancer treatment may be complicated. Growth of pVHL-deficient mouse liver hemangiomas was similarly shown to be specifically dependent on HIF2 α , but not HIF1 α ⁴⁶

HIF1 α and HIF2 α deficiency in tumor-associated stromal cells also revealed isoform-specific effects on cancer progression (Figure 1). Initial gene expression studies revealed independent effects of HIF1 α and HIF2 α in primary human macrophages, as well as cultured murine macrophages⁴⁷. HIF1 α deletion in macrophages reduced overall tumor growth in a murine breast cancer model by reducing inducible nitric oxide synthase (iNOS) expression and consequent production of NO, which inhibits T cell responses *in vitro* and immune surveillance *in vivo*⁴⁸. Intriguingly, HIF2 α drives macrophage expression of arginase⁴⁹, which catabolizes and thereby reduces pools of L-arginine, from which NO is produced. The two HIF α isoforms therefore appear to oppose one another to properly regulate overall macrophage NO levels. Interestingly, deletion of HIF2 α (but not HIF1 α) in mouse macrophages also significantly inhibits the expression of cytokine and chemokine receptors, including the macrophage colony-stimulating factor 1 receptor (M-CSFR, also known as CSF1 R) and CXCR4⁵⁰, thereby limiting macrophage migration into autochthonous liver and colorectal cancers and reducing overall tumor burden.

Loss of either HIF1 α or HIF2 α in mouse vascular endothelial cells (ECs) reduces tumor expansion in xenograft models, although through different mechanisms. EC-specific HIF1 α deletion reduces VEGFR2 receptor expression, thereby inhibiting VEGF signaling and EC proliferation, survival and expansion in hypoxic tumor zones⁵¹. In contrast, loss of HIF2 α function in ECs reduced expression of ephrin A1⁵², delta-like ligand 4 (DLL4) and angiopoietin 2 (ANG2)⁵³, which correlated with unproductive sprouting and aberrant vessel remodeling and xenograft tumor growth. Collectively, these results reveal complex roles for HIF1 α and HIF2 α in distinct tumor and stromal cell types, although it will be important to test their function in additional tumor models.

Differential regulation of HIF α isoform expression

What molecular mechanisms contribute to the differential regulation of HIF1 α and HIF2 α ? Control of HIF activity has been traditionally attributed to O₂-dependent posttranslational stabilization of HIF α subunits; however, recent data indicate that control of HIF1 α and HIF2 α expression can be selectively regulated at the level of transcription, translation, and protein stability (summarized in Figure 2A).

Differential transcription

Surprisingly, relatively little is known about the transcriptional regulation of the *Hif1a* and *Epas1* (encoding HIF2 α) genes. Nuclear factor- κ B (NF- κ B) regulates the transcription of the *Hif1a* gene⁵⁴⁻⁵⁷. Moreover, Th1 cytokines stimulate this NF- κ B-HIF1 α pathway to activate a range of HIF1 α target genes, whereas Th2 cytokines interleukin-4 (IL-4) and IL-10 differentially activate *Epas1* expression⁴⁹, although the precise mechanisms involved are not clear. Expression of the *Hif1a* locus, in contrast to *Epas1*, is also regulated by the SWIISNF chromatin remodeling protein BAF57⁵⁸. Additional investigation into differential *Hif1a* and *Epas1* transcription is certainly warranted.

Differential mRNA translation

It is well established that elevated HIF α mRNA translation rates increase HIF α protein levels and activity, particularly in cells with activated PI3K/AKT/mTOR signaling, a common feature of cancer cells (reviewed in⁶). Intriguingly, HIF1 α expression in RCC cell lines appears to be regulated by both mTORC1 and mTORC2 kinase complexes, whereas HIF2 α expression is mTORC2-dependent and mTORC1-independent⁵⁹. Other forms of differential translation control have been reported for HIF α proteins^{60, 61}. For example, the iron response element binding protein 1 (IREBP1) was shown to bind a canonical iron response element (IRE) in the HIF2 α 5' UTR, thereby inhibiting translation⁶¹. This effect appears to be specific for HIF2 α , as IREBP1 fails to bind the HIF1 α transcript or regulate its translation, despite the presence of a near-consensus IRE in the HIF1 α 5' UTR⁶². This regulation is also consistent with the identification of HIF2 α as the primary regulator of erythropoiesis and cellular iron metabolism in vivo^{42, 63-60}.

Differential stability

As HIF1 α and HIF2 α protein levels are both modulated in a similar way by PHD-pVHL-dependent mechanisms (Box 1), the observation that HIF1 α and HIF2 α proteins accumulate at different O₂ levels in specific cell types came as a surprise. Pahlman and colleagues first demonstrated that HIF2 α protein is stabilized at moderate (2-5% O₂) levels, whereas HIF1 α accumulates only at lower (0-2% O₂) levels in HeLa and neuroblastoma cells^{67, 68} (similar results were later reported for glioma cells⁶⁹). Hypoxic neuroblastoma⁶⁸ and lung adenocarcinoma cells⁶⁰ maintain elevated HIF2 α protein levels during long-term hypoxic culture (48 hours); in contrast, HIF1 α levels increase acutely upon hypoxic exposure, but then decline after several hours. The HIF-mediated expression of antisense transcripts from the *Hif1a* (but not *Epas1*) locus, results in *Hif1a* mRNA destabilization and may explain the gradual and specific reduction of HIF1 α protein⁶⁰.

Two HIF1 α specific E3 ubiquitin ligases have been described recently that may also contribute to the differential stability of HIF1 α and HIF2 α . HIF-associated factor (HAF) binds and destabilizes HIF1 α under normoxic and hypoxic conditions in a pVHL-independent, proteasome-dependent manner, but has no effect on HIF2 α levels⁷⁰. Instead, HAF binds HIF2 α at a distinct C-terminal region and promotes HIF2 α transcriptional activity, effectively switching cells from a HIF1 α to a HIF2 α transcriptional program⁷¹. In addition, heat shock protein 70 (HSP70) and carboxyl terminus of Hsc70-interaction protein

(CHIP), a recently identified E3-ubiquitin ligase, were shown to bind and degrade HIF1 α (but not HIF2 α) under conditions of prolonged hypoxia in cultured cells, whereas rapid reoxygenation destabilized both HIF1 α and HIF2 α proteins in a PHD-pVHL-dependent manner⁷² Precisely how these novel ubiquitylation events are regulated, either by hypoxia or other stimuli, and how they affect HIF activity in cancer progression is not yet known.

Posttranslational modifications and differential HIF α activity

The regulation of HIF α subunits by posttranslational proline and asparagine hydroxylation, catalyzed by PHD and factor inhibiting HIF (FIH, also known as HIF1AN) enzymes, respectively (Box 1), has been extensively reviewed elsewhere^{4, 63, 73-70}. Interestingly, specific PHD enzymes exhibit biased activity toward HIF1 α and HIF2 α ; for example, PHD3 preferentially hydroxylates HIF2 α in multiple cell lines⁷⁶. Peet and colleagues have also shown that FIH preferentially hydroxylates HIF1 α in certain cell lines, owing to the identity of the amino acid immediately C-terminal to the hydroxylated asparagine (valine in HIF1 α , alanine in HIF2 α)⁷⁷. These results suggest that differential N-hydroxylation might regulate HIF1 α and HIF2 α activity, although the largely HIF-independent neurological phenotypes of FIH-deficient mice⁷⁸ indicate that other factors are likely involved.

In addition to hydroxylation, both HIF1 α and HIF2 α are subject to an array of distinct, O₂-independent posttranslational modifications, and growing evidence indicates that at least some of these are specific for either HIF1 α or HIF2 α , and may promote their differential activity (Figure 2B), These include:

Phosphorylation

Early work showed that both HIF1 α and HIF2 α are phosphorylated^{79, 80}, and recent work suggests that isoform-specific phosphorylation may impact tumor progression. Specifically, Huang and colleagues demonstrated that HIF1 α represses Myc-dependent expression of the DNA damage repair protein nibrin (NBS1) by displacing the SP1 transcription factor from the MYC transcriptional complex⁸¹, HIF2 α , in contrast, is inhibited from interacting with SP1 through phosphorylation on T324 by protein kinase D1 (PKD1), a modification dependent on a neighboring proline residue unique to HIF2 α (Figure 2C), When a proline residue was introduced into the corresponding position in HIF1 α , PKD1 also phosphorylated the modified HIF1 α protein, which consequently lost the ability to displace SP1 from MYC⁸¹, Other specific phosphorylation events catalyzed by MAPK⁸², casein kinase 1 (CK1)⁸³ and ataxia telangectasia mutated (ATM)⁸⁴ have been demonstrated to modulate HIF1 α activity, although it is not yet known whether these also occur in HIF2 α . It will be important to determine the degree to which these various phosphorylation events distinguish HIF1 α or HIF2 α activation, and whether they represent another mechanism of parallel regulation in cancer cells.

Acetylation

HIF α activity is also modulated by multiple sirtuins, a family of redox-sensitive, NAD⁺-dependent deacetylases and/or ADP-ribosyltransferases. Mammalian cells express a family of sirtuins (SIRT1-7) that regulate complex changes in gene expression, metabolism, and cellular redox status, and have also been implicated in controlling longevity, although this idea remains highly controversial⁸⁵. SIRT1 forms a complex with HIF2 α and deacetylates conserved lysine residues in the N-TAD, which enhances HIF2 α transcriptional activity *in vitro* and *in vivo*⁸⁶ SIRT1 was also reported to deacetylate lysine residues in HIF1 α , which resulted in HIF1 α transcriptional repression⁸⁷ although this effect was not universally observed⁸⁶ (Figure 3).

The apparently opposing effects of SIRT1 on HIF1 α and HIF2 α could skew cells toward either HIF1 α or HIF2 α transcriptional programs in response to changing metabolic activity in hypoxic tumors. Park and colleagues⁸⁷ proposed a positive feedback mechanism in which HIF1 α promotes glycolysis, reducing NAD⁺/NADH ratios under hypoxia and inhibiting SIRT1, thereby further augmenting HIF1 α activity. Presumably, inhibiting SIRT1 under these conditions would also decrease HIF2 α activity, although the relative sensitivity of endogenous HIF1 α and HIF2 α proteins to SIRT1-mediated effects over a range of O₂ levels is not yet clear, and the kinetics of these responses may differ. It would be interesting to determine whether deacetylation by SIRT1 contributes to the high relative abundance of HIF2 α at intermediate O₂ levels. There appear to be yet more wrinkles in this story, as both HIF1 α and HIF2 α were shown to bind the *SIRT1* gene promoter and induce its expression under hypoxia⁸⁸, and AKT activity can induce both HIF1 α and SIRT1 expression by downregulating miR199a-5p expression⁸⁹.

Other sirtuins have also been shown to regulate HIF α activity. Mostoslavsky and colleagues identified SIRT6 as a HIF1 α repressor, and showed that SIRT6 deficiency increased HIF1 α -dependent glucose uptake and glycolytic activity at the expense of mitochondrial respiration⁹⁰. Although the precise mechanisms regulating interactions between SIRT6 and HIF1 α are not yet clear, SIRT6 deficiency increases both HIF1 α synthesis and stability, suggesting that the effects of SIRT6 may be at least partly indirect. In addition, the mitochondrial SIRT3 deacetylase indirectly regulates HIF α stabilization by suppressing the formation of mitochondrial reactive oxygen species (ROS)⁹¹ which, in turn, promote HIF1 α stabilization⁹²⁻⁹⁴. For this reason, SIRT3-deficient cells display HIF1 α -dependent increases in glucose transport, glycolysis and proliferation^{95,96}. The implications of these findings for tumor progression have not been explored in depth, but are likely to be both complex and important.

It is possible that other acetylation and deacetylation events regulate HIF activity: for example, the mouse arrest defective-1 (mARD1) protein was reported to destabilize HIF1 α by acetylating K532⁹⁷, an event apparently reversed by recruitment of HDAC1 to HIF1 α by metastasis-associated protein 1 (MTA1)⁹⁸. Other researchers, in contrast, observed neither interaction between mARD1 and HIF1 α , nor any effects of hypoxia on mARD1 activity, and the importance of this regulatory event remains in dispute⁹⁹. Finally, a growing number of reports indicate that HIF α proteins are subject to numerous other posttranslational modifications, including sumoylation, S-nitrosylation, and neddylation¹⁰⁰⁻¹⁰⁶ although whether any of these differentially regulate HIF1 α and HIF2 α is as yet unknown.

HIFs, oncogenes, and tumor suppressors - balancing HIF1 α and HIF2 α

Although HIF1 α and HIF2 α clearly influence tumor progression by directly regulating unique and shared target genes (Table 2), recent evidence indicates that these HIF α proteins also affect tumor progression by exerting distinct, often opposing effects on critical oncoproteins and tumor suppressors including MYC, p53, and mTOR.

HIF α and c-Myc

In many cell types, hypoxia suppresses proliferation. Koshiji et al. were the first to demonstrate that acute HIF1 α stabilization at 1% O₂ produces cell cycle arrest by inhibiting the protooncoprotein MYC¹⁰⁷, a bHLH/leucine zipper (bHLH/LZ) transcription factor that is overexpressed in >40% of human cancers. MYC controls the *G1/S* cell cycle transition by forming heterodimers with the related protein MAX, binding conserved E-box sequences (CTCGAG), and promoting expression of genes encoding cyclin D2 (*CCND2*), E2F1, and ornithine decarboxylase 1 (*ODC1*), for example. MYC simultaneously inhibits the expression of *CDKN1A* and *CDKN1B* genes encoding cyclin-dependent kinase inhibitors

(CKIs) p21 and p27, respectively¹⁰⁸, in part by displacing the SP1 protein from the transcription factor MIZ1. MYC also promotes proliferation by inducing the expression of essentially all glycolytic enzymes and enhancing protein synthesis, thereby increasing cell growth.

Under hypoxic conditions, HIF1 α binds to SP1, displacing MYC from multiple target genes including *CDKN1A*, *MSH2*, *MSH6*, and *NBS1*^{81,109}. (Figure 3B). Gordan et al. subsequently showed that HIF1 α rapidly disrupted the association of MYC with MAX and MIZ1, thus reducing MYC promoter occupancy at the *CDKN1A*, *CDKN1B*, *CDKN2B* (which encodes p15), *ODC1*, *CCND2*, and *E2F1* genes¹¹⁰. A more chronic adaptation results from HIF1 α mediated induction of MXI1, which interacts with MAX at E-boxes to inhibit the expression of *ODC1* and peroxisome proliferator-activated receptor- γ coactivator 1 β (*PGC-1 β*)^{111, 112}, suppressing mitochondrial biogenesis and function. Moreover, HIF1 α promotes MYC degradation under chronic hypoxia^{111,112}. Through these multiple mechanisms, HIF1 α effectively limits MYC-dependent anabolic metabolism, protein synthesis and cell division, an important hypoxic adaptation. Intriguingly, HIF1 α also drives expression of the glycolytic pathway genes, permitting hypoxic cells to inhibit MYC driven macromolecular synthesis whilst producing ATP from glycolysis.

Surprisingly, transformed cells expressing HIF2 α exclusively exhibit enhanced MYC activity, with more rapid entry into S phase of the cell cycle, increased *CCND2*, *E2F1*, and *ODC1* gene expression, and elevated MYC promoter occupancy¹¹⁰. Moreover, HIF2 α promotes cell cycle progression in hypoxic cells via transcriptional effects on both MYC activated (*CCND2*, *E2F1*) and repressed (*p21*, *p27*) target genes, and interactions with MAX, SP1, and MIZ1. This impact on MYC likely contributes to HIF2 α -mediated neoplastic progression of renal clear cell carcinoma (RCC) tumorigenesis following loss of the *VHL* tumor suppressor¹¹³. Of note, RCC cells exclusively expressing HIF2 α also displayed reduced genomic instability, correlating with increased MYC-dependent expression of genes encoding DNA repair proteins (including BRCA 1, BARD1, XRCC2, BUB1, and CENPE)¹¹³. These results reveal a critical collaborative role for HIF2 α and MYC in promoting genomic integrity and resistance to replication stress.

How do HIF1 α and HIF2 α exert these opposing roles on MYC? Multiple mechanisms appear to be involved: for example, HIF1 α binds to SP1 via the PAS-B domain, whereas HIF2 α fails to do so because it is phosphorylated by PKD1, blocking its ability to interact with SP1⁸¹. In contrast, HIF2 α forms a complex with MAX, causing a dose-dependent stabilization of MYC-MAX and MYC-MAX-SP1 complexes, resulting in increased MYC-MAX binding at *CCND2*, *E2F1*, *p21*, and *p27*¹¹⁰. These effects occur rapidly and can be detected after only 1-2 hours at 0.5% O₂, suggesting they are independent of HIF2 α transcriptional activity, which peaks at approximately 16 hours at 0.5% O₂. A specific role for MXI1 in this differential regulation is currently unclear, as both HIF1 α and HIF2 α appear to contribute to MXI1 expression in *VHL*-deficient RCC cells¹¹². How the “competition” between HIF1 α and HIF2 α is moderated in a given cell type, in terms of their respective influence on MYC activity, is equally mysterious at present.

The relative expression levels of MYC and HIF α proteins also play an important role in regulating tumor cell proliferation and metabolism. Many cancer cells exhibit subtle alterations in MYC levels as a consequence of elevated oncogenic signaling, whereas other cells express MYC at very high levels due to chromosomal amplifications, translocations, and mutations within *MYC* coding exons¹⁰⁸. It appears that high levels of MYC sequester and tightly bind MAX, thereby relieving potential inhibition by HIF1 α ¹¹⁴. For example, most genes induced by ectopic *MYC* expression were not transcriptionally repressed by hypoxia in a B-cell tumor model. The picture is more complex, however, as HIF1 α can

actually cooperate with MYC to induce the expression of specific target genes, including those encoding the glycolytic enzyme hexokinase 2 (*HK2*), pyruvate dehydrogenase kinase 1 (*PDK1*), and VEGFA¹¹⁴. Similarly, high levels of NMYC override HIF1 α inhibition of cell cycle progression while cooperating with HIF1 α to promote phosphoglycerate kinase 1 (*PGK1*), (*HK2*), and lactate dehydrogenase A (*LDHA*) expression in neuroblastomas with *MYCN* gene amplification¹¹⁵. In summary, when *MYC* family members are highly overexpressed, they not only overcome the inhibitory effects of HIF1 α , but MYC and HIF1 α collaborate to favor glycolysis and continued proliferation under decreased O₂ availability. In contrast, tumors with lower MYC levels are susceptible to HIF1 α inhibition, explaining the anti-tumorigenic effects of HIF1 α in certain cancers such as RCC¹¹⁶.

HIF1 α , HIF2 α , and p53

Low O₂ and other stresses associated with tumor growth (such as growth factor withdrawal, nutrient deprivation, and acidosis) activate p53, a critical tumor suppressor that is mutated or silenced in a majority of human cancers¹¹⁷. While it is maintained at low levels in normal cells by MDM2-mediated degradation, p53 is posttranslationally modified and stabilized in response to numerous stimuli, including abnormal proliferation signals, osmotic stress, DNA damage, and hypoxia¹¹⁸. p53 forms homotetramers that bind and regulate numerous genes involved in metabolism, DNA repair, cell cycle arrest, and cell death, thereby coordinating cellular responses to microenvironmental stress¹¹⁷

HIF1 α and HIF2 α display opposing effects on the p53 pathway. Numerous studies have shown that p53 accumulation occurs within hypoxic regions of solid tumors, and correlates with cells undergoing apoptosis, although this may only occur when also accompanied by acidosis and nutrient deprivation¹¹⁹. An et al. originally suggested that transcriptionally active wild type p53 is stabilized through a physical association with HIF1 α .¹²⁰ Sanchez-Puig further reported that the HIF1 α ODD and N-terminal TAD domains bind to p53 tetramers under physiological conditions¹²¹; however, subsequent reports suggested that MDM2 mediates the interaction between p53 and HIF1 α by acting as a bridge between the two transcription factors¹²². Whereas HIF1 α fails to bind p53 *in vitro*, it directly binds MDM2, suppressing MDM2-dependent ubiquitylation of p53 *in vivo* and p53 nuclear export. Surprisingly, MDM2 overexpression actually promotes p53 accumulation and target gene stimulation when HIF1 α is activated in hypoxic cells¹²². Furthermore, HIF1 α appears to enhance p53 activation by ionizing radiation (IR), resulting in increased p53 phosphorylation and p53-mediated apoptosis¹²³. IR significantly increases HIF1 α activity in tumors due to increased reactive oxygen and nitrogen species, and radiation combined with hypoxia lead to increased p53 phosphorylation in a HIF1 α dependent manner, by a mechanism that remains unclear.

It should also be noted that the relationship between HIF1 α and p53 provides a potential negative feedback loop for HIF1 α activity. Ravi et al. have suggested that p53 can induce HIF1 α turnover, by promoting its MDM2-mediated ubiquitylation and proteasomal degradation¹²⁴. Therefore, p53 loss in colon cancer cells enhanced HIF1 α levels and augmented VEGFA expression and tumor angiogenesis, suggesting that inactivating p53 mutations can contribute to the “angiogenic switch” during colorectal tumorigenesis.

In contrast to HIF1 α , HIF2 α does not bind MDM2¹²⁵, and appears to inhibit p53 indirectly by multiple mechanisms. Bertout et al. demonstrated that elevated HIF2 α expression inhibits p53 phosphorylation and stabilization in ReG cell lines, whereas knocking down HIF2 α expression increases p53 transcriptional activity and target gene expression¹²⁵. Furthermore, HIF2 α deficient cells exhibit elevated ATM activity and DNA double strand break formation, as well as increased levels of ROS after IR. HIF2 α has been reported to regulate antioxidants such as superoxide dismutase 1 (SOD1), SOD2, glutathione peroxidase

1, and catalase in developing embryos and neonates⁴¹. However, in RGG cells, HIF2 α instead decreases ROS accumulation by regulating the expression of distinct antioxidant enzymes (heme oxygenase 1, ceruloplasmin, glutathione peroxidase 8, and peroxiredoxin 3). Importantly, HIF2 α expression in RCC tumor samples correlates with decreased p53 phosphorylation and target gene expression, and may contribute to radioresistance in HIF2 α expressing RCCs¹²⁵.

In parallel studies, Roberts et al. showed that HIF2 α also suppresses p53 expression and function via indirect effects on MDM2¹²⁶. AKT-mediated phosphorylation of MDM2 promotes its nuclear localization and enhanced p53 degradation, and represents an important pro-survival effect of AKT. AKT activation occurs downstream of growth factor receptors like EGFR and platelet-derived growth factor receptor (PDGFR), which are stimulated by transforming growth factor- α (TGF- α) and PDGF β , specific transcriptional targets of HIF2 α in RCC cells. Thus, HIF2 α overexpression in *VHL*-deficient RCC can inhibit p53 through a growth factor receptor-AKT-MDM2 pathway, in addition to maintaining redox homeostasis. In aggregate, these findings suggest that HIF2 α likely contributes to RCC tumor cell survival during both radiation and chemotherapy by multiple mechanisms.

HIFs regulate mTOR

Cell division requires high levels of protein synthesis and anabolic metabolism, which is regulated by the serine/threonine kinase mTOR in response to nutrient and growth factor availability. mTORC1 promotes ribosome biogenesis, mRNA translation, and nutrient import, while inhibiting autophagy¹²⁷. Elevated mTORC1 activity is observed in the majority of human tumors, due to activation of upstream oncogenes (PI3K, AKT) and/or loss of tumor suppressors (PTEN, LKB1)¹²⁸. In particular, the tuberous sclerosis proteins TSC1 and TSC2 together inhibit mTORC1 activity to limit cell growth under conditions of environmental stress, including reduced growth factor, glucose, amino acid, and O₂ levels¹²⁷.

Hypoxia suppresses mTORC1 through multiple mechanisms. For example, decreased ATP levels in severely hypoxic cells activate AMP-activated kinase (AMPK)¹²⁹, which phosphorylates TSC2 (as well as the mTORC1-associated factor RAPTOR) to inhibit mTORC1 activity. In addition, HIF1 α (but not HIF2 α) induces expression of the *DDIT4* gene¹³⁰, which encodes REDD1, a protein that represses mTORC1 by promoting the release of sequestered TSC2 from 14-3-3 proteins¹³¹. Finally, the hypoxia-inducible proautophagic protein BNIP3 binds and inhibits RAS homolog enriched in brain (RHEB), resulting in decreased mTORC1 activity¹³². HIF1 α dependent inhibition of mTORC1 may benefit cells by reducing ATP-intensive protein synthesis, while increasing autophagy, under conditions of hypoxic stress.

Growing evidence suggests that HIF2 α may, in contrast, stimulate mTORC1 to promote cellular proliferation in O₂-deprived cells. The focal adhesion kinase (FAK) family interacting protein of *200 kd* (FIP200) gene has been identified as a HIF2 α target through microarray studies²⁷ and FIP200 has been proposed to interact with TSC1, thereby disrupting TSC1/TSC2 complexes and promoting mTORC1 activation¹³³. In addition, FIP200 may promote TSC1 degradation by the ubiquitin-proteasome pathway¹³⁴. HIF2 α could also selectively enhance mTORC1 activity by positive effects on growth factor signaling, as HIF2 α induces the expression of TGF- α , PDGF- β , and IGF-1, leading to AKT and mTORC1 activation in renal cancer cells¹²⁶. Although additional work is clearly needed to further elucidate the molecular mechanisms by which HIF2 α promotes mTORC1 functions, these results reveal another example in which HIF1 α and HIF2 α antagonize one another to balance hypoxic responses in key growth regulatory pathways.

HIF α and growth control

Why would the two HIF α subunits result in opposite effects on the c-Myc, p53, and mTORC1 pathways? The inhibitory activity of HIF1 α towards these growth regulatory systems represents important energy conservation mechanisms in light of decreased ATP production during periods of O₂ limitation, which are likely to be compounded by decreased availability of nutrients (glucose, amino acids, lipids) and growth factors in hypoxic subdomains of solid tumors. In contrast, the “pro-growth” effects of HIF2 α may contribute to the ability of endothelial cells to proliferate during neoangiogenesis in ischemic tissues. It is interesting that HIF2 α accumulates at higher O₂ levels than HIF1 α , which may allow its selective activation in blood vessels. In addition, the ability of HIF2 α to promote cell growth in RCCs may explain why HIF1 α expression is often silenced in these tumors. Selective¹³⁵, as well as genome-wide¹³⁶, sequence and copy number analyses have identified truncating *Hif1a* mutations in a small percentage of RCCs, as well as *Hif1a* heterozygosity in others¹³⁷, supporting the hypothesis that inhibition of HIF1 α function is a selective advantage for some RCCs. It is also tempting to speculate that the recent identification of *Epas1* single nucleotide polymorphisms (SNPs) as a predisposing factor for RCC development¹³⁶ could reveal genetic alterations that increase or expand HIF2 α function.

Therapy

As HIF complexes are instrumental in cancer cell adaptation to hypoxic tumor microenvironments, the ability to *selectively* inhibit HIF activity would appear to be of clinical benefit^{1, 139}. Historically, DNA-binding proteins *have* been difficult to target, but a large collection of compounds *have* been reported to inhibit HIF transcriptional activity, either directly or indirectly. For example, compounds including topoisomerase inhibitors (camptothecin, topotecan)¹⁴⁰ and DNA intercalators (echinomycin, daunorubicin, doxorubicin)¹⁴¹ block HIF heterodimerization and transcriptional activation, and interfere with xenograft tumor growth in a HIF-dependent manner. These observations are particularly interesting, given their frequent use as sequence non-specific DNA damaging agents in chemotherapy. Oncogenic signal transduction pathways also promote mTORC1-dependent HIF1 α mRNA translation; consequently, receptor tyrosine kinase inhibitors (Herceptin, *Gleevec*, erlotinib, gefitinib) and mTOR inhibitors (rapamycin, temsirolimus, everolimus)^{142,143} are thought to reduce tumor angiogenesis, and possibly other hypoxic responses, by indirectly reducing HIF α protein synthesis. Other drugs have been shown to increase HIF α degradation, including HDAC inhibitors^{144,145}, and compounds that disrupt HIF α binding to HSP90 (geldanamycin)^{146, 147}. These studies indicate that HIF activity is susceptible to inhibition using a variety of drugs already approved for cancer treatment; however, the extent to which these drugs limit the growth and progression by specifically inhibiting HIF activity in autochthonous tumors is as yet unknown, and needs to be investigated.

Given the disparate effects of HIF1 α and HIF2 α on tumor growth and progression described in this review, it will also be critical to determine whether potential HIF inhibitors affect both HIF α subunits equally. There may be situations where *selective* inhibition of either HIF α protein would be especially beneficial; for example, inhibiting HIF1 α may be particularly advantageous for highly glycolytic hypoxic tumors, whereas inhibiting only HIF2 α is likely to be useful in treating RCCs. Intriguingly, Iliopoulos and colleagues identified a series of small compounds that interfere with HIF2 α mRNA translation by enhancing IREBP1 binding to the iron response element found in the 5' UTR of HIF2 α , but not HIF1 α ⁶². Selective agents of this kind could be particularly useful in cancers that express both HIF1 α and HIF2 α , where they *have* distinct roles. Furthermore, Dewhirst et al *have* shown that radiation induces HIF1 α and VEGFA, protecting endothelial cells from

radiation-mediated apoptosis¹⁴⁸. Treatment of mice harboring tumors with the HIF inhibitor YC-1 enhanced vessel destruction and slowed tumor growth; in another study¹⁴⁸, the HIF inhibitor PX-478 reduced VEGFA expression, rendering xenografts more sensitive to ionizing radiation. As stated previously, endothelial VEGFA expression appears to be regulated primarily by HIF1 α , suggesting that its selective inhibition would be beneficial.

Finally, HIF inhibition may be advantageous only up to a certain point. As discussed earlier, HIF2 α overexpression, as well as HIF2 α deletion, increases the growth of KRAS driven murine lung tumors, although by different mechanisms. Too much HIF2 α increases VEGF and SNAIL expression, promoting angiogenesis and tumor invasion, whereas complete loss of HIF2 α reduces the expression of the tumor suppressor *Scgb3a1*, a direct HIF2 α target gene. These data suggest that successful HIF inhibition in cancer treatment may involve a narrower therapeutic window than initially envisioned. Although currently in early stages, the prospect of pharmacological HIF inhibition for cancer treatment, whether targeting HIF1 α and HIF2 α together, or either subunit individually, is an exciting one.

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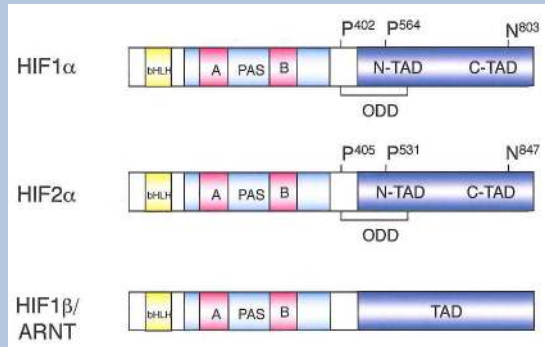
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Box 1. O₂-dependent HIF regulation

Using molecular O₂ and 2-oxoglutarate as substrates, **HIF** prolyl hydroxylase (PHD) enzymes⁴ hydroxylate two specific proline residues that reside in the O₂-dependent degradation domain (ODD) of HIF- α proteins. These hydroxylation events occur on P402 and P564 in HIF1 α , and P405 and P531 in HIF2 α , respectively, and are required for the Von Hippel-Lindau (pVHL) tumor suppressor protein, the recognition component of an E3-ubiquitin ligase, to bind and degrade HIF α subunits under normoxic conditions. Hypoxia inhibits PHD activity through a number of mechanisms, including substrate limitation (reviewed in⁴), resulting in HIF α stabilization, heterodimerization with HIF1 β /ARNT, and increased **HIF** transcriptional activity. Hypoxic conditions also inhibit a second hydroxylation of a conserved HIF α C-terminal asparagine residue by the FIH hydroxylase, an event that blocks the interaction between HIF α and the transcriptional co-activators p300/CBP¹⁴⁹⁻¹⁵¹. Thus, whereas PHD-mediated hydroxylation destabilizes HIF α subunits, FIH-mediated hydroxylation inhibits their transcriptional activity.



Box 2. HIFs in normal and cancer stem cells

Stem cells reside in complex microenvironments or niches, and multiple studies revealed that O₂ levels influence the ability of stem and/or progenitor cells to remain quiescent or undergo differentiation, depending on cell type¹⁵². Again, HIF1 α and HIF2 α exhibit distinct roles in stem cell regulation. HIF1 α appears to play a dominant role in modulating¹⁵². WNT- β -catenin signaling in hypoxic ES cells and isolated neural stem cells (NSCs) of the embryonic mesencephalon and adult hippocampus¹⁵³. WNT- β -catenin activity is closely associated with low O₂ regions in the subgranular zone of the hippocampus, an important NSC niche, and *Hif1 α* deletion impairs WNT-dependent processes, such as NSC proliferation, differentiation, and neuronal maturation. It should be noted that the opposite result has been reported for colon cancer cells, where HIF1 α inhibits WNT- β -catenin activity¹⁵⁴, indicating that the interaction between HIF1 α and WNT in stem cells is functionally distinct from more differentiated cells, including neoplastic cells. The basis for this difference is currently unknown. HIF1 α has also been proposed to increase the intracellular stability of activated NOTCH1 and to promote NOTCH target gene activation of myogenic and neural precursor cells¹⁵⁵. This has been extended to thymic lymphomas in p53 mutant mice where HIF1 α promotes NOTCH1 activation and target gene expression¹⁵⁶. However, the data on neuroblastoma stem cells suggest that both HIF1 α and HIF2 α can augment NOTCH pathway signaling.

In contrast, HIF2 α (but not HIF1 α) regulates the POU transcription factor OCT4 (also known as POU5F1)¹⁵⁷. OCT4 is essential for maintaining an undifferentiated cell fate in embryonic stem (ES) cells, the embryonic epiblast, and primordial germ cells (PGCs). Finally, HIF2 α is selectively expressed in CD133⁺ glioblastoma “stem” cells, whereas HIF1 α is detected in both tumorigenic (i.e. stem) and non-tumorigenic populations, suggesting HIF2 α has a unique role in the CD133⁺ fraction⁶⁹. Similarly, human neuroblastomas exhibit small numbers of tumor initiating/stem cells expressing neural crest markers (ID2, NOTCH1, HES1, and Vimentin) and HIF2 α ¹⁵⁸. Upon HIF2 α inhibition, these cells undergo early sympathetic neuronal differentiation, and express markers such as HASH1 (also known as ASCL1), ISL1, and SCG10 (also known as STMN2). It is noteworthy that the CD133⁺ glioblastoma and putative neuroblastoma tumor initiating/stem cells express high levels of HIF2 α , although they reside in periendothelial niches¹⁵⁹. While the extent of O₂ saturation within these capillaries is unknown, the data are consistent with the idea that HIF2 α accumulates at higher levels of O₂ than HIF1 α . Alternatively, HIF α expression in distinct cancer cell subpopulations may be controlled by non-hypoxic stimuli, such as aberrant metabolism¹⁶⁰.

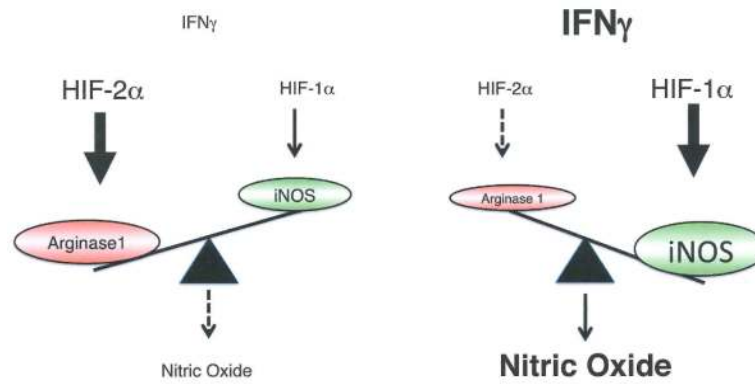


Figure 1. HIF1 α and HIF2 α exhibit antagonistic functions in nitric oxide (NO) production
 Under low IFN γ conditions, HIF2 α is more abundant and induces arginase1 expression, resulting in NO production. Under high IFN γ conditions, HIF2 α is diminished and HIF1 α dominates so that iNOS can utilize arginine for NO generation. These physiologically antagonistic functions allow the HIF α subunits to coordinately regulate NO production in a cytokine-induced and transcription-dependent fashion.

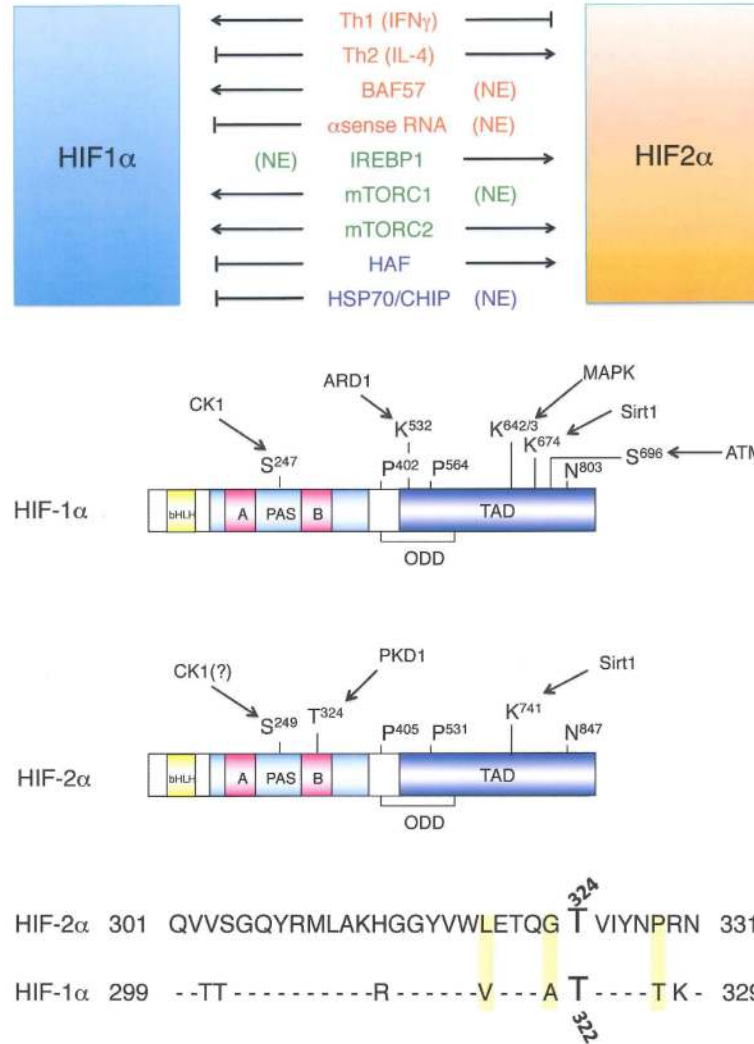


Figure 2. HIF1 α and HIF2 α are post-translationally modified, and differentially regulated by multiple mechanisms

(A) Multiple mechanisms differentially regulate HIF1 α and HIF2 α at the levels of transcription or mRNA stability (red), mRNA translation (green), and protein stability (blue). In most cases, these regulatory events have opposite effects on HIF α expression, or appear to be specific for only one HIF α isoform. See text for details. (NE), no effect. (B) Summary of phosphorylation, acetylation, and hydroxylation of the two HIF α subunits by CK1, ARD1, PHDs, FIH, MAPK, SIRT1, PKD1, and ATM. It should be noted that ARD1 acetylates HIF1 α , while SIRT1 deacetylates both HIF1 α and HIF2 α . (C) Sequence alignment of HIF2 α residues 301-331 with a similar region of HIF1 α ; shaded residues are unique to HIF2 α and allow the selective phosphorylation of HIF2 α T324 by PKD1.

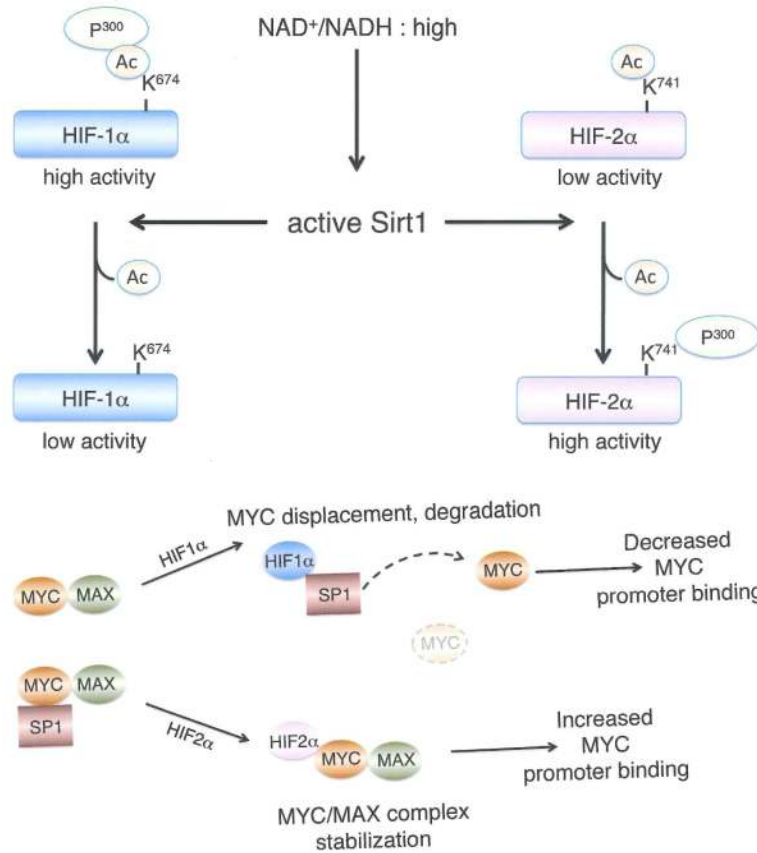


Figure 3. Differential regulation of HIF1α and HIF2α by SIRT1

(A) High levels of NAD⁺ inactivate SIRT1, resulting in decreased HIF1α transcriptional activity and enhanced HIF2α stimulation of target genes like erythropoietin. (8) Distinct effects of HIF1α and HIF2α on MYC complex formation and promoter occupancy. Hypoxic cells exclusively expressing HIF1α exhibit decreased MYC activity due to diminished association with MAX and SP1, as well as reduced MYC stability. HIF1α also induces MXI1 expression, which inhibits MYC target gene expression (see text for details). Cells expressing HIF2α exhibit increased MYC complex formation and target gene activation, although the mechanisms involved are not fully understood.

Table 1Correlation between HIF α protein expression and poor prognosis in human cancers *

Cancer type	HIF1 α	HIF2 α	References
Astrocytoma	+	+	161,162
Bladder	+	ND	163
Breast	+	+	164,165
Cervical	+	+ ^a	166,167
Colorectal	+	+	168
Gastric	+	NC	169, 170
Gastric	+	ND	171
GIST	+	ND	172
Glioblastoma	ND	+	162
Glioma	NC ^b	+ ^b	69
Head/neck	+	+	173,174
Hepatocellular	ND	+	175
Lung (NSCLC)	+	+	176
Lung (NSCLC)	+	ND	177
Lung (NSCLC)	NC	+	178
Melanoma	+	+	179
Neuroblastoma	FP	+	180
Ovarian	+	ND	181
Ovarian	+	+ ^d	182
Pancreatic	+	ND	183,184
Prostate	+	+ ^d	185
Renal	FP	ND	186
Renal	+	ND	187

NC, no correlation; ND, not determined; FP, correlation between HIF α expression and favorable prognosis

* Also see references (3) and (138).

^a HIF2 α expression in macrophages^b Correlation using mRNA levels^c Correlation of HIF1 α expression with favorable prognosis^d Correlation with cytoplasmic HIF2 α expression

Table 2Representative shared and unique target genes regulated by HIF1 α and HIF2 α

Gene	Function	HIF1 α	HIF2 α	Cell type
GLUT1	Glucose transport	+	+	RCC ²⁷ , mouse ES ^{36, 37}
ADRP	Lipid metabolism	+	+	RCC ²⁷
CAXII	pH homeostasis	+	+	RCC ²⁷
FILAG	Cytoskeletal structure	+	+	RCC ²⁷
IL-6	Immune cytokine	+	+	RCC ²⁷
ADM1	Angiogenesis	+	+	RCC ²⁷
VEGF	Angiogenesis	+	+	RCC, Hep3B ²⁷⁻²⁹
VEGF	Angiogenesis	+	-	Mouse EC ⁵¹ , mouseES ^{36,37}
BNIP3	Autophagy, apoptosis	+	-	RCC ²⁸
HK1	Glycolysis	+	-	mouse ES ^{36, 37}
HK2	Glycolysis	+	-	RCC ²⁷ , mouse ES ^{36, 37}
PFK	Glycolysis	+	-	RCC ²⁷ , mouse ES ^{36,37}
ALDA	Glycolysis	+	-	RCC ²⁷ , mouse ES ^{36, 37}
PGK1	Glycolysis	+	-	RCC ²⁷ , mouse ES ^{36, 37}
LDHA	Glycolysis	+	-	RCC ²⁷ , mouse ES ^{36, 37}
INOS	NO production	+	-	Macrophages ⁴⁹
ARG	Inhibitor of NO production	-	+	Macrophages ⁴⁹
EPO	Erythropoiesis	-	+	Kidney ^{41, 42, 65} , liver ¹⁸⁸
OCT4	Stem cell identity	-	+	Mouse ES ¹⁵⁷
SCGB3A1	Secretoglobin 3A1	-	+	NSCLC ²⁶
TGF α	Growth Factor	-	+	RCC ^{28, 189}
CCND1	Cell cycle progression	-	+	RCC ²⁸
DLL4	NOTCH signaling, EC branching	-	+	Mouse ECS ⁵³
ANG2	Blood vessel remodeling	-	+	Mouse ECS ⁵³

Table 3
Mouse models testing altered expression of HIF α proteins in tumour growth and progression

Tumour or cell type	HIF1 α status	HIF2 α status	Phenotypes	Refs
<i>Xenograft tumours</i>				
Teratoma	Loss-of-function knockout	Wild-type	Reduced growth and angiogenesis	37,190
Teratoma	Wild-type	Loss-of-function knockout	Increased growth	25
Fibrosarcoma	Loss-of-function knockout	Wild-type	Reduced growth	191
RCC	Gain of function	Wild-type	Reduced growth	23,28
RCC	Wild-type	Gain of function	Increased growth	28,192
<i>Autochthonous tumours</i>				
MMIV-PyMT mammary tumours	Conditional knockout	Wild-type	Reduced metastasis	43
KRAS-driven NSCLC	Conditional knockout	Wild-type	No effect	26
KRAS-driven NSCLC	Wild-type	Conditional knockout	Increased tumour burden and progression	26
p53-driven thymic lymphoma	Heterozygous germline knockout	Wild-type	Decreased tumour incidence	156
<i>Tumour-associated stromal cells</i>				
Tumour-associated macrophages	Conditional knockout	Wild-type	Reduced NO production. increased T cell-mediated tumour immunosurveillance and reduced autochthonous mammary tumour growth	48,49
Tumour-associated macrophages	Wild-type	Conditional knockout	Decreased macrophage infiltration into autochthonous liver and colon tumours and decreased tumour growth	50
Vascular ECs	Conditional knockout	Wild-type	Decreased xenograft tumour angiogenesis and growth	51
Vascular ECs	Wild-type	Conditional knockout	Non-productive angiogenic sprouting and impaired vessel remodelling	53

EC, endothelial cell; MMTV, mouse mammary tumour virus; NO, nitric oxide; NSCLC, non-small-cell lung cancer; PyMT, polyoma middle T antigen; RCC, renal cell carcinoma.