The Role of Hypoxia-Inducible Factors in Cancer Resistance

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Abstract

Diminished oxygen availability (hypoxia) is a hallmark of the tumor microenvironment. A major regulator of cellular adaptation to hypoxia is the hypoxia-inducible factor (HIF) family of transcription factors, which play key roles in many crucial aspects of cancer biology including angiogenesis, stem cell maintenance, metabolic reprogramming, resistance to apoptosis, autocrine growth factor signaling, the EMT program, invasion and metastasis.

Resistance to chemotherapy/radiotherapy is the primary cause for treatment failure in clinical oncology. Hypoxia and accumulation of hypoxia-inducible factors (HIFs) in solid tumors have been associated with resistance to treatment and poor prognosis. HIFs causes autophagy establishment to promote survival of cancer cells, and is also associated with the promotion and maintenance of cancer stem cells, a minority subpopulation within the tumor responsible for tumor recurrence and resistance to chemotherapy.

In this review, we provide a concise comparative description of the structure, regulation, transcribed genes and roles played by each HIFα subunit in coordinating the transcriptional responses to hypoxia and on the roles they play in the promotion of resistance to anti-cancer therapy.

Keywords: Hypoxia; Hypoxia-inducible factors; Cancer resistance; HIF1α; HIF2α; HIF3α; chemotherapy-resistance

Introduction

Hypoxia is a fundamental physiological stimulus that induces adaptive responses to maintain a homeostatic state. It was defined as a reduction in O₂ availability in one condition compared with another at different spatial or temporal conditions. For that reason, a constant O₂ supply, maintained by the vascular system in mammals, is critical for proper tissue development, homeostasis, and function [1].

Another condition that causes localized hypoxia is the rapid cellular division during embryonic development because hypoxia is not always a pathological entity; there is increasing evidence that it is an important component of some cellular niches, particularly those of stem and progenitor cells [2]. In addition, an abnormal condition such as the rapid tumor growth induces O₂ deprivation in intratumoral regions [3].

Hypoxia-inducible factors (HIFs) are a family of mammalian transcription factors that regulate the expression of a wide array of hypoxia-inducible genes to deal with physiologically O₂ changing concentrations. They have been characterized as α/β heterodimers of basic-helix-loop-helix DNA binding proteins of the PER-ARNT-SIM family (bHLH-PAS), in which the β-subunit is constitutive expressed and the α-subunit is regulated by oxygen levels. The genes regulated by HIFs are involved in the cellular adaptive response to hypoxia including erythropoiesis, apoptosis, angiogenesis, proliferation, and also in tumorigenesis [4,5]. In this respect, it has been demonstrated that HIFs regulate multiple steps of tumorigenesis and are typically associated in cancer cells with changes in metabolic reprogramming, neo-vascularization, invasion, metastasis, autophagy induction, drug resistance, and poor clinical outcomes [6].

Until now, three oxygen-dependent different alpha subunits encoded by different genes have been reported in human and other vertebrate species: HIF1α, first described by Semenza and colleagues in 1992 [7], HIF2α described by several groups in 1997, and HIF3α, discovered in mouse by Gu et al. in 1998 [8] and in humans by Makino et al. [9]. But while it has been clearly established that HIF1α and HIF2α function as master regulators of the response to hypoxia and are critical regulators of tumorigenesis, the roles played by HIF3α under hypoxia and in cancer biology are far less clear. The reasons of this reside not only on the initial discovery of a large array of HIF3α variants which has posed enormous challenges to study HIF3α-mediated physiological roles, but mainly on the prevailing view of HIF3α as a negative regulator of HIF1α and HIF2α actions, on the basis of the initial finding that two of its variants, lacking transactivation domains, act as negative regulators of HIF1α and HIF2α transactivating gene functions [9-11]. However, this dogma was refuted in 2014 by Zhang et al. [12], which demonstrated that HIF3α functions as a transcriptional activator in zebrafish embryos. They showed that under hypoxia, HIF3α stabilizes and binds to HREs in the promoters of its target genes and upregulates their expression. When tested in human cells, these authors found that both human HIF3α-9 and zebrafish Hif-3α were capable of upregulating target gene expression, suggesting that the function of this transcription factor is evolutionarily conserved, and providing unequivocal evidence that Hif-3α functions as an oxygen-dependent transcriptional activator in vivo.

The HIF1β subunits, also known as aryl hydrocarbon receptor nuclear translocators (ARNTs), are encoded by two genes ARNT1 and ARNT2. Under conditions of normal oxygen tension, the alpha subunits are hydroxylated at key proline and asparagine residues, which inhibits their transactivation function and targets them for proteasomal degradation. Upon hypoxia, the HIF alpha subunits are stabilized and accumulate in the nucleus, where they dimerize with HIF1β, allowing them to bind to DNA and stimulate the transcription of their target genes [13]. However, multiple oxygen-independent mechanisms can also...
The mechanisms underlying these oxygen-independent roles of HIFs, however, are not well understood. In this review, we provide a concise comparative description of the structure, regulation, transcribed genes and roles played by each HIFα subunit in coordinating the transcriptional responses to hypoxia and on the roles they play in the promotion of resistance to anti-cancer therapy.

**Protein Structure of the Oxygen-Labile Alpha Subunits**

The HIF1α, HIF2α and HIF3α subunits conserve similarities in their protein structure, since they have several well conserved domains. As shown in Figure 1, all three subunits contain a basic helix-loop-helix (bHLH) domain that is necessary for DNA binding at a consensus hexanucleotide E box [18,19].

They also contain two repeats of Per-Arnt-Sim (PAS) domain, derived its name from the proteins found containing it: Drosophila Period (Per), the human aryl hydrocarbon receptor nuclear translocator (ARNT), which has a bHLH domain, PAS-A and PAS-B domains, and only one domain at the C-terminal region of the protein. HIF3α lacks C-terminal transactivation domain and instead contains LZIP domain. HIF1α and HIF2α have nuclear localization signals (NLS). HIF1β is the aryl hydrocarbon receptor nuclear translocator (ARNT), which has a bHLH domain, PAS-A and PAS-B domains, and only one C-terminal transactivation domain. Modified from Pasanen et al. and Maynard et al.

![Figure 1: Hypoxia-Inducible factors.](image-url)

**DNA binding**

Dimerization degradation transactivation

HIF-1α bHLH PAS-A PAS-B PAC ODD N-TAD C-TAD 826 aa

HIF-2α bHLH PAS-A PAS-B PAC ODD N-TAD C-TAD 870 aa

HIF-3α bHLH PAS-A PAS-B PAC ODD N-TAD LZIP 667 aa

ARNT/HIF-1β bHLH PAS-A PAS-B C-TAD 789 aa

Figure 1: Hypoxia-Inducible factors. Structural alignment of HIF1α, HIF2α and HIF3α subunits. All of them contain a basic helix-loop-helix (bHLH) and two Per-ARNT-Sim (PAS-A and PAS-B) domains that mediate DNA binding and dimerization, respectively. They also contain a PAS-associated C-terminal (PAC) domain, and an oxygen-dependent degradation domain (ODD) that is required for oxygen-dependent hydroxylation and degradation under normoxia conditions. The transactivation domains (N- and C-Terminal TADs) are responsible for the transcriptional activity of HIF1α and of HIF2α. N-TAD domain is located within the ODD domain and C-TAD domain at the C-terminal region of the protein. HIF3α lacks C-terminal transactivation domain and instead contains LZIP domain. HIF1α and HIF2α have nuclear localization signals (NLS). HIF1β is the aryl hydrocarbon receptor nuclear translocator (ARNT), which has a bHLH domain, PAS-A and PAS-B domains, and only one C-terminal transactivation domain. Modified from Pasanen et al. and Maynard et al.
Transcriptional and Post-transcriptional Regulation of Alpha Subunits

A salient feature in both HIF2α and HIF3α subunits regulation is that, in addition to altering protein stability, hypoxia also increases their levels by inducing their gene transcription. This mechanism distinguishes the HIF1α gene from the HIF2α and HIF3α genes. In this respect, Lin et al. [34] reported that transcription of HIF1α and HIF2α is differentially regulated under hypoxia in neuroblastoma cell lines. They found that while transcription of HIF1α was consistently repressed by both acute and chronic hypoxia, transcription of HIF2α was consistently upregulated under the same hypoxic conditions. Their observations thus suggest that expression of HIF1α and HIF2α is differentially regulated in the hypoxic tumor microenvironment, although the underlying mechanisms remain to be fully investigated.

In the case of HIF3α, there is also experimental evidence that hypoxia induces HIF3α gene transcription and has been documented in human cells, mice, rats, and zebrafish. Interestingly, the hypoxic induction of HIF3α gene expression is mediated by HIF1α and HIF2α in a tissue-specific fashion [35]. The hypoxic induction of HIF3α gene expression may be particularly important for the HIF3α variants lacking an ODD domain.

Post-Translational Modifications

The HIF alpha subunit is subjected to posttranslational modifications, whose affect its stability and its gene transactivating functions. The oxygen-dependent stability and activity of the HIFα subunits are traditionally associated with post-translational modifications such as hydroxylation, acetylation, ubiquitination, and phosphorylation. The most characterized of them are the hydroxylations, which are catalyzed by 2-oxoglutarate and iron (Fe²⁺)-dependent family of dioxygenases that employ molecular oxygen as co-substrate and ascorbate as cofactor, and are called prolyl hydroxylases (PDHs) [36,37]. Proline hydroxylation allows binding of the von Hippel-Lindau (VHL) tumor suppressor protein, which in conjunction with elongins B and C functions as an E3 ubiquitin ligase complex, which poly-ubiquitinitates the HIFα subunits targeting them for proteasomal degradation.

The HIF1α subunit has two specific proline residues at its ODD domain that are hydroxylated: the proline 564 [38,39] and the proline 402 [36]. These both sites are necessary for optimal targeting of the HIF1α to the proteasome and the hydroxylation order is important since Pro564 controls the efficient hydroxylation of proline 402 [40]. The HIF2α subunit is also hydroxylated by PHDs at the ODD domain, but in different positions of proline residues: Pro405 and Pro531. In the case of HIF3α, it has been demonstrated that pVHL recognizes the hydroxylated Pro490 at the ODD domain of HIF3α. So at least, one site of this α-subunit has been demonstrated to be subject to regulation by oxygen levels [26].

Whereas PHDs mediate the hydroxylation of conserved ODD domain prolyl residues, the activity of the C-TAD of HIF1α and HIF2α is additionally regulated by hydroxylation of a single conserved asparaginyl residue (in HIF1α: Asn803 and in HIF2α: Asn851) to prevent its physical interaction with the transcriptional coactivator.
CBP/p300, resulting in silencing of HIFs transcriptional ability. This hydroxylation is catalyzed by another member of the 2-oxoglutarate and iron-dependent family of dioxygenases, called factor-inhibiting HIF (FIH) [41]. Despite FIH and PDHs are members of the same dioxygenases family, the estimated Km values of FIH-1 and the PHDs for molecular oxygen are different [42]. FIH has a lower Km for oxygen than the PHDs, meaning that the PHDs would be inactivated first, while FIH-1 would require more severe hypoxia to lose activity. FIH-1 can still exert a catalytic effect at 0.2% oxygen concentration, whereas the PHDs are inactive under the same conditions [43]. Nevertheless, the oxygen-dependent sensitivity of FIH-1 apparently fluctuates between different cell types independently of the PHDs [44]. Another interesting data is that the FIH action appears to require the association with VHL [41]. Since HIF3α lacks the C-TAD, the FIH-mediated hydroxylation mechanism is unlikely involved in HIF3α regulation.

Under hypoxic conditions, the PHD enzyme cannot hydroxylate HIF, and therefore HIFs are not recognized by pVHL. As mentioned before, In addition to oxygen, PHDs require Fe²⁺, 2-oxoglutarate, and ascorbate for prolyl-hydroxylase activity. Thus, their action can enhances HIF2α transcriptional activity [45]. HIF2α protein levels and enhances target gene activation [55,56]. In addition, it has also been reported that ERK1 directly phosphorylates the ODD domain of HIF1α, and it also has a different sequence after the ODD/N-TAD domains, but lacks the LZIP domain and the second LXXLL motif. HIF1α-3 lacks the bHLH and LZIP domains. HIF3α-4, which presents 636 aminoacid residues, is similar to the mouse IPAS in structure and it has the bHLH, PAS-A, and PAS-B domain, but lacks the LZIP domain and the second LXXLL motif. HIF1β, decreasing HIF1α -induced gene expression [51,52]. Glycogen synthase -3β (GSK-3β), which translocate into the VHL, recruit coactivators such as P300/CBP, and bind to hypoxia response elements (HRE: [A/G]CGTG) in the promoter regions of the target genes [47].

Phosphorylation of HIF1α and HIF2α subunits has been demonstrated to enhance transactivation of target genes by either disrupting interaction with VHL and thereby stabilizing HIFs, or by increasing the affinity of HIFs for transcriptional coactivators [13]. In this regard, it has been reported that caskin kinase II phosphorylates both HIF1α and HIF2α at conserved theonine residues in their C-TADs and that the mutation of these residues diminished their activity [48,49]. In addition, it has also been reported that ERIK1 directly phosphorylates the C-terminal domain of HIF1α (at Ser-641 and Ser-643) and that these modifications mask a nuclear export signal leading to increased nuclear accumulation and transcriptional activity [50]. There are also examples in which the phosphorylation of HIFs decreases HIF stability or activity. For example, the phosphorylation of HIF1α within the PAS-B domain at Ser-247 by caskin kinase 1, inhibits its association with HIF1β, decreasing HIF1α -induced gene expression [51,52]. Glycogen synthase -3β (GSK-3β) phosphorylates HIF1α at several serine residues within its ODD domain inducing its degradation via proteasome in a VHL-independent manner [53].

HIFs are also regulated by lysine acetylation both positively and negatively, depending on the location of the modified lysine. Acetylation of amino-terminal HIF1α lysines negatively affects HIF1α stability and impairs activation of its target genes [54]. However, the acetylation of lysines located at the carboxy-terminal region of HIF1α increases HIF1α protein levels and enhances target gene activation [55,56]. In addition, it has been reported opposing effects of SIRT1 on HIF1α and HIF2α transcriptional activities: whereas SIRT1 forms a complex with HIF2α and deacetylates conserved lysine residues in the N-TAD which enhances HIF2α transcriptional activity in vitro and in vivo, SIRT1 was reported to deacetylate lysine residues in HIF1α resulting in HIF1α transcriptional repression [57].

**Post-Transcriptional Modification by Alternative Splicing**

The HIF3α gene has been found subjected to complex regulation producing a large number of mRNA variants due to the utilization of different promoters, different transcription initiation sites, and alternative splicing in mammals. However, as pointed by Duan C in 2016 [35], the existence of multiple variants may not be unique to the HIF3α gene or restricted to vertebrates, since human HIF1α and HIF2α genes can both give rise to several variants by the same mechanisms. According to NCBI and Ensemble databases, there may be several different human HIF1 and HIF2 transcripts, which have not been explored.

The human HIF3α gene has 10 predicted variants, as depicted in Figure 2. The gene spans 43 kb and contains 17 exons. It has 3 different promoters so HIF3α can be transcribed with an amino-terminal a, b or c type. Furthermore, different mRNAs can be produced by alternative splicing [26], as shown in Figure 2. The HIF3α-1 contains the NH₂-terminal type c, and presents the exons 1 to 17, so once translated, possesses 667 aminoacid residues. HIF3α-9 contains 669 residues and differs from HIF3α-1 only by several amino acids of the very NH₂-terminal end. This HIF3α-9 is considered as full-length canonical protein because it has all of the six characteristic domains. HIF3α-2 has 632 residues and contains the bHLH, PAS-A and -B, PAS, ODD, and N-TAD domains, but lacks the LZIP domain and the second LXXLL motif. HIF3α-3 lacks the bHLH and LZIP domains. HIF3α-4, which presents 636 aminoacid residues, is similar to the mouse IPAS in structure and it has the bHLH, PAS-A, and PAS-B domain, but lacks the ODD and LZIP domains. HIF3α-5 only has the PAS-A, PAS-B, and PAS domains. HIF3α-6 should present 237 residues and was thought to be an artifact. HIF3α-7, with 607 aminoacid residues, lacks the bHLH domain and it also has a different sequence after the ODD/N-TAD domain due to the utilization of a unique exon 15. HIF3α-8 lacks only the bHLH domain and has 613 aminoacid residues. HIF3α-10 retains only the intron 1, so it encodes a 7 aminoacid peptide [26,58,59].

The HIF3α isoforms are often expressed in different tissues, at different developmental stages, and are differentially regulated. They have distinct or even opposite functions when tested by overexpression approaches [35]. For instance, while the full-length human HIF3α-1 can stimulate HRE-dependent reporter construct activity and up-regulate unique target genes [8,12], human HIF3α-4 isoform, a shorter isoform that lacks the TAD domain, inhibits the activity of HIF-1α and HIF-2α [11,60] in a similar manner as mouse IPAS was shown to inhibit HIF-1α activity [9].

Recently, Zhang et al. [17] have identified a novel zebrafish Hif3α spliced variant, termed Hif3α-2 isoform, as an oxygen-insensitive nuclear protein. Despite its lack of the bHLH and PAS domains, Hif3α-2 has HRE-dependent transcriptional activity. They investigated the in vivo role of Hif3α-2 using transgenesis and CRISPR/Cas9-mediated gene editing and showed that Hif3α-2 inhibits canonical Wnt signaling by binding to β-catenin and destabilizing the nuclear β-catenin complex, in a manner independent of its HRE-dependent transcriptional activity [17].

**Genes Regulated By Hif Alpha Subunits**

The three HIFα subunits are regulated in a similar fashion by hypoxia, bind to HIF and to same HREs, and share many overlapping genes and functions. However, HIFs are non-redundant and regulate both overlapping and unique downstream target genes as it can be seen
The genes regulated by each HIFα subunit, and the reported functions for each one are shown in (Tables 1-3). The reported genes regulated by both HIF1α and HIF2α, or by both HIF1α and HIF3α, are shown in (Tables 4 and 5), respectively. Although a highly structural homology exists between HIF1α and HIF2α, these transcription factors may also be differently regulated and transactivate common and unique target gene products in a cancer cell-dependent manner under normoxic and hypoxic conditions. As it can be seen in (Tables 1 and 2), in general, HIF1α may specifically induce the enhanced expression of glycolytic enzymes such as hexokinase-2, aldolase A, phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M (PKM), whereas HIF2α appears to preferentially up-regulate the gene products including TGF-α, cyclin D1 and embryonic stem cell-like markers such as OCT4, SOX2, and NANOG.

### Table 1: Representative genes only regulated by HIF1α.

<table>
<thead>
<tr>
<th>GENE</th>
<th>CELL FUNCTION</th>
<th>CELL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARG1</td>
<td>Inhibitor of NO production</td>
<td>Macrophages</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoiesis</td>
<td>Kidney, liver</td>
</tr>
<tr>
<td>OCT4</td>
<td>pluripotency</td>
<td>Mouse ES, hESC</td>
</tr>
<tr>
<td>SCGB3A1</td>
<td>Secretoglobin 3A1</td>
<td>NSCLC</td>
</tr>
<tr>
<td>TGfα</td>
<td>Growth Factor</td>
<td>RCC, HCC (HepG2, Huh7 cells)</td>
</tr>
<tr>
<td>CCND1</td>
<td>Cell cycle progression</td>
<td>RCC, HCC (HepG2 cells)</td>
</tr>
<tr>
<td>DLL4</td>
<td>NOTCH signaling, EC branching</td>
<td>Mouse ECS</td>
</tr>
<tr>
<td>ANG2</td>
<td>Blood vessel remodeling</td>
<td>Mouse ECS</td>
</tr>
<tr>
<td>NANO2</td>
<td>pluripotency</td>
<td>hESC</td>
</tr>
<tr>
<td>SOX2</td>
<td>pluripotency</td>
<td>hESC</td>
</tr>
<tr>
<td>MMP9</td>
<td>invasion</td>
<td>RCC</td>
</tr>
<tr>
<td>PAI-1</td>
<td>fibrinolysis</td>
<td>RCC, LN229</td>
</tr>
<tr>
<td>uPAR</td>
<td>invasion</td>
<td>RCC</td>
</tr>
<tr>
<td>DMT-1</td>
<td>iron metabolism and Lysosome</td>
<td>Mouse intestine</td>
</tr>
<tr>
<td>FBN1</td>
<td>iron transporter</td>
<td>Mouse intestine</td>
</tr>
</tbody>
</table>

### Table 2: Representative genes only regulated By HIF 2α.

ARG1: Arginase 1; EPO: Erythropoietin; OCT4: Octamer-Binding Protein 4; SCGB3A1: Secretoglobin Family 3A Member 1; TGfα: Transforming Growth Factor Alpha; CCND1: Cyclin D1; DLL4: Delta Like Canonical Notch Ligand 4; ANG2: Angiopoietin 2; NANO2 (Homeobox Transcription Factor Nanog); SOX2: SRY-Related HMG-Box Gene 2; MMP9: matrix metalloproteinases-9; PAI-1: plasminogen activator inhibitor-1; uPAR: urokinase-type plasminogen activator receptor; DMT-1: (SLC11A2: Solute Carrier Family 11 Member 2); FBN1: Fibrillin 1; hESC: human embryonic stem cell.

HK-II: Hexokinase 2; PFK1: Phosphofructokinase; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; PGK1: Phosphoglycerate Kinase 1; PGM1: Phosphoglyceromutase 1; Enolase 1 (C-Myc Promoter-Binding Protein); LDHA: Lactate Dehydrogenase A; HK1: Hexokinase 1; HK2: Hexokinase 2; PKM: Pyruvate kinase M; ALKBH5: AlkB Homolog 5 (RNA Demethylase); L1CAM: L1 Cell Adhesion Molecule; RHODA: Ras Homolog Family Member A; ROCK1: Rho Associated Coiled-Coil Containing Protein Kinase 1.
## Table 3: Genes only regulated by HIF 3α.

<table>
<thead>
<tr>
<th>GENE</th>
<th>CELL FUNCTION</th>
<th>CELL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDRG-1</td>
<td>Stress responses, hormone responses, cell growth, and differentiation</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>DMXL-1</td>
<td>Regulatory functions</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transport</td>
<td>RCC [91, 92, 93]</td>
</tr>
<tr>
<td>ADRP</td>
<td>Lipid metabolism</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>CAXII</td>
<td>pH homeostasis</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>FILAG</td>
<td>Cytoskeletal structure</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Immune cytokine</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>ADM1</td>
<td>Angiogenesis</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>VEGF</td>
<td>Angiogenesis</td>
<td>RCC [91]</td>
</tr>
<tr>
<td>LOX</td>
<td>Cross-linking of extracellular matrix</td>
<td>HEAK293T [114]</td>
</tr>
<tr>
<td>PFKFB4</td>
<td>Metabolism</td>
<td>HEAK293T [114]</td>
</tr>
<tr>
<td>BNIP3</td>
<td>Autophagy, apoptosis</td>
<td>HEAK293T, RCC [112, 12]</td>
</tr>
<tr>
<td>RAB20</td>
<td>Autophagy</td>
<td>HEAK293T [112]</td>
</tr>
<tr>
<td>ANGPTL4</td>
<td>Erythropoiesis</td>
<td>MDA-MB-231 cells [91]</td>
</tr>
<tr>
<td>HIF3α</td>
<td>Transcription factor</td>
<td>RCC [91]</td>
</tr>
</tbody>
</table>

## Table 4: Representative genes regulated by both HIF-1α and HIF-2α.

<table>
<thead>
<tr>
<th>GENE</th>
<th>CELL FUNCTION</th>
<th>CELL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>myhα2*</td>
<td>Cytoskeleton organization</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>susd5</td>
<td>Hyaluronic acid binding</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>atxn1b*</td>
<td>Involved in transcriptional repression and to regulate developmental processes.</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>mplc3*</td>
<td>Autophagy</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>zgc:153723</td>
<td>Sulfoxidation activity</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>zgc:153126*</td>
<td>Cytoskeleton organization</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>fmn2b</td>
<td>Segmentation, central nervous system</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>htra1a</td>
<td>Development</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>wu:fc3e06</td>
<td></td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>wsb1*</td>
<td>Probable substrate-recognition component of a SCF-like ECS (elongin-Cullin-SOCS-box protein) E3 ubiquitin ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>casp62</td>
<td>Cysteine-type endopeptidase activity</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>coxβ2</td>
<td>Cytoskeleton-c oxide activity</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>s2ca1b</td>
<td>D-glucose transmembrane transporter activity; sprouting angiogenesis</td>
<td>Zebrash [15]</td>
</tr>
</tbody>
</table>

**Myhα2**: myosin, heavy polypeptide 2*; susd5: Sushi Domain Containing 5; attn1b: Spincocerebellar Ataxia Type 1 Protein*; mplc3: Microtubule Associated Protein 1 Light Chain 3 Gamma* (ortholog of the yeast autophagosome protein Atg8); zgc:153723 (sulfoxidation enzyme family 5A, member 1); zgc:153126 (Formin Homology 2 domain containing 3*); fmn2b: formin 2b; htra1a: HtrA serine peptidase 1a; wu:fc3e06 (integral component of membrane: EST); wsb1: WD Repeat And SOCS Box Containing 1*; casp62: caspase 6, apoptosis-related cysteine peptidase, like 2; zgc:162126 (ppr15α: protein phosphatase 1, regulatory subunit 15A); coxβ2: cytochrome c oxidase subunit Vb 2; s2ca1b: solute carrier family 2 (facilitated glucose transporter), member 1b. (*) Exist a human ortholog gene.

## Table 5: Representatives genes regulated by both HIF-3α and HIF-1α.

<table>
<thead>
<tr>
<th>GENE</th>
<th>CELL FUNCTION</th>
<th>CELL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>REDD1</td>
<td>Involved in mTOR pathway</td>
<td>HEK293 cells [10]</td>
</tr>
<tr>
<td>LC3c</td>
<td>Autophagy</td>
<td>HEK293 cells [10]</td>
</tr>
<tr>
<td>SQDRL</td>
<td>Metabolism</td>
<td>HEK293 cells [10]</td>
</tr>
<tr>
<td>zp3v2</td>
<td>Embryogenesis</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>isg15*</td>
<td>Chemotactic activity towards neutrophils, direction of ligated target proteins to intermediates, cell-to-cell signaling, and anti viral activity during viral infections</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>sqd8</td>
<td>Metabolism: catalyze the conversion of sulfide to persulfides, thereby decreasing toxic concentrations of sulfide</td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>EH546362</td>
<td></td>
<td>Zebrash [15]</td>
</tr>
<tr>
<td>gcnt7*</td>
<td>Metabolism and O-linked glycosylation</td>
<td>Zebrash [15]</td>
</tr>
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<td>mchtb*</td>
<td>BCL2 family Apoptosis Regulator</td>
<td>Zebrash [15]</td>
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<td>casp8*</td>
<td>Signaling pathways of apoptosis, necrosis and inflammation</td>
<td>Zebrash [15]</td>
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**REDD1**: DNA Damage Inducible Transcript 4; **LC3c**: (Autophagy-Related Protein LC3 C); **SQDRL**: Sulfide Quinone Reductase-Like (Yeast); **zp3v2**: zona pellucida glycoprotein 3d tandem duplicate 2; **isg15**: Interferon-Stimulated Protein, 15 KDa.; **sqd8**: Sulfide Quinone Reductase-Like; **EH546362** (cystatin 14b, tandem duplicate 2); **gcnt7**: Glucosaminyl (N-Acetyl) Transferase Family Member 7*; **mchtb**: myeloid cell leukemia 1b*; **casp8**: Caspase 8, Apoptosis-Related Cysteine Peptidase*; (*) Exist a human ortholog gene.
as Oct-3/4, Sox-2 and/or Nanog in cancer cells under normoxic and hypoxic conditions. Expression of HIF3α-regulated genes are involved in nitrogen metabolism, methane metabolism, the Jak-STAT signaling pathway, and NOD-like receptor signaling. Interestingly, genes involved in erythropoiesis an angiogenesis induction are regulated by both HIF1α and HIF2α, but not by HIF3α subunit.

The Involvement of HIFs in Cancer Treatment Resistance

The inherent or developed resistance of many cancer cells to chemotherapy, targeted types of therapy, and irradiation, is the primary cause for treatment failure in clinical oncology. This problem represents a complex and multifactorial phenomenon related to tumor microenvironment, such as hypoxia, acidosis, nutrient starvation and inflammation [61]. HIFs overexpression is associated with therapeutic resistance or decreased survival in many cancer types and it has been demonstrated that they contribute to resistance via multiple mechanisms. Some of these mechanisms are well documented, such as the induction of efflux pumps expression, apoptosis inhibition, cell survival promotion, autophagy induction, stemness promotion and also inducing epigenetic changes to regulate gene expression in cancer cells.

HIFs Induce Chemoresistance by Efflux Pump Expression

Prevention of toxin absorption by efflux pump mechanisms is very effective and important to induce chemical resistance in cells. Several studies in different cancer cell types have demonstrated that the multidrug resistance gene (MDR1), encoding the transmembrane P-glycoprotein (Pgp), which belongs to the ATP-binding cassette superfamily of transport proteins, is induced by hypoxia [62-64]. Consistent with this, HIF1α expression inhibition by antisense oligonucleotides resulted in significant hypoxia-inducible MDR1 expression suppression and a nearly complete loss of basal MDR1 expression. Ding et al. as it has been observed the relationship between HIF-1α and MDR1/P-glycoprotein in human colon carcinoma tissues. The expression of both HIF1α protein and P-gp were significantly higher in tissue samples classified as Duke's stages C or D, involving lymph node metastasis, than in samples classified as Duke's stages A or B, indicating that HIF-1α was involved in tumor invasion and metastasis. They also used cultured human colon carcinoma cells (HCT-116, HT-29, LoVo, and SW480), and observed that HIF-1α expression was significantly associated with MDR1/P-gp expression in human colon carcinoma cells as well [65]. In addition, the expression of the multidrug resistance-associated protein 1 (MRP1), another ABC transporter, is also induced by hypoxia, and have been reported in the majority of brain tumors, including glioblastomas [66].

HIF-Mediated Survival after Ionizing Radiation Therapy

Ionizing radiation, such as that used in radiotherapy, kills cells by producing DNA damage, particularly DNA double strand breaks. This damage results from ionizations in or very close to the DNA that produces a radical on the DNA. It has been demonstrated that the more hypoxic tumors are more radio-resistant than the less hypoxic tumors [67].

Hypoxic tumor cells are more resistant to radiotherapy as a consequence of the interference of hypoxia with the fixation of free radical-induced DNA damage. HIF1α pathway is involved in the tumor-protective response to radiotherapy both via vascular protection post-irradiation, and via enhancing the tumor antioxidant capacity through initiating a glycolytic tumor metabolism. Targeting HIF1α and tumor glucose metabolism affects the tumor microenvironment, induces metabolic alterations, and sensitizes various solid tumors to irradiation (reviewed by Meijer et al. [68]). HIF2α also contributes to tumor cell survival after ionizing radiation treatment by reducing ROS levels generated from normal metabolic processes as well as irradiation treatment, promoting tumor cell survival. HIF2α deficiency promotes p53 phosphorylation in ccRCC and lung carcinoma cells in culture, which enhances ccRCC cell death by disrupting cellular redox balance, thereby promoting DNA damage. HIF2α also affected p53 in the absence of irradiation, suggesting that HIF2α inhibition may be effective alone or in combination with therapies other than radiation [69].

HIFs and Cell Death Pathways: Apoptosis and Autophagy

In the vast majority of transformed cells, HIF1α functions as a robust suppressor of apoptosis and functional interference with HIF1α results in enhanced cell death upon treatment with chemotherapeutic agents in tumors of different origins [70]. The molecular nature of this phenomenon was mostly explained by HIF1α anti-apoptotic target gene induction (Bak, Bax, Bcl-xl, Bcl-2, Bid, Mcl-1, NF-xB, p53 and survivin), but also by suppression of p53 activation in response to chemotherapeutic agents [71], as it can be seen in Figure 3. HIF2α was recently shown to act in a similar manner, namely suppressing p53 activation and apoptosis in response to radiation-induced DNA damage in clear cell kidney cancer cells [69]. Interestingly, hypoxia selects for tumor cells that have lost sensitivity to p53-mediated apoptosis or that are deficient in DNA mismatch repair resulting in apoptosis resistance and genomic instability [72,73].

Besides apoptosis, the process of autophagy is increasingly recognized as an important regulator of cellular viability under stressful conditions. Autophagy is a highly conserved catabolic process whereby long-lived or damaged proteins and organelles are engulfed in double-membrane structures called autophagosomes and targeted to the lysosomes for degradation for energy production. In addition, autophagy plays other key cellular functions such as adaptation to nutrient depletion, extension of lifespan, cellular development and anti-aging [74].

Autophagy seems to play a role at multiple levels of tumor development and it has been reported that have a protective role in carcinogenesis. Autophagy is also a consequence of cytotoxic drug treatment, and more recently, has been appreciated as a means by which cells might survive the stress of cellular insults, and so become resistant to treatment.

Enhanced autophagy has been associated with the elevated level of HIF1α in several cancer types. In this regard, it has been observed that hypoxia-mediated failure of cytotoxic treatment in vitro can be conferred via HIF1α-dependent induction of autophagy [75]. This process is mediated by the atypical BH3-only proteins such as the Bcl-2/E1B 19 kDa-interacting protein 3 (BNIP3/BINP3L (NIX)) that are induced by HIF1α [74]. These mitochondrial associated BNIP proteins also mediate mitophagy, a metabolic adaptation for survival that is able to control reactive oxygen species (ROS) production and DNA damage [74]. However, it has been established that cells lacking expression of two HIF isofoms, HIF1α or HIF2α, elicited a lower autophagic response under hypoxic conditions. Therefore, HIF1α and HIF2α appear to be evenly matched in their capacity to induce autophagy.
HIFs Promote Stemness and Cancer Aggressiveness

A growing body of experimental evidence has indicated that cancer-and metastasis-initiating cells with stem cell-like features typically display a higher resistance than the bulk mass of differentiated cancer cells to radiation therapy and chemotherapy, and thereby they can be responsible for disease relapse [76,77]. Cancer stem cells (CSC) are a small subpopulation of cells within various tumor types, which have the dual properties of self-renewal and differentiation by giving rise to both daughter cancer stem cells and bulk (non-stem) cancer cells. It has been demonstrated that CSCs are involved in initiating and sustaining tumor growth in many cancer types.

Hypoxia is associated with the promotion and maintenance of cancer stem cells. Many recent studies have shown that hypoxia inhibits differentiation of embryonic stem cells, progenitor cells [78], and mesenchymal stem/progenitor cells [79]. Under hypoxic conditions, tumor cells show increased clonogenic potential and Semenza’s group [77], demonstrated that the exposure of breast cancer cells to hypoxia increases the percentage of breast cancer stem cells, which are required for tumor initiation and metastasis. Importantly, they showed that this response is dependent on the activity of hypoxia-inducible factors (HIFs) which induce the increase in cancer stem cell markers expression. They found that NANOG, SOX2, and OCT4 expression increased in all human breast cancer cells they analyzed in response to chemotherapy or hypoxia [77].

Interestingly, it has been proposed that the switch from HIF1α to HIF2α-dependent signaling plays an important role in the promotion of stemness, aggressive tumor growth, and tumor progression [80,81]. Tumor cells are subjected to a range of oxygen tensions and experience periods of acute/intermittent hypoxia or chronic/prolonged hypoxia. The variability in hypoxic intensity and duration needs distinct sets of cellular responses appropriate for each condition. In this respect, HIF1α seems to have the dominant role in controlling responses to acute hypoxia, whereas HIF2α drives the response to chronic hypoxia [80,82]. Consistent with this, it has been reported that HIF2α drives tumor progression in renal carcinoma cells in which there is a gradual shift from HIF1α to HIF2α expression with increasing tumor grade [83]. HIF2α (but not HIF1α) has been shown to cooperate with a number of oncoproteins frequently deregulated in cancer such as c-Myc, epidermal growth factor receptor (EGFR), and K-Ras [84-86] and has been linked to increased tumor aggressiveness through the promotion of self-renewal and epithelial to mesenchymal transition (EMT). In addition, Koh et al. [80] identified the hypoxia-associated factor (HAF) as an E3 ubiquitin ligase that binds to and ubiquitinates HIF1α by an oxygen- and pVHL-independent mechanism, targeting HIF1α for proteasomal degradation. But interestingly, they found that HAF binding to HIF2α does not lead to its degradation but instead, increases HIF2α transactivating activity. Thus, HAF expression switches the hypoxia response of the cancer cell from HIF1α – to the HIF2α –dependent transcription of genes such as MMP9 and OCT-3/4.
They also showed that this switch by HAF promotes the cancer stem cell phenotype and invasion, resulting in highly aggressive tumors in vivo. As mention before, there is experimental evidence that whereas the transcription of HIF2α and HIF3α is consistently increased by hypoxia, transcription of HIF1α is not and can show variable levels of repression. Altogether, these data suggest an important role of HIF2α and HIF3α in the regulation of tumor progression under chronic hypoxia.

These interesting observations have lead to a new paradigm that tumor hypoxia may facilitate the emergence of malignant clones by maintaining cancer stem cells in their undifferentiated stem cell state. Thus, the complete eradication of the total mass of tumor cells, including hypoxic and normoxic cancer stem/progenitor cells and their differentiated progenies, by targeting the HIF signalling network might be crucial to improve current cancer therapies and prevent disease relapse.

**Hypoxia Induces Epigenetic Changes to Regulate Target Gene Expression**

There is experimental evidence that hypoxia induces epigenetic regulation of transcription of many HIF target genes, by different mechanisms that results in enhancement of expression of some target genes, or in the transcriptional repression of other target genes [87]. With respect to the mechanisms involved, it has been reported that both histone methylation and acetylation status change in the promoters of HIF target genes. Epigenetic changes that promote hypoxia-induced negative regulation of transcription can be produced by the interaction between Reptin and HIF-1α leading to recruitment of histone deacetylase 1 (HDAC1) to some HIF target genes [88].

Epigenetic changes that promote positive regulation of transcription can be mediated by HIF-dependent recruitment of co-activators such as p300/CBP histone acetyltransferases to interact with HIF at HIF target promoters, increasing transcription [87]. Enhancement of transcription can also occur by hypoxia-induced changes in the histone methylation status at promoters of hypoxia-inducible genes: oxygen deprivation activates JMJD1A and inhibits JARID1A histone demethylases, promoting respectively a decrease in H3K9me2, and an increase in H3K4me2 levels. Hypoxia also provokes an increase in H3K9me2 levels as a result of G9a up-regulation [89]. Mechanistically, JMJD1A is not essential for stem cell self-renewal but is crucial for tumor suppression, whereas H3K9 methyltransferase G9a is linked with tumor growth and poor diagnosis. In addition, JMJD1A and G9a differentially drive the expression of antiangiogenic factors. As apart from depositing H3K9me2, G9a and its partner protein GLP also form a complex and play a role in the maintenance of DNA methylation at specific loci [90], it is possible that the aberrant G9a-linked DNA methylation downregulates tumor suppressors and leads to malignancy, although its associated H3K9me2 could be erased by JMJD1A. Therefore, investigation of the levels of G9a and the patterns of its associated DNA methylation might create a new direction for the prognosis and treatment of carcinomas in humans [91-95].

**Concluding Remarks**

Diminished oxygen availability (hypoxia) is a hallmark of the tumor microenvironment. It is well known that cancer cells exhibit several biological properties called as "hallmarks of cancer" that they acquire during the multistep development of cancer. Tumor hypoxia is involved in each hallmark and enabling characteristic displayed by cancer cells.

Cellular adaptation to hypoxia is mediated by the HIF family of transcription factors: three oxygen-dependent different alpha subunits HIF1α, HIF2α and HIF3α and two oxygen-independent HIFβ subunits [96-105]. In contrast to the well-established importance of HIF1α and HIF2α in cancer biology, the functional significance of HIF3α is remarkably understudied. Models should be developed to accurately study the temporal regulation of HIF1α, HIF2α and HIF3α.

Enhanced expression and activation of hypoxia-inducible factors (HIFs) frequently occur in cancer cells during cancer progression and is associated with their acquisition of a more malignant behaviour, treatment resistance and poor outcome of cancer patients. Thus, targeting hypoxia is an exciting prospect to improve current anti-cancer therapy and prevent disease relapse [106-112].

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