Title: Molecular Responses to Hypoxia Inducible Factor-1α and beyond

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Title: Molecular Responses to Hypoxia Inducible Factor-1α and beyond.

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Abstract:

Cellular response to changes in oxygen tension during normal development or pathological processes is, in part, regulated by hypoxia-inducible factor (HIF), an oxygen sensitive transcription factor. HIF activity is primarily controlled through post-translational modifications and stabilization of HIF-1α and HIF-2α proteins, and is regulated by a number of cellular pathways involving both oxygen-dependent and oxygen-independent mechanisms. Stabilization of HIF-1α, in particular, activates transcription of genes that participate in key pathways in carcinogenesis, such as angiogenesis, dedifferentiation, and invasion. Since its discovery more than two decades ago, HIF-1α has become a hot topic in molecular research and has been implicated not only in disease pathology, but also in prognosis. In this review, we will focus on recent insights into HIF-1α regulation, function and gene expression. We will also discuss emerging data on HIFs involvement in cancer prognosis and therapeutic interventions.
Introduction

Oxygen (O₂) is an indispensible component of eukaryotic metabolic processes. When oxygen demand exceeds its cellular supply, cells and tissues often become hypoxic. Hypoxia is an important factor in the pathology of a number of human diseases, including cancer, diabetes, ageing, and stroke/ischemia (Melvin and Rocha, 2012; Semenza, 2012). Hypoxia can also lead to the production of oxygen radicals in a variety of experimental systems via electron attack of molecular oxygen in the inactive mitochondria (Favaro et al.; Kolamunne et al.; Selivanov et al.). The roles of these mitochondria-generated free radicals are especially important in hypoxia signaling pathways, which have important implications for cancer, inflammation and a variety of other diseases (Poyton et al., 2009).

Hypoxic regions can often be found in cancer tissue due to high cellular proliferation rate coupled with the development of abnormal vasculature. Solid tumors, for example, often become hypoxic because the normal tissue vasculature can only support tumor growth within a diameter of ~2 mm (Folkman, 1971). Cellular response to changes in oxygen tension during normal development or pathological processes is in part regulated by hypoxia-inducible factor (HIF). HIFs are DNA-binding transcription factors that mediate cellular responses to reduced oxygen availability through transcriptional activation of a multitude of genes that encode proteins needed for tissue O₂ delivery and energy metabolism (Elvidge et al., 2006; Manalo et al., 2005). HIFs are basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins that form heterodimers, composed of an oxygen liable α subunit (HIFα) and a stable β-subunit (HIFβ), also known
as aryl hydrocarbon nuclear translocator (ARNT)). Together, these subunits bind hypoxia-responsive elements (HREs), which are similar to Enhancer box (E-box) motifs and have the consensus sequence G/ACGTG. In humans there are three distinct HIFα isoforms: HIF-1α, encoded by $HIF1A$; HIF-2α, encoded by $EPAS1$, and HIF-3α, which is expressed by multiple HIF3α splice variants (Ke and Costa, 2006). HIF-1α, HIF-2α, and HIF-3α splice variants 1–3 possess an oxygen-dependent degradation domain (ODDD) and an N-terminal transactivation domain (NTAD), while HIF-1α and HIF-2α possess a C-terminal transactivation domain (CTAD) (Ke and Costa, 2006). HIF activity is primarily controlled through post-translational modifications and stabilization of HIF-1α and HIF-2α proteins. However, HIF-1α mRNA contains an internal ribosome entry site (IRES), the presence of which allows translation to be maintained under conditions that are inhibitory to cap-dependent translation, which occurs during hypoxia (Lang et al., 2002). In this review, we will focus on recent insights into HIF-1α regulation, function and gene expression. We will also discuss emerging data on HIFs involvement in cancer prognosis and therapeutic interventions.

Oxygen-dependent regulation of HIF signaling

2-OG-dependent dioxygenases

Under normal oxygen tension (normoxia) HIFα becomes hydroxylated on one or (both) of the two highly conserved proline residues within the ODDD domain by prolyl-hydroxylase domain (PHDs)-containing enzymes. Hydroxylated HIFα is then recognized by the β-domain of von Hippel-Lindau tumor suppressor protein (pVHL) and ubiquitinated by the Elongin BC/Cul2/pVHL (E3) ubiquitin–ligase complex. The
ubiquitination then marks HIF\(\alpha\) for proteasomal degradation (Greer et al., 2012; Ke and Costa, 2006). There are three HIF- prolyl hydroxylases known in mammals and they are encoded by separate genes: \(PHD1\), \(PHD2\), and \(PHD3\) (Myllyharju and Koivunen, 2013). Like all 2-OG-dependent dioxygenases, PHDs, require oxygen for hydroxylation, as well as, the tricarboxylic acid (TCA) cycle intermediate, 2-oxoglutarate (\(\alpha\)-ketoglutarate), iron (Fe\(^{2+}\)), and ascorbate as cofactors (Schofield and Zhang, 1999). When oxygen levels are low, HIF\(\alpha\) escapes PHD hydroxylation and recognition by the pVHL ubiquitin–ligase complex and translocates into the nucleus where it transcriptionally activates hundreds of genes involved in erythropoiesis, angiogenesis, autophagy, and energy metabolism (Kaelin and Ratcliffe, 2008). Another dioxygenase that can influence HIF activity is the factor inhibiting hypoxia inducible factor (FIH). When oxygen is available, FIH hydroxylates a conserved asparagine residue within the C-terminus trans-activation domains of HIF\(\alpha\), creating a steric clash and preventing binding to co-activators such as p300/CBP, thus limiting HIF transcriptional activity (Ruas et al., 2005). FIH remains active at lower oxygen concentrations as compared to PHDs and is capable of suppressing the activity of HIF\(\alpha\) that escaped proteasomal degradation under moderate hypoxia (Dayan et al., 2006).

Given that PHDs and FIHs require \(\alpha\)-ketoglutarate as a co-substrate, they have been reported to be inhibited by several citric acid cycle metabolic intermediates, such as fumarate and succinate, which competitively inhibit all three PHDs with similar IC\(_{50}\)s, and citrate and oxaloacetate, which competitively inhibit FIH (Koivunen et al., 2007). This inhibition of HIF hydroxylases may explain why highly vascular tumors develop in the absence of VHL mutations. Elevated levels of fumarate and succinate, in fumarate
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hydratase (FH) and succinate dehydrogenase-deficient tumors were shown to inhibit all three PHDs resulting in consequent stabilization/upregulation of HIFαs and their product genes (Isaacs et al., 2005; Selak et al., 2005).

In addition to a playing vital role in tumorigenesis, FIH has been reported to be essential regulator of metabolism and epithelial differentiation. Fih1−/− mice displayed a range of metabolic phenotypes, such as decreased adiposity, hyperventilation and increased insulin sensitivity. When placed on a high fat diet, these animals were also less likely to develop insulin resistance, weight gain, and hepatic steatosis (or fatty liver) (Zhang et al., 2010). FIH1 was shown to negatively regulate corneal epithelial glycogen metabolism in a HIF-1α–independent manner, and suppression of miR-31 in human corneal epithelial keratinocytes (HCEKs) led to increased FIH1 protein levels, decreased Notch activity, and diminished differentiation (Peng et al., 2012a; Peng et al., 2012b). Moreover, FIH1 expression was markedly increased in the epidermis of patients with psoriasis and in the corneal epithelium of patients with diabetic keratopathies (Peng et al., 2012a). Collectively, these data suggest that FIH may play a role in obesity and related diseases (i.e. diabetes and non-alcoholic fatty liver disease), making it a potential target of therapeutic intervention.

Glycolytic isoenzyme pyruvate kinase-M2 (PMK2)

Another enzyme that has been recently implicated in HIF-1α regulation is pyruvate kinase (ATP: pyruvate 2-O-phosphotransferase, EC 2.7.1.40 ), which is the final enzyme in glycolysis. It catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP to create pyruvate and ATP. There are several
different isoenzymes of pyruvate kinase: pyruvate kinase isoenzyme type L (L-PK), which is expressed in tissues with gluconeogenesis, such as liver, kidney and intestine, and pyruvate kinase isoenzyme type M1 and M2 (Mazurek, 2011). In tumors, M2 variant is the predominant form of pyruvate kinase that is expressed, and HIF-1α has been reported to activate PKM2 transcription (Christofk et al., 2008; Luo et al., 2011).

Specifically, PHD3-mediated hydroxylation of PKM2 increased the HIF-1α binding to HREs at target genes, recruitment of co-activator p300, histone acetylation, and subsequent transactivation of HIF-1 target genes (Luo et al., 2011). PHD3 knockdown inhibited PKM2 co-activator function, reduced glucose uptake and lactate production, and increased O2 consumption in cancer cells. Moreover, PKM2 hydroxylation did not appear to be affected by 1% O2, suggesting that PHD3 is still active under hypoxic conditions. PKM2-stimulated expression of HIF-1α target genes promoted the shift from oxidative phosphorylation to glycolytic metabolism and increased expression of vascular endothelial growth factor (VEGF) gene (Luo et al., 2011). PKM2, therefore, may play a more extensive role in promoting HIF-mediated cancer progression than previously thought.

**Runt-related transcription factors (RUNX)**

HIF-1α is known to be an important regulator of angiogenesis. HIF-1α regulation and stability during angiogenesis is mediated by the runt-related transcription factor (RUNX). *RUNX* genes (*RUNX1, RUNX2, and RUNX3*) also known as the acute myeloid leukemia (AML), are developmental regulators and play a role in human cancers (Ito, 2004). All three *RUNX* genes appear to be involved in HIF-1α regulation and stability during angiogenesis. RUNX1 and HIF-1α proteins were demonstrated to directly interact
with one another, primarily via the Runt homology domain of RUNX1, with dual effects. RUNX1 overexpression inhibited DNA-binding and transcriptional activity of HIF-1α protein with reduced expression of a HIF1-targeted gene, VEGF, while silencing of RUNX1 expression by specific small interfering RNA significantly increased transcriptional activity of HIF-1α protein, suggesting that RUNX1 inhibited transcription-dependent function of HIF-1α (Peng et al., 2008). RUNX2 was reported to compete with VHL tumor suppressor protein, by directly binding to HIF-1α oxygen-dependent degradation domain (ODDD) and significantly inhibiting the ubiquitination of HIF-1α, without changing its hydroxylation status. Moreover, overexpression of RUNX2 enhanced in vitro and in vivo angiogenesis through enhanced secretion of VEGF (Lee et al., 2012).

The effect of RUNX3 on HIF-1α stability was also recently investigated. RUNX3 overexpression was found to attenuate HIF-1α stability under normoxic and hypoxic conditions in gastric cancer cells. Under hypoxic conditions, RUNX3 was shown to regulate the stability of HIF-1α at the post-translational level. Treatments by 5-aza-2-deoxycytidine, an inhibitor of DNA methyltransferase, and by trichostatin A (TSA), an HDAC inhibitor, resulted in the recovery of RUNX3 and suppression of both HIF-1α and VEGF. Furthermore, RUNX3 was shown to directly interact with the C-terminal activation domain of HIF-1α and PHD2, potentiate proline hydroxylation, and subsequently promote the degradation HIF-1α. RUNX3 overexpression, also markedly inhibited hypoxia-induced angiogenesis in vitro and in vivo (Lee et al., 2013). Ultimately, RUNX3 suppresses hypoxia-mediated angiogenesis by destabilizing HIF-1α protein via promoting its proline hydroxylation.
Oxygen-independent regulation of HIF signaling

Although HIF regulation has primarily thought to be subject to oxygen tension, multiple studies have also reported that HIF-1α can undergo oxygen-independent regulation. As noted earlier the oxygen-dependent degradation of the HIF-1α subunit is mediated by PHDs, VHL, Elongin C/Elongin B E3 ubiquitin ligase complex, and the proteasome. However, inhibition of heat-shock protein 90 (HSP90) was shown to lead to oxygen/PHD/VHL-independent proteasomal degradation. Receptor for the activated protein kinase C (RACK1) is a HIF-1α-interacting protein that competes with HSP90 for binding to HIF-1α and is required for O2-independent and HSP90 inhibitor-induced degradation of HIF-1α (Liu et al., 2007). Hypoxia-associated factor (HAF), a novel E3-ubiquitin ligase, was also reported to bind to HIF-1α, resulting in its proteasomal degradation irrespective of cellular oxygen tension (Koh et al., 2008). HAF and HIF-1α interact in vitro and in vivo through binding of HAF residues 654 to 800 to HIF-1α residues 296 to 400 and this binding is not oxygen dependent and does not require prolyl hydroxylation (Koh et al., 2008). Therefore, HAF is capable of negatively regulating HIF-1α levels under conditions in which the pVHL-E3 ligase complex is inactive, like hypoxia.

HIF-1α and Gene Expression

HIFs are the key regulators in the major transcriptional cascade involved in the cellular response to changes in oxygen tension. Recently, several groups have used chromatin immunoprecipitation coupled to next-generation high-throughput sequencing
(ChIP-seq) to examine the binding of HIFα subunits across the genome (Schödel et al., 2011; Tanimoto et al., 2010). Analysis of HIF-binding motifs confirmed the 5’-RCGTG-3’ (where ‘R’ denotes a purine residue) core binding sequence (Simon and Keith, 2008) and revealed no additional absolute sequence requirement for HIF binding (Schödel et al., 2011; Xia et al., 2009a). HIF and several other bHLH transcription factors bind to the E-box binding site, CACGTG. When a cytosine precedes the HIF consensus site, the E-box site is formed (Benita et al., 2009). Moreover, these analyses also revealed that the HIFs bind to ~ 500 high-affinity target sites across the genome, many of which are located at considerable distances (>100 kb) away from the genes that they regulate (Schödel et al., 2011; Tanimoto et al., 2010). The Nucleosome-Seq and ChIP-Seq analyses of histone modifications (H3K4me3, H3Ac, and H3K27me3) and binding status of RNA polymerase II revealed that the chromatin formed an open structure in regions surrounding the HIF-1α binding sites, but this event occurred prior to the actual binding of HIF-1α (Tanimoto et al., 2010). DNase I hypersensitivity also revealed that HIFs appear to be recruited to genes that are already expressed under normoxic conditions, indicating that it is unlikely that they direct the hypoxia- induced changes in the chromatin structure of target genes. Therefore, epigenetic regulation of chromatin may have a central role in defining the hypoxic response (Schödel et al., 2011). The limited concordance (40–60%) between the HIF binding sites detected in breast cancer (MCF7) and renal cell carcinoma (RCC) cell lines may indicate that the range of HIF target genes may largely be determined by the underlying cell-type-specific patterns of chromatin structure (Schödel et al., 2011).
HIF-1α and Jumonji-domain-containing histone demethylases (JMJDHs)

Multiple groups have also reported that HIF-1α can bind to and regulate the expression of multiple Jumonji-domain-containing histone demethylases (JMJDHs). JmjC domain lysine demethylases are members of the dioxygenase superfamily of enzymes, which contain iron and are 2-oxoglutarate-dependent enzymes (Klose et al., 2006). Many of these histone demethylases have HREs in their promoters and are induced by HIF-1α (Yang et al., 2009). For example, JARID1B (KDM5B), JMJD1A (KDM3A), JMJD2B (KDM4B) and JMJD2C (KDM4C) are known to be direct HIF-1α target genes with robust HIF-1α binding to HREs in their promoters and up-regulated expression under hypoxic conditions (Guo et al.; Krieg et al.; Pollard et al., 2008; Sar et al., 2009). Under hypoxia, cells ectopically expressing JARID1B had decreased levels of histone 3 lysine 4 (H3K4) methylation (Xia et al., 2009b). Furthermore, JMJD1A was shown to regulate a subset of hypoxia-induced genes, including ADM and GDF15, by maintaining a lower level of histone 3 lysine 9 di-methylation (H3K9me2) at their promoter regions. JMJD1A was also important for tumor growth in the hypoxic microenvironment of tumor xenografts (Krieg et al., 2010). In addition to up-regulating the expression of certain demethylases, hypoxia can also directly inhibit their enzyme activity because they require oxygen, α-ketoglutarate, and ascorbate as cofactors to carry out their enzymatic function. Demethylases are not completely inactive during hypoxia. Their enzymatic activity is less so an increased amount of the enzyme is needed (Chen and Costa, 2009). Moreover, in human cancer cell lines and tumors, miR-210, a robust target of HIF and master miRNA of the hypoxia response, was found to target the mitochondrial iron-sulfur scaffold (ISCU) protein, down-regulation of which was the
major cause of formation of reactive oxygen species (ROS) in hypoxia (Favaro et al., 2010). Therefore, the hypoxia-induced production of free radical oxygen species may also diminish demethylase activity, by depleting ascorbate levels or oxidizing iron (Fe) necessary for the demethylase activity.

HIF-1α and Cancer Prognosis

Increased expression of HIF-1α has been observed in a broad range of human cancers, often correlating with poor prognosis (Table 1). HIF-1α and VEGF status were significantly associated with tumor stage, lymph nodes and liver metastases in patients with colorectal cancer (CRC). Patients with positive HIF-1α and VEGF tumors tended to have a poorer prognosis and shorter survival time (Cao et al., 2009). HIF-1α overexpression was also positively correlated with colorectal liver metastasis (CRLM) and phosphoinositide-3-kinase catalytic subunit alpha (PIK3CA) mutation status and served as an independent risk factor for malignancy recurrence after curative resection of CRLM (Shimomura et al., 2013). Elevated expression of HIF-1α was associated with vascular invasion and a poor prognosis for patients with hepatocellular carcinoma (HCC) (Zheng et al., 2013). In patients with esophageal squamous cell carcinoma (ESCC), high HIF-1α expression was also associated with lymph-node metastasis, serosa infiltration, tumor stage, and overall survival time in ESCC patients (Chai et al., 2013). HIF-1α expression was also correlated with angiogenesis and unfavorable prognosis in bladder cancer, gastrointestinal stromal tumor of the stomach, early-stage invasive cervical cancer, pancreatic cancer, head and neck cancer, non-small cell lung cancer (NSCLC), melanoma and breast and ovarian cancers (Birner et al., 2000; Daponte et al., 2008; Giatromanolaki, 2001; Giatromanolaki et al., 2001; Giatromanolaki et al., 2003; Klatte et
The above studies indicate that increased expression of HIF-1α proteins in tumor cells, whether induced by hypoxia or aberrant oncogenic signaling, is responsible for driving tumor growth and progression, most likely by regulating the expression of crucial target genes which are involved in carcinogenesis.

While increased HIF-1α levels are often correlated with poor prognosis, some studies have shown the opposite to be true. Fillies et al. found that HIF-1α overexpression provides a better prognosis for patients with early stage squamous cell carcinoma of the oral floor (Fillies et al., 2005). In a study by Lidgren et al., high HIF-1α levels in renal cell carcinoma was associated with a better prognosis (Lidgren et al., 2006).

The controversy regarding HIF-1α levels and prognosis is best illustrated in a study by Vleugel et al. The group examined HIF-1α concentrations in invasive breast cancers with two overexpression patterns: 1) HIF-1α overexpressed in perinecrotic regions, and 2) diffusely overexpressed HIF-1α. They found that the perinecrotic expression patterns were associated with the expression of hypoxia-associated genes-carbonic anhydrase IX (CAIX) and glucose transporter 1 (GLUT1) – and this correlated with poor prognosis. Tumors with HIF-1α diffusely expressed were not associated with CAIX and GLUT1 expression and these patients had a better prognosis (Vleugel et al., 2005). The contradicting conclusions predicting HIF-1α’s role in cancer prognosis further emphasizes the complexity of HIFs involvement in cancer.
HIF-1α and Therapeutic Interventions

Given its wide scoping role in the pathology of human disease, HIF-1α targeting therapeutic agents have gained considerable traction and attention among the medical and scientific community. A number of potential cancer therapeutic agents have recently been shown to function by directly or indirectly interacting with mammalian target of rapamycin (mTOR) signaling cascade. mTOR is a serine/threonine protein kinase that regulates protein synthesis, cell growth, and cell survival and is involved in both positive and negative feedback loops with HIF-1α (Demidenko and Blagosklonny, 2011). Phosphorylation of mTOR and its downstream effectors 4E-BP1 and p70S6K were reported to raise cellular levels of HIF-1α by stimulating its translation. Specifically, HIF-1α was shown to bind with HIF-1β and activate transcription of growth factors and cytokines that stimulated the RTK-PI3K-Akt pathway, which resulted in further activation of mTOR (Agani and Jiang, 2013; Finlay et al., 2012). HIF-1α was also shown to stabilize DNA-damage-inducible transcript 4 (REDD1). Once stabilized, REDD1 can bind and activate TSC2, resulting in an indirect inactivation of mTOR (Horak et al., 2010; Kucejova et al., 2011). Below we will discuss several drugs that inhibit mTOR and/or HIF-1α and this information is presented in Table 2. We would like to note here that this is not a comprehensive list of all HIF-1α inhibiting drugs and the reader is referred to reviews by (Hu et al., 2013; Tang and Yu, 2013; Xia et al., 2012) for more information on this topic.

Inhibition of HIF-1α protein synthesis via suppression of the PI3K-Akt-mTOR pathway appears to be a common mode of action for a number of the recently characterized anti-cancer drug candidates (i.e. magnolol, sorafenib, YC-1 and BEZ235).
In human bladder cancer cells, the phenolic compound magnolol was shown to suppress the PI3K-Akt-mTOR pathway and subsequently reduced HIF-1α accumulation by inhibiting hypoxia-induced reactive oxygen species (ROS) and VEGFR2, two known activators of the PI3K/Akt pathway (Chen et al., 2013). Sorafenib, like magnolol, was also reported to decrease synthesis of HIF-1α, but mediated its effects by decreasing phosphorylation of mTOR and its downstream effectors p70S6K and 4E-BP1 (Liu et al., 2012). YC-1 prevents HIF-1α translation by suppressing the PI3K/Akt/mTOR/4E-BP pathway. YC-1 also inhibited NFkB, a downstream target of Akt (Sun et al., 2007b). Moreover, a recent study by Karar et al. reported on the HIF-1α inhibitory effects of BEZ235, an agent that inhibits both PI3K and mTOR by interacting with their ATP binding clefts. BEZ235 proved to be a more efficient inhibitor of HIF-1α synthesis than either an mTOR inhibitor or a PI3K inhibitor alone (Karar et al., 2012).

Other mTOR inhibitors, such as deferasiro and SP600125, which may be therapeutic in the treatment of cancer, function to inactivate mTOR by increasing the levels of REDD1, a HIF-1α stabilized protein (Jin et al., 2009; Ohyashiki et al., 2009). Given the implications of HIF-1α in human cancers, it is likely that the mTOR inhibitors that function to reduce HIF-1α levels are likely to have promising treatment effects. However, further research is needed to establish mTOR inhibitors as effective chemotherapeutics.

Some other notable drugs that have shown promising results for the inhibition of HIF-1α in cancer therapy include topotecan and PX-478. Topotecan is a topoisomerase I (Top I) inhibitor and has been shown to inhibit HIF-1α. Top 1 is a monomeric enzyme that
catalyzes the conversions of the different topological states of DNA. Topotecan stabilizes Top I on DNA and induces DNA damage during replication; however, topotecan has been shown to inhibit HIF-1α in an S-phase independent manner. Topotecan inhibits HIF-1α accumulation by preventing translation of HIF-1α mRNA and Top I is required for this inhibition (Rapisarda et al., 2004). PX-478 is the first HIF-1α inhibitor to be used at a clinical stage for the treatment of solid tumors. The drug inhibits HIF-1α levels via a number of mechanisms: 1) decreasing HIF-1α mRNA levels, 2) preventing HIF-1α translation, and 3) preventing HIF-1α deubiquitination. The drug significantly decreased expression of the HIF-1α- target genes, VEGF and GLUT-1 (Lee and Kim, 2011).

Although the inhibition of HIF-1α appears to be a promising field of cancer research, only a few of the drugs have advanced past preclinical and clinical development and further investigations are needed to establish these drugs as valid tools in chemotherapy.

**Conclusion**

HIF is a transcription factor that is regulated by a number of cellular pathways involving both oxygen-dependent and oxygen- independent mechanisms. Stabilized HIF-1α activates transcription of genes that participate in angiogenesis, dedifferentiation, and invasion- all of which are key pathways in carcinogenesis. Due to its large role in cancer and its association with poor prognosis, it is not surprising that HIF-1α has become an important topic in molecular research. The complex mechanisms underlying the hypoxic microenvironment of solid tumors have yet to be fully characterized, but it is certain that HIF-1α is a key mediator. Manipulating pathways and mediators such as mTOR that are known to regulate HIF-1α may be of therapeutic value. Future research should attempt to unearth the missing links between HIF and cancer metabolism, as well as, examine the
therapeutic effects of small molecular inhibitors of HIF-1α regulators.

Authorship Contribution

Wrote or contributed to the writing of the manuscript: Brocato, Chervona, and Costa
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between pVHL and mTORC1 pathways in clear-cell renal cell carcinoma.

Molecular cancer research : MCR 9(9):1255-1265.


Footnotes

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### Tables

**Table 1: Correlation of prognosis and HIF-1α expression in human cancers**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Prognosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>Poor</td>
<td>Cao et al., 2009 Shimomura et al., 2013</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>Poor</td>
<td>Zheng et al., 2013</td>
</tr>
<tr>
<td>Esophageal</td>
<td>Poor</td>
<td>Chai et al., 2013</td>
</tr>
<tr>
<td>Bladder</td>
<td>Poor</td>
<td>Theodoropoulos et al., 2004</td>
</tr>
<tr>
<td>GIST</td>
<td>Poor</td>
<td>Takahashi et al., 2003</td>
</tr>
<tr>
<td>Cervical</td>
<td>Poor</td>
<td>Birner et al., 2000</td>
</tr>
<tr>
<td>Head and neck</td>
<td>Poor</td>
<td>Winter et al., 2006</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Poor</td>
<td>Giatromanolaki et al., 2001</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Poor</td>
<td>Koukourakis et al., 2002</td>
</tr>
<tr>
<td>Breast</td>
<td>Poor</td>
<td>Yamamoto et al., 2008</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Poor</td>
<td>Daponte et al., 2008</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Poor</td>
<td>Sun et al., 2007 Shibai et al., 2003</td>
</tr>
<tr>
<td>RCC</td>
<td>Poor</td>
<td>Klatte et al., 2007</td>
</tr>
</tbody>
</table>

GIST, gastrointestinal stromal tumor, NSCLC, non-small-cell lung cancer, RCC, renal cell carcinoma
Table 2: HIF-1α inhibitors and mechanisms of action

<table>
<thead>
<tr>
<th>HIF-1α Inhibitor</th>
<th>Mechanism</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Magnolol</td>
<td>Suppress PI3K-Akt-mTOR pathway</td>
<td>Chen et al., 2013</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Decreases phosphorylation of mTOR and its downstream effectors p70S6K and 4E-BP1</td>
<td>Liu et al., 2012</td>
</tr>
<tr>
<td>YC-1</td>
<td>Suppresses the PI3K/Akt/mTOR/4E-BP pathway</td>
<td>Sun et al., 2007</td>
</tr>
<tr>
<td>BEZ235</td>
<td>Inhibits both PI3K and mTOR by interacting with their ATP binding clefts</td>
<td>Karar et al., 2012</td>
</tr>
<tr>
<td>Metformin¹</td>
<td>Inactivates mTOR by increasing the levels of REDD1</td>
<td>Ben Sahra et al., 2011</td>
</tr>
<tr>
<td>Deferasiro¹</td>
<td>Inactivates mTOR by increasing the levels of REDD1</td>
<td>Ohyashiki et al., 2009</td>
</tr>
<tr>
<td>SP600125¹</td>
<td>Inactivates mTOR by increasing the levels of REDD1</td>
<td>Jin et al., 2009</td>
</tr>
<tr>
<td>Topotecan</td>
<td>Prevents translation of HIF-1α mRNA</td>
<td>Rapisarda et al., 2004</td>
</tr>
<tr>
<td>PX-478</td>
<td>1) Decreases HIF-1α mRNA levels, 2) Prevents HIF-1α translation, and 3) Prevents HIF-1α deubiquitination</td>
<td>Lee et al., 2011</td>
</tr>
</tbody>
</table>

¹May actually increase HIF-1α levels.

PI3K, phosphoinositide 3 kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; 4E-BP, 4E-binding protein; HIF-1α, hypoxia inducible factor 1α; REDD1, DNA-damage-inducible transcript 4.