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REVIEW

Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics

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Adaptation of cancer cells to their microenvironment is an important driving force in the clonal selection that leads to invasive and metastatic disease. O_2 concentrations are markedly reduced in many human cancers compared with normal tissue, and a major mechanism mediating adaptive responses to reduced O_2 availability (hypoxia) is the regulation of transcription by hypoxia-inducible factor 1 (HIF-1). This review summarizes the current state of knowledge regarding the molecular mechanisms by which HIF-1 contributes to cancer progression, focusing on (1) clinical data associating increased HIF-1 levels with patient mortality; (2) preclinical data linking HIF-1 activity with tumor growth; (3) molecular data linking specific HIF-1 target gene products to critical aspects of cancer biology and (4) pharmacological data showing anticancer effects of HIF-1 inhibitors in mouse models of human cancer.
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Introduction

Human cancers frequently contain areas of necrosis in which cancer cells have died due to inadequate oxygenation (Harris, 2002; Brahimi-Horn *et al*., 2007). Cells closest to a perfused blood vessel are exposed to relatively high O_2 concentrations, which decline as distance from the vessel increases. Although such gradients exist in normal tissues, in cancers the gradients are much steeper and O_2 concentrations drop to near zero in areas of necrosis. In addition to physical gradients, temporal fluctuations in oxygenation also commonly occur within tumors (Dewhirst *et al*., 2008). Most physiological functions of cells are modulated according to the cellular O_2 concentration. A major mechanism mediating adaptive responses to reduced O_2 availability (hypoxia) is the regulation of transcription by hypoxia-inducible factor 1 (HIF-1) (Semenza, 2009a). These adaptive responses are co-opted by cancer cells, in which normal feedback mechanisms have been disrupted by somatic mutation and epigenetic changes. As a result, the adaptation to hypoxia promotes many key aspects of cancer progression that ultimately lead to patient mortality (Harris, 2002).

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HIF-1α and HIF-2α levels are increased in many human cancers

HIF-1 is a heterodimeric protein that is composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit (Wang and Semenza, 1995; Wang *et al*., 1995). HIF-1α is subjected to O_2-dependent hydroxylation on proline residue 402 and/or 564 by prolyl hydroxylase domain protein 2 (PHD2) and this modification creates an interface for interaction with the von Hippel–Lindau tumor suppressor protein (VHL), which recruits an E3 ubiquitin-protein ligase that catalyzes polyubiquitination of HIF-1α, thereby targeting it for proteasomal degradation (Kaelin and Ratcliffe, 2008). Under hypoxic conditions, hydroxylation is inhibited and HIF-1α rapidly accumulates, dimerizes with HIF-1β, binds to the core DNA binding sequence 5′-RCGTG-3′ (R, purine (A or G)) in target genes, recruits co-activators and activates transcription. O_2-dependent hydroxylation of asparagine-803 by factor inhibiting HIF-1 (FIH-1) blocks interaction of HIF-1α with the co-activators P300 and CBP under normoxic conditions (Lando *et al*., 2002). Both PHD2 and FIH-1 use O_2 and α-ketoglutarate as substrates and generate CO_2 and succinate as by-products of the hydroxylation reaction. HIF-2α is a protein with extensive sequence similarity to HIF-1α that is also regulated by proline and asparagine hydroxylation, dimerizes with HIF-1β and activates transcription of a group of target genes that overlaps with, but is distinct from, those regulated by HIF-1α (Lau *et al*., 2007). HIF-3α is an inhibitor of HIF-1 that may be involved in feedback regulation because its expression is transcriptionally regulated by HIF-1 (Makino *et al*., 2007).

Immunohistochemical analysis of human cancer biopsies revealed increased levels (relative to surrounding normal tissue) of HIF-1α or HIF-2α protein (or both) in the majority of primary human cancers and their metastases (Zhong *et al*., 1999; Talks *et al*., 2000). Intratumoral hypoxia is a major mechanism underlying the increased levels of HIF-1α and HIF-2α in cancer and stromal cells. For example, the median PO_2 level measured in breast cancers was 10 mm Hg, as compared to 65 mm Hg in normal breast tissue (Vaupel *et al*., 2004). Other inducers of HIF-1α in the tumor microenvironment include reactive oxygen and nitrogen species, which also inhibit proteasomal degradation of HIF-1α (Quintero *et al*., 2006; Gao *et al*., 2007; Li *et al*., 2007; Dewhirst *et al*., 2008). Complementing these mechanisms for blocking HIF-1α degradation, activation of the phosphatidylinositol-3-kinase and MAP kinase pathways (either as a result of oncogenic mutation or increased signaling from receptor tyrosine kinases and G-protein-coupled receptors) increases HIF-1α synthesis, primarily through the action of mTOR (Laughner *et al*., 2001).

HIF-1α and HIF-2α protein levels can also be increased in cancer cells due to loss of function (LOF) of many different tumor suppressors, which results in either increased HIF-1α synthesis or decreased HIF-1α degradation (Table 1). Remarkably, a large number of proteins encoded by transforming viruses that cause tumors in humans also induce HIF-1 activity (Table 2). Most remarkable is the case of Kaposi's sarcoma, a highly vascularized tumor that is caused by a infection with a herpesvirus, the genome of which encodes three different proteins that together increase HIF-1α protein half-life, nuclear localization and transactivation under nonhypoxic conditions, thereby mimicking the effect of hypoxia. The finding that, in addition to intratumor hypoxia, major genetic and epigenetic alterations resulting in oncogene gain of function (GOF) or tumor suppressor gene LOF lead to increased HIF-1 activity suggests that increased HIF-1 activity represents a final common pathway in cancer pathogenesis, that is, clonal selection favors those cancer cells in which HIF-1 activity is increased (Semenza, 2000; Vogelstein and Kinzler, 2004; Gillies and Gatenby, 2007; Gatenby and Gillies, 2008).

Clinical data linking HIF-1α and HIF-2α levels to cancer mortality

Taken together, the observed effects of tumor suppressor LOF and transforming virus protein expression provide compelling evidence that HIF-1 activation promotes oncogenesis and/or cancer progression. In support of this hypothesis, a large body of clinical data shows an association between increased levels of HIF-1α or HIF-2α protein, as determined by immunohistochemistry of standard formalin-fixed, paraffin-embedded tumor biopsy sections, with increased patient mortality in many human cancers (Table 3). The data from studies of early stage breast, cervical and endometrial cancers are particularly striking. Early stage disease is usually associated with a good prognosis,

Table 1 Tumor suppressor gene loss of function contributes to increased levels of HIF-1α in human cancers

TSG	*Tumor(s) with TSG loss of function*	*Effect on HIF-1α*	*Reference*
VHL	Renal carcinoma, hemangioblastoma	Decr ubiquitination	Maxwell *et al*. (1999)
SDHB	Paraganglioma	Decr hydroxylation	Selak *et al*. (2005)
SDHC	Paraganglioma	Decr hydroxylation	Selak *et al*. (2005)
SDHD	Paraganglioma	Decr hydroxylation	Selak *et al*. (2005)
FH	Leiomyoma, renal carcinoma	Decr hydroxylation	Isaacs *et al*. (2005)
IDH1	Glioblastoma	Decr hydroxylation	Zhao *et al*. (2009)
P53	Many	Decr ubiquitination	Ravi *et al*. (2000)
TSC2	Tuberous sclerosis	Incr synthesis	Brugarolas *et al*. (2003)
PTEN	Glioblastoma, others	Incr synthesis	Zhong *et al*. (2000) Zundel *et al*. (2000)
LKB1	Gastrointestinal hamartoma	Incr synthesis	Shackelford *et al*. (2009)

Abbreviations: Decr, decreased; Incr, increased; TSG, tumor suppressor gene.

Table 2 Proteins of oncogenic viruses increase HIF-1 activity

Viral oncoprotein	*Effect on HIF-1α*	*Reference*
EBV latent membrane protein 1	Decr degradation	Kondo *et al*. (2006)
Hepatitis B virus X protein	Decr degradation	Yoo *et al*. (2004)
Human papillomavirus E6/E7 proteins	Incr HIF-1α protein	Nakamura *et al*. (2009)
Human T-cell leukemia virus tat protein	Incr HIF-1α protein	Tomita *et al*. (2007)
KSHV G-protein-coupled receptor	Incr transactivation	Sodhi *et al*. (2000)
KSHV latency-associated nuclear antigen	Incr nuclear localization	Cai *et al*. (2007)
KSHV latency-associated nuclear antigen	Decr degradation	Cai *et al*. (2006)
KSHV viral interferon regulatory factor 3	Decr degradation	Shin *et al*. (2008)

Abbreviations: EBV, Epstein–Barr virus; KSHV, Kaposi's sarcoma herpesvirus.

Table 3 Immunohistochemical studies in which increased levels of HIF-1α (or HIF-2α) protein in diagnostic tumor biopsies were associated with decreased patient survival

Cancer type	*Reference*
Astrocytoma, diffuse	Korkolopoulou *et al.* (2004)
Bladder, superficial urothelial[a]	Theodoropoulos *et al.* (2005)
Bladder, transitional cell	Theodoropoulos *et al.* (2004)
Breast	Dales *et al.* (2005); Kronblad *et al.* (2006); Trastour *et al.* (2007); Vleugel *et al.* (2005); Yamamoto *et al.* (2008)
Breast, c-erbB-2 positive	Giatromanolaki *et al.* (2004)
Breast, ER positive	Generali *et al.* (2006)
Breast, LN positive	Schindl *et al.* (2002)
Breast, LN negative	Bos *et al.* (2003)
Cervix, early stage	Birner *et al.* (2000)
Cervix, RTX	Bachtiary *et al.* (2003); Burri *et al.* (2003)
Colorectal	Rajaganeshan *et al.* (2008); Schmitz *et al.* (2009)
Colorectal[b]	Cleven *et al.* (2007)
Endometrial	Sivridis *et al.* (2002)
Esophageal SCC	Tzao *et al.* (2008)
Gastric	Griffiths *et al.* (2007)
GIST, stomach	Takahashi *et al.* (2003)
Head-and-neck SCC[c]	Koukourakis *et al.* (2002)
Laryngeal	Schrijvers *et al.* (2008)
Lung, NSCLC	Swinson *et al.* (2004)
Lung, NSCLC[d]	Giatromanolaki *et al.* (2001)
Malignant melanoma[d]	Giatromanolaki *et al.* (2003)
Oligodendroglioma	Birner *et al.* (2001a)
Oropharynx SCC	Aebersold *et al.* (2001)
Ovarian[a]	Birner *et al.* (2001b)
Ovarian, serous	Daponte *et al.* (2008)
Pancreatic	Sun *et al.* (2007)
Prostate[e]	Nanni *et al.* (2009)
Rectal	Rasheed *et al.* (2009)

Abbreviations: ER, estrogen receptor; GIST, gastrointestinal stromal tumor; LN, lymph node; NSCLC, non-small-cell lung carcinoma; RTX, radiation therapy; SCC, squamous cell carcinoma.
[a]Combination of high HIF-1α and mutant p53 was associated with mortality.
[b]HIF-2α expression in stromal cells (not in cancer cells) was associated with mortality.
[c]High HIF-1α and high HIF-2α were each associated with mortality.
[d]High HIF-2α (not HIF-1α) was associated with mortality.
[e]Combination of high HIF-2α and nuclear localization of endothelial nitric oxide synthase was associated with mortality.

but the subset of patients whose tumors contained high levels of HIF-1α had a significantly increased mortality rate. Thus, HIF-1α immunohistochemistry has the potential to identify patients for whom more aggressive therapy may be indicated. Testing this hypothesis by clinical trials is warranted, especially now that anticancer drugs that target HIF-1 are available, as described below.

Experimental data linking HIF-1α and HIF-2α levels to cancer progression

Complementing the clinical data is a large body of experimental data showing that HIF-1α LOF results in decreased tumor growth, vascularization and metastasis, whereas HIF-1α GOF has the opposite effects (Table 4). Whereas the clinical data are by their nature correlative, the experimental data show causation, using many different cancer cell types and many different techniques to modulate the levels of HIF-1α or HIF-2α. Whereas the overall conclusion of these experiments is that HIF-1α and HIF-2α promote cancer progression, the association is not absolute. For example, whereas HIF-1α LOF decreased the growth of SW480 colorectal carcinoma xenografts, HIF-2α LOF increased xenograft growth and, remarkably, immunohistochemical analysis of human colon cancer biopsies revealed a significant association between loss of HIF-2α protein expression and advanced tumor stage (Imamura *et al.*, 2009). In contrast, HIF-2α GOF increased the growth of 786-O renal cell carcinoma xenografts whereas HIF-1α GOF decreased xenograft growth (Raval *et al.*, 2005).

The xenograft studies have many limitations, not the least of which is that LOF is achieved in the tumor cells but not in the host stromal cells, in which HIF-1 activity contributes to tumor progression. For example, tumor growth is impaired in mice with mutant p53 and germ-line heterozygosity for an HIF-1α knockout allele as compared to mutant p53 mice that are wild type for HIF-1α (Bertout *et al.*, 2009) and in mice with conditional knockout of HIF-1α in vascular endothelial cells (Tang *et al.*, 2004). There are no published examples of combined HIF-1α and HIF-2α inhibition in tumor and stroma resulting in increased tumor growth. Thus, inhibitors that target HIF-1α and HIF-2α will have the highest probability of therapeutic efficacy across a broad range of human cancers.

HIF-1 regulates the expression of genes encoding proteins with key roles in cancer biology

HIF-1α and HIF-2α exert their effects on cancer progression by binding to and activating the transcription of target genes with *cis*-acting hypoxia response elements containing the consensus binding site 5′-RCGTG-3′. The hundreds of genes that are induced by hypoxia in an HIF-1-dependent manner encode proteins from A to Z that have key roles in every critical aspect of cancer biology (Table 5), including angiogenesis (Liao and Johnson, 2007), cell survival, chemotherapy and radiation resistance (Moeller *et al.*, 2007), genetic instability (Bindra *et al.*, 2007), immortalization, immune evasion (Lukashev *et al.*, 2007), invasion and metastasis (Chan and Giaccia, 2007; Sullivan and Graham, 2007), proliferation, metabolism and pH regulation (Swietach *et al.*, 2007; Chiche *et al.*, 2009; Semenza, 2009b), and stem cell maintenance (Barnhart and Simon, 2007). Among these functions, the most fundamental may be angiogenesis and glucose/energy metabolism, which control oxygen delivery and use, respectively. Angiogenesis has been a major focus of cancer biology and therapy over the past decade, whereas tumor metabolism is likely to attract similar attention over the next decade.

Table 4 Selected experimental studies linking HIF-1 activity with cancer progression

Cancer cell line	*Manipulation*	*Model*	*Outcome measure*	*Reference*
Hepa1 hepatoma	HIF-1β LOF[a]	Xenograft	Decr growth	Jiang *et al.* (1997)
Hepa1 hepatoma	HIF-1β LOF	Xenograft	Decr growth, MVD[b]	Maxwell *et al.* (1997)
HCT116 colorectal	HIF-1α GOF[c]	Xenograft	Incr growth, MVD	Ravi *et al.* (2000)
HCT116 colorectal	Gal-TAD-C[d]	Xenograft	Decr growth	Kung *et al.* (2000)
PCI-10 pancreatic	HIF-1α GOF	Xenograft	Incr growth	Akakura *et al.* (2001)
PCI-43 pancreatic	HIF-1αDN[e]	Xenograft	Decr growth, metab[f]	Chen *et al.* (2003)
786-O renal clear cell	HIF-2α shr[g]	Xenograft	Decr growth	Kondo *et al.* (2003)
TMK1 gastric	HIF-1αDN	Orthotopic	Decr growth, vessel maturity	Stoeltzing *et al.* (2004)
D54MG glioma	HIF-1α shr	Xenograft	Decr growth	Li *et al.* (2005)
786-O renal clear cell	HIF-2α GOF	Xenograft	Incr growth	Raval *et al.* (2005)
BxPc-3 pancreatic	HIF-1α AS[h]	Xenograft	Decr growth	Chang *et al.* (2006)
PC3 prostate	HIF-1α shr	Xenograft	Decr growth	Gao *et al.* (2007)
MDA-MB231 breast	HIF-1α G/L[i]	Intracardiac	Incr/Decr bone mets[j]	Hiraga *et al.* (2007)
Breast cancer	HIF-1α cKO[k]	Spontaneous	Decr lung mets	Liao *et al.* (2007)
SW480 colorectal	HIF-1α shr	Xenograft	Decr growth	Imamura *et al.* (2009)
A375 melanoma	Tf-PEI-shr[l]	Xenograft	Decr growth	Liu *et al.* (2009)
U87 glioma	HIF-1α sir[m]	Xenograft	Decr growth	Wang *et al.* (2009)

[a]Loss of function (spontaneous mutation).
[b]Microvessel density.
[c]Gain of function (stably transfected HIF-1α expression vector).
[d]GAL4 fusion protein containing HIF-1α C-terminal transactivation domain (LOF).
[e]HIF-1αDN, dominant-negative form of HIF-1α (stably transfected expression vector) LOF.
[f]Impaired glucose metabolism.
[g]Short-hairpin RNA (inducible expression vector) LOF.
[h]Antisense RNA expression vector (LOF).
[i]Gain or loss of function by constitutively active HIF-1α or HIF-1αDN.
[j]Metastases.
[k]Mice with conditional HIF-1α knockout/polyoma middle T expression in mammary epithelium.
[l]Intravenous injection of transferrin–polyethylenimine–HIF-1α shRNA complex (LOF).
[m]Intravenous injection of short-interfering RNA nanoparticles (LOF).

When a given cell type is exposed to hypoxia, increased expression of several hundred mRNAs is induced and expression levels of a roughly equal number of mRNAs are repressed in an HIF-1-dependent manner (Manalo *et al.*, 2005). The hypoxia-induced binding of HIF-1 to many target genes that are activated by hypoxia has been shown by chromatin immunoprecipitation assays, whereas binding of HIF-1 to hypoxia-repressed genes is not observed, suggesting an indirect, but HIF-1-dependent mechanism for repression (Mole *et al.*, 2009). For example, loss of E-cadherin expression is essential for cancer cell invasion and metastasis (Cavallaro and Christofori, 2004). In the renal cell carcinoma RCC4 line, HIF-1 activates transcription of the genes encoding TCF3 (also known as E12/E47), ZFHX1A (also known as δEF1 or ZEB1) and ZFHX1B (also known as SIP1 or ZEB2), which are known to bind to the promoter of the gene encoding E-cadherin to repress its transcription (Krishnamachary *et al.*, 2006). HIF-1 may also promote genetic instability by repressing transcription of the *MSH2* and *MSH6* genes, which encode subunits of the DNA mismatch repair protein MutSα, by blocking the interaction of MYC with SP1, an effect that does not require direct DNA binding or even the presence of HIF-1β (Koshiji *et al.*, 2005).

Within any given cancer, only a subset of these genes will be activated by HIF-1, which increases the transcription of genes that are already active within a cell type. In addition, subsets of these genes are differentially regulated by HIF-1α and HIF-2α. Thus, the biological consequences of HIF-1 activation in a cancer will vary depending on the battery of target genes that respond. This heterogeneity is in no way unique to HIF-1 (Yu *et al.*, 1999) and represents one of the greatest obstacles to improving cancer therapy. The major conclusion to be drawn from these data is that HIF-1 has an incredibly broad and important role in cancer biology and that the delineation of the HIF-1 transcriptome has provided a molecular basis for the association between intratumoral hypoxia and invasion, metastasis and patient mortality (Vaupel *et al.*, 2004).

Anticancer drugs inhibit HIF-1

Many of the novel anticancer drugs that target specific pathways have been shown to have antiangiogenic effects that appear to be due, in large part, to their inhibition of HIF-1 activity (Table 6), including the BCR-ABL inhibitor imatinib/Gleevec (Mayerhofer *et al.*, 2002); epidermal growth factor receptor inhibitors gefitinib/Iressa, erlotinib/Tarceva and cetuximab/C225 (Luwor *et al.*, 2005; Pore *et al.*, 2006); $HER2^{neu}$ inhibitor trastuzumab/Herceptin (Laughner *et al.*, 2001) and the mTOR inhibitors rapamycin, temsirolimus/CCI-779 and everolimus/RAD-001 (Laughner *et al.*, 2001; Majumder *et al.*, 2004; Thomas *et al.*, 2006), which all function by inhibiting mTOR-dependent translation of HIF-1α mRNA into protein (Laughner *et al.*, 2001).

Table 5 Selected HIF-1 target genes whose products contribute to cancer progression

Gene product	*Role in cancer progression*
Angiopoietin 2	Angiogenesis, lymphangiogenesis
Angiopoietin-like 4	Metastasis
Breast cancer resistance protein (ABCG2)	Multidrug transport, stem cell maintenance
Carbonic anhydrase 9 and 12	pH regulation
C-MET	Invasion
CXCR4	Metastasis
DEC1	Genomic instability
Endothelin 1	Invasion
Fibronectin 1	Invasion
Glucose phosphate isomerase	Cell motility, glucose metabolism, immortalization
Glucose transporter 1	Glucose uptake
Hexokinase 1 and 2	Glucose phosphorylation, cell survival
Inhibitor of differentiation 2	Angiogenesis, proliferation
Insulin-like growth factor 2	Cell survival, proliferation
JARID1B	Stem cell maintenance
Kit ligand (stem cell factor)	Angiogenesis, stem cell maintenance
Lactate dehydrogenase A	Glucose metabolism
Lysyl oxidase	Metastasis
Matrix metalloproteinase 2 and 14	Invasion
NT5E (ecto-5′-nucleotidase/CD73)	Immune evasion, multidrug resistance
OCT4	Stem cell maintenance
Placental growth factor	Angiogenesis
Platelet-derived growth factor B	Cell proliferation/survival, angiogenesis
Pyruvate dehydrogenase kinase 1	Glucose metabolism
Pyruvate kinase M2	Glucose metabolism
Stromal-derived factor 1	Angiogenesis
Survivin	Cell survival
Telomerase	Immortalization
Transforming growth factor-α	Cell proliferation/survival
TWIST1	Epithelial–mesenchymal transition
Urokinase plasminogen activator receptor	Invasion
Vascular endothelial growth factor	Angiogenesis
WSB1	Cell survival
ZEB1 (ZFHX1A), *ZEB2* (ZFHX1B)	Epithelial–mesenchymal transition

For references, see Semenza (2009a).

Inhibition of HIF-1 activity leads to decreased vascular endothelial growth factor (VEGF) expression. Drugs that inhibit phosphatidylinositol-3-kinase and ERK signal transduction also inhibit HIF-1 and SP1, both of which bind to the *VEGF* gene promoter (Pagès and Pouyssègur, 2005).

Another novel class of molecularly targeted anticancer agents consists of inhibitors of heat shock protein 90 (HSP90), such as geldanamycin, 17-allylaminogeldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin, which target HIF-1α for proteasomal degradation (Isaacs *et al*., 2002). In the absence of inhibitors, HSP90 binds to HIF-1α and promotes its stability by preventing the binding of RACK1, whereas the inhibitors block HSP90 binding, thereby promoting the binding of RACK1, which triggers ubiquitination and proteasomal degradation of HIF-1α (Liu *et al*., 2007). Histone deacetylase inhibitors are another novel class of anticancer drugs that promote HIF-1α degradation (Kong *et al*., 2006; Qian *et al*., 2006) and inhibit HIF-1α transactivation domain function (Fath *et al*., 2006) at clinically relevant concentrations. Finally, the proteasome inhibitor bortezomib inhibits HIF-1 by blocking its transcriptional activity (Kaluz *et al*., 2006). Bortezomib may promote binding of the asparagine hydroxylase FIH-1 to HIF-1α (Shin *et al*., 2008) although this finding has been disputed (Kaluz *et al*., 2008). In addition to these drugs, many other chemical compounds have been shown to inhibit HIF-1 activity and are in preclinical development (Melillo, 2007).

HIF-1 inhibitors block tumor growth and vascularization

Cell-based screening assays have been performed to identify inhibitors of HIF-1 transcriptional activity. These assays have revealed that several traditional chemotherapeutic agents are potent HIF-1 inhibitors, including topoisomerase I inhibitors, such as topotecan (Rapisarda *et al*., 2002) and microtubule-targeting drugs, such as taxotere (Escuin *et al*., 2005). DNA

Table 6 Selected classes of drugs that inhibit HIF-1 activity and tumor xenograft growth

Drug class	*Example*	*Effect*[a]	*Reference*
Anthracyclines	Doxorubicin	A	Lee *et al*. (2009)
EGFR inhibitors	Cetuximab	B	Luwor *et al*. (2005); Pore *et al*. (2006)
Cardiac glycosides	Digoxin	B	Zhang *et al*. (2008)
Histone deacetylases	TSA[b]	C	Kong *et al*. (2006); Qian *et al*. (2006)
		D	Fath *et al*. (2006)
HSP90 inhibitors	17-AAG[c]	C	Isaacs *et al*. (2002); Liu *et al*. (2007)
Microtubule targeting agents	Taxotere	B	Escuin *et al*. (2005)
mTOR inhibitors	Rapamycin	B	Zhong *et al*. (2000); Laughner *et al*. (2001)
Proteasome inhibitors	Bortezomib	D	Kaluz *et al*. (2006)
Topoisomerase I inhibitors	Topotecan	B	Rapisarda *et al*. (2002)

[a]Mechanisms of action that lead to decreased HIF-1 transcriptional activity are as follows: A, decreased HIF-1 DNA binding; B, decreased HIF-1α synthesis; C, increased HIF-1α degradation; D, decreased HIF-1α transactivation.
[b]Trichostatin A.
[c]17-Allylaminogeldanamycin.

intercalating drugs, including echinomycin (Kong *et al*., 2005) and anthracyclines, such as doxorubicin (Adriamycin) and daunorubicin (Lee *et al*., 2009), inhibit HIF-1 transcriptional activity by blocking its binding to DNA. Because extensive clinical experience has already defined the use and limitations of these drugs, one might wonder why this is at all noteworthy. The key point is the use of these drugs as HIF-1 inhibitors engenders an entirely new treatment paradigm. Traditional chemotherapies have been administered to patients at maximum tolerated doses as cytotoxic agents intended to kill as many dividing cancer cells as possible. Unfortunately, these drugs also kill dividing hematopoietic cells and, as a result, they are administered episodically followed by drug-free intervals of 2–3 weeks during which bone marrow recovery occurs. Unfortunately, tumor recovery often occurs as well and eventually leads to the selection of a resistant clone of cancer cells that metastasizes and kills the patient (note also that cell proliferation is inhibited under conditions of reduced O_2 availability, thereby protecting hypoxic cancer cells from chemotherapy).

The laboratory of the late Judah Folkman discovered that administering the chemotherapy agent cyclophosphamide at reduced doses on a more frequent and regular dosing interval prevents bone marrow toxicity and blocks tumor growth by interfering with angiogenesis (Hahnfeldt *et al*., 2003), which was thought to result from either inducing apoptosis or decreasing proliferation of endothelial cells (Kerbel and Kamen, 2004). Treatment with doxorubicin for 5 days inhibited HIF-1-dependent transcription and significantly reduced the levels of mRNAs encoding the angiogenic cytokines stem cell factor (also known as Kit ligand), stromal-derived factor 1 (SDF-1), and VEGF in human prostate cancer xenografts (Lee *et al*., 2009). These secreted proteins activate resident endothelial cells and also increase the numbers of circulating angiogenic cells, which home to the tumor and promote vascularization. Treatment of tumor-bearing mice with doxorubicin for 5 days reduced blood SDF-1 levels and the number of circulating angiogenic cells to levels similar to those observed in nontumor-bearing mice, and significantly reduced tumor vascularization, leading to growth arrest (Lee *et al*., 2009).

HIF-1 inhibitors sensitize tumors to radiation therapy

It is known that the hypoxic fraction of human cancers is resistant to radiation therapy due to reduced generation of oxygen radicals (Gray *et al*., 1953; Moeller *et al*., 2007). A major paradigm shift occurred with the discovery that the tumor vasculature represents an important target of radiation therapy and a major determinant of the clinical response (Garcia-Barros *et al*., 2003). Radiation was shown to induce HIF-1 activity, leading to the production of VEGF and other angiogenic cytokines that protect the endothelial cells of the tumor vasculature from radiation-induced death (Moeller *et al*., 2004). Treatment of tumor-bearing mice with the HIF-1 inhibitor YC-1 (3-(5′-hydroxymethyl-2′furyl)-1-benzylindazole) dramatically increased radiation-induced vessel destruction and tumor control (Moeller *et al*., 2004). The HIF-1 inhibitor PX-478 (*S*-2-amino-3-[4′-*N*,*N*,-bis(chloroethyl)amino]phenylpropionic acid *N*-oxide dihydrochloride) also blocked radiation-induced VEGF expression and thereby sensitized tumor xenografts to radiation therapy (Schwartz *et al*., 2009). Among patients with oropharyngeal cancer, those whose tumor biopsies showed the highest HIF-1α protein levels by immunohistochemistry had a significantly increased incidence of failure to achieve complete remission after radiation therapy (Aebersold *et al*., 2001). Taken together, these experimental and clinical data delineate an important role for HIF-1 in mediating radiation resistance and provide a compelling rationale for clinical trials that combine radiation therapy with HIF-1 inhibitors.

Perspective

It has only been 10 years since the first report of HIF-1α overexpression in primary human cancers and their metastases (Zhong *et al*., 1999), but during that time a large body of scientific data has been amassed delineating the mechanisms and consequences of increased HIF-1 activity during human cancer progression. Most recently, a growing number of drugs that inhibit HIF-1 have been identified and validated as anticancer agents. The challenge now is to identify on a patient-by-patient basis: (1) those cancers in which HIF-1 is having a critical role in disease pathogenesis; (2) those combinations of drugs or other treatment modalities to which addition of a HIF-1 inhibitor will have additive or even synergistic effects; and (3) clinical assays that will show an effect of HIF-1 inhibitors (or lack thereof) on critical aspects of cancer biology such as tumor metabolism and vascularization. Close collaborations between basic scientists and clinical oncologists will be needed to devise, evaluate and refine strategies for translating research knowledge into safe and effective cancer therapies.

Conflict of interest

The author declares no conflict of interest.

References

Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH *et al*. (2001). Expression of hypoxia-inducible factor-1α: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Res* **61**: 2911–2916.

Akakura N, Kobayashi M, Horiuchi I, Suzuki A, Wang J, Chen J *et al*. (2001). Constitutive expression of hypoxia-inducible factor-1α renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. *Cancer Res* **61**: 6548–6554.

Bachtiary B, Schindl M, Pötter R, Dreier B, Knocke TH, Hainfellner JA *et al*. (2003). Overexpression of hypoxia-inducible factor 1α indicates diminished response to radiotherapy and unfavorable prognosis in patients receiving radical radiotherapy for cervical cancer. *Clin Cancer Res* **9**: 2234–2240.

Barnhart BC, Simon MC. (2007). Metastasis and stem cell pathways. *Cancer Metastasis Rev* **26**: 261–271.

Bertout JA, Patel SA, Fryer BH, Durham AC, Covello KL, Olive KP *et al*. (2009). Heterozygosity for hypoxia inducible factor 1α decreases the incidence of thymic lymphomas in a p53 mutant mouse model. *Cancer Res* **69**: 3213–3220.

Bindra RS, Crosby ME, Glazer PM. (2007). Regulation of DNA repair in hypoxic cancer cells. *Cancer Metastasis Rev* **26**: 249–260.

Birner P, Gatterbauer B, Oberhuber G, Schindl M, Rössler K, Prodinger A *et al*. (2001a). Expression of hypoxia-inducible factor-1α in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. *Cancer* **92**: 165–171.

Birner P, Schindl M, Obermair A, Breitenecker G, Oberhuber G. (2001b). Expression of hypoxia-inducible factor 1α in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. *Clin Cancer Res* **7**: 1661–1668.

Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. (2000). Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. *Cancer Res* **60**: 4693–4696.

Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM *et al*. (2003). Levels of hypoxia-inducible factor-1α independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer* **97**: 1573–1581.

Brahimi-Horn MC, Chiche J, Pouysségur J. (2007). Hypoxia and cancer. *J Mol Med* **85**: 1301–1307.

Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin Jr WG. (2003). TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* **4**: 147–158.

Burri P, Djonov V, Aebersold DM, Lindel K, Studer U, Altermatt HJ *et al*. (2003). Significant correlation of hypoxia-inducible factor-1α with treatment outcome in cervical cancer treated with radical radiotherapy. *Int J Radiat Oncol Biol Phys* **56**: 494–501.

Cai Q, Murakami M, Si H, Robertson ES. (2007). A potential α-helix motif in the amino terminus of LANA encoded by Kaposi's sarcoma-associated herpesvirus is critical for nuclear accumulation of HIF-1α in normoxia. *J Virol* **81**: 10413–10423.

Cai QL, Knight JS, Verma SC, Zald P, Robertson ES. (2006). EC5S ubiquitin complex is recruited by KSHV latent antigen LANA for degradation of the VHL and p53 tumor suppressors. *PLoS Pathog* **2**: e116.

Cavallaro U, Christofori G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* **4**: 118–132.

Chan DA, Giaccia AJ. (2007). Hypoxia, gene expression, and metastasis. *Cancer Metastasis Rev* **26**: 333–339.

Chang Q, Qin R, Huang T, Gao J, Feng Y. (2006). Effect of antisense hypoxia-inducible factor 1α on progression, metastasis, and chemosensitivity of pancreatic cancer. *Pancreas* **32**: 297–305.

Chen J, Zhao S, Nakada K, Kuge Y, Tamaki N, Okada F *et al*. (2003). Dominant-negative hypoxia-inducible factor-1α reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. *Am J Pathol* **162**: 1283–1291.

Chiche J, Ilc K, Laferrière J, Trottier E, Dayan F, Mazure NM *et al*. (2009). Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res* **69**: 358–368.

Cleven AH, van Engeland M, Wouters BG, de Bruine AP. (2007). Stromal expression of hypoxia regulated proteins is an adverse prognostic factor in colorectal carcinomas. *Cell Oncol* **29**: 229–240.

Dales JP, Garcia S, Meunier-Carpentier S, Andrac-Meyer L, Haddad O, Lavaut MN *et al*. (2005). Overexpression of hypoxia-inducible factor HIF-1α predicts early relapse in breast cancer: retrospective study in a series of 745 patients. *Int J Cancer* **116**: 734–739.

Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE *et al*. (2008). Prognostic significance of hypoxia-inducible factor 1α (HIF-1α) expression in serous ovarian cancer: an immunohistochemical study. *BMC Cancer* **8**: 335.

Dewhirst MW, Cao Y, Moeller B. (2008). Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* **8**: 425–437.

Escuin D, Kline ER, Giannakakou P. (2005). Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1α accumulation and activity by disrupting microtubule function. *Cancer Res* **65**: 9021–9028.

Fath DM, Kong X, Liang D, Lin Z, Chou A, Jiang Y *et al*. (2006). Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF-α. *J Biol Chem* **281**: 13612–13619.

Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V *et al*. (2007). HIF-dependent antitumorigenic effect of antioxidants *in vivo*. *Cancer Cell* **12**: 230–238.

Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A *et al*. (2003). Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science* **300**: 1155–1159.

Gatenby RA, Gillies RJ. (2008). A microenvironmental model of carcinogenesis. *Nat Rev Cancer* **8**: 56–61.

Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S *et al*. (2006). Hypoxia-inducible factor-1α expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. *Clin Cancer Res* **12**: 4562–4568.

Giatromanolaki A, Koukourakis MI, Simopoulos C, Polychronidis A, Gatter KC, Harris AL *et al*. (2004). c-erbB-2 related aggressiveness in breast cancer is hypoxia inducible factor-1α dependent. *Clin Cancer Res* **10**: 7972–7977.

Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F *et al*. (2001). Relation of hypoxia inducible factor 1α and 2α in operable non-small cell lung cancer to angiogenic/molecular profile of tumors and survival. *Br J Cancer* **85**: 881–890.

Giatromanolaki A, Sivridis E, Kouskoukis C, Gatter KC, Harris AL, Koukourakis MI. (2003). Hypoxia-inducible factors 1α and 2α are related to vascular endothelial growth factor expression and a poorer prognosis in nodular malignant melanomas of the skin. *Melanoma Res* **13**: 493–501.

Gillies RJ, Gatenby RA. (2007). Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev* **26**: 311–317.

Gray LH, Conger AO, Ebert M, Hornsey S, Scott OCA. (1953). The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol* **26**: 638–648.

Griffiths EA, Pritchard SA, Valentine HR, Whitchelo N, Bishop PW, Ebert MP *et al*. (2007). Hypoxia-inducible factor-1α expression in the gastric carcinogenesis sequence and its prognostic role in gastric and gastro-oesophageal adenocarcinomas. *Br J Cancer* **96**: 95–103.

Hahnfeldt P, Folkman J, Hlatky L. (2003). Minimizing long-term tumor burden: the logic for metronomic chemotherapeutic dosing and its antiangiogenic basis. *J Theor Biol* **220**: 545–554.

Harris AL. (2002). Hypoxia—a key regulatory factor in tumor growth. *Nat Rev Cancer* **2**: 38–47.

Hiraga T, Kizaka-Kondoh S, Hirota K, Hiraoka M, Yoneda T. (2007). Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer. *Cancer Res* **67**: 4157–4163.

Imamura T, Kikuchi H, Herraiz MT, Park DY, Mizukami Y, Mino-Kenduson M *et al*. (2009). HIF-1α and HIF-2α have divergent roles in colon cancer. *Int J Cancer* **124**: 763–771.

Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. (2002). Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1α-degradative pathway. *J Biol Chem* **277**: 29936–29944.

Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL *et al*. (2005). HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* **8**: 143–153.

Jiang BH, Agani F, Passaniti A, Semenza GL. (1997). V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. *Cancer Res* **57**: 5328–5335.

Kaelin Jr WG, Ratcliffe PJ. (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* **30**: 393–402.

Kaluz S, Kaluzov M, Stanbridge EJ. (2006). Proteasomal inhibition attenuates transcriptional activity of hypoxia-inducible factor 1 (HIF-1) via specific effect on the HIF-1α C-terminal activation domain. *Mol Cell Biol* **26**: 5895–5907.

Kaluz S, Kaluzov M, Stanbridge EJ. (2008). Comment on the role of FIH in the inhibitory effect of bortezomib on hypoxia-inducible factor-1. *Blood* **111**: 5258–5259.

Kerbel RS, Kamen BA. (2004). The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer* **4**: 423–436.

Kondo K, Kim WY, Lechpammer M, Kaelin Jr WG. (2003). Inhibition of HIF-2α is sufficient to suppress pVHL-defective tumor growth. *PLoS Biol* **1**: e83.

Kondo S, Seo SY, Yoshizaki T, Wakisaka N, Furukawa M, Joab I *et al*. (2006). EBV latent membrane protein 1 up-regulates hypoxia-inducible factor 1α through Siah1-mediated down-regulation of prolyl hydroxylases 1 and 3 in nasopharyngeal epithelial cells. *Cancer Res* **66**: 9870–9877.

Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, Monks A *et al*. (2005). Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Res* **65**: 9047–9055.

Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J. (2006). Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1α. *Mol Cell Biol* **26**: 2019–2028.

Korkolopoulou P, Patsouris E, Konstantinidou AE, Pavlopoulos PM, Kavantzas N, Boviatsis E *et al*. (2004). Hypoxia-inducible factor 1α/vascular endothelial growth factor axis in astrocytomas. Associations with microvessel morphometry, proliferation and prognosis. *Neuropathol Appl Neurobiol* **30**: 267–278.

Koshiji M, To KK, Hammer S, Kumamoto K, Harris AL, Modrich P *et al*. (2005). HIF-1α induces genetic instability by transcriptionally downregulating MutSα expression. *Mol Cell* **17**: 793–803.

Koukourakis MI, Giatromanolaki A, Sivridis E, Simopoulos C, Turley H, Talks K *et al*. (2002). Hypoxia-inducible factor (HIF1A and HIF2A), angiogenesis, and chemoradiotherapy outcome of squamous cell head-and-neck cancer. *Int J Radiat Oncol Biol Phys* **53**: 1192–1202.

Krishnamachary B, Zagzag D, Nagasawa H, Rainey K, Okuyama H, Baek JH *et al*. (2006). Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel–Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. *Cancer Res* **66**: 2725–2731.

Kronblad A, Jirstrom K, Ryden L, Nordenskjold B, Landberg G. (2006). Hypoxia inducible factor-1α is a prognostic marker in premenopausal patients with intermediate to highly differentiated breast cancer but not a predictive marker for tamoxifen response. *Int J Cancer* **118**: 2609–2616.

Kung AL, Wang S, Klco JM, Kaelin WG, Livingston DM. (2000). Suppression of tumor growth through disruption of hypoxia-inducible transcription. *Nat Med* **6**: 1335–1340.

Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. (2002). FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* **16**: 1466–1471.

Lau KW, Tian YM, Raval RR, Ratcliffe PJ, Pugh CW. (2007). Target gene selectivity of hypoxia-inducible factor-α in renal cancer cells is conveyed by post-DNA-binding mechanisms. *Br J Cancer* **96**: 1284–1292.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. (2001). HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1α (HIF-1α) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* **21**: 3995–4004.

Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL. (2009). Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. *Proc Natl Acad Sci USA* **106**: 2353–2358.

Li F, Sonveaux P, Rabbani ZN, Liu S, Yan B, Huang Q *et al*. (2007). Regulation of HIF-1α stability through *S*-nitrosylation. *Mol Cell* **26**: 63–74.

Li L, Lin X, Staver M, Shoemaker A, Semizarov D, Fesik SW *et al*. (2005). Evaluating hypoxia-inducible factor-1α as a cancer therapeutic target via inducible RNA interference *in vivo*. *Cancer Res* **65**: 7249–7258.

Liao D, Corle C, Seagroves TN, Johnson RS. (2007). Hypoxia-inducible factor-1α is a key regulator of metastasis in a transgenic model of cancer initiation and progression. *Cancer Res* **67**: 563–572.

Liao D, Johnson RS. (2007). Hypoxia: a key regulator of angiogenesis in cancer. *Cancer Metastasis Rev* **26**: 281–290.

Liu Y, Tao J, Li Y, Yang J, Yu Y, Wang M *et al*. (2009). Targeting hypoxia-inducible factor-1α with Tf–PEI–shRNA complex via transferrin receptor-mediated endocytosis inhibits melanoma growth. *Mol Ther* **17**: 269–277.

Liu YV, Baek JH, Zhang H, Diez R, Cole RN, Semenza GL. (2007). RACK1 competes with HSP90 for binding to HIF-1α and is required for O_2-independent and HSP90 inhibitor-induced degradation of HIF-1α. *Mol Cell* **25**: 207–217.

Lukashev D, Ohta A, Sitkovsky M. (2007). Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues. *Cancer Metastasis Rev* **26**: 273–279.

Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z. (2005). The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1α, leading to transcriptional inhibition of vascular endothelial growth factor expression. *Oncogene* **24**: 4433–4441.

Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, Xue Q *et al*. (2004). mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med* **10**: 594–601.

Makino Y, Uenishi R, Okamoto K, Isoe T, Hosono O, Tanaka H *et al*. (2007). Transcriptional up-regulation of inhibitory PAS domain protein gene expression by hypoxia-inducible factor 1 (HIF-1): a negative feedback regulatory circuit in HIF-1-mediated signaling in hypoxic cells. *J Biol Chem* **282**: 14073–14082.

Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ *et al*. (2005). Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* **105**: 659–669.

Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ *et al*. (1997). Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci USA* **94**: 8104–8109.

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME *et al*. (1999). The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**: 271–275.

Mayerhofer M, Valent P, Sperr WR, Griffin JD, Sillaber C. (2002). BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1α, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood* **100**: 3767–3775.

Melillo G. (2007). Targeting hypoxia cell signaling for cancer therapy. *Cancer Metastasis Rev* **26**: 341–352.

Moeller BJ, Cao Y, Li CY, Dewhirst MW. (2004). Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. *Cancer Cell* **5**: 429–441.

Moeller BJ, Richardson RA, Dewhirst MW. (2007). Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev* **26**: 241–248.

Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J *et al*. (2009). Genome-wide association of hypoxia-inducible factor (HIF)-1α and HIF-2α DNA binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem* **284**: 16767–16775.

Nakamura M, Bodily JM, Beglin M, Kyo S, Inoue M, Laimins LA. (2009). Hypoxia-specific stabilization of HIF-1α by human papillomaviruses. *Virology* **387**: 442–448.

Nanni S, Benvenuti V, Grasselli A, Priolo C, Aiello A, Mattiussi S *et al*. (2009). Endothelial NOS, estrogen receptor beta, and HIFs cooperate in the activation of a prognostic transcriptional pattern in aggressive human prostate cancer. *J Clin Invest* **119**: 1093–1108.

Pagès G, Pouyssègur J. (2005). Transcriptional regulation of the vascular endothelial growth factor gene—a concert of activating factors. *Cardiovasc Res* **65**: 564–573.

Pore N, Jiang Z, Gupta A, Cerniglia G, Kao GD, Maity A. (2006). EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. *Cancer Res* **66**: 3197–3204.

Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, Atadja P *et al*. (2006). Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1α. *Cancer Res* **66**: 8814–8821.

Quintero M, Brennan PA, Thomas GJ, Moncada S. (2006). Nitric oxide is a factor in the stabilization of hypoxia-inducible factor-1α in cancer: role of free radical formation. *Cancer Res* **66**: 770–774.

Rajaganeshan R, Prasad R, Guillou PJ, Poston G, Scott N, Jayne DG. (2008). The role of hypoxia in recurrence following resection of Dukes' B colorectal cancer. *Int J Colorectal Dis* **23**: 1049–1055.

Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH *et al*. (2002). Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res* **62**: 4316–4324.

Rasheed S, Harris AL, Tekkis PP, Turley H, Silver A, McDonald PJ *et al*. (2009). Hypoxia-inducible factor-1α and -2α are expressed in most rectal cancers but only hypoxia-inducible factor-1α is associated with prognosis. *Br J Cancer* **100**: 1666–1673.

Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, LI J-L *et al*. (2005). Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel–Lindau-associated renal cell carcinoma. *Mol Cell Biol* **25**: 5675–5686.

Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q *et al*. (2000). Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1α. *Genes Dev* **14**: 34–44.

Schindl M, Schoppmann SF, Samonigg H, Hausmaninger H, Kwasny W, Gnant M *et al*. (2002). Overexpression of hypoxia-inducible factor 1α is associated with an unfavorable prognosis in lymph node-positive breast cancer. *Clin Cancer Res* **8**: 1831–1837.

Schmitz KJ, Müller CI, Reis H, Alakus H, Winde G, Baba HA *et al*. (2009). Combined analysis of hypoxia-inducible factor 1α and metallothionein indicates an aggressive subtype of colorectal carcinoma. *Int J Colorectal Dis* **24**: 1287–1296.

Schrijvers ML, van der Laan BF, de Bock GH, Pattje WJ, Mastik MF, Menkema L *et al*. (2008). Overexpression of intrinsic hypoxia markers HIF-1α and CA-IX predict for local recurrence in stage T1-T2 glottic laryngeal carcinoma treated with radiotherapy. *Int J Radiat Oncol Biol Phys* **72**: 161–169.

Schwartz DL, Powis G, Thitai-Kumar A, He Y, Bankson J, Williams R *et al*. (2009). The selective hypoxia inducible factor-1 inhibitor PX-478 provides *in vivo* radiosensitization through tumor stromal effects. *Mol Cancer Ther* **8**: 947–958.

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD *et al*. (2005). Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. *Cancer Cell* **7**: 77–85.

Semenza GL. (2000). Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. *Crit Rev Biochem Mol Biol* **35**: 71–103.

Semenza GL. (2009a). Oxygen homeostasis. *Systems Biol Med* (doi:10.1002/wsbm.69).

Semenza GL. (2009b). Regulation of cancer cell metabolism by hypoxia-inducible factor 1. *Semin Cancer Biol* **19**: 12–16.

Shackelford DB, Vasquez DS, Corbeil J, Wu S, Leblanc M, Wu CL *et al*. (2009). mTOR and HIF-1α-mediated tumor metabolism in an LKB1 mouse model of Peutz–Jeghers syndrome. *Proc Natl Acad Sci USA* **106**: 11137–11142.

Shin DH, Chun YS, Lee DS, Huang LE, Park JW. (2008). Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated repression of hypoxia-inducible factor-1. *Blood* **111**: 3131–3136.

Shin YC, Joo CH, Gack MU, Lee HR, Jung JU. (2008). Kaposi's sarcoma-associated herpesvirus viral IFN regulatory factor 3 stabilizes hypoxia-inducible factor-1α to induce vascular endothelial growth factor expression. *Cancer Res* **68**: 1751–1759.

Sivridis E, Giatromanolaki A, Gatter KC, Harris AL, Koukourakis MI. (2002). Association of hypoxia-inducible factors 1α and 2α with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. *Cancer* **95**: 1055–1063.

Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri EA *et al*. (2000). The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1α. *Cancer Res* **60**: 4873–4880.

Stoeltzing O, McCarty MF, Wey JS, Fan F, Liu W, Belcheva A *et al*. (2004). Role of hypoxia-inducible factor 1α in gastric cancer cell growth, angiogenesis, and vessel maturation. *J Natl Cancer Inst* **96**: 946–956.

Sullivan R, Graham CH. (2007). Hypoxia-driven selection of the metastatic phenotype. *Cancer Metastasis Rev* **26**: 319–331.

Sun HC, Qiu ZJ, Liu J, Sun J, Jiang T, Huang KJ *et al*. (2007). Expression of hypoxia-inducible factor-1α and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. *Int J Oncol* **30**: 1359–1367.

Swietach P, Vaughan-Jones RD, Harris AL. (2007). Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev* **26**: 299–310.

Swinson DE, Jones JL, Cox G, Richardson D, Harris AL, O'Byrne KJ. (2004). Hypoxia-inducible factor-1α in non small cell lung cancer: relation to growth factor, protease and apoptosis pathways. *Int J Cancer* **111**: 43–50.

Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ *et al*. (2000). The expression and distribution of the hypoxia-inducible factors HIF-1α and HIF-2α in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* **157**: 411–421.

Tang N, Wang L, Esko J, Giordano FJ, Huang Y, Gerber HP *et al*. (2004). Loss of HIF-1α in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. *Cancer Cell* **6**: 485–495.

Takahashi R, Tanaka S, Hiyama T, Ito M, Kitadai Y, Sumii M *et al*. (2003). Hypoxia-inducible factor-1α expression and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncol Rep* **10**: 797–802.

Theodoropoulos VE, Lazaris AC, Kastriotis I, Spiliadi C, Theodoropoulos GE, Tsoukala V *et al*. (2005). Evaluation of hypoxia-inducible factor 1α overexpression as a predictor of tumor recurrence and progression in superficial urothelial bladder carcinoma. *BJU Int* **95**: 425–431.

Theodoropoulos VE, Lazaris AC, Sofras F, Gerzelis I, Tsoukala V, Ghikonti I *et al*. (2004). Hypoxia-inducible factor 1α expression correlates with angiogenesis and unfavorable prognosis in bladder cancer. *Eur Urol* **46**: 200–208.

Thomas GV, Tran C, Mellinghoff IK, Welsbie DS, Chan E, Fueger B *et al*. (2006). Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med* **12**: 122–127.

Tomita M, Semenza GL, Michiels C, Matsuda T, Uchihara JN, Okudaira T *et al*. (2007). Activation of hypoxia-inducible factor 1 in human T-cell leukaemia virus type 1-infected cell lines and primary adult T-cell leukaemia cells. *Biochem J* **406**: 317–323.

Trastour C, Benizri E, Ettore F, Ramaioli A, Chamorey E, Pouyssegur J *et al*. (2007). HIF-1α and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. *Int J Cancer* **120**: 1443–1450.

Tzao C, Lee SC, Tung HJ, Hsu HS, Hsu WH, Sun GH *et al*. (2008). Expression of hypoxia-inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF)-D as outcome predictors in resected esophageal squamous cell carcinoma. *Dis Markers* **25**: 141–148.

Vaupel P, Mayer A, Hockel M. (2004). Tumor hypoxia and malignant progression. *Methods Enzymol* **381**: 335–354.

Vleugel MM, Greijer AE, Shvarts A, van der Groep P, van Berkel M, Aarbodem Y *et al*. (2005). Differential prognostic impact of hypoxia induced and diffuse HIF-1α expression in invasive breast cancer. *J Clin Pathol* **58**: 172–177.

Vogelstein B, Kinzler KW. (2004). Cancer genes and the pathways they control. *Nat Med* **10**: 789–799.

Wang GL, Jiang BH, Rue EA, Semenza GL. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci USA* **92**: 5510–5514.

Wang GL, Semenza GL. (1995). Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* **270**: 1230–1237.

Wang XL, Xu R, Wu X, Gillespie D, Jensen R, Lu ZR. (2009). Targeted systemic delivery of a therapeutic siRNA with a multi-functional carrier controls tumor proliferation in mice. *Mol Pharm* **6**: 738–746.

Yamamoto Y, Ibusuki M, Okumura Y, Kawasoe T, Kai K, Iyama K *et al*. (2008). Hypoxia-inducible factor 1α is closely linked to an aggressive phenotype in breast cancer. *Breast Cancer Res Treat* **110**: 465–475.

Yoo YG, Cho S, Park S, Lee MO. (2004). The carboxy-terminus of the hepatitis B virus X protein is necessary and sufficient for the activation of hypoxia-inducible factor-1α. *FEBS Lett* **577**: 121–126.

Yu J, Zhang L, Hwang PM, Rago C, Kinzler KW, Vogelstein B. (1999). Identification and classification of p53-regulated genes. *Proc Natl Acad Sci USA* **96**: 14517–14522.

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR *et al*. (2008). Digoxin and other cardiac glycosides inhibit HIF-1α synthesis and block tumor growth. *Proc Natl Acad Sci USA* **105**: 19579–19586.

Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P *et al*. (2009). Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1α. *Science* **324**: 261–265.

Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM *et al*. (2000). Modulation of hypoxia-inducible factor 1α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* **60**: 1541–1545.

Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D *et al*. (1999). Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Res* **59**: 5830–5835.

Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E *et al*. (2000). Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* **14**: 391–396.