

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. O₂ concentrations are markedly reduced in many human cancers compared with normal tissue, and a major mechanism mediating adaptive responses to reduced O₂ 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 O₂ concentrations, which decline as distance from the vessel increases. Although such gradients exist in normal tissues, in cancers the gradients are much steeper and O₂ 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 O₂ concentration. A major mechanism mediating adaptive responses to reduced O₂ 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).

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

HIF-1 is a heterodimeric protein that is composed of a constitutively expressed HIF-1 β subunit and an O₂-regulated HIF-1 α subunit (Wang and Semenza, 1995; Wang *et al.*, 1995). HIF-1 α is subjected to O₂-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-1 α , thereby targeting it for proteasomal degradation (Kaelin and Ratcliffe, 2008). Under hypoxic conditions, hydroxylation is inhibited and HIF-1 α rapidly accumulates, dimerizes with HIF-1 β , binds to the core DNA binding sequence 5'-RCGTG-3' (R, purine (A or G)) in target genes, recruits co-activators and activates transcription. O₂-dependent hydroxylation of asparagine-803 by factor inhibiting HIF-1 (FIH-1) blocks interaction of HIF-1 α with the co-activators P300 and CBP under normoxic conditions (Lando *et al.*, 2002). Both PHD2 and FIH-1 use O₂ and α -ketoglutarate as substrates and generate CO₂ and succinate as by-products of the hydroxylation reaction. HIF-2 α is a protein with extensive sequence similarity to HIF-1 α that is also regulated by proline and asparagine hydroxylation, dimerizes with HIF-1 β and activates transcription of a group of target genes that overlaps with, but is distinct from, those regulated by HIF-1 α (Lau *et al.*, 2007). HIF-3 α 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).

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Immunohistochemical analysis of human cancer biopsies revealed increased levels (relative to surrounding normal tissue) of HIF-1 α or HIF-2 α 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-1 α and HIF-2 α 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-1 α in the tumor micro-environment include reactive oxygen and nitrogen species, which also inhibit proteasomal degradation of HIF-1 α (Quintero *et al.*, 2006; Gao *et al.*, 2007; Li *et al.*, 2007; Dewhirst *et al.*, 2008). Complementing these mechanisms for blocking HIF-1 α 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-1 α synthesis, primarily through the action of mTOR (Laughner *et al.*, 2001).

HIF-1 α and HIF-2 α 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-1 α synthesis or decreased HIF-1 α 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-1 α 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-1 α and HIF-2 α 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-1 α or HIF-2 α 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-1 α in human cancers

TSG	Tumor(s) with TSG loss of function	Effect on HIF-1 α	Reference
VHL	Renal carcinoma, hemangioblastoma	Decr ubiquitination	Maxwell <i>et al.</i> (1999)
SDHB	Paranglioma	Decr hydroxylation	Selak <i>et al.</i> (2005)
SDHC	Paranglioma	Decr hydroxylation	Selak <i>et al.</i> (2005)
SDHD	Paranglioma	Decr hydroxylation	Selak <i>et al.</i> (2005)
FH	Leiomyoma, renal carcinoma	Decr hydroxylation	Isaacs <i>et al.</i> (2005)
IDH1	Glioblastoma	Decr hydroxylation	Zhao <i>et al.</i> (2009)
P53	Many	Decr ubiquitination	Ravi <i>et al.</i> (2000)
TSC2	Tuberous sclerosis	Incr synthesis	Brugarolas <i>et al.</i> (2003)
PTEN	Glioblastoma, others	Incr synthesis	Zhong <i>et al.</i> (2000)
LKB1	Gastrointestinal hamartoma	Incr synthesis	Zundel <i>et al.</i> (2000) Shackelford <i>et al.</i> (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-1 α	Reference
EBV latent membrane protein 1	Decr degradation	Kondo <i>et al.</i> (2006)
Hepatitis B virus X protein	Decr degradation	Yoo <i>et al.</i> (2004)
Human papillomavirus E6/E7 proteins	Incr HIF-1 α protein	Nakamura <i>et al.</i> (2009)
Human T-cell leukemia virus tat protein	Incr HIF-1 α protein	Tomita <i>et al.</i> (2007)
KSHV G-protein-coupled receptor	Incr transactivation	Sodhi <i>et al.</i> (2000)
KSHV latency-associated nuclear antigen	Incr nuclear localization	Cai <i>et al.</i> (2007)
KSHV latency-associated nuclear antigen	Decr degradation	Cai <i>et al.</i> (2006)
KSHV viral interferon regulatory factor 3	Decr degradation	Shin <i>et al.</i> (2008)

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

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

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

^aCombination of high HIF-1 α and mutant p53 was associated with mortality.

^bHIF-2 α expression in stromal cells (not in cancer cells) was associated with mortality.

^cHigh HIF-1 α and high HIF-2 α were each associated with mortality.

^dHigh HIF-2 α (not HIF-1 α) was associated with mortality.

^eCombination of high HIF-2 α and nuclear localization of endothelial nitric oxide synthase was associated with mortality.

but the subset of patients whose tumors contained high levels of HIF-1 α had a significantly increased mortality rate. Thus, HIF-1 α 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-1 α and HIF-2 α levels to cancer progression

Complementing the clinical data is a large body of experimental data showing that HIF-1 α LOF results in decreased tumor growth, vascularization and metastasis,

whereas HIF-1 α 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-1 α or HIF-2 α . Whereas the overall conclusion of these experiments is that HIF-1 α and HIF-2 α promote cancer progression, the association is not absolute. For example, whereas HIF-1 α LOF decreased the growth of SW480 colorectal carcinoma xenografts, HIF-2 α LOF increased xenograft growth and, remarkably, immunohistochemical analysis of human colon cancer biopsies revealed a significant association between loss of HIF-2 α protein expression and advanced tumor stage (Imamura *et al.*, 2009). In contrast, HIF-2 α GOF increased the growth of 786-O renal cell carcinoma xenografts whereas HIF-1 α 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-1 α knockout allele as compared to mutant p53 mice that are wild type for HIF-1 α (Bertout *et al.*, 2009) and in mice with conditional knockout of HIF-1 α in vascular endothelial cells (Tang *et al.*, 2004). There are no published examples of combined HIF-1 α and HIF-2 α inhibition in tumor and stroma resulting in increased tumor growth. Thus, inhibitors that target HIF-1 α and HIF-2 α 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-1 α and HIF-2 α 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
Hepal hepatoma	HIF-1 β LOF ^a	Xenograft	Decr growth	Jiang <i>et al.</i> (1997)
Hepal hepatoma	HIF-1 β LOF	Xenograft	Decr growth, MVD ^b	Maxwell <i>et al.</i> (1997)
HCT116 colorectal	HIF-1 α GOF ^c	Xenograft	Incr growth, MVD	Ravi <i>et al.</i> (2000)
HCT116 colorectal	Gal-TAD-C ^d	Xenograft	Decr growth	Kung <i>et al.</i> (2000)
PCI-10 pancreatic	HIF-1 α GOF	Xenograft	Incr growth	Akakura <i>et al.</i> (2001)
PCI-43 pancreatic	HIF-1 α DN ^e	Xenograft	Decr growth, metab ^f	Chen <i>et al.</i> (2003)
786-O renal clear cell	HIF-2 α shr ^g	Xenograft	Decr growth	Kondo <i>et al.</i> (2003)
TMK1 gastric	HIF-1 α DN	Orthotopic	Decr growth, vessel maturity	Stoeltzing <i>et al.</i> (2004)
D54MG glioma	HIF-1 α shr	Xenograft	Decr growth	Li <i>et al.</i> (2005)
786-O renal clear cell	HIF-2 α GOF	Xenograft	Incr growth	Raval <i>et al.</i> (2005)
BxPc-3 pancreatic	HIF-1 α AS ^h	Xenograft	Decr growth	Chang <i>et al.</i> (2006)
PC3 prostate	HIF-1 α shr	Xenograft	Decr growth	Gao <i>et al.</i> (2007)
MDA-MB231 breast	HIF-1 α G/L ⁱ	Intracardiac	Incr/Decr bone mets ^j	Hiraga <i>et al.</i> (2007)
Breast cancer	HIF-1 α cKO ^k	Spontaneous	Decr lung mets	Liao <i>et al.</i> (2007)
SW480 colorectal	HIF-1 α shr	Xenograft	Decr growth	Imamura <i>et al.</i> (2009)
A375 melanoma	Tf-PEI-shr ^l	Xenograft	Decr growth	Liu <i>et al.</i> (2009)
U87 glioma	HIF-1 α sir ^m	Xenograft	Decr growth	Wang <i>et al.</i> (2009)

^aLoss of function (spontaneous mutation).^bMicrovessel density.^cGain of function (stably transfected HIF-1 α expression vector).^dGAL4 fusion protein containing HIF-1 α C-terminal transactivation domain (LOF).^eHIF-1 α DN, dominant-negative form of HIF-1 α (stably transfected expression vector) LOF.^fImpaired glucose metabolism.^gShort-hairpin RNA (inducible expression vector) LOF.^hAntisense RNA expression vector (LOF).ⁱGain or loss of function by constitutively active HIF-1 α or HIF-1 α DN.^jMetastases.^kMice with conditional HIF-1 α knockout/polyoma middle T expression in mammary epithelium.^lIntravenous injection of transferrin-polyethylenimine-HIF-1 α shRNA complex (LOF).^mIntravenous 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), ZFH1A (also known as δ EF1 or ZEB1) and ZFH1B (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 MutS α , by blocking the interaction of MYC with SP1, an effect that does not require direct DNA binding or even the presence of HIF-1 β (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-1 α and HIF-2 α . 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-1 α 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
<i>C-MET</i>	Invasion
<i>CXCR4</i>	Metastasis
<i>DEC1</i>	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
<i>JARID1B</i>	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
<i>TWIST1</i>	Epithelial-mesenchymal transition
Urokinase plasminogen activator receptor	Invasion
Vascular endothelial growth factor	Angiogenesis
<i>WSB1</i>	Cell survival
<i>ZEB1</i> (ZFHX1A), <i>ZEB2</i> (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 Pouyssegur, 2005).

Another novel class of molecularly targeted anticancer agents consists of inhibitors of heat shock protein 90 (HSP90), such as geldanamycin, 17-allylamino-geldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin, which target HIF-1 α for proteasomal degradation (Isaacs *et al.*, 2002). In the absence of inhibitors, HSP90 binds to HIF-1 α 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-1 α (Liu *et al.*, 2007). Histone deacetylase inhibitors are another novel class of anticancer drugs that promote HIF-1 α degradation (Kong *et al.*, 2006; Qian *et al.*, 2006) and inhibit HIF-1 α 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-1 α (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 <i>et al.</i> (2009)
EGFR inhibitors	Cetuximab	B	Luwor <i>et al.</i> (2005); Pore <i>et al.</i> (2006)
Cardiac glycosides	Digoxin	B	Zhang <i>et al.</i> (2008)
Histone deacetylases	TSA ^b	C	Kong <i>et al.</i> (2006); Qian <i>et al.</i> (2006)
		D	Fath <i>et al.</i> (2006)
HSP90 inhibitors	17-AAG ^c	C	Isaacs <i>et al.</i> (2002); Liu <i>et al.</i> (2007)
Microtubule targeting agents	Taxotere	B	Escuin <i>et al.</i> (2005)
mTOR inhibitors	Rapamycin	B	Zhong <i>et al.</i> (2000); Laughner <i>et al.</i> (2001)
Proteasome inhibitors	Bortezomib	D	Kaluz <i>et al.</i> (2006)
Topoisomerase I inhibitors	Topotecan	B	Rapisarda <i>et al.</i> (2002)

^aMechanisms of action that lead to decreased HIF-1 transcriptional activity are as follows: A, decreased HIF-1 DNA binding; B, decreased HIF-1 α synthesis; C, increased HIF-1 α degradation; D, decreased HIF-1 α transactivation.

^bTrichostatin A.

^c17-Allylamino-geldanamycin.

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 O₂ 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-1 α 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-1 α 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.

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