Minireview

Hypoxia inducible factor-1α as a cancer drug target

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Abstract

The hypoxia inducible factor 1 (HIF-1) is a heterodimeric transcription factor that is an important regulator of the growing tumor's response to hypoxia. HIF-1 activity in tumors depends on the availability of the HIF-1α subunit, the levels of which increase under hypoxic conditions and through the activation of oncogenes and/or inactivation of tumor suppressor genes. HIF-1 activates genes that allow the cancer cell to survive and grow in the hostile hypoxic tumor environment. Increased tumor HIF-1α has been correlated with increased angiogenesis, aggressive tumor growth, and poor patient prognosis, leading to the current interest in HIF-1α as a cancer drug target. A number of anticancer agents have been reported to decrease HIF-1α or HIF-1 transactivating activity in cells in culture. However, more relevant to the agents' antitumor activity is whether HIF-1 is inhibited in tumors *in vivo*. This has been demonstrated for only a few of the reported HIF-1 inhibitors. Some of the agents are moving toward clinical trial where it will be important to demonstrate that the agents inhibit HIF-1α in patient tumors or, failing this, the downstream consequences of HIF-1 inhibition such as decreased vascular endothelial growth factor formation, and relate this inhibition to antitumor activity. Only in this way will it be possible to determine if HIF-1α is a valid cancer drug target in humans. [Mol Cancer Ther 2004; 3(5):647–54]

The Cellular Response to Hypoxia

Mammalian cells have an array of responses that maintain oxygen homeostasis; a balance between the requirement for oxygen as an energy substrate and the inherent risk of oxidative damage to cellular macromolecules. The molecular basis for a variety of cellular and systemic mechanisms of oxygen homeostasis are now being identified and the mechanisms have been found to occur at every regulatory level, including gene transcription, protein translation, posttranslational modification, and cellular localization (1).

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Hypoxic cancer cells occur for a number of reasons. Oxygen is only able to diffuse 100–180 μm from a capillary to cells before it is completely metabolized. Therefore, any cell located greater than this distance from a blood vessel will be hypoxic. Hypoxia may occur when aberrant blood vessels are shutdown by becoming compressed or obstructed by growth, a feature commonly observed during the rapid growth of tumors.

Cells that become hypoxic convert to a glycolytic metabolism, become resistant to apoptosis (programmed cell death), and are more likely to migrate to less hypoxic areas of the body (metastasis). Hypoxic cells also produce pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), which stimulate new blood vessel formation from existing vasculature, increasing tumor oxygenation and, ultimately, tumor growth. For this reason, hypoxic tumors are the most pro-angiogenic and aggressive of tumors.

The Hypoxia Inducible Transcription Factor

The most important mediator identified to date of the cell's response to reduced oxygen availability is the hypoxia inducible factor 1 (HIF-1) transcription factor (reviewed in Refs. 1, 2). HIF-1 controls the expression of a variety of genes, the protein products of which play crucial roles in the acute and chronic adaptation to oxygen deficiency, including erythropoiesis, glycolysis, promotion of cell survival, inhibition of apoptosis, inhibition of cell differentiation, and angiogenesis. Loss of HIF-1 activity dramatically decreases tumor growth, vascularization, and energy metabolism, whereas overexpression of HIF-1α increases HIF-1 transcription factor activity and promotes tumor growth.

HIF-1 is a heterodimer consisting of a HIF-1α subunit and a HIF-1β subunit, also known as the aryl hydrocarbon nuclear translocator (ARNT) (Fig. 1). Both subunits are members of the basic-helix-loop-helix (bHLH) PER, ARNT, SIM (PAS) superfamily of eukaryotic transcription factors in which DNA binding is mediated by the basic domains and subunit dimerization by the bHLH domains. Hetrodimers containing HIF-2α or HIF-3α and ARNT are designated HIF-2 and HIF-3, respectively.

Expression of HIF-1α and HIF-1α mRNA has been detected in all adult and embryonic mouse and human tissues analyzed to date. HIF-2α mRNA has a restricted distribution in vascular endothelial cells, catecholamine-producing cells, kidney, and lung during embryogenesis in addition to tumor-associated macrophages. Knockout studies show that HIF-1α, HIF-2α, and HIF-1β are required

for normal mouse development. Less is known about HIF-3α compared to other family members. HIF-3α shows high structural similarity within the bHLH and PAS domains, but lacks the structures for transactivation found in the COOH terminus of HIF-1α and HIF-2α. HIF-3α mRNA is expressed in adult thymus, lung, brain, heart, and kidney. Interestingly, although HIF-3α is up-regulated in response to hypoxia and dimerizes with ARNT, transient transfection experiments suggest that HIF-3α may be a negative regulator of hypoxia inducible gene expression. Additionally, the inhibitory PAS domain protein (IPAS), an alternatively spliced form of HIF-3α, has been shown to negatively regulate HIF-1α function.

Regulation of HIF-1α Protein Levels

The best studied mechanism of HIF-1α protein regulation is the pathway mediated by the von Hippel-Lindau (pVHL) protein (3) (Fig. 1). von Hippel-Lindau protein binds directly to the oxygen degradation domain of HIF-α which recruits an ubiquitin-protein ligase complex containing elongin B, elongin C, and cullin, resulting in ubiquitination and degradation of HIF-α by the 26S proteasome pathway. The binding of von Hippel-Lindau protein is mediated by hydroxylation of two specific proline residues (Pro402 and Pro564 in human HIF-1α) by a prolyl-4-hydroxylase (PHD). PHDs are dioxygenases that show an absolute requirement for oxygen, Fe^{2+}, and 2-oxoglutarate as substrates. There are now four identified PHDs that have distinct functions. PHD2 is the critical oxygen sensor setting the low steady-state levels of HIF-1α in normoxia, and being up-regulated by hypoxia, thus, providing an HIF-1-dependent auto-regulatory mechanism driven by oxygen tension. Under oxygen-deprived conditions, or when PHDs are inactivated by competitive substrate analogues, the HIF prolines remain unmodified preventing binding of von Hippel-Lindau protein and HIF-α levels increase.

The von Hippel-Lindau protein pathway is not the only pathway controlling levels of HIF-1α. The MDM2 ubiquitin protein ligase is recruited to HIF-1α by binding of the tumor suppressor p53 which results in a decrease in HIF-1α levels by promoting MDM-2-mediated ubiquitination and proteasomal degradation of HIF-1α (4). Indeed, loss of p53 in tumor cells enhances HIF-1α levels. Another von Hippel-Lindau protein-independent pathway for degradation of HIF-1α involves the heat shock protein 90 (Hsp-90). Hsp-90 interacts directly with HIF-1α and has been suggested to promote a conformational change of HIF-1α in response to dimerization with HIF-1β (5). Hsp-90 inhibitors such as geldanamycin promote loss of HIF-1α protein even in cell lines lacking von Hippel-Lindau protein (6). Mutation of prolines 402 and 564 in HIF-1α does not protect HIF-1α from geldanamycin-induced degradation, suggesting that Hsp-90 degradation involves a novel E3 ubiquitin ligase.

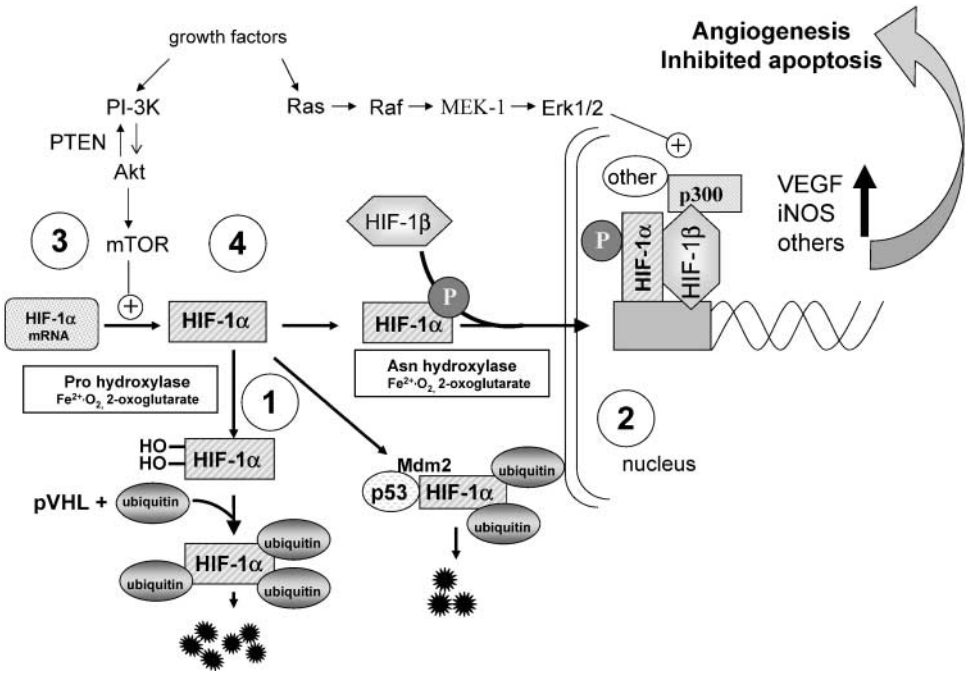

Figure 1. Hypoxia signaling through HIF-1. HIF-1α combines with HIF-1β (ARNT) to regulate the transcription of hypoxia-regulated genes involved in glycolysis, angiogenesis, cell survival, and metastasis. There are four mechanisms for regulating HIF-1α activity. *1,* Pro 4-hydroxylase hydroxylates the oxygen degradation domain of HIF-1α allowing the von Hippel-Lindeau protein (*pVHL*) to bind leading to ubiquitination and degradation of HIF-1α by the 26S proteasome; p53 binds to HIF-1α recruiting Mdm2 also leading to ubiquitination and degradation by the 26S proteasome; *2,* HIF-1 transactivating activity is decreased by Asn hydroxylase which decreases p300 transcription co-activator binding; *3,* the activation of Akt and mTOR by phosphatidylinositol 3-kinase and antagonized by PTEN, increases HIF-1α levels by increasing HIF-1α translation; and *4,* p300 phosphorylation by MAPK signaling increases HIF-1/p300 complex formation. Not shown is the Hsp-90 stabilization of HIF-1α. Hypoxia inhibits process *1* and *2*, stimulates *3*, and has no effect on *4*.

The phosphatidylinositol-3-kinase (PI3K)/AKT pathway and its downstream target mammalian target of rapamycin (mTOR) have been shown to increase levels of HIF-1α protein (7, 8). mTOR increases protein translation by phosphorylation of the mRNA cap binding protein eukaryotic initiation factor 4E (eIF4E), providing a potential mechanism for increasing HIF-1α levels under normoxic conditions. However, mTOR may increase HIF-1α levels under hypoxia by a mechanism that does not involve eIF4E, because HIF-1α translation has been shown to occur independently of eIF4E under hypoxic conditions. It should also be noted that there are contradictory reports in the literature of the effects of the PI3K/AKT pathway on HIF-1α in different cell lines (9).

Transactivation of HIF-1

Despite the designation of ARNT (HIF-1β) as a nuclear translocator, it is not required for translocation of HIF-1α into the nucleus. Rather, HIF-1α translocates into the nucleus due to the presence of two nuclear localization signals: amino acids 17–33 within the bHLH domain and amino acids 718–721 within the COOH-terminal regulatory domain (10). The transactivation domains of HIF-1α have been identified and characterized. Truncation mutants initially revealed that amino acids 390–826 of human HIF-1α mediated hypoxia inducible transactivation while a construct lacking amino acids 4–27 (the basic domain) and amino acids 391–826 (the transactivation domain) resulted in a protein that could dimerize with HIF-1β but could not bind to DNA or activate transcription (11). Transfection of a vector encoding this protein caused dose-dependent inhibition of HIF-1 reporter gene activity in hypoxic cells. Further study identified two independently regulated transactivation domains: the NH_2-terminal transactivation domain (amino acids 531–575) and the COOH-terminal transactivation domain (amino acids 786–826) (12).

The transactivation domains of HIF-1 interact with the general transcriptional machinery to activate transcription from promoters of HIF-1 target genes. These complexes form the scaffold to which co-activators possessing histone acetyltransferase activity can bind to activate chromatin remodeling for initiation of transcription. The co-activators CBP and p300 have been shown to interact with HIF-1α to activate transcription via the CH1 region of p300/CBP. This interaction can also be enhanced by SRC-1 and, at limiting concentrations, SRC-1 produces this effect synergistically with CBP. A p300-CH1 interacting protein, p35srj, has been shown to bind to p300/CBP and inhibit HIF-1 transactivation by blocking the HIF-1α/p300 CH1 interaction. Endogenous p35srj is markedly up-regulated by hypoxia via HIF-1, allowing feedback regulation of the hypoxic response. It has been proposed that, in hypoxic cells, p35srj may regulate HIF-1 transactivation by controlling access of HIF-1α to p300/CBP, and may partially sequester and functionally compartmentalize cellular p300/CBP such that a portion of p300/CBP is available for interaction with other transcription factors. However, this does not explain why a constitutively stable version of the COOH-terminal transactivation domain only activates transcription under hypoxic conditions. A critical asparagine residue (amino acid 851) within the COOH-terminal transactivation domain is hydroxylated by a process requiring 2-oxoglutarate, Fe^{2+}, and O_2 which prevents binding of the p300/CBP co-activator, preventing transcription. However, under hypoxic conditions, or in the presence of iron chelators or 2-oxoglutarate antagonists, hydroxylation does not occur and transcription can be activated. The structures of the HIF-1α COOH-terminal transactivation domain bound to either the CH1 domains of the co-activators p300 or CBP have now been solved and demonstrate that the critical asparagine residue is buried within a hydrophobic pocket allowing the transcriptional complex to form. This interaction is disrupted by hydroxylation of the asparagine residue. Asparagine hydroxylase was originally discovered as functional inhibitor of HIF-1 (FIH-1), a HIF-binding protein that repressed COOH-terminal transactivation domain function. Uncertainty remains over the importance of asparagine hydroxylation as several groups have reported that hypoxia inducible gene expression is not markedly increased by hypoxia in von Hippel-Lindau protein-defective cells.

Control of HIF-1 by Oncogenic Signaling Pathways

Many growth factors and cytokines stabilize HIF-1α under normoxic conditions. Examples include insulin, insulin-like growth factors 1 and 2, epidermal growth factor, fibroblast growth factor 2, tumor necrosis factor-α, and platelet-derived growth factor. Although at first glance there is great diversity among these growth factors, it seems likely that most stabilize HIF-1α via common kinase pathways activated by cell-specific receptors. The PI3K/AKT and mitogen-activated protein kinase (MAPK) pathways are examples. Cross-talk between pathways would also allow for the cell- and target gene-specific fine-tuning of the hypoxic response.

Phosphorylation of p300 by the MAPK pathway (p42/p44; Erk1/Erk2) increases HIF-1 transcriptional activity by increasing HIF-1/p300 complex formation (13). However, hypoxia *per se* probably does not activate the MAPK pathway. The PI3K/AKT pathway is also implicated in the HIF-1 response as previously discussed. Loss of the tumor suppressor gene *PTEN*, a negative regulator of AKT phosphorylation and activation, has been shown to increase HIF-1-mediated gene expression (14).

Redox Control of HIF-1

A number of studies have suggested a role for redox (reduction-oxidation)-dependent processes in the control of HIF-1. Treatment of purified HIF-1 with diamide, hydrogen peroxide, or *N*-ethyl-maleimide, a specific alkylator of cysteine sulfhydryl groups, results in complete loss of DNA binding activity (15). This can be prevented by prior addition of DTT. Increasing the cellular levels of the small redox

protein thioredoxin-1 (Trx-1) by transfection increases HIF-1α protein under both normoxic and hypoxic conditions (16). The increase is associated with increased HIF-1 transactivation, and the expression of downstream targets such as VEGF leading to increased angiogenesis in tumor xenografts. Expression of a redox inactive mutant Trx-1 decreases HIF-1α protein levels and HIF-1 transactivating activity leading to decreased production of VEGF. Trx-1 has also been reported to promote the binding of the transcription co-activator complex CBP/p300 to the COOH-terminal transactivation domain of HIF-1α resulting in increased HIF-1 transactivation acting through the dual function DNA repair endonuclease and redox regulatory protein redox-factor-1 (Ref-1) (17). Activation of HIF-1 by redox-factor-1 involves reduction of a cysteine residue in the COOH-terminal transactivation domain of HIF-1α. However, the functional importance of this cysteine residue has not been confirmed by mutation experiments and the significance of the redox-dependent binding of CBP/p300 remains questionable.

HIF-1 and Cancer

HIF-1α expression has been detected in the majority of solid tumors examined including brain, bladder, breast, colon, ovarian, pancreatic, renal, and prostate, whereas no expression was detected in surrounding normal tissue nor was it detected in benign tumors such as breast fibroadenoma and uterine leiomyoma (18, 19). Clinically, HIF-1α overexpression has been shown to be a marker of highly aggressive disease and has been associated with poor prognosis and treatment failure in a number of cancers including breast, ovarian, cervical, oligodendroglioma, esophageal, and oropharyngeal cancer (20–23). In lymph-node-negative breast cancer, HIF-1α overexpression identifies a subset of patients at increased risk of treatment failure and death, despite the fact that their tumors are histopathologically classified as low grade (22). A similar trend has been observed in oropharyngeal cancers where patients with HIF-1α overexpression in >10% of their tumor cells were 3-fold more likely to fail to achieve complete remission following radiation therapy, compared to patients with HIF-1α overexpression in <10% of their tumor cells (23).

In many cancers, overexpression of HIF-1α is seen at an early stage of tumor development with expression correlating with vascular density of the lesions. Ductal carcinoma *in situ*, the early pre-invasive stage of breast cancer, shows overexpression of HIF-1α which correlates with lesion vascular density (24). The same phenomenon is seen in brain tumors and ovarian carcinoma (21, 25), suggesting that HIF-1 activity contributes to the angiogenic switch, most likely mediated by increased formation of pro-angiogenic factors such as VEGF.

The presence of HIF-1α also correlates with tumor grade and vascularity in more advanced tumors, most strikingly in brain tumors (25). The pattern of HIF-1α protein expression in glioblastoma multiforme is identical to that described for VEGF mRNA (26). High-grade glioblastoma multiforme has significantly higher levels of VEGF expression and neovascularization compared with low-grade gliomas (27). Together, the data show that HIF-1 mediates hypoxia-induced VEGF expression in tumors leading to highly aggressive tumor growth.

Increased HIF-1α levels can also occur independently of hypoxia in human tumors. HIF-1α is expressed in the majority of tumor cells in hemangioblastoma, despite the highly vascularized nature of this tumor (19, 25). HIF-1α is also found expressed immediately adjacent to tumor blood vessels where its expression is unlikely to be hypoxia induced. Clear cell renal carcinoma and hemangioblastoma both have inactivation of the von Hippel-Lindau protein gene (28) and show high levels of HIF-1α (19, 25). In renal cell carcinoma cells lacking von Hippel-Lindau protein, expression of both HIF-1α and HIF-2α proteins is constitutively expressed at high levels under non-hypoxic conditions. The expression is associated with increased transcription of target genes such as *VEGF*. HIF-1α is also increased in tumors when the PI3K/AKT signaling pathway is activated. This is clearly demonstrated in prostate cancer cells where inactivation of *PTEN* facilitates HIF-1-mediated gene expression leading to increased tumor growth and tumor vascularity compared to cells expressing *PTEN* (14). *PTEN* mutations frequently occur in the progression from early to advanced prostate cancer and correlate with increased angiogenesis.

The correlation between HIF-1α overexpression, resistance to apoptosis, and poor prognosis is not, however, universal. In one study of non-small cell lung cancer, HIF-1α overexpression was found to be correlated with tumor apoptosis and patient survival (29), although another study did not repeat this finding (20). HIF-1α has been reported to promote apoptosis in mouse embryonic stem cells exposed to oxygen and glucose deprivation (30). This might be explained by the interaction of HIF-1α with p53 resulting in stabilization of p53 (4), or by the up-regulation of pro-apoptotic genes such as *NIP3* (31). The combined overexpression of HIF-1α and the anti-apoptotic protein Bcl-2, or combined expression of HIF-1α and mutant p53 have shown significant association with treatment failure and patient mortality, respectively (21, 32).

A question has been raised whether HIF-1α is a good cancer drug target if HIF-1 inhibition can lead to decreased expression of pro-apoptotic genes such as *NIP3* which might increase tumor growth (33). This does not appear to be the case, presumably because the proliferative, pro-angiogenic, and anti-apoptotic effects of HIF-1 in tumors outweigh any pro-apoptotic effects. Furthermore, the putative inhibitors of HIF-1 thus far identified inhibit and do not stimulate tumor growth (see below).

While most work has focused on the role of HIF-1α in increasing tumor growth, HIF-2α is also up-regulated in human cancer, for example, in renal cancer (34). However, studies in mouse embryo fibroblasts have shown that unlike HIF-1α, HIF-2α is a primarily cytoplasmic and constitutively expressed protein that does not exhibit oxygen-dependent degradation (35). Endogenous HIF-2α, at least, does not stimulate the transcription of typical HIF-1 target genes. It has been suggested that HIF-2α is primarily responsible for hypoglycemia-induced gene expression (36).

HIF-1α Inhibitors as Anticancer Drugs

Although natural antagonists of HIF-1 activity, such as p35srj and antisense therapy against HIF-1α has shown inhibition of tumor growth in a mouse model (37), the potential of HIF-1α as a target for cancer therapy lies with the development of small molecule inhibitors (38). A variety of anticancer drugs, most of which were not developed as HIF-1 inhibitors, have been reported to inhibit HIF-1 (Table 1).

Inhibitors of Hsp-90 such as radicicol; KF58333, a radicicol analogue; and geldanamycin and its analogue 17-allylamino-17-desmethoxygeldanamycin (17-AAG) have been reported to lower HIF-1α levels and to decrease HIF-1 transactivating activity in cells in culture in an oxygen and von Hippel-Lindau protein-independent manner (6, 39–41). While it is clear that geldanamycin decreases HIF-1α levels by promoting its degradation (6), the precise mechanism and the role of other Hsp-90 client proteins in the degradation of HIF-1α remains to be determined. In one study where HIF-1α levels were studied by immunohistochemical staining in human tumor xenografts *in vivo*, the radicicol analogue KF5833 did not lower HIF-1α (40).

Vincristine, taxol, and 2-methoxyestradiol, drugs that cause disruption of the interphase microtubule cytoskeleton, have been reported to decrease hypoxia-induced HIF-1α levels and HIF-1 transactivating activity (42). In contrast, under normoxic conditions, the microtubule depolymerizing agents colchicine and vinblastine, but not taxol, a microtubule stabilizing agent, stabilize HIF-1α and increase its levels by an NF-κB-dependent mechanism (43). 2-Methoxyestradiol inhibits hypoxia-induced HIF-1 transactivating activity in cultured cells by a translation-dependent mechanism without a change in HIF-1α transcription or ubiquitin-dependent proteasomal degradation (42). The decrease in HIF-1α by 2-methoxyestradiol is associated with inhibition of downstream targets such as VEGF production, glucose transporter-1 (Glut-1), and endothelin-1.

Table 1. Compounds reported to inhibit HIF-1α

Compound	HIF-1α Levels IC_{50} (μM)	HIF-1 Activity IC_{50} (μM)	Cytotoxicity IC_{50} (μM)	Mechanism of HIF-1α Inhibition	*In Vivo* HIF-1 Levels	Ref.
Hsp-90 inhibitor						
Radicicol	1.0	1.4	1.4	DNA binding		(39)
KF58333 (radicicol analogue)	0.1–0.5		0.1		not decrease	(40)
Geldanamycin	5.0			breakdown		(6)
		10.0	0.05	breakdown		(41)
Flavonoid						
Genestein	100					(56)
	150	150				(57)
Topoisomerase inhibitor						
Topotecan (topo-I)	0.10	0.05	0.04–1.0	translation		(44, 45)
GL331 (topo-II)	10.0		0.2–2.0	transcription		(46)
Antimicrotubule						
Taxol	0.01–0.1		0.001	translation		(42)
Vincristine	0.01–0.1					(42)
2-Methoxyestradiol	1.0–5.0	100	1.0	translation		(42)
Histone deacetylase inhibitor						
FK228	2–20 nM		0.3 nM	transcription	not decrease	(47)
PI-3-kinase pathway inhibitor						
LY294002	10.0–50.0		10–20			(48)
	40					(58)
Wortmannin	0.01–0.10		5.0–20.0			(48)
Rapamycin	0.01–0.05		0.005	translation		(8, 48)
MEK1 Inhibitor						
PD98059	100		>10			(58)
	100	100		transactivation		(57)
Soluble guanyl cyclase stimulator						
YC-1	5.0–10.0		5.0–50.0	transcription	decrease	(53)
Thioredoxin inhibitor						
PX-12	7.2	11.5	2.0–3.0		decrease	(54)
Pleurotin	7.6	9.0	1.0		decrease	(54)
Other						
UCNO-1 (ser/thre kinase inhibitor)		0.1	0.2–1.0	kinase inhibition		(59)
Diphenylene iodonium	1.0			redox inhibition		(60)
Carboxyamido-triazole (Ca^{2+} blocker)	1.0–5.0		5.0	transcription		(61)
PX-478	5.0–25.0	5.0–25.0	5.0–25.0	breakdown	decrease	(55)

Inhibitors of topoisomerase I and II are also able to decrease HIF-1α levels. A cell-based reporter assay used to screen the National Cancer Institute's drug library identified several camptothecin analogues, including topotecan, as inhibitors of HIF-1α transactivating activity and topotecan decreased hypoxia-induced HIF-1α protein levels (44). Subsequent studies showed that topotecan did not alter HIF-1α protein degradation or mRNA accumulation but inhibited translation through a topoisomerase I-dependent mechanism (45). The topoisomerase II inhibitor GL331, a podophyllotoxin derivative, decreased HIF-1α protein and mRNA levels, suggesting, in this case, transcriptional repression (46). The cell-based reporter screen also identified DX-2-1, a carbomycin derivative, as a HIF-1 inhibitor, although DX-2-1 inhibits a number of transcription factors in addition to HIF-1 (44). The histone deacetylase inhibitor FK228 has been found to inhibit HIF-1α in cells by a transcription-dependent mechanism but did not decrease HIF-1α in tumor xenografts (47).

Inhibitors of PI3K such as LY294002 and wortmannin decrease basal and mitogen and hypoxia-induced levels of HIF-1α, inhibit HIF-1 transactivation, decrease VEGF formation by cells, and inhibit tumor angiogenesis (48). PI3K inhibitors may be working through inhibition of mTOR which is known to stimulate the translation of HIF-1α mRNA into protein (see previously). The mTOR inhibitor rapamycin decreases HIF-1α levels and inhibits VEGF production by cells in culture (8, 48, 49). Treatment of cells with the MEK1 inhibitor PD098059 decreases HIF-1α protein, HIF-1 DNA binding activity, HIF-1 reporter gene transcription, as well as expression of the HIF-1 downstream targets VEGF and iNOS induced by treatment with mersalyl, an organomercurial compound which has insulin-mimetic effects by acting through the insulin-like growth factor-1 receptor resulting in signaling to MEK1 (50). In contrast, transcriptional activation mediated by HIF-2α was blocked by PD098059 without any change in HIF-2α protein expression (51).

It should be noted that in nearly every report of these agents' ability to lower HIF-1α protein levels, the effect occurred at a concentration of the agent an order of magnitude or more higher than the concentration required to inhibit cell growth. Even allowing for relatively short drug exposures to inhibit HIF-1α (4–24 h) compared to exposures of several days for cytotoxicity, it is likely that inhibiting HIF-1α in cells in culture does not itself lead to cell death.

Probably more relevant in identifying effective HIF inhibitors is to seek agents that inhibit tumor HIF-1α or decrease HIF-1 activity *in vivo*, where HIF-1α is known to be a key factor in tumor growth, and at doses that also produce inhibition of tumor growth. This has been done for relatively few agents. YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole], a drug originally developed for circulatory disorders through inhibition of platelet aggregation and vascular contraction by activating soluble guanyl cyclase, decreases hypoxia-induced HIF-1α levels *in vitro*, HIF-1 transactivating activity, and the expression of HIF-1 downstream target genes including VEGF and erythropoietin (52, 53). YC-1 decreases tumor HIF-1α levels and shows antitumor activity against human tumor xenografts in animals (53). However, the potential of YC-1 to increase bleeding time and to cause hypotension and penile erection may limit its clinical usefulness.

Another group of agents affecting HIF-1 activity includes compounds inhibiting redox signaling by inhibiting Trx-1. The Trx-1 inhibitor PX-12, recently evaluated in a Phase I clinical trial as an antitumor agent, and the thioredoxin reductase inhibitor pleurotin, both decrease constitutive and hypoxia-induced HIF-1α levels, as well as HIF-1 transactivating activity and the expression of VEGF in cells (54). PX-12 decreases tumor HIF-1α levels, tumor VEGF, and tumor angiogenesis in human tumor xenografts in mice. However, PX-12 has effects through inhibition of Trx-1 on other signaling pathways and the contribution of HIF-1α inhibition to the antitumor activity of PX-12 is not known.

A novel inhibitor of HIF-1α is PX-478 which decreases cellular HIF-1α protein levels under both normoxic and hypoxic conditions, as well as hypoxia-induced HIF-1 transactivation and VEGF protein, in a variety of cancer cell lines. PX-478 decreases the half-life of HIF-1α protein with increased ubiquitination and degradation by a mechanism that remains to be determined. PX-478 decreases tumor HIF-1α within an hour of administration to animals, with a corresponding decrease in plasma VEGF and a long-term decrease in the expression of tumor glucose transporter-1. PX-478 shows potent antitumor effects with tumor regression, long-term growth delay and in some cases cure, in a range of well-established human tumor xenografts in mice (55). The antitumor activity of PX-478 correlated positively with tumor xenograft HIF-1α levels.

Conclusions

It is clear that the hypoxia-mediated increase in HIF-1α plays a critical role in both the establishment and progression of many common cancers through the HIF-1-dependent activation of genes that allow cancer cells to survive and metastasize in the hostile hypoxic tumor environment. Additionally, increased HIF-1 activity arises through the activation of oncogenes and/or inactivation of tumor suppressor genes. Increased HIF-1α is correlated with the increased expression of survival factors such as VEGF, aggressive tumor growth, and poor patient prognosis. The interest in HIF-1α as a cancer drug target stems from associations such as this. A number of agents with anticancer activity have been reported to decrease HIF-1α or HIF-1 transactivating activity in cells. This has been proposed, often on the basis of limited evidence, to contribute to the agents' antitumor activity, for example, through decreased formation of angiogenic factors such as VEGF. However, it is not always clear that HIF-1 inhibition can occur at therapeutically relevant concentrations of the agents. Not infrequently the concentration of the agents required to inhibit HIF-1 is considerably higher than the concentration necessary to inhibit cell growth. A more stringent and relevant test is whether the agents inhibit

HIF-1α or HIF-1 transactivating activity in tumors *in vivo*, because this is where HIF-1 is essential for tumor growth. With an anticancer drug, this should occur at doses of drug that produce antitumor activity. This has been demonstrated for only a few of the reported HIF-1 inhibitors and for only one agent has antitumor activity in human tumor xenografts been correlated with HIF-1α levels in the tumor. HIF-1 inhibitors are moving toward clinical trials where it will be important to demonstrate that the agents can inhibit HIF-1 in patient tumors or, failing this, inhibit the downstream consequences of HIF-1 inhibition such as VEGF formation. Because HIF-1α levels are variable between patients, it will be important to demonstrate that the tumor response is correlated with tumor HIF-1α levels and/or HIF-1 activity. Only in this way will it be possible to determine if HIF-1 is a valid cancer drug target in humans.

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