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Review

Targeting Hypoxia and Angiogenesis through HIF-1α Inhibition

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ABSTRACT

Hypoxia is an important phenomenon in the tumor microenvironment. Hypoxic tumors are more aggressive and resistant to anti-neoplastic treatments. HIF-1α plays a major role in the response of tumors to hypoxia, and it is mainly responsible for the "angiogenic switch". HIF-1α contributes to tumor aggressiveness, invasiveness and resistance to radiotherapy and chemotherapy. Targeting HIF-1α is an attractive strategy, with the potential for disrupting multiple pathways crucial for tumor growth. We review recent findings on the potential efficacy of small molecules to downregulate HIF-1α. These promising drugs inhibit HIF-1α synthesis or transcriptional activity by blocking a variety of steps in several different signaling pathways. Blocking HIF-1α activity should not only downregulate tumor angiogenesis, but also interfere with glycolytic metabolism and tumor cell growth. This strategy could also improve the efficiency of established tumor therapies.

ABBREVIATIONS

17-AAG, 17-allylamino, 17-demethoxygeldanamycin; 17-DAMG, 17-dimethylaminoethylamino, 17-demethoxygeldanamycin; 2ME2, 2-methoxyestradiol; ARNT, aryl hydrocarbon receptor nuclear translocator; ATP, adenosine triphosphate; CA9, carbonic anhydrase; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; EGFR, epidermal growth factor receptor; EPO, erythropoietin; FTI, farnesyl transferase inhibitors; GA, geldanamycin; GC, guanil cyclase; GLUT-1, glucose transporter 1; HIF-1, hypoxia inducible factor-1; HRE, hypoxia response elements; Hsp90, heat shock protein 90; IL-1, interleukin 1; ILGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; mTOR, Mammalian target of rapamycin; NCI, National Cancer institute; NF-κB, nuclear factor kappa-beta; NO, nitric oxide; PDGF, B platelet-derived growth factor; PET, positron emission tomography; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; pVHL, Von Hippel-Lindau tumor suppressor protein; TK, tyrosine kinase; Topo-1, topoisomerase I; TPT, topotecan; Trx-1, thioredoxin-1; VEGF, vascular endothelial growth factor

INTRODUCTION

HIF-1α as key regulator of response to hypoxia. Hypoxia plays a critical role in tumor development and aggressiveness, and is an important prognostic factor for resistance to antineoplasic treatments. Moreover, hypoxia is a key player in the "angiogenic switch" during tumor development. Therefore, targeting the critical steps in tumor angiogenesis, including hypoxia-induced pathways, has become an important area in basic, translational and clinical cancer research.[1]

Hypoxia inducible factor-1 (HIF-1) is one of the main regulators of the molecular mechanisms involved in the response to hypoxia. HIF-1 is a heterodimeric basic-helix-loop-helix-PAS domain transcription factor composed of a stable and constitutively expressed subunit (HIF-1β or ARNT) and an inducible subunit called HIF-1α, whose levels are controlled by oxygen tension. HIF-1α is rapidly degraded under normoxic conditions,[2] where it is hydroxylated at several proline residues, and acetylated at lysine 5328.[3] This post-translational modification serves as recognition motif for the subsequent ubiquitination by the von Hippel-Lindau tumor suppressor protein (pVHL) thus targeting it for degradation by the proteosome.[4] Loss of pVHL or hypoxic conditions, inhibits prolyl-hydroxylation, leading to accumulation of the HIF-1α protein in the cytoplasm. Translocation to the nucleus results in a concomitant increase in transcriptional activity and expression of target genes.

The transcriptional activity of HIF-1 requires binding of the HIF-1 heterodimer complex to hypoxia response elements (HRE) located in the promoters of various target genes, of which there are more than sixty. However, the presence of a HIF-1-binding site is necessary, but not sufficient to activate gene expression: HIF-1α interacts with coactivators such as CBP, p300, SRC-1 and TIF2[5] and this association is regulated by both oxygen concentration and redox state. For this reason it has been assumed that HIF-1 must interact with other transcription factors to be fully active. Some examples of the adaptive events regulated by HIF-1α are: the induction of angiogenesis, through activation of the expression of vascular growth factors (i.e.: VEGF and PDGF B); alterations in the glycolytic metabolism towards anaerobic routes (GLUT-1); pH regulation (CA9); and inhibition of apoptosis and cell cycle arrest.[6] These events generate a higher invasive capacity, increase tumor growth and so enhance tumor hypoxia, in this manner creating a vicious circle.

HIF-1α synthesis and transactivation can also be activated by nonhypoxia-mediated mechanisms present in many human tumors. For instance, the activation and stabilization of HIF-1α can be mediated through the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling pathway. It has also been shown that the mitogen-activated protein kinase (MAPK) pathway enhances HIF-1α transcriptional activity.

In summary, HIF-1α regulates the transcription of many genes that confer a more aggressive phenotype, and HIF-1α activation is a clear and definitive event in the tumor angiogenesis.[7] Thus, the downregulation of the activity of HIF-1α could have an immediate effect on its target genes, causing a detrimental effect on tumor growth and maintenance of the aggressive phenotype.

HIF-1α as prognostic factor. HIF-1α has been associated with an unfavorable prognosis in patients with a wide range of tumors. Immunohistochemical analyses using monoclonal antibodies revealed that HIF-1α is overexpressed in many human cancers.[8] For instance, in oligodendroma, breast, ovary, cervix, lung, colon and head and neck cancer an apparent correlation between HIF-1α overexpression and a poor outcome has been described.[9-20]

HIF-1α expression confers resistance to radiation therapy and to chemotherapy.[21] In clinical studies, it had a negative impact on response to radiotherapy and chemotherapy in patients with head and neck cancer.[14,18] Furthermore, links between radiation exposure and HIF-1 signaling activation have been demonstrated.[22] There are several ways through which HIF-1α contributes to create a resistant phenotype, mainly by the direct effects of severe hypoxia. The expression of HIF-1α regulated proteins Ca9 and VEGF has been associated with worse prognosis in a variety of tumors.[23,24] HIF-1α dysregulation can activate the expression of genes like MDR1 or topoisomerase II. These phenomena confer increased target gene expression as well as increased resistance to chemotherapy.[25,26] Additionally, HIF-1α activation is shown to be a crucial step in the radiotherapy-induced radioprotection of tumor vasculature, an important mechanism of radioresistance.[27] Thus, inhibition of HIF-1α synthesis or activity can contribute to blocking tumor angiogenesis, glycolysis and tumor cell growth. Furthermore, this strategy could also improve the efficiency of classical tumor therapies. For these reasons, HIF-1α has much potential as a new target for cancer treatment.

INHIBITING HIF-1α

A potential clinically applicable strategy for targeting HIF-1α is based on the use of small molecules acting on key steps of the pathways that regulate HIF-1α synthesis and activity. In the next pages, we review the current status of research in terms of HIF-1α inhibition with small molecules. For a more rational overview, we will follow the different pathways regulating HIF-1α (Fig. 1), and will focus on the molecules or pathways that have been demonstrated as inhibitors of HIF-1α synthesis or activity. Many of the reviewed compounds are not exclusive inhibitors of HIF-1α, but are also involved in the blockage of related pathways. This is a potential caveat to take into account, especially in evaluating the specific causal relationship between HIF-1α inhibition and anti-tumor effect. The most important HIF-1α regulatory systems reviewed are: PI3K/Akt/mTOR, MAPK, the Hsp90 system, the enzymatic complexes Topo-1 isomerase and redox protein thioredoxin-1. HIF-1α is also affected by small molecules that disrupt the microtubule polymerization and miscellaneous compounds with less known mechanisms of action (Table 1).

PI3K/Akt and HIF-1α. In response to cell stimulation by growth factors and hormones, the phosphorylation of PI3K activates the PI3K/Akt pathway, leading to cell cycle entry, cell division, migration and survival.[28] One of the major downstream targets of PI3K is Akt, which regulates the transmission of angiogenic and oncogenic signals. PI3K/Akt regulates the phosphorylation and activation of several protein families like Forkhead BAD, GSK3 and mTOR. Multiple mechanisms for the activation of the PI3K-Akt pathway have been identified in a range of cancers. PTEN is a phosphatase that limits activity of PI3K pathway. Loss of PTEN function also leads to PI3K/Akt signaling activation. The PI3K/Akt pathway has been recently (reviewed in refs. 29–31).

There are abundant data showing the relationship between Akt and HIF-1α activity. Growth factors like EGF, HER2,[32] androgens,[33] ILGF[34] or IL-1,[35] increase the HIF-1α synthesis under normoxic conditions through the PI3K/Akt activation. Under hypoxic and normoxic conditions, Akt activation leads to a decrease in the HIF-1α protein degradation.[36] Additionally, the loss of PTEN function facilitates the activity of HIF-1α.[37] The PI3K pathway appears to be cell-type specific, and this may be why some studies have questioned its role in the hypoxic response.[38,39]

Several molecules are able to downregulate HIF-1α protein by interfering with the PI3K/Akt pathway:

LY294002 is a chemical inhibitor of PI3K. This drug has reduced HIF-1α protein levels in vitro with a consequent reduction of transcription and expression of HIF-1α target genes.[36,40,41] However, LY294002 has a small therapeutic window and is not suitable for clinical use.

9-β-D-arabinofuranosyl-2-fluoroadenine (FARA-A), is a nucleoside analog which induces DNA damage in S-phase cells. FARA-A inhibits HIF-1α and VEGF expression in ovarian cancer cell lines by inhibiting Akt activation,[42] but it does not affect cell viability.

SU5416 (Semaxanib®) is a potent and selective inhibitor of the VEGF receptor (Flk-1/KDR). This compound facilitates the anti-angiogenic effect of radiotherapy.[43,44] SU5416 has been tested in clinical trials either in monotherapy or in combination with chemotherapy. SU5416 produces a decrease in HIF-1α protein and VEGF mRNA through PI3K/Akt pathway in cell lines of ovarian carcinoma.[45]

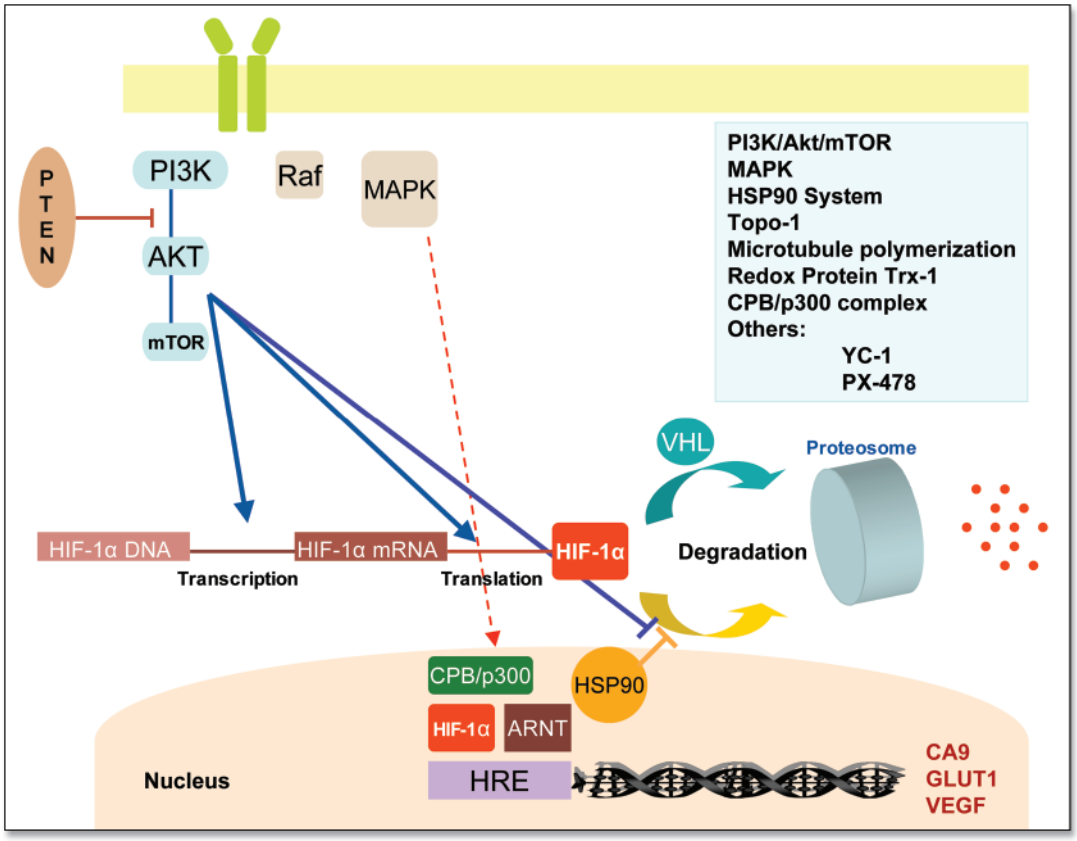

Figure 1. HIF-1α molecular pathway. Small molecules acting at different steps are able to downregulate HIF-1α synthesis and activity.

mTOR Inhibitors: HIF-1α has also been shown to be regulated by mammalian target of rapamycin (mTOR). mTOR promotes increased translation of HIF-1α mRNA into protein[32,41] and HIF-1α stabilization.[46] The regulation of HIF-1α by mTOR is Akt dependent, but Akt-independent regulation has also been described.[47] Moreover, there are in vivo data showing that Akt-dependent induction of HIF-1α is entirely mTOR dependent.[48] Rapamycin is a specific inhibitor of mTOR. Rapamycin inhibits both the stabilization and the transcriptional activity of HIF-1α in hypoxic cancer cells[36] and this effect is directly related to the disruption of mTOR-dependent signaling functions. TSC2 also inhibits mTOR, and HIF-1 upregulation in TSC2-deficient cells occurs as the result of increased mTOR activity. In TSC2-deficient cells, HIF-1α levels can be returned to normal by treatment with rapamycin.[49]

The prostate of transgenic AKT1 mice (a model where Akt is activated) treated with mTOR inhibitor RAD001 showed decreased expression of HIF-1α mRNA and HIF-1α regulated genes like GLUT-1.[48]

The current hypothesis is that in cancer patients PI3K/Akt and mTOR inhibitors (Rapamycin, CCI-779, RAD001, AP23573, AP23481 and ABT-578) could be effective inhibitors of hypoxic adaptation in developing tumors, with relevance to tumor growth, invasiveness, and metastatic potential. These effects could be especially relevant in tumors with loss of PTEN function.

MAPK pathway and HIF-1α. The MAPK pathway plays a role in facilitating the transactivation activity of HIF-1 which requires the recruitment of p300/CBP. Some authors suggested that MAPK increased the phosphorylation of HIF-1α, leading to increased transcription of a variety of proangiogenic genes, like VEGF. However, it has recently been shown that MAPK activation affects the transactivation of p300 and the HIF-p300/CBP interaction, and it is this mechanism, rather than direct phosphorylation of HIF-1α that is responsible for the effects of the MAPK pathway on HIF-1α.[50]

Farnesyl transferase inhibitors (FTI) downregulate HIF-1α. Another promising approach is to inhibit the upstream MAPK pathway activators Ras and Rho by blocking their farnesylation. Several FTIs have been tested in phase I-II clinical trials for different types of cancer with dissimilar outcomes. R115777 (tipifarnib, Zarnestra®) and SCH66336 (lonafarnib, Sarasar®) are the only two FTIs evaluated in phase III clinical trials in hematological and solid tumors. R115777 enhanced the radiosensitivity of a T24 tumor cell xenograft model with activated H-Ras[51] and part of this effect may be due to a reduction of hypoxia in tumors expressing H-Ras mutation.[52] In human glioblastoma xenografts, FTI reduced cellular HIF-1α levels and also produced changes in blood vessel morphology and density.[53]

Heat shock proteins (Hsp90). Among other heat shock proteins, Hsp90 plays an important role in mediating the correct folding and activation of its client proteins. Hsp90 also cooperates with the proteasomal pathway to eliminate miss-folded cellular proteins.[54] Client proteins bind to Hsp90 in an intermediate complex needed to reach their mature conformation. Hsp90 targets are numerous and many of them are strongly implicated in cancer development. Some of the more important are steroid receptors, tyrosine kinases (v-src, Bcr-Abl, Her2/neu, EGFR) and serine/threonine kinases (Raf-1, Akt).[55-58]

Hsp90 inhibitors and HIF-1α. Hsp90 also plays a role in HIF-1α protein folding, protects against the degradation of HIF-1α protein by the proteasome, and stabilizes the interaction between HIF-1α and ARNT,[59] regulating HIF-1α transcriptional activity[60] and influencing the angiogenic response to hypoxia.[60,61]

Hsp90-inhibitor drugs bind to the N-terminal ATP-binding pocket of Hsp90 and inhibit ATP binding and hydrolysis, inhibiting binding of the client proteins, which are instead ubiquitinated and targeted to the proteasome for degradation.[58]

Geldanamycin (GA) is a natural ansamycin antibiotic. GA binds to Hsp90 preventing the stabilization of Hsp90 client proteins leading to their degradation by the proteasome.[62-64] GA has been shown to reduce HIF1α protein levels via its effects on Hsp90,[60] and to inhibit HIF-1α transcriptional activity, particularly decreasing transcription

Table 1 **Small molecules inhibiting HIF-1α**

Molecule	Mechanism	Effect	Model	Clinical Trial	Ref.
PI3K/Akt/mTOR					
FARA-A	∇ Akt	↓ HIF-1α, VEGF	In vitro: ovarian	No	42
SU5416	∇ PI3K/Akt	↓ HIF-1α, VEGF	In vitro: ovarian	Yes	45
Rapamycin	∇ mTOR	↓ HIF-1α	In vitro: prostate	Yes	49
RAD001	∇ mTOR	↓ HIF-1α, GLUT-1	In vivo: prostate	Yes	48
Ras/MAPK					
R115777	∇ MAPK	↓ HIF-1α, vascularity	In vivo: Glioblastoma	Yes	53
Hsp90 system					
GA	∇ Hsp90-HIF1a	↓ HIF-1α, VEGF	In vitro: Renal, Prostate, Glioma	Yes*	60, 61, 65
Topo-1					
TPT	∇ Topo-1	↓ HIF-1α, vascularity	In vivo: Glioblastoma	Approved	80
Microtubule polymerization					
2ME2	∇ Microtubule polymerization	↓ HIF-1α, vascularity	In vivo: Breast, Head&Neck	Yes	89, 85
Redox Protein Thioredoxin-1					
Px-12	∇ Trx-1	↓ HIF-1α, VEGF, vascularity	In vivo: Breast	Yes	92
Others					
Chetomin	∇ p300-HIF1a	↓ VEGF, GLUT-1, EPO	In vivo: Colorectal	No	96
YC-1	Unknown	↓ HIF-1α, VEGF, vascularity	In vivo: several	No	97
PX-478	Unknown	↓ HIF-1α, VEGF, GLUT-1	In vivo: several	No	99

∇, Downregulation; * GA Derivatives 17-AAG and 17-DMAG.

of VEGF, antagonizing hypoxia-induced angiogenesis.[60,61] GA also blocked HIF-1α induction and VEGF expression in human glioblastoma cell lines.[65] In this study, GA induced inhibition of cell migration, and this phenomenon was PTEN- and Akt-independent and associated with HIF-1α downregulation. The connection between HIF-1α downregulation, decreased VEGF expression and inhibition of cell migration inhibition seems promising for clinical studies with Hsp90 inhibitors. However, GA is extensively metabolized by the liver and is not suitable for clinical practice, because of a small therapeutic window.

The GA derivative 17-allylamino, 17-demethoxygeldanamycin (17-AAG) is currently undergoing phase II clinical trials. This small molecule has an improved therapeutic index and showed tumor growth inhibition in cell lines,[66] and in several human xenograft models.[66-68] An adequate profile of toxicity and activity in phase I clinical trials has also been shown for this drug.[69,70] An interesting therapeutic point is that 17-AAG enhances the in vitro radiosensitivity of several cell lines.[71,72] However, this compound seems to have some difficulties with its administration vehicle and substantial interindividual variability in pharmacokinetic parameters.[73]

17-dimethylaminoethylamino, 17-demethoxygeldanamycin (17-DMAG) was developed to be administered orally, is water soluble, has better formulation properties and does not give rise to potentially toxic metabolites.[74] 17-DMAG shows at least equivalent antitumor activity to 17-AAG both in vitro and in vivo.[75] 17-DMAG has just begun a phase I clinical trial. Moreover, 17-DMAG enhances the in vitro and in vivo radiosensitivity of human tumor cells.[76]

Topoisomerase inhibitors: A novel connection between Topo-1 and HIF-1α. One of the approaches to identify new molecular targeted drugs is to screen large libraries of small molecules using a high throughput (HTS) cell-based assay.[77] In a HIF-1-targeted HTS screening of the NCI Diversity Set, four compounds were found to specifically inhibit HIF-1α transcriptional activity in a glioblastoma cell line. Three of these four molecules were camptothecin analogs and topoisomerase I inhibitors. These drugs also inhibited the hypoxic induction of VEGF at both transcriptional and translational level.

Topotecan (NSC-609699, TPT) was the best studied compound. TPT specifically inhibited hypoxic induction of HIF-1α protein and DNA binding activity.[78] TPT acts at the translational level, and HIF-1α mRNA is not modified. Topo-1 is required for the inhibition of HIF-1α and TPT downregulates HIF-1α independently of DNA replication-dependent DNA damage. The dissociation between the mechanisms of cytotoxicity and HIF-1α inhibition[79] suggests a new pathway connecting Topo-1 and HIF-1α protein accumulation. Studies with glioblastoma xenografts showed that daily administration of TPT (1mg/kg qdx10) causes a tumor growth delay associated with a decrease of HIF-1α protein levels and a reduction of angiogenesis. An intermittent schedule of TPT (10 mg/kg q4dx3) did not produce this effect, suggesting that a sustained rather than a transient inhibition of HIF-1α is required.[80] These results provide a rationale for clinical trials of metronomic therapy with TPT to target HIF-1α.

SN38, the active metabolite of CPT11 (Irinotecan), was tested in glioblastoma cell lines with three other antineoplasic agents: ACNU, cisplatin, and etoposide for antiangiogenic affects. SN38 decreased HIF-1α and VEGF in glioma cells and also selectively inhibited endothelial cell proliferation. In this study the other chemotherapeutic

agents did not reduce the neovascularization, except for weak inhibition of vessel branch-points by etoposide.[81]

Disruption of microtubule polymerization. 2-methoxyestradiol (2ME2) is an endogenous metabolite of estradiol that has shown promising activity in a number of tumor models. 2ME2 and some derivative compounds inhibit tubulin polymerization,[82] resulting in a G_2/M arrest in a variety of tumor models.[83-85] Like other microtubule inhibitors, 2ME2 enhances radiosensitivity in vitro.[86] There is also evidence of synergism between 2ME2 and other modifiers of tubulin stabilization as paclitaxel and vincristine.[85,87] 2ME2 is known for its significant antiangiogenic activity.[88] Although the mechanism for this has not been fully explained, 2ME2 has been shown to act on HIF-1α and VEGF.

2ME2 and HIF-1α downregulation. 2ME2 inhibited HIF-1α protein levels and the subsequent VEGF expression in prostate and breast cancer cells. 2ME2 inhibits HIF-1α at the translational level. Perhaps the most interesting point is the link between microtubule cytoskeleton disruptions and HIF-1α function inhibition. Depolymerization of microtubules by 2ME2 is required to inhibit HIF-1α accumulation. This effect seems to be independent of the mitotic arrest.[89] In a mouse orthotopic breast tumor model, 2ME2 affected microtubule polymerization and subsequently HIF-1α downregulation, leading to inhibition of tumor vascularization,[89] and this has also been demonstrated in a model of head and neck cancer.[85] This fact is especially interesting because of the extensive evidence that tumor hypoxia and HIF-1α overexpression are poor prognostic factors in these tumors. In a head and neck squamous cell carcinoma xenograft, 2ME2 inhibited tumor vessel formation, delayed tumor growth and improved the paclitaxel efficacy (an agent that is particularly active in this type of cancer, and that can also act as a radiosensitizer).[85]

Taken together, these data provide evidence for a connection between microtubule cytoskeleton disruption, HIF-1α dysregulation, and angiogenesis inhibition. This scenario suggests an exciting strategy for clinical practice. 2ME2 has been recently registered as Panzem® and is currently being tested in clinical trials, both in hematologic and solid tumors.

Redox protein thioredoxin-1. Thioredoxin-1 (Trx-1) is a small redox protein that has many functions: as a cofactor in DNA synthesis; regulation of transcription factor activity (HIF-1α, NF-kB, glucocorticoid and estrogen receptors, AP1 and AP2, p53) and the regulation of enzyme activity by heterodimer formation. Trx-1 stimulates cell growth and is an apoptosis inhibitor. Increased levels of Trx-1 are found in many human tumors, where it is associated with aggressive tumor growth (reviewed in ref. 90). Higher Trx-1 expression was correlated with an increase of HIF-1α protein. In addition, Trx-1 increases the transcriptional activity of HIF-1α, along with increased VEGF production and enhanced tumor angiogenesis.[91]

PX-12 and Pleurotin are both Trx-1 inhibitors. Both molecules decreased HIF-1α protein, HIF-1α trans-activating activity and expression of the downstream targets VEGF, and iNOS. These inhibitors also decreased expression of HIF-1α and VEGF proteins and microvessel density in several tumor xenograft models, with potent anti-tumor activity.[92] PX-12 had been tested in a phase I clinical trial in patients with advanced solid malignancies. Trx-1 participates in many processes involved in cancer development. The anti-tumor activity of Trx-1 inhibitors may be mediated, in part, by HIF-1α inhibition.

Inhibition of the DNA binding site: targeting p300. Since binding of HIF-1 to coactivator p300/CBP is required for transcriptional activation, disruption of HIF-1α interaction with p300/CBP could serve as a point of intervention in the hypoxia-response pathway.[93-95]

Chetomin is a small peptide derived from the fungus *Chaetomium*. Chetomin specifically disrupts the CH1 domain of p300 preventing its binding to HIF-1α and HIF-2α. Chetomin attenuates hypoxia-inducible transcription of HIF-1α in vitro and downregulates genes like GLUT-1, VEGF, and EPO in vivo when systemically administered in mice. In addition, Chetomin attenuates tumor growth in vivo. Nevertheless, the use of Chetomin in clinical practice may be limited by its local toxicity.[96]

Other small molecules. YC-1. YC-1 is a soluble guanylyl cyclase (GC) stimulator. Stimulators of GC have been developed as antihypertensive and anti-platelet function agents. Nevertheless, YC-1 demonstrated HIF-1α inhibition and an anti-angiogenic effect in a guanylyl cyclase independent pathway. YC-1 produced tumor growth delay in mice xenograft models. A decrease of vascularization, lower levels of HIF1α protein, and VEGF mRNA and protein in the treated tumor correlated with the inhibitory effect on tumor growth. No anti-platelet aggregation effect of YC-1 was detected.[97] Despite the promising results of this compound, difficulties regarding the exact mechanism of action have been mentioned, especially regarding the role of NO in angiogenesis regulation, and because of the many effects attributed to this molecule.

PX-478. PX-478 is a small molecule that inhibits HIF-1α levels and HIF-1-related gene expression in vitro and in vivo. PX-478 given to mice suppressed HIF-1α levels in xenografts and inhibited the expression of HIF-1α targeted genes (VEGF, GLUT-1). Additionally, this compound produced growth delay and regression in several tumor models. The anti-tumor response was positively correlated with HIF-1α tumor levels.[98]

MONITORING RESPONSE: MOLECULAR IMAGING

Molecular targeted therapies demand the development of accurate noninvasive methods of molecular imaging. Molecular imaging is a vital tool in identifying the specific molecular effect of new drugs. Molecular imaging can be defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular levels.[99] However, many of the molecular targeted therapies have been developed, even the most recent, with only indirect evidence of their specific action in tumors. Recently, advances have been made in incorporating imaging modalities into clinical drug development, such as positron emission tomography (PET) to predict response after administration of tyrosine kinase inhibitors, or the use of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to monitor the physiologic process following anti-angiogenic agents.[100,101]

The link between specific pathways and induction of particular protein products able to be imaged with radiolabeled antibodies may have clinical relevance.[102,103] Another strategy is to improve preclinical models using reporter gene systems, which allows the monitoring of new drugs. In this context, the possibility of imaging intratumoral processes, such as hypoxia or angiogenesis, may assist the development of therapeutic HIF-1α inhibition.

In the case of HIF-1α, some studies have used PET imaging in rodent tumor models to detect activation of a thymidine kinase reporter gene construct, placed under the control of HIF-1α. Thus, by using ^{124}I-FIAU or ^{18}F-FEAU, drugs that are phosphorylated by the TK protein and preferentially trapped in cells expressing HIF-1α, tumor hypoxia can be detected noninvasively. Moreover,

uptake of these tracers can be analyzed at the level of autoradiography and compared using immunohistochemistry to established hypoxia markers such as pimonidazole. This comparison will help link the molecular events of tumor hypoxia and noninvasive nuclear imaging.[104,105] Additionally, the direct effect of HIF-1α inhibitors could be tested in vivo with this methodology.

CONCLUSIONS AND CHALLENGES

HIF-1α plays a major role in the tumor response to hypoxia, and is the link between hypoxia and angiogenesis. HIF-1α is involved in mechanisms of tumor aggressiveness, invasion capacity, and resistance to radiotherapy and chemotherapy. Targeting HIF-1α is an attractive strategy, with potential for synergism with other therapies.

Many small molecules, some of them currently being evaluated in clinical trials, have shown specific effects in the inhibition of HIF-1α activity. Most of these compounds do not exclusively inhibit HIF-1α, and in some cases they exert their effects through pathways that had not been previously linked to HIF-1α. Thus, PI3K/Akt inhibitors increase the apoptotic activity, decrease the glycolytic rate, and produce cell cycle arrest in the G_1 phase; Hsp90 inhibitors have a role in the regulation of a variety of critical cancer pathways (e.g., Her2/neu and androgen receptor expression). Others show more specific HIF-1α inhibition mechanisms, in some cases as an additional effect to their intrinsic anti-tumor activity (e.g., Topo-1 inhibitors, 2ME2). Most of these small molecules, through the effect on HIF-1α expression and activity, decrease the expression of proangiogenic factors like VEGF and induce an antiangiogenic environment. The lack of specificity of most of these HIF-1α inhibitors may limit the precise evaluation of their mechanism of action. However, the multitarget properties of these compounds could in fact be an advantage in the clinical setting. The concept of combining therapies directed against different targets is currently growing. In the case of many of the HIF-1α inhibitors discussed in the present review, a multiple pathway effect could be achieved, although this may require very finely tuned dosing analysis to minimize the side effects to the different pathways in the normal cells. These drugs also offer the interesting possibility of enhancing the effect of radiotherapy. Some of the HIF-directed compounds are also radiosensitizers due to their primary mechanism of action (e.g., Hsp90 inhibitors, topo-1 inhibitors, microtubule polymerization inhibitors). In addition, reduction of HIF-1α expression and subsequent effects on the vasculature could lead to further radiosensitization.

Development of drugs against specific molecular targets is an important component of current cancer research. Especial efforts are going on to identify new molecules able to block critical pathways for tumor progression, with an adequate therapeutic ratio. As HIF-1α is a key transcriptional factor linking several important pathways in tumor biology, the possibility of using more specific HIF-1α inhibitors may offer a promising approach to cancer control. Additionally, the possibility of using these new drugs to improve the effectiveness of conventional anti-cancer treatments, as chemotherapy and radiotherapy, makes this approach even more exciting.

References

1. Hockel M, Vaupel P. Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001; 93:266-76.
2. Semenza GL. HIF-1 and human disease: One highly involved factor. Genes Dev 2000; 14:1983-91.
3. Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW. Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 2002; 111:709-20.
4. Kaelin Jr WG. How oxygen makes its presence felt. Genes Dev 2002; 16:1441-5.
5. Semenza GL. Hypoxia-inducible factor 1: Oxygen homeostasis and disease pathophysiology. Trends in Molecular Medicine 2001; 7:345-50.
6. Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev Cancer 2002; 2:38-47.
7. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: Role of the HIF system. Nat Med 2003; 9:677-84.
8. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of Hypoxia-inducible Factor 1{{alpha}} in Common Human Cancers and Their Metastases. Cancer Res 1999; 59:5830-5.
9. Birner P, Gatterbauer B, Oberhuber G, Schindl M, Rossler K, Prodinger A, Budka H, Hainfellner JA. Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: Its impact on prognosis and on neoangiogenesis. Cancer 2001; 92:165-71.
10. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of Hypoxia-inducible factor 1{{alpha}} is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000; 60:4693-6.
11. Bos R, van der GP, Greijer AE, Shvarts A, Meijer S, Pinedo HM, Semenza GL, van Diest PJ, van der WE. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 2003; 97:1573-81.
12. Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Vasko J, Ljungberg B. The expression of hypoxia-inducible factor 1{alpha} is a favorable independent prognostic factor in renal Cell Carcinoma. Clin Cancer Res 2005; 11:1129-35.
13. Schindl M, Schoppmann SF, Samonigg H, Hausmaninger H, Kwasny W, Gnant M, Jakesz R, Kubista E, Birner P, Oberhuber G. Overexpression of hypoxia-inducible factor 1{alpha} is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin Cancer Res 2002; 8:1831-7.
14. Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, Semenza GL. Expression of hypoxia-inducible factor-1alpha: A novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001; 61:2911-6.
15. Bachtiary B, Schindl M, Potter R, Dreier B, Knocke TH, Hainfellner JA, Horvat R, Birner P. Overexpression of hypoxia-inducible factor 1{alpha} indicates diminished response to radiotherapy and unfavorable prognosis in patients receiving radical radiotherapy for cervical cancer. Clin Cancer Res 2003; 9:2234-40.
16. Beasley NJP, Leek R, Alam M, Turley H, Cox GJ, Gatter K, Millard P, Fuggle S, Harris AL. Hypoxia-inducible factors HIF-1{alpha} and HIF-2{alpha} in head and neck cancer: Relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res 2002; 62:2493-7.
17. Hui EP, Chan ATC, Pezzella F, Turley H, To KF, Poon TCW, Zee B, Mo F, Teo PML, Huang DP, Gatter KC, Johnson PJ, Harris AL. Coexpression of hypoxia-inducible factors 1{alpha} and 2{alpha}, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival. Clin Cancer Res 2002; 8:2595-604.
18. Koukourakis MI, Giatromanolaki A, Sivridis E, Simopoulos C, Turley H, Talks K, Gatter KC, Harris AL. Hypoxia-inducible factor (HIF1A and HIF2A), angiogenesis, and chemoradiotherapy outcome of squamous cell head-and-neck cancer. Int J Radiat Oncol Biol Phys 2002; 53:1192-202.
19. Kuwai T, Kitadai Y, Tanaka S, Onogawa S, Matsutani N, Kaio E, Ito M, Chayama K. Expression of hypoxia-inducible factor-1alpha is associated with tumor vascularization in human colorectal carcinoma. Int J Cancer 2003; 105:176-81.
20. Swinson DE, Jones JL, Cox G, Richardson D, Harris AL, O'Byrne KJ. Hypoxia-inducible factor-1 alpha in non small cell lung cancer: Relation to growth factor, protease and apoptosis pathways. Int J Cancer 2004; 111:43-50.
21. Unruh A, Ressel A, Mohamed HG, Johnson RS, Nadrowitz R, Richter E, Katschinski DM, Wenger RH. The hypoxia-inducible factor-1 alpha is a negative factor for tumor therapy. Oncogene 2003; 22:3213-20.
22. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: Role of reoxygenation, free radicals, and stress granules. Cancer Cell 2004; 5:429-41.
23. Toi M, Matsumoto T, Bando H. Vascular endothelial growth factor: Its prognostic, predictive, and therapeutic implications. Lancet Oncol 2001; 2:667-73.
24. Potter C, Harris AL. Hypoxia inducible carbonic anhydrase IX, marker of tumour hypoxia, survival pathway and therapy target. Cell Cycle 2004; 3:164-7.
25. Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (*MDR1*) gene. Cancer Res 2002; 62:3387-94.
26. Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, Miller C, Demonacos C, Stratford IJ, Dive C. Hypoxia-mediated downregulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Mol Cell Biol 2004; 24:2875-89.
27. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, Fuks Z, Kolesnick R. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003; 300:1155-9.
28. Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002; 296:1655-7.
29. Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol 2004; 22:2954-63.
30. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002; 2:489-501.
31. Fresno Vara JA, Casado E, de CJ, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev 2004; 30:193-204.

32. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: Novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001; 21:3995-4004.
33. Mabjeesh NJ, Willard MT, Frederickson CE, Zhong H, Simons JW. Androgens stimulate hypoxia-inducible factor 1 activation via autocrine loop of tyrosine kinase receptor/phosphatidylinositol 3'-kinase/protein kinase B in prostate cancer cells. Clin Cancer Res 2003; 9:2416-25.
34. Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 2002; 277:38205-11.
35. Stiehl DP, Jelkmann W, Wenger RH, Hellwig-Burgel T. Normoxic induction of the hypoxia-inducible factor 1alpha by insulin and interleukin-1beta involves the phosphatidylinositol 3-kinase pathway. FEBS Lett 2002; 512:157-62.
36. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL. Modulation of hypoxia-inducible factor 1alpha 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 2000; 60:1541-5.
37. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D, Giaccia AJ. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 2000; 14:391-6.
38. Alvarez-Tejado M, Alfranca A, Aragones J, Vara A, Landazuri MO, del PL. Lack of evidence for the involvement of the phosphoinositide 3-kinase/Akt pathway in the activation of hypoxia-inducible factors by low oxygen tension. J Biol Chem 2002; 277:13508-17.
39. Arsham AM, Plas DR, Thompson CB, Simon MC. Phosphatidylinositol 3-kinase/Akt signaling is neither required for hypoxic stabilization of HIF-1 alpha nor sufficient for HIF-1-dependent target gene transcription. J Biol Chem 2002; 277:15162-70.
40. Sandau KB, Faus HG, Brune B. Induction of hypoxia-inducible-factor 1 by nitric oxide is mediated via the PI 3K pathway. Biochem Biophys Res Commun 2000; 278:263-7.
41. Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van OE. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. J Biol Chem 2002; 277:27975-81.
42. Fang J, Cao Z, Chen YC, Reed E, Jiang BH. 9-beta-D-arabinofuranosyl-2-fluoroadenine inhibits expression of vascular endothelial growth factor through hypoxia-inducible factor-1 in human ovarian cancer cells. Mol Pharmacol 2004; 66:178-86.
43. Geng L, Donnelly E, McMahon G, Lin PC, Sierra-Rivera E, Oshinka H, Hallahan DE. Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. Cancer Res 2001; 61:2413-9.
44. Schuuring J, Bussink J, Bernsen HJ, Peeters W, van Der Kogel AJ. Irradiation combined with SU5416: Microvascular changes and growth delay in a human xenograft glioblastoma tumor line. Int J Radiat Oncol Biol Phys 2005; 61:529-34.
45. Zhong XS, Zheng JZ, Reed E, Jiang BH. SU5416 inhibited VEGF and HIF-1alpha expression through the PI3K/AKT/p70S6K1 signaling pathway. Biochem Biophys Res Commun 2004; 324:471-80.
46. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002; 22:7004-14.
47. Abraham RT. mTOR as a positive regulator of tumor cell responses to hypoxia. Curr Top Microbiol Immunol 2004; 279:299-319.
48. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 2004; 10:594-601.
49. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin Jr WG. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 2003; 4:147-158.
50. Sang N, Stiehl DP, Bohensky J, Leshchinsky I, Srinivas V, Caro J. MAPK signaling upregulates the activity of hypoxia-inducible factors by its effects on p300. J Biol Chem 2003; 278:14013-9.
51. Cohen-Jonathan E, Muschel RJ, Gillies MW, Evans SM, Cerniglia G, Mick R, Kusewitt D, Sebti SM, Hamilton AD, Oliff A, Kohl N, Gibbs JB, Bernhard EJ. Farnesyltransferase inhibitors potentiate the antitumor effect of radiation on a human tumor xenograft expressing activated HRAS. Radiat Res 2000; 154:125-32.
52. Cohen-Jonathan E, Evans SM, Koch CJ, Muschel RJ, McKenna WG, Wu J, Bernhard EJ. The farnesyltransferase inhibitor L744,832 reduces hypoxia in tumors expressing activated H-ras. Cancer Res 2001; 61:2289-93.
53. Delmas C, End D, Rochaix P, Favre G, Toulas C, Cohen-Jonathan E. The farnesyltransferase inhibitor R115777 reduces hypoxia and matrix metalloproteinase 2 expression in human glioma xenograft. Clinical Cancer Research 2003; 9:6062-8.
54. Hohfeld J, Cyr DM, Patterson C. From the cradle to the grave: Molecular chaperones that may choose between folding and degradation. EMBO Rep 2001; 2:885-90.
55. Isaacs JS, Xu W, Neckers L. Heat shock protein 90 as a molecular target for cancer therapeutics. Cancer Cell 2003; 3:213-17.
56. Maloney A, Workman P. HSP90 as a new therapeutic target for cancer therapy: The story unfolds. Expert Opin Biol Ther 2002; 2:3-24.
57. Workman P. Altered states: Selectively drugging the Hsp90 cancer chaperone. Trends Mol Med 2004; 10:47-51.
58. Kamal A, Boehm MF, Burrows FJ. Therapeutic and diagnostic implications of Hsp90 activation. Trends Mol Med 2004; 10:283-90.
59. Isaacs JS, Jung YJ, Neckers L. Aryl hydrocarbon nuclear translocator (ARNT) promotes oxygen-independent stabilization of hypoxia-inducible factor-1alpha by modulating an Hsp90-dependent regulatory pathway. J Biol Chem 2004; 279:16128-35.
60. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. J Biol Chem 2002; 277:29936-44.
61. Mabjeesh NJ, Post DE, Willard MT, Kaur B, Van Meir EG, Simons JW, Zhong H. Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. Cancer Res 2002; 62:2478-82.
62. Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, Krutzsch H, Ochel HJ, Schulte TW, Sausville E, Neckers LM, Toft DO. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. J Biol Chem 1997; 272:23843-50.
63. Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: Targeting of a protein chaperone by an antitumor agent. Cell 1997; 89:239-50.
64. Neckers L, Schulte TW, Mimnaugh E. Geldanamycin as a potential anti-cancer agent: Its molecular target and biochemical activity. Invest New Drugs 1999; 17:361-73.
65. Zagzag D, Nomura M, Friedlander DR, Blanco CY, Gagner JP, Nomura N, Newcomb EW. Geldanamycin inhibits migration of glioma cells in vitro: A potential role for hypoxia-inducible factor (HIF-1alpha) in glioma cell invasion. J Cell Physiol 2003; 196:394-402.
66. Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P. DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst 1999; 91:1940-9.
67. Basso AD, Solit DB, Munster PN, Rosen N. Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. Oncogene 2002; 21:1159-66.
68. Solit DB, Zheng FF, Drobnjak M, Munster PN, Higgins B, Verbel D, Heller G, Tong W, Cordon-Cardo C, Agus DB, Scher HI, Rosen N. 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. Clin Cancer Res 2002; 8:986-93.
69. Goetz MP, Toft D, Reid J, Ames M, Stensgard B, Safgren S, Adjei AA, Sloan J, Atherton P, Vasile V, Salazaar S, Adjei A, Croghan G, Erlichman C. Phase I Trial of 17-Allylamino-17-Demethoxygeldanamycin in Patients With Advanced Cancer. J Clin Oncol 2005; 23:1078-87.
70. Sausville EA, Tomaszewski JE, Ivy P. Clinical development of 17-allylamino, 17-demethoxygeldanamycin. Curr Cancer Drug Targets 2003; 3:377-83.
71. Russell JS, Burgan W, Oswald KA, Camphausen K, Tofilon PJ. Enhanced cell killing induced by the combination of radiation and the heat shock protein 90 inhibitor 17-allylamino-17- demethoxygeldanamycin: A multitarget approach to radiosensitization. Clin Cancer Res 2003; 9:3749-55.
72. Bisht KS, Bradbury CM, Mattson D, Kaushal A, Sowers A, Markovina S, Ortiz KL, Sieck LK, Isaacs JS, Brechbiel MW, Mitchell JB, Neckers LM, Gius D. Geldanamycin and 17-allylamino-17-demethoxygeldanamycin potentiate the in vitro and in vivo radiation response of cervical tumor cells via the heat shock protein 90-mediated intracellular signaling and cytotoxicity. Cancer Res 2003; 63:8984-95.
73. Chen X, Bies RR, Ramanathan RK, Zuhowski EG, Trump DL, Egorin MJ. Population pharmacokinetic analysis of 17-(allylamino)-17-demethoxygeldanamycin (17AAG) in adult patients with advanced malignancies. Cancer Chemother Pharmacol 2005; 55:237-43.
74. Egorin MJ, Rosen DM, Wolff JH, Callery PS, Musser SM, Eiseman JL. Metabolism of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) by murine and human hepatic preparations. Cancer Res 1998; 58:2385-96.
75. Hollingshead M, Alley M, Burger AM, Borgel S, Pacula-Cox C, Fiebig HH, Sausville EA. In vivo antitumor efficacy of 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride), a water-soluble geldanamycin derivative. Cancer Chemother Pharmacol 2005; 56:115-25.
76. Bull EE, Dote H, Brady KJ, Burgan WE, Carter DJ, Cerra MA, Oswald KA, Hollingshead MG, Camphausen K, Tofilon PJ. Enhanced tumor cell radiosensitivity and abrogation of G_2 and S phase arrest by the Hsp90 inhibitor 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin. Clin Cancer Res 2004; 10:8077-84.
77. Shoemaker RH, Scudiero DA, Melillo G, Currens MJ, Monks AP, Rabow AA, Covell DG, Sausville EA. Application of high-throughput, molecular-targeted screening to anticancer drug discovery. Curr Top Med Chem 2002; 2:229-46.
78. Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, Melillo G. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res 2002; 62:4316-24.
79. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, Melillo G. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: Mechanism and therapeutic implications. Cancer Res 2004; 64:1475-82.
80. Rapisarda A, Zalek J, Hollingshead M, Braunschweig T, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, Hewitt SM, Shoemaker RH, Melillo G. Schedule-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. Cancer Res 2004; 64:6845-8.

81. Kamiyama H, Takano S, Tsuboi K, Matsumura A. Anti-angiogenic effects of SN38 (active metabolite of irinotecan): Inhibition of hypoxia-inducible factor 1 alpha (HIF-1alpha)/vascular endothelial growth factor (VEGF) expression of glioma and growth of endothelial cells. J Cancer Res Clin Oncol 2005; 131:205-13.
82. Edsall AB, Mohanakrishnan AK, Yang D, Fanwick PE, Hamel E, Hanson AD, Agoston GE, Cushman M. Effects of altering the electronics of 2-methoxyestradiol on cell proliferation, on cytotoxicity in human cancer cell cultures, and on tubulin polymerization. J Med Chem 2004; 47:5126-39.
83. Zhou NN, Zhu XF, Zhou JM, Li MZ, Zhang XS, Huang P, Jiang WQ. 2-methoxyestradiol induces cell cycle arrest and apoptosis of nasopharyngeal carcinoma cells. Acta Pharmacol Sin 2004; 25:1515-20.
84. Lis A, Ciesielski MJ, Barone TA, Scott BE, Fenstermaker RA, Plunkett RJ. 2-methoxyestradiol inhibits proliferation of normal and neoplastic glial cells, and induces cell death, in vitro. Cancer Lett 2004; 213:57-65.
85. Ricker JL, Chen Z, Yang XP, Pribluda VS, Swartz GM, Van WC. 2-methoxyestradiol inhibits hypoxia-inducible factor 1alpha, tumor growth, and angiogenesis and augments paclitaxel efficacy in head and neck squamous cell carcinoma. Clin Cancer Res 2004; 10:8665-73.
86. Amorino GP, Freeman ML, Choy H. Enhancement of radiation effects in vitro by the estrogen metabolite 2-methoxyestradiol. Radiat Res 2000; 153:384-91.
87. Han GZ, Liu ZJ, Shimoi K, Zhu BT. Synergism between the anticancer actions of 2-methoxyestradiol and microtubule-disrupting agents in human breast cancer. Cancer Res 2005; 65:387-93.
88. Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 1994; 368:237-9.
89. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell 2003; 3:363-75.
90. Powis G, Montfort WR. Properties and biological activities of thioredoxins. Annu Rev Pharmacol Toxicol 2001; 41:261-95.
91. Welsh SJ, Bellamy WT, Briehl MM, Powis G. The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 2002; 62:5089-95.
92. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation. Mol Cancer Ther 2003; 2:235-43.
93. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, Livingston DM. An essential role for p300/CBP in the cellular response to hypoxia. Proc Natl Acad Sci USA 1996; 93:12969-73.
94. Kung AL, Wang S, Klco JM, Kaelin WG, Livingston DM. Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat Med 2000; 6:1335-40.
95. Dames SA, Martinez-Yamout M, De Guzman RN, Dyson HJ, Wright PE. Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response. Proc Natl Acad Sci USA 2002; 99:5271-6.
96. Kung AL, Zabludoff SD, France DS, Freedman SJ, Tanner EA, Vieira A, Cornell-Kennon S, Lee J, Wang B, Wang J, Memmert K, Naegeli HU, Petersen F, Eck MJ, Bair KW, Wood AW, Livingston DM. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 2004; 6:33-43.
97. Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, Kim MS, Park JW. YC-1: A potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 2003; 95:516-25.
98. Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G. Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1alpha. Mol Cancer Ther 2004; 3:233-44.
99. Weissleder R, Mahmood U. Molecular imaging. Radiology 2001; 219:316-33.
100. Waldherr C, Mellinghoff IK, Tran C, Halpern BS, Rozengurt N, Safaei A, Weber WA, Stout D, Satyamurthy N, Barrio J, Phelps ME, Silverman DH, Sawyers CL, Czernin J. Monitoring antiproliferative responses to kinase inhibitor therapy in mice with 3'-deoxy-3'-18F-fluorothymidine PET. J Nucl Med 2005; 46:114-20.
101. Liu G, Rugo HS, Wilding G, McShane TM, Evelhoch JL, Ng C, Jackson E, Kelcz F, Yeh BM, Lee Jr FT. Charnsangavej C, Park JW, Ashton EA, Steinfeldt HM, Pithavala YK, Reich SD, Herbst RS. Dynamic contrast-enhanced magnetic resonance imaging as a pharmacodynamic measure of response after acute dosing of AG-013736, an oral angiogenesis inhibitor, in patients with advanced solid tumors: Results from a phase I study. J Clin Oncol 2005; 23:5464-73.
102. Smith-Jones PM, Solit DB, Akhurst T, Afroze F, Rosen N, Larson SM. Imaging the pharmacodynamics of HER2 degradation in response to Hsp90 inhibitors. Nat Biotechnol 2004; 22:701-6.
103. Koch CJ, Evans SM. Noninvasive PET and SPECT imaging of tissue hypoxia using isotopically labeled 2-nitroimidazoles. Adv Exp Med Biol 2003; 510:285-92.
104. Serganova I, Doubrovin M, Vider J, Ponomarev V, Soghomonyan S, Beresten T, Ageyeva L, Serganov A, Cai S, Balatoni J, Blasberg R, Gelovani J. Molecular imaging of temporal dynamics and spatial heterogeneity of hypoxia-inducible factor-1 signal transduction activity in tumors in living mice. Cancer Res 2004; 64:6101-8.
105. Wen B, Burgman P, Zanzonico P, O'donoghue J, Cai S, Finn R, Serganova I, Blasberg R, Gelovani J, Li GC, Ling CC. A preclinical model for noninvasive imaging of hypoxia-induced gene expression; comparison with an exogenous marker of tumor hypoxia. Eur J Nucl Med Mol Imaging 2004; 31:1530-8.