Development of HIF-1 inhibitors for cancer therapy

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Abstract

Intratumour hypoxia has long been considered a driving force of tumour progression and a negative prognostic factor in human cancers. The discovery of hypoxia inducible factors (HIFs), which mediate transcriptional responses to changes in oxygen levels, has renewed enthusiasm for the discovery and development of targeted therapies exploiting the hypoxic tumour microenvironment. In spite of an ever increasing number of putative small molecule inhibitors of HIF, only few progress through pre-clinical and early clinical development. In this review, we will focus primarily on: (1) HIF inhibitors that have been more recently described and (2) small molecules targeting HIF that are being tested in early clinical trials or that are already approved for use in patients. A rigorous ‘validation’ of HIF targeted therapies in relevant pre-clinical models and eventually in pharmacodynamic-based early clinical trials is essential for ‘credentialing’ HIF-1 as a legitimate target that can be pharmacologically modulated in cancer patients.

Keywords: HIF-1 • hypoxia • cancer therapy

Introduction

The identification of novel molecular targets for cancer therapy has led to a paradigm shift in drug development, with more emphasis placed now on small molecules that can effectively inhibit signalling pathways deregulated in cancer cells. Of the many survival pathways activated by cancer cells, hypoxic cell signalling has attracted significant interest for a number of years, based on the assumption that normal tissues do not experience the same extreme variations in oxygen levels present in the tumour microenvironment. Cancer cells are exposed to a gradient of oxygen levels that fluctuate in time and space, which trigger the activation of survival pathways that are not usually induced in normal tissues and that can be potentially targeted for therapeutic purposes [1].

The discovery of hypoxia inducible factor (HIF)-1 in the early 1990s provided a molecular target associated with intratumour hypoxia that could be used for the development of novel cancer therapeutics [2]. Despite the intrinsic challenges associated with the discovery and development of pharmacological inhibitors of transcription factors, more so in the absence of structural information that could facilitate drug design, many academic groups and pharmaceutical companies have attempted to identify HIF-1 inhibitors [3–6]. For the most part, efforts have been based on high throughput screening assays aimed at identification of inhibitors of HIF-1 expression and/or transcriptional activity [4]. After several years of attempts and many HIF-1 inhibitors described in the literature, there are several conclusions that can be drawn and considerations that can be made, which might help in shaping future directions in this field.

The common denominator of most, if not all, HIF-1 inhibitors described so far is the lack of specificity, which indicates the fact that they inhibit multiple targets and that HIF-1 inhibition cannot be easily separated from other activities exerted by these agents.
This feature of HIF-1 inhibitors may have hampered efforts in validating HIF-1 as a target using pharmacological approaches; nevertheless HIF-1 inhibitors they may still have potential applications for therapeutic purposes. A challenge that HIF-1 inhibitors must face to be ‘validated’ as potential therapeutic tools is the evidence that they inhibit the intended target in relevant \textit{in vivo} models and more so in patients with cancer. Indeed, inhibition of HIF-1 expression and/or activity in cell culture is hardly predictive of their potential usefulness as therapeutic agents. However, validation of HIF-1 inhibitors in pre-clinical models is hindered by the lack of established biomarkers that can be consistently associated with HIF-1 inhibition in tumour tissue. Different end-points have been measured to assess HIF-1 inhibition in published studies, including but not limited to IHC and/or Western blot analysis of HIF-1 protein expression, mRNA expression of HIF-1 target genes and more indirect, surrogate end-points of HIF inhibition, \textit{e.g.} angiogenesis and microvessels density. Despite these challenges, efforts to validate HIF-1 inhibitors in appropriate \textit{in vivo} models are essential to move these potential therapeutic agents to the clinical setting. This is even more relevant in light of the potential lack of antitumour activity of HIF-1 inhibitors used as single agents. In fact, antitumour activity cannot be and should not be used as a surrogate end-point for the validation of HIF-1 inhibition, as it is conceptually difficult to envision how HIF-1 inhibition alone may be associated with dramatic tumour shrinkage in xenograft models in which HIF-1 expression in tumour tissue is heterogeneous and focal in nature.

Even more challenging is, of course, to generate evidence of HIF-1 inhibition in the clinical setting. However, this is a necessary path for the validation of HIF-1 inhibitors in early clinical trials and for the development of this strategy in combination approaches, which appears to be a more promising avenue for the application of HIF-1 inhibitors.

In this review, we will discuss more in detail HIF-1 inhibitors that have been recently described, referring to previously published reviews for a more systematic description of HIF-1 inhibitors \cite{5, 6}. In particular, we will emphasize those agents for which validation of HIF-1 inhibition in pre-clinical models has been provided and/or agents that are in early clinical development. It is hoped that results of ongoing clinical trials with HIF-1 inhibitors may provide in the near future sufficient information that should aid in the design of future strategies aimed at targeting hypoxic cell signalling.

\section*{Mechanisms of action of HIF-1 inhibitors}

An ever increasing number of agents are constantly being reported that inhibit HIF-1 expression and/or activity. We will attempt to discuss these agents based on their putative mechanism of action (Fig. 1), which could provide some useful insights for their clinical development. It should also be noted that the information published so far relates for the most part to HIF-1\(\alpha\) although many of these agents may also affect HIF-2\(\alpha\). Both sub-units are potential targets of small molecule inhibitors and no clear selectivity, capable of discriminating between inhibition of HIF-1\(\alpha\) or HIF-2\(\alpha\), has been so far convincingly demonstrated.

According to their putative mechanism of action and although this is an obviously simplified classification, HIF inhibitors could be tentatively divided in agents that modulate:

1. HIF-1\(\alpha\) mRNA expression,
2. HIF-1\(\alpha\) protein translation,
3. HIF-1\(\alpha\) protein degradation,
4. HIF-1\(\alpha\) DNA binding and
5. HIF-1\(\alpha\) transcriptional activity.

\section*{Inhibitors of HIF-1\(\alpha\) mRNA expression}

HIF-1\(\alpha\) accumulation is controlled primarily at the level of protein degradation or protein translation and most of the HIF-1\(\alpha\) inhibitors identified so far target these pathways. However, it has also been suggested that, under hypoxic conditions, levels of HIF-1\(\alpha\) mRNA may be a limiting factor affecting the rate of protein translation \cite{7} and it is presumable that small molecule inhibitors might affect HIF-1\(\alpha\) mRNA expression \cite{8} and as a consequence the rate of HIF-1 translation.

An interesting approach that might add specificity to HIF-1\(\alpha\) inhibition is the use of an antisense oligonucleotide targeting HIF-1\(\alpha\) (EZN-2698) \cite{9}. EZN-2968 is highly specific and binds HIF-1\(\alpha\) mRNA with high affinity causing its down-regulation and consequent reduction of HIF-1\(\alpha\) protein levels, both \textit{in vitro} and \textit{in vivo}. Treatment with EZN-2968 results in tumour cell growth inhibition, down-regulation of HIF-1\(\alpha\) target genes and impaired ability of HUVEC cells to form tubes \textit{in vitro}. In vivo, EZN-2968 administration decreased endogenous HIF-1\(\alpha\) and vascular endothelial growth factor (VEGF) mRNA levels in the liver of normal mice and showed antitumour activity in xenograft models of human prostate cancer (DU145). Preliminary results of ongoing phase I clinical trials in patients with advanced solid tumours indicate that EZN-2968 can be given safely and that potential activity has been observed in one patient with metastatic renal cell carcinoma (RCC). Future studies will be required to address a main limitation of antisense oligonucleotide approaches, which is the delivery to tumour tissues.

Another agent that appears to affect HIF-1\(\alpha\) mRNA expression is aminoflavone. AF is a ligand of the aryl-hydrocarbon receptor (AhR) and is currently in phase I clinical trials in patients with metastatic cancer. Based on the notion that AhR dimerizes with HIF-1\(\beta\), it was interesting to test whether pharmacological activation of the AhR pathway using AF might affect HIF-1\(\alpha\) levels. Results of these studies have shown that AF does indeed inhibit HIF-1\(\alpha\) accumulation, although in an AhR-independent fashion. The proposed mechanism of HIF-1 inhibition by AF is modulation of HIF-1\(\alpha\) mRNA expression, although the exact mechanism remains to be fully elucidated.
Inhibitors of HIF-1α protein translation

Although the mechanism(s) underlying hypoxic regulation HIF-1α translation are still poorly understood, several agents have been described that may affect the rate of HIF-1α protein synthesis, including but not limited to inhibitors of topoisomerase I and II [10–12], receptor tyrosine kinase [13–15], cyclin-dependent kinase [16], oncogenic pathways [17–21], thioredoxin reductase [22], activators of p53 [23] and microtubule disrupting agents [24].

One of the first agents described that may affect HIF-1α protein translation is topotecan, an FDA approved chemotherapeutic agent currently used as second line therapy for patients with small cell lung cancer or ovarian cancer. Topotecan was originally identified at the National Cancer Institute in a high throughput screen using a cell-based assay of HIF-1α transcriptional activity [25]. Topotecan is a camptothecin analogue that poisons topoisomerase I by inducing the formation of stable Top1-DNA cleavage complexes, which in the presence of DNA replication generate double strand DNA breaks and cytotoxicity. Interestingly, topotecan inhibited HIF-1α translation by a Top1-dependent but DNA damage-independent mechanism, suggesting that cytotoxicity and HIF-1α inhibition could be mechanistically distinguished [26]. Indeed, daily low dose administration of topotecan in a mouse xenograft glioma model caused inhibition of HIF-1α protein expression, angiogenesis and tumour growth [27]. More recently, it has been shown that administration of daily topotecan in combination with the anti-VEGF antibody bevacizumab exerts synergistic antitumour activity in xenograft models, providing a rationale for clinical development of this combination strategy [28]. A pilot study ongoing at NCI in which daily oral topotecan is given to patients with metastatic refractory cancers should provide evidence as to whether this agents is able to affect HIF-1 signalling in tumour tissue (http://clinicaltrials.gov/ct2/show/NCT00182676).

The ability to inhibit HIF-1α protein translation appears to be shared by all the agents that inhibit Top1. Because topotecan has a short half-life when administered to patients, it is conceivable that other topoisomerase 1 inhibitors with more favourable
pharmacokinetics may be more suitable for chronic suppression of the HIF-1 pathway. In this regard an interesting agent is EZN-2208, a PEGylated form of SN38, the active component of CPT-11 (Irinotecan Pfizer, New York, NY, USA; Yakult Honsha, Tokyo, Japan), characterized by improved pharmacokinetics and by remarkable antitumour activity in pre-clinical models of solid tumours and lymphomas, including CPT-11-resistant tumours [29]. The demonstrated activity of EZN-2208 in CPT-11 refractory tumours could be potentially explained by the ability of this agent to inhibit HIF-1α accumulation, thus acting on the tumour microenvironment rather than only on cancer cells [30]. EZN-2208 is currently in phase I clinical trials and phase II as well as combination studies are being planned.

Cardiac glycosides are another class of agents that have been recently reported to affect HIF-1α protein translation. In particular digoxin was identified as a potent inhibitor of HIF-1 activity in a cell-based screen of a chemical library of FDA approved agents [31]. Digoxin inhibited the translation of HIF-1α by an mTOR-independent mechanism and showed antitumour activity in xenograft models. Interestingly, digoxin inhibited tumour growth in established PC3 and P493-Myc tumour xenografts, yet did not affect the growth of xenografts expressing a constitutively active form of HIF-1α, implicating HIF-1 in the antitumour activity of digoxin. Interestingly, digoxin, which is routinely used for the treatment of heart failure and arrhythmias, is currently being tested in a phase I clinical trial as a potential anticancer agent. Consistent with these results, fractionation of an organic solvent extract of the plant Crossosoma bigelovii led to the discovery of a new strophanthidin glycoside that also inhibited HIF-1α transcriptional activity [32]. Whether cardiac glycosides may effectively be used to inhibit HIF-1 in cancer patients, in the absence of unacceptable adverse events, remains to be established.

Another HIF-1 inhibitor currently in phase I clinical trials in patients with advanced metastatic cancer is PX-478. This agent showed remarkable antitumour activity in a variety of human tumour xenograft models, which seemed to correlate with levels of expression of HIF-1α [33]. PX-478 inhibited constitutive and hypoxia-induced HIF-1α expression in a pHVL and p53 independent fashion. The inhibition seems to occur at multiple levels, since three different mechanisms have been proposed that might contribute to the decrease of HIF-1α accumulation. Indeed, it has been suggested that PX-478 inhibits HIF-1α deubiquitination, leading to increased degradation of polyubiquitinated HIF-1α, reduces HIF-1α mRNA expression and also affects HIF-1α translation [34]. Results of an ongoing phase I trial should be available in the near future and might provide interesting information regarding antitumour activity and modulation of HIF-1α levels in cancer patients.

A signalling pathway that has been implicated in growth factor-dependent induction of HIF-1α translation is mTOR [35, 36]. However, mTOR and global protein synthesis are inhibited under severe hypoxia, thus the contribution of these pathways to HIF-1α translation under hypoxic conditions is still poorly understood [37]. Several mTOR inhibitors, including temsirolimus and everolimus that are FDA approved agents for the treatment of renal cancer, have been shown to inhibit HIF-1α [38–40]. Clinical trials have demonstrated efficacy of these agents in the treatment of RCC [41]. Up to 75% of sporadic clear cell RCCs are VHL-deficient therefore HIF-1α is stabilized under normoxic conditions [42]. In this context mTOR may contribute to increase HIF-1α protein levels and this might contribute to the efficacy of mTOR inhibitors in RCC [43]. In a phase II clinical study, temsirolimus as a single agent significantly improved overall survival of patients with advanced RCC and poor prognosis compared with IFN-α treatment [44]. The administration of everolimus to patients with metastatic RCC that progressed after VEGF-targeted therapies resulted in a prolongation of progression-free survival compared with placebo in a randomized phase III clinical trial [45]. Clinical trials are ongoing to evaluate the potential of mTOR inhibitors, as single agents or in combination studies, for the treatment of other solid malignancies. Whether HIF-1α inhibition may contribute to the therapeutic activity of this class of agents in malignancies other than renal cancer remains to be established.

**Inhibitors that affect HIF-1α degradation pathway**

Hsp90 is a molecular chaperone that controls the folding and regulates the function of different client proteins, including receptor tyrosine kinases, serine/threonine kinases, transcription factors and activated oncoproteins [46]. HIF-1α protein stability is also affected by its interaction with Hsp90. In the presence of Hsp90 inhibitors HIF-1α undergoes VHL-independent proteasomal degradation [47]; moreover HIF-1α heterodimers may not acquire the proper conformation and fail to recruit cofactors important for HIF-1-mediated transcriptional activity [48]. The development of Hsp90 inhibitors started with the discovery of the natural product galdanamycin, a benzoquinone ansamycin antibiotic that inhibits Hsp90 by competing with the ATP binding site. Galdanamycin was found to induce HIF-1α degradation under both hypoxic and normoxic conditions in several cell lines [49]. The first Hsp90 inhibitors to enter clinical trials were 17-AAG and 17-DMAG and currently, a large number of second generation Hsp90 inhibitors are in clinical development as anticancer agents [50]. However, given the range of client proteins that may be affected by Hsp90 inhibition, it is difficult to determine whether and to what extent their antitumour activity may be related to HIF inhibition, more so in the absence of clinical trial aimed at assessing the specific effects of Hsp90 inhibition on HIF-1 signalling pathway.

Histone deacetylase inhibitors have also been implicated in the regulation of HIF-1 activity by several potential mechanisms, including induction of HIF-1α protein degradation and regulation of HIF-1 transcriptional activity [51]. Although a direct role of acetylation in the regulation of HIF-1α protein remains controversial, recent evidence indicates that Sirtuin 1 (Sirt1), a redox-sensing deacetylase, selectively stimulates activity of HIF-2α during hypoxia [52]. Histone deacetylase inhibitors are currently being
Inhibitors of HIF-1 binding to DNA

Inhibition of HIF-1 DNA binding to the hypoxia responsive element (HRE), a step required for induction of transcription, is also a potential mechanism by which small molecules may inhibit HIF-1 activity [53–55].

Proof of principle that this mechanism may effectively inhibit HIF-1 transcriptional activity was provided by the identification of echinomycin, a cyclic peptide of the family of quinoxaline antibiotics originally isolated from Streptomyces echinatus, which was known to bind DNA in a sequence-specific fashion. Echinomycin was shown in chromatin immunoprecipitation experiments to inhibit HIF-1, but not AP-1 or NF-κB, binding to DNA, providing evidence of a fairly selective inhibition based on recognition of DNA sequences [56]. Echinomycin clinical development was halted in the late 1980s following extensive testing as cytotoxic agent in phase I-II trials, which failed to show significant activity.

More recently, anthracyclines were found to inhibit HIF-1 activity [57]. Anthracyclines exert their cytotoxic activities by a number of different mechanisms, including DNA intercalation, and are among the most effective chemotherapeutic agents used to treat a wide range of cancers. Recent evidence indicated that doxorubicin (DXR) and daunorubicin (DNR) inhibit HIF-1α transcriptional activity by blocking its binding to the HRE sequence. Administration of DXR or DNR to mice bearing human prostate cancer xenografts significantly inhibited tumour growth and vascularization, along with a decrease of circulating angiogenic cells (CAC). The mobilization of CACs in the bloodstream was shown to be mediated by HIF-1-induced genes encoding pro-angiogenic cytokines, which were significantly down-regulated in anthracycline-treated mice. These results raise the possibility that metronomic administration of anthracyclines may exert antitumour activity by inhibiting HIF-1 and angiogenesis and that HIF inhibition may be one of the potential mechanisms contributing to the activity of metronomic chemotherapy.

Inhibitors of HIF-1α transcriptional activity

Chetomin was originally identified as an inhibitor of HIF-1 transcriptional activity by interfering with the interaction of HIF-1α with the co-activator p300 [58]. However, because of toxicity the development of chetomin has not been further pursued.

Inhibition of the proteasome leads to normoxic accumulation of HIF-1α [59]. Paradoxically, HIF-1α that accumulates in the presence of proteasome blockade is transcriptionally inactive [60]. Bortezomib (PS-341) is a proteasome inhibitor FDA approved for treatment of patients with multiple myeloma and patients with mantle cell lymphoma who had received at least one prior therapy [61, 62]. Interestingly, the antitumour activity of bortezomib may correlate with its ability to repress HIF-1α transcriptional activity [63]. At low nanomolar concentrations bortezomib was able to impair the p300-HIF-1α interaction, by enhancing the binding of FIH to HIF-1α [64]. FIH is a dioxygenase that hydroxylates Asn803 in the C-terminal transactivation domain of HIF-1α, thus preventing the recruitment of the co-activator p300. Interestingly, the concentrations of bortezomib able to inhibit HIF-1α activity are much lower than those required to impair proteasome function, suggesting that the mechanism of HIF inhibition by bortezomib may be independent from proteasome inhibition [65].

Conclusions

Significant progress in our understanding of the molecular mechanism(s) underlying intratumour hypoxia has fuelled interest in developing strategies targeting hypoxic cell signalling for cancer therapy. In addition, therapy-induced hypoxia, which may be caused by anti-VEGF therapies, has provided challenges but also opportunities for the development of combination strategies incorporating HIF-1-targeting agents. The excitement for innovative approaches targeting previously poorly characterized pathways triggered by hypoxia in the tumour microenvironment has been so far tempered by the lack of specific and effective small molecules that may be thought of as ‘gold standard’ for HIF-1 inhibition. Nevertheless, validation of available small molecules in preclinical models and more importantly in early clinical trials may provide a unique opportunity for the pharmacological inhibition of hypoxia-induced pathways that can be clinically exploitable. Efforts should be devoted to implementing well-designed, pharmacodynamic-based early clinical trials of promising HIF-1 inhibitors to validate their activity and identify agents that can be used in combination strategies.

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References

1. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer. 2004; 4: 437–47.

2. Wang GL, Jiang BH, Rue EA, et al. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA. 1995; 92: 5510–14.

3. Giaccia A, Slim BG, Johnson RS. HIF-1 as a target for drug development. Nat Rev Drug Discov. 2003; 2: 803–11.

4. Melillo G. Hypoxia-inducible factor 1 inhibitors. Methods Enzymol. 2007; 435: 385–402.

5. Semenza GL. Evaluation of HIF-1 inhibitors as anticancer agents. Drug Discov Today. 2007; 12: 853–9.

6. Melillo G. Targeting hypoxia cell signaling for cancer therapy. Cancer Metastasis Rev. 2007; 26: 341–52.

7. Young RM, Wang SJ, Gordan JD, et al. Hypoxia-mediated selective mRNA translation by an internal ribosome entry site-independent mechanism. J Biol Chem. 2008; 283: 16309–19.

8. Chang H, Shyu KG, Lee CC, et al. GL331 inhibits HIF-1alpha expression in a lung cancer model. Biochem Biophys Res Commun. 2003; 302: 95–100.

9. Greenberger LM, Horak ID, Filpula D, et al. A RNA antagonist of hypoxia-inducible factor-1alpha, EZN-2968, inhibits tumor cell growth. Mol Cancer Ther. 2008; 7: 3598–608.

10. Rapisarda A, Uranchimeg B, Sordet O, et al. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. Cancer Res. 2004; 64: 1475–82.

11. Creighton-Gutteridge M, Cardellina JH, Stephen AG, et al. Cell type-specific, topoisomerase II-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation by NCI 64221. Clin Cancer Res. 2007; 13: 1010–8.

12. Sapra P, Zhao H, Mehlig M, et al. Novel delivery of SN38 markedly inhibits tumor growth in xenografts, including a campothecin-11-refractory model. Clin Cancer Res. 2008; 14: 1888–88.

13. Laughner E, Taghavi P, Chiles K, et al. 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.
Majumder PK, Febbo PG, Bikoff R, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med.* 2004; 10: 594–601.

Wouters BG, Koritzinsky M. Hypoxia signaling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer.* 2008; 8: 851–64.

Del BD, Ciuffreda L, Trisciuoglio D, et al. Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. Cancer Res. 2006; 66: 5549–54.

Wan X, Shen N, Mendoza A, et al. CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1alpha/VEGF signaling. *Neoplasia.* 2006; 8: 394–401.

Majumder PK, Febbo PG, Bikoff R, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med.* 2004; 10: 594–601.

Kaper A, Figlin RA. Targeted inhibition of mammalian target of rapamycin for the treatment of advanced renal cell carcinoma. *Cancer.* 2009; 115: 3618–30.

Kaelin WG Jr. The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin Cancer Res.* 2007; 13: 680s–4s.

Thomas GV, Tran C, Mellinghoff IK, et al. Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med.* 2006; 12: 122–7.

Motzer RJ, Bacik J, Murphy BA, et al. Interferon-alpha as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol.* 2002; 20: 289–96.

Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet.* 2008; 372: 449–56.

Neckers L. Heat shock protein 90: the cancer chaperone. *J Biol Chem.* 2007; 282: 29936–44.

Huir E, Kim HH, Choi SM, et al. Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1alpha/aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. *Mol Pharmacol.* 2002; 62: 975–82.

Isaacs JS, Jung YJ, Mimaugh EG, et al. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem.* 2002; 277: 29936–44.

Neckers L. Using natural product inhibitors to validate Hsp90 as a molecular target. *Curr Top Med Chem.* 2006; 6: 1163–71.

Ellis L, Hammers H, Pili R. Targeting tumor angiogenesis with histone deacetylase inhibitors. *Cancer Lett.* 2008; 280: 145–53.

Dieum EM, Chen R, Alexander MS, et al. Regulation of hypoxia-inducible factor 2alpha signaling by the stress-responsive deacetylase sirtuin 1. *Science.* 2009; 324: 1289–93.

Kong D, Park EJ, Stephen AG, et al. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Res.* 2005; 65: 9047–55.

Lee K, Qian DZ, Rey S, et al. Anthraccline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. *Proc Natl Acad Sci USA.* 2009; 106: 2353–8.

Kung AL, Zabludoff SD, France DS, et al. Small molecule blockade of transcriptional cocactivation of the hypoxia-inducible factor pathway. *Cancer Cell.* 2004; 6: 33–43.

Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem.* 1997; 272: 22642–7.

Kaluz S, Kaluzova M, Stanbridge EJ. Proteasome inhibition attenuates transcriptional activity of hypoxia-inducible factor 1 (HIF-1) via specific effect on the HIF-1alpha C-terminal activation domain. *Mol Cell Biol.* 2006; 26: 5895–907.

Richardson PG, Hideshima T, Anderson KC. Bortezomib (PS-341): a novel, first-in-class proteasome inhibitor for the treatment of multiple myeloma and other cancers. *Control Cancer.* 2003; 10: 361–9.

Adams J, Kauffman M. Development of the proteasome inhibitor Velcade (Bortezomib). *Cancer Invest.* 2004; 22: 304–11.

Mackay H, Hedley D, Major P, et al. A phase II trial with pharmacodynamic endpoints of the proteasome inhibitor bortezomib in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2005; 11: 5526–33.

Shin DH, Chun YS, Lee DS, et al. Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FHI-mediated repression of hypoxia-inducible factor-1. *Blood.* 2008; 111: 3131–6.

Shin DH, Chun YS, Lee DS, et al. Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FHI-mediated repression of hypoxia-inducible factor-1. *Blood.* 2008; 111: 3131–6.