Targeting the hypoxia-inducible factor (HIF) pathway in cancer

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The central component of hypoxia sensing in the cell is the hypoxia-inducible factor (HIF) transcriptional complex. HIF activity is deregulated in many human cancers, especially those that are highly hypoxic. Hypoxic tumour cells are usually resistant to radiotherapy and most conventional chemotherapeutic agents, rendering them highly aggressive and metastatic. Overexpression of HIF-α, the regulatory subunit of HIF, is associated with increased vascular density, severity of tumour grade, treatment failure and a poor prognostic outcome with conventional therapies. Therefore HIF is an attractive, although challenging, therapeutic target, and several different strategies have been developed to target HIF directly or indirectly in recent years. This review outlines the preclinical and clinical advances in this arena and discusses which cancers may benefit from HIF-targeted therapy.

Hypoxia is a common characteristic of all solid tumours (Ref. 1) and is a condition in which proliferating tumour cells are deprived of oxygen due to a limited blood supply from abnormal tumour microvasculature (Fig. 1). Hypoxic cells are at risk of stress-induced insults including oxidative DNA damage, DNA strand breaks and genetic aberrations, which can restrain growth and ultimately result in cell death. However, cancer cells show a range of genetic changes that improve survival and enable them to adapt to hypoxic conditions. As a result, hypoxic tumour cells continue to proliferate, are associated with a more invasive and metastatic phenotype and are usually resistant to conventional treatments such as radiotherapy and chemotherapy (Refs 2, 3, 4, 5, 6). Therefore, gaining a clearer understanding of the underlying molecular mechanisms involved in hypoxia signalling in cancer cells and how these processes might become deregulated in different cancer types is likely to result in better targeting of hypoxic tumour cells and more-effective treatments for solid tumours. A central component of hypoxia signalling in the cell is hypoxia-inducible factor (HIF), which is critically involved in both sensing and responding to changes in cellular oxygen (Ref. 7). This review outlines the therapeutic strategies used to target HIF/hypoxic signalling in tumour cells and discusses recent advances made in this area.

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Accession information: doi:10.1017/S1462399409001173; Vol. 11; e26; August 2009 © Cambridge University Press 2009
The HIF pathway

HIF is a transcriptional complex activated in response to changes in cellular oxygen levels and mediates the expression of many genes (Refs 8, 9). HIF target genes encode proteins that are involved in the regulation of various aspects of tumour biology, including oxygen transport, iron metabolism, glycolysis, glucose transport, cell survival and proliferation, angiogenesis, invasion and metastasis. HIF activity is deregulated in many human cancers, and this is most commonly due to the overexpression of HIF-α, the regulatory subunit of the HIF complex. Overexpression of HIF-α is usually associated with increased vascular density, severity of tumour grade, treatment failure and a poor prognostic outcome (Refs 10, 11). Blocking HIF activity or targeting HIF-1α expression in tumours has been shown to significantly slow tumour growth in xenograft.
models (Ref. 12) and render hypoxic cells more susceptible to killing by conventional therapies (Refs 13, 14, 15, 16).

**HIF-1: structure and regulation**

The HIFs belong to a family of structurally related basic-helix–loop–helix (bHLH)-containing proteins (Ref. 7). The prototype of the family is HIF-1. HIF-1 consists of two subunits: the regulatory HIF-1α subunit and the ubiquitously expressed HIF-1β subunit (also known as aryl hydrocarbon receptor nuclear translocator, ARNT). HIF-1α protein is composed of four functional domains: a bHLH domain and a PER–ARNT–SIM (PAS) domain (involved in dimerisation and DNA binding), an oxygen-dependent degradation (ODD) domain (required for targeting to the proteasome and degradation), and two transactivation domains (N-TAD and C-TAD) required for transcriptional activation (Ref. 17) (Fig. 2). HIF-1β contains bHLH, PAS and transactivation domains (Ref. 18).

Whereas HIF-1β is constitutively expressed in cells, the availability of HIF-1α is dependent on cellular oxygen levels. In normoxia (21% oxygen levels), HIF-1α protein is rapidly and continuously expressed and degraded (for review, see Ref. 9). The synthesis of HIF-1α protein is regulated by oxygen-independent mechanisms involving growth-factor-mediated activation of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (Refs 19, 20). HIF-1α protein degradation is controlled by the ODD domain, and deletion of the entire ODD region renders HIF-1α stable even in the absence of hypoxia signalling (Ref. 21). Hydroxylation of proline residues 402 and 564 within the ODD domain of HIF-1α mediates its interaction with the von Hippel–Lindau tumour suppressor protein (pVHL), which is the recognition component of an E3 ubiquitin ligase, leading to HIF-1α ubiquitination and subsequent degradation by the 26S proteasome (Refs 22, 23, 24, 25). The hydroxylation process is governed by three evolutionarily conserved HIF prolyl hydroxylases – PHD1 (EGLN2), PHD2 (EGLN1) and PHD3 (EGLN3) – and their activity depends on the availability of oxygen, iron, 2-oxoglutarate and ascorbate (Refs 26, 27). Interestingly, it has been shown by using small interfering RNA (siRNA) techniques that PHD2 plays a predominant role in controlling HIF-1α levels (Ref. 28).

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**Figure 2. Schematic representation of HIF-1α and its functional domains.** The basic-helix–loop–helix (bHLH) and PER–ARNT–SIM (PAS) domains of hypoxia-inducible factor 1α (HIF-1α) are involved in dimerisation and DNA binding; the oxygen-dependent degradation (ODD) domain is required for degradation via the proteasome; and the transactivation domains (N-TAD and C-TAD) are involved in transactivation activity. Hydroxylation of proline residues 402 and 564 within the ODD domain mediates its interaction with the von Hippel–Lindau tumour suppressor protein (pVHL). Hydroxylation of asparagine 803 in the C-TAD blocks its association with transcriptional coactivator p300/CBP.
Under hypoxic conditions, prolyl hydroxylation within the ODD domain is inhibited and the interaction of HIF-1α with pVHL is prevented. As a result, HIF-1α ubiquitination and degradation is blocked and consequently the level of the protein increases. The accumulated HIF-1α translocates to the nucleus where it dimerises with HIF-1β (Ref. 7) via the bHLH and part of the PAS domain to form the HIF-1 complex. HIF-1 recruits transcriptional coactivators such as p300/CBP (p300/CREB-binding protein) (Ref. 29) and binds to the hypoxia-response element (HRE) within the promoter region of HIF-1-responsive target genes (Ref. 30), thereby mediating their transcriptional activation.

The transcriptional activity of HIF-1 is also controlled by an asparagine hydroxylase known as FIH-1 (factor inhibiting HIF-1) (Refs 31, 32, 33, 34, 35). In normoxia, hydroxylation of Asn803 in the C-TAD of HIF-1α blocks its association with p300/CBP. FIH-1 was also reported to interact with pVHL to modulate HIF-1α protein stabilisation (Ref. 35). Thus, FIH-1 acts as a negative regulator of HIF-1α to suppress transcriptional activity.

Other HIF-α family members

Two other HIF-α isoforms have been identified: HIF-2α and HIF-3α (Ref. 36). HIF-2α has a similar structure to HIF-1α (Ref. 37). Like HIF-1α, HIF-2α is rapidly induced in response to hypoxia, negatively regulated by the pVHL ubiquitin E3 ligase complex, and can mediate the transcriptional activation of a number of known HIF-1 target genes (Ref. 38). However, the expression of HIF-2α is cell-type specific and it has a distinct biological role from HIF-1α (Refs 39, 40, 41, 42, 43), with particular importance in renal cancer and vascular biology. In development, HIF-1α and HIF-2α were demonstrated to have nonoverlapping functions: HIF-1α+/− and HIF-2α+/− mouse embryos have different phenotypes and developmental defects (Ref. 40). HIF-2α is also expressed at a higher level than HIF-1α in several pVHL-defective renal carcinoma cell lines (Refs 42, 44), and various groups have reported that HIF-1 and HIF-2 can regulate both overlapping and distinct target genes (Refs 39, 41, 43).

The function of HIF-3α is not well understood. Several splice variants of HIF-3α have been identified (Ref. 45). One of the HIF-3α splice variants, known as the inhibitory PAS domain protein (IPAS), can function as a dominant negative regulator of hypoxia-inducible gene expression: it binds to the HIF-1α subunit to form a nonfunctional complex in the nucleus, impairing the expression of HIF-1 target genes under hypoxic conditions (Ref. 46).

Interestingly, it has been reported recently that IPAS gene expression is induced in response to hypoxia and is regulated directly by HIF-1 binding, forming a further level of negative feedback in the hypoxia-response system (Ref. 47).

**HIF and cancer**

In addition to hypoxia, loss-of-function mutations in several genes involved in the oxygen-sensing mechanism have also been shown to contribute to the overexpression of HIF-α (HIF-1α and HIF-2α) and activation of the HIF pathway in tumour cells (Fig. 3). For example, loss-of-function mutations in VHL have been shown to increase HIF-1α and HIF-2α expression in clear-cell renal carcinoma, haemangioblastoma and other VHL-associated tumours due to the lack of HIF-α ubiquitination and degradation (Ref. 44).

Mutations in succinate dehydrogenase (SDH) and fumarate hydratase (FH) inhibit prolyl hydroxylase activity, resulting in abnormal stabilisation of HIF-1α and upregulation of HIF target genes such as vascular endothelial growth factor (VEGF) in several cancers, namely paragangliomas, phaeochromocytomas, leiomyomas and renal cell cancers (Refs 48, 49).

Dysregulation of key signal transduction pathways also contributes to the overexpression of HIF-1α and activation of HIF-1 in cancer. Tumour cells with constitutive activation of the Ras–MAPK pathway (Ref. 50), Src (Ref. 51) or the PI3K–AKT(PKB)–mTOR (mammalian target of rapamycin) pathway (Refs 52, 53) have elevated expression of HIF-1α protein. Loss of function of tumour suppressor proteins such as PTEN (which leads to constitutive activation of AKT) (Refs 54, 55) and p53 can also result in increased HIF-1 activity (Fig. 3).

Since HIF-α (HIF-1α and HIF-2α) is induced in cancer cells in response to hypoxia and growth factors, and as a result of known genetic abnormalities, it is no surprise then that HIF-α protein has been shown to be overexpressed in human tumour biopsy samples. Immunohistochemical analyses of paraffin-embedded tissue sections have shown HIF-1α...
HIF-1α expression is deregulated in cancer
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Overexpression of hypoxia-inducible factor 1α (HIF-1α) and activation of the HIF pathway in cancer is caused by a combination of microenvironmental changes, such as changes in oxygen levels (hypoxia), pH and nutrients (deprivation), increases in growth factors, and genetic abnormalities leading to loss of tumour suppressor function, oncogenic activation or deregulated mitochondrial function. Increased HIF-α protein in cancer cells translocates to the nucleus, binds to HIF-1β, recruits coactivators (e.g. p300/CBP) and activates the transcription of multiple genes involved in angiogenesis (e.g. VEGF), metabolic adaptation (e.g. GLUT-1), cell survival (e.g. IGF-1) and metastasis (e.g. LOX, PAI-1) – thereby driving tumour progression. Abbreviations: FH, fumarate hydratase; GLUT-1, glucose transporter 1; HRG, heregulin; IGF-1, insulin-like growth factor 1; LOX, lysyl oxidase; p300/CBP, p300/CREB-binding protein; p14ARF, alternate reading frame (ARF) product of CDKN2A (cyclin-dependent kinase inhibitor 2A) locus; PAI-1, plasminogen activator inhibitor 1; PTEN, phosphatase and tensin homologue; pVHL, von Hippel–Lindau tumour suppressor protein; SDH, succinate dehydrogenase; VEGF, vascular endothelial growth factor.

(nuclear) to be highly expressed in many tumour types including pancreatic (Ref. 56), head and neck (Refs 57, 58), oropharyngeal (Ref. 59), breast (Refs 60, 61), renal (Ref. 62), ovarian (Ref. 63), urothelial (Ref. 64), bladder, brain, colorectal and prostate (Ref. 65). Several independent studies have revealed a strong correlation between HIF-1α overexpression and patient mortality. High HIF-1α expression has also been associated with low survival rates in pancreatic carcinoma (Ref. 56), head and neck squamous cell carcinoma (Ref. 58), clear-cell
renal cell carcinoma (Ref. 62) and breast carcinoma (Refs 60, 61). This might be because the overexpression of HIF-1α, which often indicates significant levels of tumour hypoxia, is involved in mediating cellular adaptive responses that enable tumour cells to survive. Tumour hypoxia and HIF-1α overexpression is reported to correlate with an increased aggressiveness of tumour cell behaviour, angiogenesis (Ref. 57) and metastasis (Ref. 61) and can be used as a marker to predict outcome in patients with metastatic disease. Interestingly, a study in clear-cell renal cell carcinoma has shown that HIF-1α expression directly correlates with markers of apoptosis (p53) and growth inhibition (p21), the mTOR pathway (AKT, p27), the chemokine receptors CXCR3 and CXCR4, and proteins of the VEGF family (Ref. 62). Therefore, induction of HIF-1α in many cancer types results in several consequences that could enable tumour cells to survive and continue to proliferate.

Surprisingly, not all tumours that exhibit HIF-1α overexpression have been found to be associated with decreased patient survival rates (Ref. 66). For example, in early-stage squamous cell carcinoma of the oral floor HIF-1α overexpression is associated with improved survival rates (Ref. 66). This difference may arise from the fact that HIF-1α could function by having a dual role in early carcinogenesis. On the one hand, HIF-1α promotes tumour angiogenesis and cell survival when mediating an adaptive response, while on the other hand, in response to cellular stress HIF-1α cooperates with the apoptotic machinery (via induction of apoptotic genes or crosstalk to p53) to mediate tumour cell death (Ref. 67). Indeed, the function of the HIFs in tumour progression might depend on the cell type and cellular context as well as the stage of carcinogenesis, and further work is needed to clarify this in order to establish when best to target the HIF pathway in cancer and whether certain cancer types would prove more or less sensitive to a HIF inhibitor.

**Strategies to target the HIF pathway in cancer**

In recent years, several strategies have been developed to identify direct and indirect inhibitors of HIF-α that function by blocking HIF-α (HIF-1α or HIF-2α) expression levels and/or HIF (HIF-1 or HIF-2) activity. These include cell-based reporter screens, antisense approaches, targeting key protein–protein interactions, increasing HIF-1α protein turnover or utilising a HIF oligonucleotide decoy (Table 1). In addition, therapeutic exploitation of other key pathways and mechanisms that are known to regulate HIF-1α protein availability (stability and synthesis) and HIF-1 activity could also potentially be utilised to target the HIF pathway in cancer (Fig. 4). While the HIF transcriptional complex itself is a challenging therapeutic target, blockade of the HIF pathway and inhibition of HIF-α expression is therapeutically attractive because of its pivotal role in driving angiogenesis and tumour progression. Overexpression of HIF-1α in many cancers and deregulation of HIF activity offers a degree of selectivity for tumour cells over normal tissue (Ref. 68), and blocking HIF-1α especially when in combination with conventional therapies has a significant impact on tumour growth (Ref. 69).

**Targeting HIF-1α directly**

As it functions as part of a transcriptional complex, targeting HIF-1α directly is challenging. Specific antisense approaches have been used to reduce HIF-1α expression and transcriptional activity (Ref. 70), and a dominant negative form of HIF-1α has also been used (Ref. 71). Another approach is to inhibit HIF-1 transcriptional activity by blocking HIF-1α protein–protein interactions (Ref. 72). For example, the binding between HIF-1α and the coactivator p300/CBP, and hence hypoxia-inducible transcription, has been attenuated by retroviral expression of a polypeptide (Ref. 73), by the small-molecule chetomin (Refs 13, 74) or by the use of the indazole compound YC-1 (Refs 75, 76). In addition, small molecules such as rolitetracycline (a semisynthetic pyrrolidnomethyltetracycline) that block HIF-1α–HIF-1β dimerisation by targeting the PAS domain offer a strategy to block HIF-1-mediated activity in tumour cells by inhibiting the formation of the HIF-1 complex (Ref. 77).

**Targeting HIF-1α expression and/or HIF-1 activity indirectly**

**Prolyl hydroxylases**

Mechanisms that regulate HIF-1α protein stability provide indirect means to target HIF-1α protein...
levels in tumour cells (Fig. 4). For example, overexpression of PHDs (Ref. 78) enhances HIF-1α protein turnover and results in reduced HIF-1α protein availability in tumour cells. Thus, small-molecule activators of the PHDs – such as KRH1020053 – have been developed to reduce HIF-1α protein levels in tumour cells (Ref. 79). Alternatively, genetic blockade of SIAH2, which encodes an E3 ubiquitin ligase that ubiquinates PHD2 in hypoxia, leads to reduced HIF-1α availability (Ref. 80) and provides another potential mechanism for targeting HIF-1α protein stability (Ref. 81).

**The p53 tumour suppressor protein**

Understanding the relationship between HIF and other key signalling pathways can provide valuable therapeutic insight for developing strategies to target HIF indirectly (Fig. 4). For example, considerable progress has been made to our understanding of the molecular mechanisms by which HIF is regulated by the tumour suppressor protein p53, a transcription factor that plays a crucial role in monitoring cellular integrity. When the cell is stressed, p53 protein rapidly accumulates leading to either cell cycle arrest or apoptosis. However, p53 is mutated in over 50% of human cancers (Ref. 82). Mutated p53 is unable to transactivate downstream targets and is associated with malignant progression and metastasis (Refs 83, 84). While hypoxia induces cells to undergo p53-dependent apoptosis under some circumstances, cancer cells with dysregulated p53 function are able to survive (Refs 85, 86, 87). p53 is involved in negatively regulating HIF-1α expression and HIF-1 activity (Refs 88, 89, 90, 91). While the molecular crosstalk between HIF-1 and p53 is complex, HIF-1α has been observed to bind to p53 in some cellular settings (Ref. 88). An in vitro study has provided biophysical evidence supporting the direct binding of p53 with

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Abbreviations: HIF, hypoxia-inducible factor; HRE, hypoxia-response element; PHD, prolyl hydroxylase.
Strategies to target the HIF-1 pathway in cancer

Figure 4. Strategies to target the HIF-1 pathway in cancer. (a–c) Several strategies to specifically target hypoxia-inducible factor 1α (HIF-1α) protein levels and HIF-1 activity in cancer cells have been developed (shown in red), including: (a) inhibition of protein–protein interactions (e.g. HIF-1 dimerisation or coactivator recruitment); (b) inhibition of HRE transcriptional activity (e.g. using small-molecules identified in HRE cell-based reporter screens); and (c) activation of HIF-1α protein degradation (e.g. using PHD activators). (d–f) In addition, therapeutic targets currently in development that are known regulators of the HIF-1 pathway provide an alternative means for blocking HIF-1α protein availability (stability and synthesis) and HIF-1 activity in tumour cells (shown in green), including: (d) inhibition of signalling pathways upstream of HIF (PI3K–AKT–mTOR/HDM2 and Ras–MAPK); (e) inhibition of chaperone proteins (e.g. HSP90); and (f) activation of the tumour suppressor p53. Direct downstream targets are indicated by solid lines, and downstream effectors are indicated by dashed lines. Abbreviations: AKT, AKT/protein kinase B; HDM2, human homologue of MDM2 (E3 ubiquitin ligase; p53-binding protein); HRE, hypoxia-response element; HSP90, heat shock protein 90; MAPK, mitogen-activated protein kinase (also known as extracellular-signal-regulated kinase, ERK); MEK, MAPK kinase; mTOR, mammalian target of rapamycin; p300/CBP, p300/CREB-binding protein; PHD, prolyl hydroxylation domain protein; PI3K, phosphoinositide 3-kinase; pVHL, von Hippel–Lindau tumour suppressor protein; RTK, receptor tyrosine kinase; SIAH1a/2, seven in absentia homologue 1a/2; Ub, ubiquitination.
HIF-1α via the ODD domain within HIF-1α (Ref. 92). This interaction was originally proposed to result in p53 stabilisation (Ref. 88) as well as inhibition of HIF-1 activity (Refs 88, 89, 92, 93). However, further work has shown that HIF-1α induced in hypoxia does not affect p53 stabilisation (Ref. 90), and it has been proposed that the direct interaction of p53 with HIF-1α leads to HDM2-mediated degradation of HIF-1α (Ref. 90), resulting in hypoxia-induced p53-dependent apoptosis (Refs 88, 89, 94). Indeed, recent work has shown that the apoptotic function of p53 can be regulated by the status of HIF-1α in cells and that blocking HIF-1α expression can drive p53-mediated tumour cell death in hypoxia (Ref. 91).

It has been suggested that p53 can block HIF-1α transcriptional activity by competing with HIF-1α for p300 (Ref. 93). p53, which itself is a transcription factor, requires the recruitment of p300 for its activity. Using an HRE–luciferase reporter assay to measure the transactivation of HIF-1, it has been demonstrated that low levels of exogenous p53 can block HIF-1α transcriptional activity and this effect can be relieved by overexpression of p300 in the cell (Ref. 93). This competitive binding of p53 for p300 was also confirmed in a separate in vitro transcription assay (Ref. 93). While low levels of p53 can affect HIF-1α activity, high levels of p53 have been reported by several independent studies to block HIF-1α protein accumulation (Refs 54, 90, 93, 94). Furthermore, loss of p53 has been observed to correlate with increased HIF-1α protein level and increased HIF-1α activity (Ref. 90). Forced expression of HIF-1α in p53-expressing tumour cells upregulates VEGF expression (Refs 90, 95), neovascularisation and the growth of tumour xenografts (Ref. 90). Taken together, these studies clearly demonstrate that p53 negatively regulates HIF-1α transcriptional activity and HIF-1α protein levels and highlights the possibility that reactivating p53 may provide a means to target the HIF pathway in cancer (Fig. 4).

Efforts have been made to identify agents that reactivate mutant p53 (Refs 96, 97) or activate wild-type p53 (Refs 98, 99) in cancer cells (Table 2). Recently, a small-molecule activator of p53, RITA (reactivation of p53 and induction of tumour cell apoptosis), was demonstrated to induce and activate p53, resulting in tumour cell apoptosis (Refs 91, 98). Interestingly, RITA was also observed to block HIF-1α expression, resulting in downregulation of VEGF expression, and antiangiogenic effects in vivo (Ref. 91). The ability of a single agent to activate p53-dependent apoptosis and simultaneously suppress tumour angiogenesis represents a novel and promising therapeutic strategy for targeting the HIF pathway in solid tumours (Ref. 91).

Other mechanisms for targeting HIF
Several high-throughput cell-based screening approaches using HRE–luciferase reporter systems have identified numerous HIF-1α small-molecule inhibitors that block tumour cell growth by blocking HIF-1α protein accumulation and HIF activity (Refs 100, 101), although their mechanism of actions remain to be understood. For example, topotecan – a topoiso- merase inhibitor and known cytotoxic agent (Table 2) – was found to block HRE activity in a cell-based screen and was subsequently shown to suppress HIF-1α protein translation (Refs 102, 103). This inhibitor is currently being launched in the clinic to target ovarian and small-cell lung cancer cells. Interestingly, PX-478, another inhibitor of HIF-1α protein translation (although not identified through a cell-based HRE–luciferase reporter screen) is in Phase I clinical trials (Refs 104, 105, 106); however, the precise cellular target of PX-478 that is responsible for HIF-1α inhibition is not certain, and thus the development of clear clinical endpoints may prove challenging.

In addition to strategic efforts being developed to target the HIF pathway, many recognised anticancer drugs that target known regulators of HIF function have also been shown to block HIF-1α protein levels and/or HIF-1 activity (Table 2). For example, the histone deacetylase (HDAC) inhibitors trichostatin A and FK228 inhibit HIF-1α induction and HIF-1 activity (Refs 107, 108, 109, 110, 111). Geldanamycin and 17-AAG, which are HSP90 antagonists, are also effective at inhibiting HIF-1α expression levels (Refs 112, 113, 114, 115, 116), and recent work has identified digoxin as a potent inhibitor of HIF-1α synthesis and tumour growth (Ref. 117). Other tractable HIF regulators that have emerged recently include c-Myc (Refs 118, 119), c-Met (Refs 120, 121, 122) and components of the Ras (Ref. 123) and Wnt (Refs 124, 125, 126)
Table 2. Anticancer agents that decrease HIF-1α and target the HIF-1 pathway

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(continued on next page)
Which HIF-α subunit to inhibit?
When developing new agents to target HIF-α in cancer, the specific effects mediated by a particular HIF-α (HIF-1α or HIF-2α) isofrom in different cell types should be taken into consideration (Ref. 39). Targeting a single HIF-α subunit may not necessarily give the desired effects because it has been demonstrated that different subunits may play distinct roles in different cellular contexts (Refs 39, 41, 127, 128). For example, using siRNA techniques, HIF-1α was reported to be the primary hypoxia-induced transcription factor in breast carcinoma and endothelial cells (Ref. 129), whereas in renal carcinoma cells HIF-2α was shown to be primarily responsible for the induction of hypoxic genes (Ref. 39). These findings were supported by two separate studies that confirmed HIF-1α primarily regulates the transcription of hypoxia-regulated target genes in MCF-7 breast carcinoma cells, while HIF-2α controls the transcription of target genes such as glucose transporter 1 (GLUT-1) as well as tumour progression in renal carcinoma cells that have lost pVHL function (Refs 39, 43, 130). Interestingly, in pVHL-defective renal carcinoma cells, HIF-1α was found to play a tumour suppressor role. Because the tumour-promoting HIF-α subunit in these cell lines is HIF-2α (Refs 43, 118, 130), treatment targeting this subunit may be more beneficial (Ref. 39). It is important to assess whether targeting both HIF-1α and HIF-2α or either subunit selectively will provide better therapeutic effects in vivo.

Recent work has revealed that pVHL-defective renal carcinoma cells can be further subdivided into tumours with detectable HIF-1α and HIF-2α, or just HIF-2α exclusively (Ref. 119). Accordingly, tumours with detectable HIF-1α and HIF-2α exhibit enhanced activation of AKT–mTOR and MAPK pathways and γH2AX.

<table>
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<tr>
<th>Target pathways/mechanisms</th>
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<tbody>
<tr>
<td>Translation</td>
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<td></td>
<td>Tunicamycin</td>
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<td>Topoisomerase I/II</td>
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<tr>
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<td>Mitochondria</td>
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<td>Others</td>
<td>Pleurotin</td>
<td>218</td>
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<tr>
<td>RNA polymerase</td>
<td>TAS106 (ECyd)</td>
<td>220</td>
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<td>DNA synthesis/repair</td>
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<td>Multiple signalling pathways (Ras–MAPK, P13K–AKT)</td>
<td>Resveratrol</td>
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<td>CDK</td>
<td>Flavopiridol</td>
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Abbreviations: CDK, cyclin-dependent kinase; COX-2, cyclooxygenase 2; EGFR, epidermal growth factor receptor; HIF, hypoxia-inducible factor; HSP90, heat shock protein 90; MAPK, mitogen-activated protein kinase; 2-ME2, 2-methoxy estradiol; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; RITA, reactivation of p53 and induction of tumour cell apoptosis; VEGFR, vascular endothelial growth factor receptor.
Translation of HIF inhibitors into the clinic

Hypoxic tumours are usually resistant to radiotherapy, as a result of the low level of oxygen molecules available to generate DNA strand breaks, and to chemotherapy, because of their slow divisional rate, abnormal vasculature and the upregulation of many genes that contribute to their aggressive phenotype. However, blocking HIF-1α renders tumour cells more susceptible to radiotherapy and conventional chemotherapeutic agents (Refs 13, 14). The recent advances to our understanding of the HIF pathway have helped us to clarify its role in cancer and consequently enable the identification and design of novel therapies. Inhibitors that target the HIF pathway directly or indirectly are attractive and should prove useful in the treatment of most solid tumours including breast, prostate and renal carcinomas. Understanding the mechanism of action of different HIF pathway inhibitors is of particular importance when deciding when and how they would best be used in combination.

Which cancer types to target?

HIF-1α protein levels can be used as a prognosis marker in various cancers (Refs 131, 132, 133, 134), as well as a predictive biomarker when designing new treatment regimes. Patients with VHL-mutated sporadic clear-cell renal carcinomas exhibit high basal HIF-α expression and subsequently increased expression of VEGF and platelet-derived growth factor (PDGF). To date, the small-molecule tyrosine kinase inhibitors sunitinib and sorafenib that block VEGF and PDGF signalling have been the only agents shown to stabilise the disease, although increases in overall survival are disappointingly low (Refs 69, 135, 136, 137). Both sunitinib and sorafenib have been approved for the treatment of renal cell carcinoma, where sunitinib is currently recommended as a first-line treatment and sorafenib as a second-line treatment option for people with advanced metastatic renal cell carcinoma (Refs 69, 138). Clinical trials are under way to investigate the efficacy of these drugs in combination with conventional treatments or other small-molecule inhibitors and antibody therapies. Interestingly, a recent cell-based assay approach using renal carcinoma cells has identified STF-62247, an agent that functions to selectively induce cytotoxic and antitumour effects in pVHL-deficient renal carcinoma cells by inducing autophagy (Ref. 139). The identification of STF-62247 illustrates the possibility of developing therapeutic agents that specifically target pVHL-deficient renal cancers (with high basal HIF-α expression), which account for around 75% of renal cell carcinomas (Ref. 139). Moreover, a pilot synthetic lethal screen has identified that inhibition of kinases such as CDK4/6 enhanced growth inhibition of pVHL-deficient renal cell carcinomas compared with matched pVHL-proficient renal cell carcinomas (Ref. 140), further highlighting those tractable targets for therapeutic intervention in renal cell carcinoma. It will be of particular interest to establish whether HIF inhibitors when used either alone or in combination with these other agents can provide a better therapeutic outcome for patients with advanced metastatic renal cell carcinoma.

Imaging hypoxia as a clinical tool

Effective imaging of hypoxia is important for cancer detection and diagnosis, assessment of therapy, as well as drug development. Imaging also provides a prognostic basis by which the effects of tumour hypoxia could be evaluated in personalised cancer treatment. For example, patients could be selected for hypoxia and high HIF-1α protein levels in their tumours, in order to enter them into clinical trials involving HIF inhibitors.

Several direct and indirect methods for measuring hypoxia have been developed. Direct pO2 measurement can be performed by an Eppendorf probe (a polarographic needle microelectrode) by direct insertion into tissues (Refs 141, 142). Exogenous markers of hypoxia (such as pimonidazole and EF5) provide a more reliable measurement than using Eppendorf probes and can be used to detect the distribution of hypoxia in a small fraction of tumour (Refs 143, 144, 145). Positron emission tomography (PET) tracers such as [18F]fluoromisonidazole have also been used...
to measure hypoxia throughout the body (Refs 146, 147, 148). PET imaging is sensitive and has a spatial and temporal resolution suitable for accessing the heterogeneity of local pO₂. This technique was shown to have the ability to predict outcomes from radiation therapy by imaging hypoxic tissues (Refs 149, 150, 151, 152). Several magnetic resonance (MR)-based imaging (MRI) and spectroscopy strategies have been developed to assess tumour hypoxia directly or indirectly – namely ¹⁹F-MRI and spectroscopy, high molecular weight dynamic contrast-enhanced MRI, electron paramagnetic resonance imaging and electron paramagnetic resonance oximetry. These methods can reliably and precisely reveal heterogeneity of oxygen distribution within tissues. As well as locating tumour and assessing treatment, MRI-based techniques can detect detailed metabolic and physiological information and PET imaging can reveal biochemical characteristics of the tumour, including metastases. With the preclinical development of many new HIF inhibitors being pursued currently, one challenge will be to define robust preclinical noninvasive imaging endpoints that are not only consistent with measurable effects on the HIF pathway in vivo, but that also directly relate to the mechanism of action and provide clear therapeutic insight with respect to the hypoxic tumour compartment.

Conclusions
Hypoxic tumours are usually resistant to killing by radiotherapy and conventional chemotherapies, rendering them highly aggressive and metastatic. Oxygen homeostasis in cells and the response to hypoxic stress is largely mediated by the HIF pathway. However, dysregulation of the HIF pathway occurs in many human cancers and usually correlates with a poor prognostic outcome using conventional treatments. Therefore, targeting the HIF pathway provides an attractive strategy to treat hypoxic and highly angiogenic tumours. The combination of HIF inhibitors with existing treatments or new targeted therapies may prove to be useful clinically.

However, the development of appropriate imaging strategies to accurately measure hypoxia in tumours along with the identification of suitable biomarker endpoints will accelerate the translation of new therapeutic combinations into the clinic as well as provide prognostic information that helps tailor specific treatments to different cancer types. HIF inhibitors are in preclinical and clinical development and these offer a novel and attractive approach for the treatment of solid tumours.

Acknowledgements and funding
We thank Dr Jan Bussink (Department of Radiation Oncology 874, Radboud University Nijmegen, The Netherlands) for kindly providing the data presented in Figure 1. We also thank Dr Yann Jamin (Institute of Cancer Research, Sutton, Surrey, UK) for his advice on imaging, and Prof. Patrick Maxwell (University College London, UK) for critical review of our manuscript. E.P. is funded by Cancer Research UK project grant C7358/A9958. We also thank the reviewers for their insightful comments and helpful suggestions.

References

Accession information: doi:10.1017/S1462399409001173; Vol. 11; e26; August 2009 © Cambridge University Press 2009
10 Bos, R. et al. (2001) Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis. Journal of the National Cancer Institute 93, 309-314


13 Staab, A. et al. (2007) Effects of HIF-1 inhibition by chetomin on hypoxia-related transcription and radiosensitivity in HT 1080 human fibrosarcoma cells. BMC Cancer 7, 213

14 Williams, K.J. et al. (2005) Enhanced response to radiotherapy in tumours deficient in the function of hypoxia-inducible factor-1. Radiotherapy and Oncology 75, 89-98


19 Fukuda, R. et al. (2002) 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. Journal of Biological Chemistry 277, 38205-38211

20 Laughner, E. et al. (2001) 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. Molecular and Cellular Biology 21, 3995-4004

21 Huang, L.E. et al. (1998) Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proceedings of the National Academy of Sciences of the United States of America 95, 7987-7992


27 Epstein, A.C. et al. (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107, 43-54

28 Berra, E. et al. (2003) HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO Journal 22, 4082-4090

29 Kallio, P.J. et al. (1998) Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha EMBO Journal 17, 6573-6586


31 Hewitson, K.S. et al. (2002) Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. Journal of Biological Chemistry 277, 26351-26355

32 Lando, D. et al. (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes and Development 16, 1466-1471


Accession information: doi:10.1017/S1462399409001173; Vol. 11; e26; August 2009 © Cambridge University Press 2009
<table>
<thead>
<tr>
<th>Page</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>Hu, C.J. et al. (2003) Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Molecular and Cellular Biology 23, 9361-9374</td>
</tr>
<tr>
<td>43</td>
<td>Raval, R.R. et al. (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Molecular and Cellular Biology 25, 5675-5686</td>
</tr>
<tr>
<td>46</td>
<td>Makino, Y. et al. (2001) Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 414, 550-554</td>
</tr>
<tr>
<td>47</td>
<td>Makino, Y. et al. (2007) Transcriptional up-regulation of inhibitory PAS domain protein gene expression by hypoxia-inducible factor 1 (HIF-1): a negative feedback regulatory circuit in HIF-1-mediated signaling in hypoxic cells. Journal of Biological Chemistry 282, 14073-14082</td>
</tr>
<tr>
<td>49</td>
<td>Pollard, P.J. et al. (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Human Molecular Genetics 14, 2231-2239</td>
</tr>
<tr>
<td>51</td>
<td>Karni, R. et al. (2002) Activated pp60c-Src leads to elevated hypoxia-inducible factor (HIF)-1alpha expression under normoxia. Journal of Biological Chemistry 277, 42919-42925</td>
</tr>
<tr>
<td>52</td>
<td>Lee, B.L. et al. (2008) A hypoxia-independent up-regulation of hypoxia-inducible factor-1 by AKT contributes to angiogenesis in human gastric cancer. Carcinogenesis 29, 44-51</td>
</tr>
</tbody>
</table>
60 Bos, R. et al. (2003) Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 97, 1573-1581
63 Osada, R. et al. (2007) Expression of hypoxia-inducible factor 1alpha, hypoxia-inducible factor 2alpha, and von Hippel-Lindau protein in epithelial ovarian neoplasms and allelic loss of von Hippel-Lindau gene: nuclear expression of hypoxia-inducible factor 1alpha is an independent prognostic factor in ovarian carcinoma. Human Pathology 38, 1310-1320
66 Fillies, T. et al. (2005) HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. BMC Cancer 5, 84
67 Sumiyoshi, Y. et al. (2006) Overexpression of hypoxia-inducible factor 1alpha and p53 is a marker for an unfavorable prognosis in gastric cancer. Clinical Cancer Research 12, 5112-5117
76 Yeo, E.J. et al. (2003) YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. Journal of the National Cancer Institute 95, 516-525
77 Park, E.J. et al. (2006) Targeting the PAS-A domain of HIF-1alpha for development of small molecule inhibitors of HIF-1. Cell Cycle 5, 1847-1853
Targeting the hypoxia-inducible factor (HIF) pathway in cancer

85 Denko, N.C. et al. (2000) p53 checkpoint-defective cells are sensitive to X rays, but not hypoxia. Experimental Cell Research 258, 82-91
90 Ravi, R. et al. (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes and Development 14, 34-44
91 Yang, J. et al. (2009) Small molecule activation of p53 blocks HIF-1alpha and VEGF expression in vivo and leads to tumor cell apoptosis in normoxia and hypoxia. Molecular and Cellular Biology 29, 2243-2253
100 Chau, N.M. et al. (2005) Identification of novel small molecule inhibitors of hypoxia-inducible factor-1 that differentially block hypoxia-inducible factor-1 activity and hypoxia-inducible factor-1alpha induction in response to hypoxic stress and growth factors. Cancer Research 65, 4918-4928
103 Koh, M.Y. et al. (2008) Molecular mechanisms for the activity of PX-478, an antitumor inhibitor of the hypoxia-inducible factor-1alpha. Molecular Cancer Therapeutics 7, 90-100
107 Manabe, H. et al. (2008) Inhibition of histone deacetylase down-regulates the expression of hypoxia-induced vascular endothelial growth factor by rheumatoid synovial fibroblasts. Inflammation Research 57, 4-10
108 Mie Lee, Y. et al. (2003) Inhibition of hypoxia-induced angiogenesis by FK228, a specific histone deacetylase inhibitor, via suppression of HIF-1alpha activity. Biochemical and Biophysical Research Communications 300, 241-246
110 Sasakawa, Y. et al. (2003) Antitumor efficacy of FK228, a novel histone deacetylase inhibitor, depends on the effect on expression of angiogenesis factors. Biochemical Pharmacology 66, 897-906

Accession information: doi:10.1017/S1462399409001173; Vol. 11; e26; August 2009 © Cambridge University Press 2009


116 Liu, Y.V. et al. (2007) RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O2-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. Molecular Cell 25, 207-217


118 Gordan, J.D. et al. (2007) HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. Cancer Cell 11, 335-347


122 Hayashi, M. et al. (2005) Up-regulation of c-met protooncogene product expression through hypoxia-inducible factor-1alpha is involved in trophoblast invasion under low-oxygen tension. Endocrinology 146, 4682-4689


124 Giles, R.H. et al. (2006) Interplay between VHL/HIF1alpha and Wnt/beta-catenin pathways during colorectal tumorigenesis. Oncogene 25, 3065-3070

125 Jiang, Y. et al. (2007) Hypoxia induces transforming growth factor-beta1 gene expression in the pulmonary artery of rats via hypoxia-inducible factor-1alpha. Acta Biochimica et Biophysica Sinica (Shanghai) 39, 73-80


128 Hu, C.J. et al. (2006) Differential regulation of the transcriptional activities of hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha in stem cells. Molecular and Cellular Biology 26, 3514-3526


130 Rankin, E.B. et al. (2008) Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. Oncogene 27, 5354-5358


134 Trastour, C. et al. (2007) HIF-1alpha and CA IX staining in invasive breast carcinomas: prognosis
and treatment outcome. International Journal of Cancer 120, 1451-1458
158 Greenberger, L.M. et al. (2008) A RNA antagonist of hypoxia-inducible factor-1alpha, EZN-2968,
inhibits tumor cell growth. Molecular Cancer Therapeutics 7, 3598-3608
163 Sandau, K.B. et al. (2001) Regulation of the hypoxia-inducible factor 1alpha by the inflammatory mediators nitric oxide and tumor necrosis factor-alpha in contrast to desferoxamine and phenylarsine oxide. Journal of Biological Chemistry 276, 39805-39819
164 Wang, B. et al. (2005) Genistein inhibited hypoxia-inducible factor-1alpha expression induced by hypoxia and cobalt chloride in human retinal pigment epithelium cells. Methods and Findings in Experimental and Clinical Pharmacology 27, 179-184
166 Lu, Y. et al. (2007) Responses of cancer cells with wild-type or tyrosine kinase domain-mutated epidermal growth factor receptor (EGFR) to EGFR-targeted therapy are linked to downregulation of hypoxia-inducible factor-1alpha. Molecular Cancer 6, 63
167 Luwor, R.B. et al. (2005) The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. Oncogene 24, 4433-4441
168 Pore, N. et al. (2006) EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. Cancer Research 66, 3197-3204
169 Alam, H. et al. (2009) Role of the phosphatidylinositol-3-kinase and extracellular regulated kinase pathways in the induction of hypoxia-inducible factor (HIF)-1 activity and the HIF-1 target vascular endothelial growth factor in ovarian granulosa cells in response to follicle-stimulating hormone. Endocrinology 150, 915-928
178 Wan, X. et al. (2006) CCI-779 inhibits rhodomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/HIF-1alpha/VEGF signaling. Neoplasia 8, 394-401
179 Cezka, D. et al. (2008) Everolimus (RAD001) and anti-angiogenic cyclophosphamide show long-


183 Mirzoeva, S. et al. (2008) Inhibition of HIF-1 alpha and VEGF expression by the chemopreventive flavonoid apigenin is accompanied by Akt inhibition in human prostate carcinoma PC3-M cells. Molecular Carcinogenesis 47, 686-700


186 Lau, C.K. et al. (2006) Suppression of hypoxia-inducible factor-1alpha (HIF-1alpha) by YC-1 is dependent on murine double minute 2 (Mdm2). Biochemical and Biophysical Research Communications 348, 1443-1448

187 Sun, H.L. et al. (2007) YC-1 inhibits HIF-1 expression in prostate cancer cells: contribution of Akt/NF-kappaB signaling to HIF-1alpha accumulation during hypoxia. Oncogene 26, 3941-3951

188 Zhao, Q. et al. (2007) Effects of YC-1 on hypoxia-inducible factor 1-driven transcription activity, cell proliferative vitality, and apoptosis in hypoxic human pancreatic cancer cells. Pancreas 34, 242-247

189 Huang, S.P. et al. (2005) Cyclooxygenase-2 increases hypoxia-inducible factor-1 and vascular endothelial growth factor to promote angiogenesis in gastric carcinoma. Journal of Biomedical Science 12, 229-241


194 Qian, D.Z. et al. (2004) The histone deacetylase inhibitor NVP-LAQ824 inhibits angiogenesis and has a greater antitumor effect in combination with the vascular endothelial growth factor receptor tyrosine kinase inhibitor PTK787/ZK222584. Cancer Research 64, 6626-6634

195 Thomas, S.L. et al. (2008) EF24, a novel curcumin analog, disrupts the microtubule cytoskeleton and inhibits HIF-1. Cell Cycle 7, 2409-2417


202 Vlaminck, B. et al. (2007) Dual effect of echinomycin on hypoxia-inducible factor-1
activity under normoxic and hypoxic conditions.


206 Jordan, B.F. et al. (2005) Metabolite changes in HT-29 xenograft tumors following HIF-1alpha inhibition with PX-478 as studied by MR spectroscopy in vivo and ex vivo. NMR in Biomedicine 18, 430-439


214 Kamiyama, H. et al. (2005) 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. Journal of Cancer Research and Clinical Oncology 131, 205-213


221 Zeng, L. et al. (2008) TS-1 enhances the effect of radiotherapy by suppressing radiation-induced hypoxia-inducible factor-1 activation and inducing endothelial cell apoptosis. Cancer Science 99, 2327-2335


One of the most widely pursued therapeutic strategies in cancer therapy has been to target the angiogenic process, which is regulated by the hypoxia/HIF pathway in tumour progression. This book comprehensively reviews the progress made in antiangiogenic cancer therapies in recent years.

Online sources of general information on cancer research:

http://www.cancer.gov (US National Cancer Institute)
http://www.cancer.org.uk (Cancer Research UK)
http://www.oncolink.org (web-based cancer resource, University of Pennsylvania, USA)
http://www.icr.ac.uk/ (The Institute of Cancer Research, UK)
http://www.ucl.ac.uk/cancer/ (UCL Cancer Institute, UK)

Features associated with this article

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Figure 4. Strategies to target the HIF-1 pathway in cancer.

Tables
Table 1. Strategies to identify inhibitors of HIF-1α and the HIF pathway.
Table 2. Anticancer agents that decrease HIF-1α and target the HIF-1 pathway.

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