Hypoxia-inducible factor-1 in human breast and prostate cancer

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Abstract

The tumor microenvironment is best characterized as a fluctuation of hypoxia and nutrient deprivation, which leads to epigenetic and genetic adaptation of clones and increased invasiveness and metastasis. In turn, these hypoxic adaptations make the tumors more difficult to treat and confer increased resistance to current therapies. Part of this adaptation is the regulation of gene products in response to hypoxia. Many of these hypoxia-regulated genes are mediated by the hypoxia-inducible factor 1 (HIF-1) complex, which is composed of a heterodimer pair of HIF-1 α and HIF-1 β . This heterodimer binds to the promoter of hypoxia-responsive genes, while interacting with other transcription factors, such as p300, signal and transducer of transcription 3, and Redox effector factor 1/apurinic/aprimidinic endonuclease. HIF-1 α levels itself can be regulated by hypoxia transcriptionally and post-translationally through ubiquitination; but the magnitude of the response is modulated by several other pathways, including free radicals that affect crosstalk with HIF-1 α /HIF-1 β transcriptional activities. HIF-1 α has emerged as an important transcription factor in breast cancer and prostate cancer biology, and is expressed in the early stages of mammary and prostate carcinogenesis. Its expression is correlated with diagnostic and prognostic indicators for early relapse and metastatic disease, thus making HIF-1 α a potential prognostic biomarker in proteomic assessments of breast and prostate cancers. The importance of HIF-1 α in tumor progression makes it a logical target for chemoprevention strategies in patients at higher genetic risk of breast and prostate cancer with Cox 2 inhibitors or 2-methoxyestradiol, as well as a target for new approaches to inhibiting angiogenesis. The crosstalk between estrogen signaling pathways and HIF-1 α is still not fully defined in breast cancer, but downstream estrogen receptor signaling may be a candidate for estrogen modulation of HIF-1 α levels. In prostate cancer, androgens upregulate HIF-1 α through androgen-regulated autocrine receptor tyrosine kinase receptor signaling. This review will put into perspective the role of HIF-1 α in endocrine oncology and present new data on HIF-1 α signaling and the potential for targeted therapies, including combinatory hormonal therapies.

Endocrine-Related Cancer (2006) 13 739–749

Introduction

Lethal clones of human cancer have the ability to adapt to hypoxic environments in primary or metastatic sites. Epigenetic and genetic mechanisms of adaptation to hypoxia, such as genetic instability, aerobic glycolysis, loss of cell cycle control, and loss of normal apoptotic signals are hallmarks of human malignancy. Hypoxia is defined as a loss of oxygen in tissues and is widespread in solid tumors (epithelial or mesenchymal stem cell origin) due to the tumors ability to outgrow the existing vasculature. Oxygen tension in normal tissues has a mean of approximately 7% oxygen; in

tumors, the mean oxygen tension is approximately about 1.5% oxygen (Vaupel *et al.* 2001, Vaupel 2004). Tumor cells must survive by adapting to the low pO₂ or by increasing vascularization, or both. Many gene products are involved in tumor neoangiogenesis. One of the most investigated and ‘drugable’ targets is vascular endothelial growth factor (VEGF), which is secreted by hypoxic tumor cells, with the anti-VEGF antibody (Avastin). In addition to increased vascularization, hypoxia, initiates multiple cellular responses, such as activation of proto-oncogenes (c-Src; Gleadale & Ratcliffe 1997), increase in glucose transportation

(GLUT-1; Airley *et al.* 2001), induction of glycolytic enzymes (ENO1; Semenza *et al.* 1996), and induction of various apoptotic-related genes (GADD45; Price & Calderwood 1992). These adaptive changes contribute to alterations that lend towards a surviving phenotype with clinical aggressiveness (Acs *et al.* 2004). Tumor hypoxia tends to result in poorer prognosis at diagnosis (Vleugel *et al.* 2005a) in several types of cancer. In turn, these hypoxic adaptations make the tumors more difficult to treat and confer increased resistance to death from chemotherapy and radiotherapy. We present below a focused review of what has been learned about a pivotal gene in the cancer biology of hypoxic adaptation and angiogenesis: the hypoxia-inducible factor 1 (HIF-1) complex. HIF-1 is involved in the cancer biology of many endocrine tumors. This review will concentrate on endocrine oncology: HIF-1 in breast cancer and prostate cancer, specifically. New data on HIF-1 signaling and the potential for targeted therapies, including combinations of hormonal therapies for cancer and selective investigational HIF-1 α inhibiting small molecules will be discussed.

Basic biology and regulation of HIF-1 α

Many hypoxia-regulated genes are mediated by HIF, which is composed of a heterodimer pair of HIF-1 α and HIF-1 β . It is estimated that perhaps as much as 1% of the genome is hypoxia regulated (Semenza 2003). The HIF transcription factor complex is a member of the basic helix-loop-helix (bHLH)-PER-ARNT-Sim

(PAS) family of transcription factors. They share a conserved bHLH region for DNA binding, and two PAS domains for target gene specificity and dimerization. HIF-1 α is regulated post-transcriptionally in normoxia by ubiquitination and interaction with the von Hippel-Lindau tumor suppressor protein (pVHL) then degraded by the 26S proteasome. After HIF-1 α is hydroxylated at the proline residues 402 and 577, it is recognized by pVHL, a member of the E₃ ubiquitination complex. This process is mediated by prolyl hydroxylase domain-containing (PHD) proteins. The PHDs require O₂ as a co-factor for activity (Fig. 1).

Biochemical detection of intracellular O₂ levels is directly mediated by the PHD enzymes, which regulate HIF-1 α protein levels. When cells are hypoxic, PHD activity is inhibited from low availability of O₂, HIF-1 α hydroxylation and pVHL association is lowered and steady-state HIF-1 α protein levels rise. HIF-1 α protein with its nuclear localization sequence is then translocated to the nucleus; in this process, it heterodimerizes with HIF-1 β , and the HIF-1 α complex binds to hypoxic responsive elements (HREs) upstream of hypoxic-regulated genes where it acts as a transcription factor. HIF-2 α is another structurally related HIF protein, also termed EPAS1/HRF, HLF/MOP2, and has recently been implicated in the hypoxic response of endometrial carcinoma and other cancers. HIF-2 α accumulation is associated with increased expression of the angiogenic factor thymidine phosphorylase (Sivridis *et al.* 2002). Both HIF-1 α and HIF-2 α have

Figure 1 HIF-1 complex structure. HIF-1 α and HIF-1 β both possess a basic helix-loop-helix (bHLH) structure at the N-terminus allowing for heterodimerization and a PAS domain, which allows for DNA binding to the hypoxic responsive element (HRE). HIF-1 α has two TAD domains, which allow for binding to VHL and p300/CBP. Upon dimerization, the HIF-1 complex binds to p300/CBP at a HRE upstream of hypoxia-regulated genes, thus modifying transcriptional activity.

structural homology, and both are regulated by oxygen through the PHDs. HIF-2 α also regulates hypoxia-inducible genes. These gene products are often co-expressed in tumors and the functional differences between the two proteins and their tissue distribution and roles in human tumor biology are still being defined. The HIF-1 heterodimer associates with the transcription co-activators CREB-binding protein (CBP) to a HRE in the promoter and enhances transcription of the downstream target gene (Forsythe *et al.* 1996, Jiang *et al.* 1996).

It is through the ubiquitination and post-translational control that much of the hypoxic response (change in HIF-1 α levels) is mediated; however, the magnitude of the response is modulated by several other pathways that affect crosstalk with HIF-1 α /HIF-1 β transcriptional regulation. In other words, the absolute amount of HIF-1 α protein under hypoxia/cell varies by cell type. Pathways that modulate HIF-1 α protein levels include the Harvey rat sarcoma viral oncogene homology/extracellular signal-regulated kinase (RAS/ERK), Protein kinase B (AKT), signal and transducer of transcription (STAT) pathways (Mukhopadhyay *et al.* 1995, Rak *et al.* 1995, Eliceiri *et al.* 1999, Alvarez-Tejado *et al.* 2001, Chun *et al.* 2003, Zhong *et al.* 2004c).

HIF-1 α and RAS and AKT pathways

One of the most recently identified pathways modulating and influencing HIF-1 α regulation is the RAS-ERK pathway (Lim *et al.* 2004). A structurally altered Ras oncogene has been shown to be active and relevant in about 30% of human cancers (Rak *et al.* 1995). Several investigators have recently shown that RAS effects VEGF expression through HIF-1 α . This interaction is mediated through the tyrosine kinase signaling, Murine leukemia viral oncogene homolog 1/Map/ERK Kinase-1/extracellular signal-regulated kinase (Raf/MEK1/ERK), a pathway shared with AKT (Chun *et al.* 2003) (Fig. 2).

Another oncogene that has been shown to induce VEGF through HIF-1 α signaling via activation of the protein tyrosine kinase, is c-Src, and/or its downstream mediator phosphatidylinositol 3-kinase (PI3K) (Mukhopadhyay *et al.* 1995, Eliceiri *et al.* 1999, Alvarez-Tejado *et al.* 2001, Zhong *et al.* 2004c). A rapid increase in the Src activity has been seen in both tumor and normal cells under hypoxia (Namiki *et al.* 1995, Ellis *et al.* 1998). When v-Src transfected human colon adenocarcinoma cell line HT29 was grown under normoxic conditions, an accumulation of HIF-1 α and VEGF was observed. The levels of HIF-1 α and VEGF were even higher than that under normoxia in the presence of activated Src and

Figure 2 HIF-1 α signaling pathway. Hypoxia is signaled by PHDs which in turn release HIF-1 α and redirect HIF-1 α from the proteasome towards the signaling pathway to the nucleus. This pathway is a complex multi-responsive series of different phosphorylations originating from RAS to either PI3K/AKT or RAF/MEK pathway. In turn, HIF-1 α dimerizes with HIF-1 β /ARNT and translocates to the nucleus and interacts possibly with STAT3 and p300/CBP to initiate an increase in VEGF transcription.

hypoxia (Fleming *et al.* 1997, Jiang *et al.* 1997, Staley *et al.* 1997). Src and PI3K activation appear to increase the expression and the stability of HIF-1 α , therefore, increasing VEGF levels (Forsythe *et al.* 1996, Hartsfield 1999, Karni 2002). More recently, STAT3 activation has also been shown to upregulate VEGF transcription through HIF-1 α (Niu *et al.* 2002, Wei *et al.* 2003). In addition, both HIF-1 α and STAT3 bind the transcriptional co-activator CBP/p300, suggesting that simultaneous occupancy of the VEGF promoter may occur and be part of a single large transcriptional complex (Arany *et al.* 1996, Schuringa *et al.* 2001). HIF-1 α physically associates with STAT3, CBP/p300, and Redox effector factor 1/apurinic/aprimidinic endonuclease (Ref-1/APE) (Gray *et al.* 2005). Several investigators have demonstrated that optimal transcriptional control of the VEGF promoter requires binding of both HIF-1 α and STAT3, and these factors are part of a large transcriptional complex coordinated in part by the co-activators CBP/p300 and Ref-1/APE (Tacchini *et al.* 2002, Gray *et al.* 2005, Jung *et al.* 2005).

Not only is HIF-1 α activated by oncogenes, but also the loss of tumor suppressor gene function can upregulate HIF-1 α , as well. The loss of VHL tumor suppressor gene creates upregulation of HIF-1 α in renal carcinoma (Zagzag *et al.* 2005). Mutations and allelic loss of the Phosphatase and tensin homolog (PTEN) tumor suppressor gene activate HIF-1 α through increased downstream signaling from AKT-1. The loss of PTEN repression of the PI3K pathway has sparked considerable interest in this pathway relative to HIF-1 α expression and activation with regards to tumorigenesis (Zundel *et al.* 2000). Studies have shown that PTEN deficient cells display a higher HIF-1 α activation response to hypoxia, suggesting that this may play a role in the biological aggressiveness of a tumor (Zhong *et al.* 2000, Jiang *et al.* 2001, Gomez-Manzano *et al.* 2003, Majumder *et al.* 2004).

Despite being a transcription factor rather than an enzyme, HIF-1 α is a 'druggable' downstream target based on PTEN studies (Zhong *et al.* 2000, Majumder *et al.* 2004). Inhibitors of mammalian target of rapamycin (mTOR) function like rapamycin, CCI 779, and Rad 001 decrease HIF-1 α protein levels in both normoxic and hypoxic cells by reducing protein synthesis (Hudson *et al.* 2002). These findings suggest that anti-cancer activity of rapamycin and 'rapalogs' in clinical trials may be attributed, in part, to the inhibition of HIF-1 α activated downstream genes in tumors.

Investigators have correlated hypoxia-induced HIF-1 α and the anti-apoptotic pathways (Volm & Koomagi 2000). Under hypoxic conditions, cultured cells have been shown to be resistant to many

apoptotic-inducing agents. The mechanism of this resistance is associated with alterations in the Bcl-2 family of pro-apoptotic and anti-apoptotic proteins (Erler *et al.* 2004).

HIF-1 α and epigenetic factors

Tumor hypoxia itself is a strong epigenetic factor for upregulation of HIF-1 α protein. Several groups have shown that hypoxia, in addition to inhibiting the PHDs and inhibiting HIF-1 α , generates free oxygen-free radicals. *In vitro*, epigenetically oxygen-free radicals can stabilize HIF-1 α protein (Kaelin 2005). Various free radical generating catechol estrogens (CEs) are also capable of inducing HIF-1 α and VEGF (Muzandu *et al.* 2005). One of these CEs, 4-hydroxyestradiol (4-OHE2), goes through redox cycling producing reactive oxygen species, superoxide (O₂⁻), CE semiquinone, and catechol quinone (Muzandu *et al.* 2005). This epigenetic induction is sensitive to PI3K inhibitors, which suggest that 4-OHE2 might regulate HIF-1 α and VEGF through the PI3K/Akt/FRAP pathway, not the MEK pathway (Gao *et al.* 2002).

A major question in the clinic is the actual epigenetic contribution of oxygen-free radicals to HIF-1 α levels in tumor cells (Zhang *et al.* 1999). HIF-prolyl hydroxylases regulate degradation of HIF-1 α ; these hydroxylases require 2-oxoglutarate, Fe²⁺, ascorbate, and molecular oxygen for enzymatic activity. All of these components are important to the intracellular concentration of oxygen-free radicals that effect PHD enzymatic actions on HIF-1 α (Jaakkola *et al.* 2001, Kondo & Kaelin 2001, Semenza 2001, Pugh & Ratcliffe 2003, Wang *et al.* 2005).

HIF in breast cancer

Nuclear HIF-1 α and HIF-2 β proteins are over-expressed in primary breast cancers (Zhong *et al.* 1999). Upregulation of HIF-1 α is observed in both Her2/erb2 overexpressing and Her2/erb2 negative tumors. It is found overexpressed in ER+ and ER- breast cancers (Zhong *et al.* 1999, Bos *et al.* 2004). Gene amplification of the *c-erbB2* gene is associated with poor prognosis and subsequent resistance to chemotherapy, radiotherapy, and anti-estrogen therapy. Her2/c-erb2 overexpression and activation activate increased HIF-1 α expression *in vitro*. Production of VEGF was found to be elevated in HER-2/*neu*-overexpressing cells and Her2-positive breast tumors enhanced tumor vascularization may be due in part to HIF-1 α (Petit *et al.* 1997, Laughner *et al.* 2001, Yen *et al.* 2002). High levels of HIF-1 α

expression in human breast cancer can occur in the pathogenesis of breast cancer and change the molecular pathogenesis of breast cancer. This is observed in high grade ductal carcinoma *in situ* (DCIS), which manifests high HIF-1 α levels (Zhong *et al.* 1999). In the normal breast epithelium, there is an absence of high levels of HIF-1 α protein expression by immunohistochemistry. These findings suggest that HIF-1 α is expressed in the early stages of mammary carcinogenesis and expression correlates with areas of nearby necrosis in the DCISs. HIF-1 α appears also associated with VEGF-C expression in invasive ductal carcinomas. In a large retrospective study of 745 patients, high levels of HIF-1 α at diagnosis predicted for early relapse and metastatic disease (Dales *et al.* 2005). Thus, HIF-1 α may in proteomic assessments of T1/T2 tumors and positive auxiliary lymph nodes correlate to unfavorable outcomes (Gruber *et al.* 2004). Bos *et al.* (2003) suggested that high levels of HIF-1 α have a borderline significance with decreased survival in women with lymph node negative tumors. Taken together, HIF-1 α expression levels could serve as a novel predictor of poor outcome for both node-negative and-positive breast tumors. However, these high levels of gene expression are not necessarily resulting from gene amplification or mutations in the oxygen-dependent degradation domains of HIF-1 α . Such elevation in HIF-1 α is most likely to produce either an increase in transcription of the HIF-1 α gene or some alteration in the degradation pathway (Vleugel *et al.* 2004, 2005a).

Over a treatment period, hormone-sensitive breast tumors frequently become resistant to hormonal therapy, and it is hypothesized that hypoxia may promote estrogen-independent growth. Studies *in vitro* and *in vivo* suggest that hypoxia downregulates ER α expression function in breast cancer cells and tumors (Kurebayashi *et al.* 2001). Estrogen induces recruitment of both ER α and HIF-1 α to the VEGF gene promoter in chromatin immunoprecipitation assays in the rat uterus, but the role of estrogens and tamoxifen and aromatase inhibitors in the clinic and HIF-1 α modulation in breast cancer is unclear (Kazi *et al.* 2005). HIF-1 α upregulated genes may be associated in the emergence of anti-estrogen refractory breast cancer, but may not be directly causative. ER β with estrogen may act as a negative modulator of VEGF synthesis under hypoxia. In addition, ER+ breast cancers were correlated to strong HIF-1 α levels and p21 levels. High levels of HIF-1 α levels were also associated with proliferation markers ki67 and cyclin A levels (Bos *et al.* 2004). Such an association would support the role of HIF-1 α in estrogen responsiveness or supports the occurrence of

a secondary event that leads to the increase of HIF-1 α as a result of estrogen exposure.

HIF-1 α has been associated with the expression of growth factors basic fibroblast growth factor (bFGF), platelet derived growth factor, B chain (PDGF-BB), and epidermal growth factor receptor (EGFR) in invasive breast cancers. When taken together, the associations of HIF-1 α and proliferation markers, enhanced expression of growth receptors and the increase glycolic activity supports the role of HIF-1 α in breast cancers. It also opens the possibility for targeting HIF-1 with combinatory therapies to affect the proliferation rate of breast cancers (Bos *et al.* 2002, 2005).

HIF-1 and prostate cancer

We originally observed HIF-1 α was overexpressed in primary prostate cancers, compared with normal prostate epithelium (Zhong *et al.* 1999). HIF-1 α overexpression was observed in prostate cancer bone metastases as well. Hypoxic regions exist in human prostate carcinoma, and increasing levels of hypoxia are associated with higher clinical stages. In a clinical observation of high-grade prostate intraepithelial neoplasia lesions, considered the precursor of a majority of invasive prostate adenocarcinoma, showed an increase in HIF-1 α relative to the respective normal epithelium, stromal cells, and benign prostatic hyperplasia. Zhong *et al.* (2004a) suggest that the upregulation of HIF-1 α is an early event in prostate carcinogenesis, and that HIF-1 α may be a future biomarker for premalignant lesions of the prostate. Du *et al.* have confirmed the high levels of HIF-1 α in prostate cancer as compared with benign prostate hyperplasia and normal tissue (Du *et al.* 2003). These investigators found that as prostate cancer progressed the amount of intraductal microvessel density increase as a function of tumor grade which could be co-ordinated with the increase in hypoxia and subsequent VEGF production.

One possible explanation for these high levels of HIF-1 α is the possible amplification of the HIF- α gene; however, this was argued not to be the case by Saramaki *et al.* (2001) who showed only an amplification of the HIF-1 α locus in the PC3 prostate cancer cell line and not in prostate tumors. Fu *et al.* (2005) also suggest that such a high level of HIF-1 α expression is not due to an increase in stabilization resulting from mutations in the oxygen degradation domain (ODD) region of HIF-1 α .

HIF-1 α overexpression in prostate tumorigenesis has been identified in transgenic mouse models of

prostate cancer. HIF-1 α overexpression appears to be a very early event in prostate cancer pathogenesis in these systems. Studies in the Transgenic Adenocarcinoma Mouse Prostate (TRAMP) transgenic model of prostate cancer support the correlative clinical observations and the concept that an angiogenic switch is driven in part by HIF-1 α early in prostate cancer progression (Huss *et al.* 2001). In transgenic mice expressing human AKT1 in the ventral prostate, there is an mTOR-dependent survival signal that activates HIF-1 α in early tumors. Transcriptome profiling showed that HIF-1 α /HRE targets, including genes encoding most glycolytic enzymes, constituted the dominant transcriptional response to AKT activation and mTOR inhibition.

Androgens have been discovered to modulate HIF-1 α protein levels in human prostate cancer. We tested the hypothesis that HIF-1 α overexpression may not just be epigenetic from intratumoral hypoxia, but is affected by androgen receptor and androgens. We tested as well the hypothesis that the anti-angiogenic effects of anti-androgens in androgen-responsive PCA cells occurs by blocking HIF-1 α transcriptional pathway. We found that *in vitro* dihydrotestosterone (DHT) stimulates HIF-1 α protein expression, HIF-1 α transcriptional activity, and VEGF production in androgen receptor positive LNCaP cells; conversely, the anti-androgen flutamide reduced these effects. Androgen induction of HIF-1 α protein expression and function are regulated in part through an autocrine loop mechanism. Increased secretion of EGF is androgen mediated in prostate cancer *in vitro*; EGF then acts through the EGFR in the PI3K/AKT/Tor pathway in PCA cells to increase HIF-1 α levels. Thus, anti-androgen therapy, a mainstay of prostate cancer endocrine therapy for 60 years may be based in part on downregulation of HIF-1 α .

DHT has also been shown to increase the stabilization of HIF-1 α and EGF mRNAs through the AU-rich elements within the 3'-untranslated regions of these transcripts. DHT can act post-transcriptionally as well as post-translationally to modulate HIF-1 α activity (Mabjeesh *et al.* 2003b, Sheflin *et al.* 2004). Recently, Boddy *et al.* (2005) showed that the expression of HIF-1 α and HIF-1 β were significantly correlated to androgen receptor (AR) supporting the previously mentioned *in vitro* data. The correlation between hormone status and HIF-1 α expression is also supported by the evidence that some hormone refractory PCAs have mutations in the ODD of HIF-1 α thus leading to uncoordinated gene expression in the presence and absence of hypoxia. Such dysregulation could possibly contribute to the development and

maintenance of therapeutic resistance of these cancers (Anastasiadis *et al.* 2002).

PTEN-null cancers have elevated HIF-1 α , including prostate cancer (Zhong *et al.* 2000, Zundel *et al.* 2000, Jiang *et al.* 2003). The loss of PTEN function leads to activation of PI3K signaling and Akt activation, which turns on mTOR and leads to mTOR-dependent survival. Expression profiling following manipulation of the Akt-mTOR pathway showed that many of the HIF-1 α targets were modified in response to Akt activation or mTOR inhibition (Majumder *et al.* 2004). Aberrations in signaling pathways due to genetic or epigenetic factors may interact with HIF-1 α supporting the possibility that HIF-1 α overexpressing tumors may lead to or augment PTEN mutant phenotypes of prostate cancers.

HIF as therapeutic target

Transcription factors are difficult targets for anti-cancer drug discovery as they are not enzymes for the identification of active site inhibitors. Given the overexpression of HIF-1 α in so many tumor types and association of HIF-1 α with tumor angiogenesis, many approaches have been taken to identify agents that reduce HIF-1 α protein levels (Dachs *et al.* 1997, Semenza 2002, 2003, Binley *et al.* 2003, Carroll & Ashcroft 2005). An example of one such drug, PX-478 (S-2-amino-3-[4'-N,N,-bis(2-chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride) inhibits the accumulation of HIF-1 α and reduces HIF-1 α activity (Wiedmann & Caca 2005). High throughput HIF-1 α reporter gene assays have been used to start to screen diverse chemical and natural product libraries (Tan *et al.* 2005).

Other groups have sought disruption of HIF-1 α upstream signaling by attacking RAS-related proteins. Using radio-resistant human U87 glioblastoma (GBM) and a specific farnesyltransferase inhibitor R115777, Delmas *et al.* (2003) showed reduced matrix metalloproteinase 2 activities and reduced hypoxic-regulated signaling. Another group of investigators showed that the Ras inhibitor trans-farnesylthiosalicylic acid (FTS) exhibits profound anti-oncogenic effects in U87 GBM cells. FTS inhibited active Ras and its signaling to phosphatidylinositol-3-kinase and Akt pathway; hence, HIF-1 α was lowered and downstream HIF-regulated genes were affected (Blum *et al.* 2005).

Another upstream approach to inhibit HIF-1 α in normoxic tumors utilizes the anti-epidermal growth factor receptor monoclonal antibody cetuximab (C225; Erbitux), which is approved for the treatment of metastatic colorectal cancer. This monoclonal antibody

has been known to inhibit VEGF secretion *in vitro* and *in vivo*. Studies show that cetuximab reduces HIF-1 α in epidermoid carcinoma cells under both normoxic and hypoxic conditions. This inhibition is through the RAS pathway and confirms that VEGF secretion can be modulated by signal transduction inhibition of HIF-1 α protein translation (Luwor *et al.* 2005).

In addition to monoclonal antibodies, compounds that share a 2,2-dimethylbenzopyran structural motif have been shown to inhibit hypoxia-induced transcription activity. Small molecule 103D5R markedly decreased HIF-1 α protein levels induced by hypoxia or cobaltous ions in a dose- and time-dependent manner. 103D5R was shown to inhibit the phosphorylation of Akt, Erk1/2, and stress-activated protein kinase/c-jun-NH(2)-kinase, proving worthy of continued investigation of the drug as a HIF-1 α inhibitor (Tan *et al.* 2005).

HIF-1 α inhibitors are also in clinical trials of breast and prostate cancer. 2-Methoxyestradiol (2ME2) is an estradiol metabolite with significant anti-proliferative and anti-angiogenic activity independent of estrogen receptor status; it is currently in clinical trials (Panzem:Entrre Med) and *in vivo* it has been shown to inhibit tumor growth and angiogenesis at concentrations that efficiently disrupt tumor microtubules (MTs) (Mabjeesh *et al.* 2003a, Ricker *et al.* 2004). 2ME2 downregulates HIF-1 α at the post-transcriptional level and inhibits HIF-1 α -induced transcriptional activation of VEGF expression. Inhibition of HIF-1 α occurs downstream of the 2ME2/tubulin interaction, as disruption of interphase MTs is required for HIF-1 α downregulation. These data establish 2ME2 as a small molecule inhibitor of HIF-1 α and provide a mechanistic link between the disruption of the MT cytoskeleton with

drugs, such as taxol and taxotere and inhibition of angiogenesis (Mabjeesh *et al.* 2003b).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be good candidates for chemoprevention agents because, in part, of their anti-angiogenic properties on precancerous lesions. As a class of agents, cyclo-oxygenase inhibitors appear to downregulate HIF-1 α . The inhibition of HIFs by NSAIDs was COX-2 independent (Jones *et al.* 2002, Palayoor *et al.* 2003, Zhong *et al.* 2004b). A more comprehensive list of anti-neoplastic/anti-angiogenic agents that are reported to affect HIF-1 α is shown in Table 1.

Summary and future directions

HIF-1 α has emerged as an important transcription factor in breast cancer and prostate cancer tumor biology. Mouse transgenic models have validated the translational research in human tumor banks that HIF-1 α is associated with the angiogenic switch in early tumorigenesis. In both breast cancer and prostate with cancers, upregulation is early, making HIF-1 α a logical target for chemoprevention strategies in patients at higher genetic risk of breast and prostate cancer with COX-2 inhibitors or 2ME 2 as well as target for new approaches to inhibiting angiogenesis. HIF-1 α and HIF-2 upregulation is impacted by multiple factors that are epigenetic and genetic. The crosstalk between estrogen intracellular signaling pathways and HIF-1 α is still not fully defined in breast cancer. ER β downstream actions are one candidate for estrogen modulation of HIF-1 α levels. Androgens upregulate HIF-1 α in prostate cancer through androgen-regulated autocrine receptor tyrosine kinase

Table 1 Targeted HIF therapeutic compounds

Chemical	Mechanism of action	Reference
2-Methoxyestradiol	HIF-1 α activity	Mabjeesh <i>et al.</i> (2003a), Ricker <i>et al.</i> (2004)
4-O-Methylsarcenol, Manassantin A, Manassantin B1	Inhibits HIF-1 α activity and accumulation	Hossain <i>et al.</i> (2005)
NSC-134754, NSC-643735	Inhibits HIF-1 α activity and accumulation	Chau <i>et al.</i> (2005)
Topotecan	Inhibits HIF-1 α accumulation	Beppu <i>et al.</i> (2005)
SCH66336	Inhibits farnesyltransferase, inhibits HIF-1 α activity	Han <i>et al.</i> (2005)
PX-478	Inhibits HIF-1 α activity and accumulation	Macpherson & Figg (2004), Welsh <i>et al.</i> (2004)
R115777	Inhibits farnesyltransferase, inhibits HIF-1 α activity	Delmas <i>et al.</i> (2003)
Cetuximab (C225; Erbtux)	Inhibits EGFR, inhibits HIF-1 α activity	Luwor <i>et al.</i> (2005)
103D5R	Inhibits AKT, ERK1/2 phosphorylation, inhibits HIF-1 α activity	Tan <i>et al.</i> (2005)
NSAID	Inhibits HIF-1 α activity	Jones <i>et al.</i> (2002), Milas (2001), Palayoor <i>et al.</i> (2003), Tarnawski & Jones (2003)

receptor/PI3K/AKT-1/mTor signaling. HIF-1 α has been identified as a part of the lethal phenotype of endocrine cancers. As more detailed intracellular signaling pathways are identified in breast and prostate cancer, their influence on HIF levels will need assessment.

Acknowledgements

This work is supported by Jean Sindab Endowment for Breast Cancer Research Gift, Avon Foundation and NIH P60 MD 000525-04. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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