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Review

Hypoxia-inducible factor (HIF) in human tumorigenesis

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Summary. Hypoxia is a major event that occurs in most solid tumors. Intratumoral hypoxia is sufficient to activate the key transcription factor, hypoxia-inducible factor (HIF) that mediates the activation of the "survival machinery" in cancer cells. HIF can also be induced by oxygen-independent genetic alterations that activate a variety of oncogenic signaling pathways or inactivate tumor suppressors. Increased tumor HIF occurs at early stages of carcinogeniesis and is often correlated with increased angiogenesis, malignant progression, poor patient prognosis and chemoradio-resistance. HIF-α subunit, the oxygen-regulated subunit of HIF is overexpressed in a wide range of human solid tumors. Nuclear HIF-α protein immunostaining was restricted to tumor cells compared to normal tissues. Herein, we review and discuss the role of HIF in tumorigenesis and describe the overexpression of HIF-α proteins in human cancers and its association with overall clinical outcomes.

Key words: Hypoxia-inducible factor (HIF), Tumor, Immunohistochemistry, Prognosis, Patients

Introduction

It has become clear over the past years that hypoxia-inducible factor 1 (HIF-1) is the intrinsic survival factor of tumor cells to overcome O_2 and nutrient deficits during proliferation and progression. Regions of hypoxia exist in most solid tumors, and often correlate with prognosis and resistance to radio- or chemotherapies (Vaupel, 2004). Regardless of oxygen tension, cancer cells sustain high aerobic glycolytic rates and produce high levels of lactate and pyruvate. Although this phenomenon, known as the Warburg effect after its discoverer (Warburg, 1965) was first described in cancer more than seven decades ago its molecular basis has only recently been elucidated (Semenza et al., 2001). Many studies indicate that increased expression of genes encoding glucose transporters and glycolytic enzymes in tumor cells is mainly mediated by the transcription factors c-myc and HIF-1 (Semenza et al., 2001). Whereas c-myc is a direct target for oncogenic mutations, expression of HIF-1 is indirectly up-regulated via "gain-of-function" mutations in oncogenes and "loss-of-function" mutations in tumor suppressor genes that result increased HIF-1 transcriptional activity (Semenza, 2003). As a result of genetic alterations together with the intratumoral hypoxia, HIF-1 activation is enhanced in the majority of common human cancers which is strongly correlated with tumor grade, vascularity, metastasis, prognosis and overall survival (Semenza, 2003). HIF-1 can induce a vast array of gene products that control energy metabolism, neovascularization, survival, intracellular pH and cell migration (Hirota and Semenza, 2006), all of which are promoters of tumor growth. A better understanding of the role of HIF regulation in theses cellular processes is not only crucial for understanding "hypoxic tumor biology" but also for developing novel anticancer therapies. In this review we summarize the expression profile of HIF and its relationship with prognostic parameters of common human cancers.

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Molecular mechanism of HIF-1 transcriptional activation

HIF-1 is a heterodimeric transcription factor composed of a HIF-1α subunit and a HIF-1ß subunit (Wang and Semenza, 1995). Both HIF-1 subunits are members of the basic helix-loop-helix (HLH)-containing PER-ARNT-SIM (PAS)-domain family of transcription factors (Wang et al., 1995). The HLH and PAS domains mediate heterodimer formation between the HIF-1α and HIF-1ß subunits, which is necessary for DNA binding by the basic domains (Wang et al., 1995).

In humans, 3 HIF genes have been identified; the *HIF1A*, *EPAS1*, and *HIF3A* that encode *HIF-1*α, HIF-2α, and HIF-3α, respectively. In contrast to HIF-1α, HIF-2α and HIF-3α have more restricted expression patterns (Tian et al., 1997; Gu et al., 1998). HIF-1α and HIF-2α share similar structure, function and regulatory pathways whereas HIF-3α (also known as IPAS) functions as an inhibitor of transcriptional responses to hypoxia (Hirota and Semenza, 2006). To date, the

mechanism and the pattern of expression in human specimens of HIF-1α isoform is the most studied.

HIF-1α is constitutively produced and degraded under normal O_2 conditions (normoxia) while HIF-1ß expression is unaffected by O_2 levels (Hirota and Semenza, 2006). The process is triggered by posttranslational HIF-1α hydroxylation on specific proline residues (proline 402 and 564) within the O_2-dependent degradation domain (ODDD) via prolyl hydroxylases in the presence of O_2, iron and 2-oxoglutarate (Bruick and McKnight, 2001; Epstein et al., 2001; Ivan et al., 2001; Metzen et al., 2003). The hydroxylated protein is then recognized by von Hippel Lindau protein (pVHL), which is a part of an E3 ubiquitin ligase complex, ubiquitinated and targeted for proteasomal degradation. Concurrently, hydroxylation of the asparagine residue 803 catalyzed by an asparaginyl hydroxylase (FIH-1), which, in turn, blocks the coactivators p300 and CBP from binding to the HIF-1α subunit (Mahon et al., 2001; Hewitson et al., 2002; Lando et al., 2002). Under hypoxic conditions, HIF-1α remains unhydroxylated and does not interact with pVHL but preferentially binds to the transcription coactivators, CBP and p300. Following this hypoxic stabilization, HIF-1α translocates to the nucleus where it heterodimerizes with HIF-1ß to bind to an enhancer element (the "hypoxia-response element", HRE) in target genes. The resulting activated HIF-1 drives transcription of more than 2% of all human genes either directly or indirectly as recently described in arterial endothelial cells using DNA microarrays (Manalo et al., 2005). However, the expression of over 40 genes is known to be activated at the transcriptional level by HIF-1 as defined by the most stringent criteria, including the induction of gene expression in response to hypoxia, the presence of a functionally-essential HIF-1 binding site in the gene, and an effect of HIF-1 "gain-of-function" or "loss-of-function" on expression of the gene. Among these are the glycolytic enzymes, the glucose transporter *Glut-1,* endothelin-1 (ET-1), vascular endothelial growth factor (*VEGF*), VEGF receptor-1 (*Flt-1*), carbonic anhydrase 9 (CA9) and erythropoietin (for full list see (Hirota and Semenza, 2006)).

HIF-1 activation in cancer cells

The hypoxic response pathway has been recognized as an important contributor to a wide range of human cancers, including breast, prostate, brain, lung and head and neck (Quintero et al., 2004; Brahimi-Horn and Pouyssegur, 2005). Increased levels of HIF-1 activity are often associated with increased tumor aggressiveness, therapeutic resistance and mortality (Semenza, 2003; Quintero et al., 2004). HIF-1 can be induced as a result of the high growth rate of tumor cells and intratumoral hypoxia as well as by O_2-independent genetic alterations that activate a variety of oncogenic signaling pathways or, alternatively, inactivate tumor suppressors (Kim and Kaelin, 2003).

However, the mechanisms by which oncogenes or tumor suppressor genes affect the aerobic induction of HIF function in the majority of tumors are not completely elucidated. Several mechanisms other than mutations of the tumor suppressor pVHL were shown to activate the HIF pathway in cancer cells (Fig. 1). For instance, studies from various laboratories have shown that HIF-1α protein synthesis is enhanced upon activation of the phosphatidylinositol 3-kinase (PI3K), AKT (protein kinase B), and its effector FKBP-rapamycin-associated protein (FRAP or mTOR; mammalian target of rapamycin) pathway (Minet et al., 2000; Zundel et al., 2000; Jiang et al., 2001; Laughner et al., 2001; Sodhi et al., 2001; Tacchini et al., 2001; Stiehl et al., 2002; Fan et al., 2004). Dysregulated signal transduction from receptor tyrosine kinases to PI3K/AKT/mTOR occurs via autocrine stimulation or inactivation of the tumor suppressor PTEN in many cancers. Basic fibroblast growth factor, insulin, interleukin-1, hepatocyte growth factor and heregulin induce the expression of HIF-1α as well (Jiang et al., 2001; Laughner et al., 2001; Sodhi et al., 2001; Tacchini et al., 2001; Stiehl et al., 2002; Fan et al., 2004). Regulation of HIF-1 by phosphorylation through the mitogen-activated protein kinase (MAPK) signaling pathway has also been demonstrated. Indeed, several studies showed that phosphorylation through the ERK/MAPK pathway is required for activation of the transcriptional activity but not for HIF-1α stabilization under hypoxia (Salceda et al., 1997; Minet et al., 2000; Hur et al., 2001). In particular, ERK1/2 (also known as p44/p42), two kinases of the MAPK signaling pathway, have been implicated in HIF activation (Minet et al., 2001). ERK1/2 are activated by extracellular proliferative signaling triggered by membrane-tyrosine kinases and transduced through the Ras-Raf-MEK pathway by a cascade of phosphorylation events that can be repressed by specific kinase inhibitors. In addition to growth factors, prostaglandin E2, thrombin, angiotensin II, 5-hydroxytryptamine, acetylcholine and some nitric oxide donors induce HIF-1 activation under non-hypoxic conditions via these pathways (Richard et al., 2000; Page et al., 2002; Hirota et al., 2004; Kasuno et al., 2004).

Additional mechanism that explains HIF activation in cancer is the existence of mutations in the ODDD of HIF-1α gene (*HIF1A*). We and others have identified the presence of polymorphism in the ODDD of *HIF-1A* (1772C>T), which results in an amino acid change from proline 582 to serine (P582S) (Anastasiadis et al., 2002; Tanimoto et al., 2003; Ollerenshaw et al., 2004; Chau et al., 2005; Kim et al., 2005a; Orr-Urtreger et al., 2006). Overexpression of P582S HIF-1α protein in COS7 and CV1 cells exhibited increased HIF-1 transcriptional activity compared with wild-type HIF-1α (Tanimoto et al., 2003; Fu et al., 2005). The increased HIF-1 activity was attributed to increased protein stability of the mutant protein (Fu et al., 2005). Although P582S is located within the ODDD domain of HIF-1α it does not affect the association of HIF-1α with VHL (Percy et al., 2003).

However, the exact mechanism by which this mutation enhances HIF-1 transcriptional activity is not fully clear. The effect of HIF-1 on tumor growth, however, is complex. Several genetic studies using embryonic stem (ES) cells or mouse embryonic fibroblasts (mEF) have indicated that loss of HIF-1α or disruption of HIF-1 function causes tumor growth retardation (Maxwell et al., 1997; Ryan et al., 1998, 2000), while others showed that HIF-1α acts as a tumor suppressor or "negative factor", in ES cell-derived tumors (Carmeliet et al., 1998). Subsequently, Blouw et al. (2003) showed that HIF-1α has differential roles in tumor progression that are greatly dependent upon the microenvironment existing in or surrounding the tumor (Blouw et al., 2003). Nevertheless, a number of recent studies using HIF-1α knock-down or knock-out in cancer cells confirm the role of HIF-1 as a "positive factor" in tumor growth and proliferation (Zhang et al., 2004b; Helton et al., 2005; Dang et al., 2006; Li et al., 2006; Luo et al., 2006).

HIF-1α protein expression in common human solid tumors

Prostate cancer

Results from several laboratories on human specimens of prostate cancer exhibited over-expression of HIF-1α protein (Table 1). Most (62.5-100%) of the prostate carcinoma samples were positive for nuclear HIF-1α immunostaining compared to 60-79% of high-grade prostate intraepithelial neoplasm (HG-PIN) lesions and 0-68% of benign prostate hyperplasia (BPH), while there was no detectable expression in normal prostate tissues (Table 1). It is of note that not only was HIF-1α protein level overexpressed, but the mean percentage of positive cells was also higher in prostate carcinoma than in PIN or in BPH. Animal model studies (Huss et al., 2001) and immunohistochemical analyses (Du et al., 2003; Zhong et al., 2004) show that the expression of HIF-1α protein occurs at the very early stage of prostate tumorigenesis (PIN). However, it should be kept in mind that high rates of HIF-1α expression could be detected in BPH as well (Du et al., 2003). No significant correlations between HIF-1α expression and clinical or prognostic parameters were found, with the exception of one study that reported on a positive correlation with the presence of distant metastases (Hao et al., 2004).

Breast cancer

HIF-1α expression is not detected in normal breast tissue or hyperplastic lesions but is present in well-differentiated ductal carcinoma *in situ* and in all more malignant forms of breast cancer (Table 2). HIF-1α has also been implicated as an independent prognostic

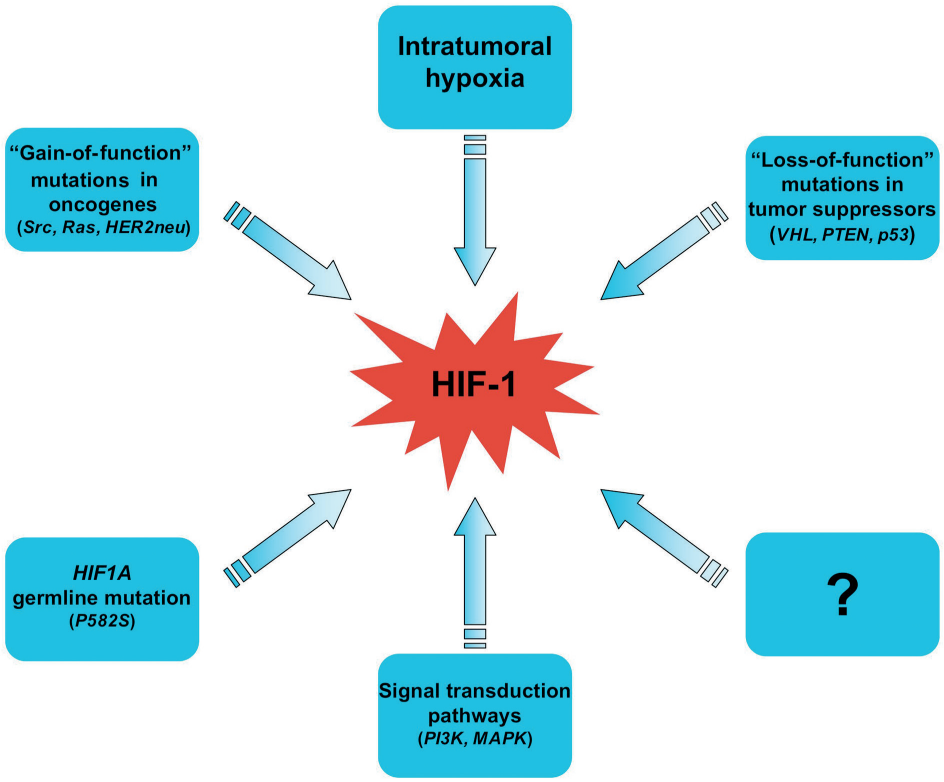

Fig. 1. Mechanisms of HIF-1α activation in cancer. High HIF-1α levels in tumors reflect the frequent presence of intratumoral hypoxia, activation of signal transduction pathways, genetic alterations in oncogenes, tumor supressoor genes and *HIF-1α* gene itself. VHL: von Hippel-Lindau; PTEN: posphatase and tensin homolog deleted on chromosome 10; p13K: phosphatidylinositol-3-kinase; HIF1A, *HIF-1α* gene; MPAK, mitogen-activated protein kinase; ?, other unknown yet mechanisms.

marker in both lymph node-negative (Bos et al., 2003) as well as lymph node-positive breast cancers (Schindl et al., 2002; Gruber et al., 2004). HIF-1α expression in breast cancer is associated with poor overall and disease-free survival in most studies (Table 2). Although high histological grade, tumor size, proliferation and the presence of necrotic regions have been linked to the presence of HIF-1α the relation between estrogen and progesterone receptor status was not consistent among the reports (Table 2). HIF-1α was associated with molecular markers of aggressive breast cancer including VEGF, EGFR, cyclin E, cyclin A and Her2 expression. Therefore, HIF-1α may be used as an adjunct to improve clinical decisions for adjuvant treatment in selected patients.

Lung cancer

HIF-1α protein expression was detected in 20.5-67% of lung cancer specimens, depending on the histological type (Table 3). Normal lung and areas distal to the tumor showed a weak or negative reactivity. HIF-1α expression was associated with HIF-2α (Giatromanolaki et al., 2001), CA9 (Swinson et al., 2004; Kim et al., 2005b) and VEGF expression or microvessel density (MVD) (Lee et al., 2003; Zhang et al., 2004a), oncogene mutant p53 (Giatromanolaki et al., 2001; Swinson et al., 2004; Zhang et al., 2004a) and MMP-9 expression (Swinson et al., 2004). A significant correlation between HIF-1α expression, apoptosis and pro-apoptotic factors, caspase-3, Fas and Fas ligand expression was found as well (Volm and Koomagi, 2000). The association between HIF-1α expression and prognosis, survival or recurrence is controversial. Volm and Koomagi found that patients with HIF-1α positive carcinomas had significantly longer median survival time than patients with HIF-1α negative carcinomas (Volm and Koomagi, 2000) while Fan et al. (2002) showed a significant association between HIF-1α expression and increased metastasis (Fan et al., 2002).

Colorectal cancer

Zhong et al. (1999) were the first to document HIF-1α protein expression in colon carcinomas compared to normal colonic mucosa without evaluating the impact on clinicopathological features and patient prognosis (Zhong et al., 1999). Several studies detected HIF-α protein expression in colorectal carcinoma using immunohistochemistry (IHC) in 45-80% of the specimens (Table 4). The staining of the colorectal carcinoma was observed both in the nucleus and the cytoplasm, in contrast to normal mucosal cells, where staining was weak and restricted to the cytoplasm (Yoshimura et al., 2004). One study showed that HIF-1α protein expression correlated closely with lymphatic,

Table 1. HIF-1α protein expression in prostate cancer.

Reference	HIF-1α positive expression No. of total (%)				Positive correlation	No correlation	Comments
	PCa	PIN	BPH	NP			
Zhong et al., 1999	9/11 (81.8)			0/12 (0)			Overexpression of HIF-1α can occur very early in carcinogenesis, before histological evidence of angiogenesis or invasion
Talks et al., 2000	2/5 (40)						Only small numbers of PCa samples were tested without comparison to normal or hyperplastic tissues
Du et al., 2003	31/34 (91)		19/28 (68)	0/13 (0)		Clinicopathological factors	
Hao et al., 2004	33/42 (78.6)	9/12 (75)		0/9 (0)	Distant metastases		Up-regulation of HIF-1α is an early event in PCa
Zhong et al., 2004	6/6 (100)	11/14 (78.6)	0/3 (0)	0/8 (0)			Up-regulation of HIF-1α is an early event in PCa
Boddy et al., 2005	119/139 (85.6)				HIF-2·, AR & VEGF expression	PSA recurrence	HIF-2α expression was significantly related to VEGF, AR and inversely related to PHD2 expression.
Wang et al., 2006	20/32 (62.5)	9/15 (60)	1/16 (6.3)	0/12 (0)			Up-regulation of HIF-1α is an early event in PCa Simultaneous higher MVD and positive rate of HIF-1α were significantly correlated with high Gleason score group than those with low Gleason score group

PCa, prostate cancer; PIN, prostate intraepithelial neoplasia; BPH, benign prostate hyperplasia; NP, normal prostate; MVD, microvessel density; AR, androgen receptor; VEGF, vascular endothelial growth factor; PHD, prolyl hydroxylase enzyme; PSA, protate-specific antigen.

Table 2. HIF-1α protein expression in breast cancer.

Authors (Ref.)	HIF-1α positive expression No. of total (%)		Positive Correlation	No Correlation	Comments
	Carcinoma	Normal			
Zhong et al., 1999	15/52 (28.8)	0/18 (0)	Metastasis		Overexpression of HIF-1α can occur very early in carcinogenesis, before histological evidence of angiogenesis or invasion
Talks et al., 2000	10/12 (83.3)		Necrosis		
Bos et al., 2001	60/80 (75)	0/20 (0)	Pathologic stage Poor differentiated lesions Proliferation ER expression VEGF expression Necrosis		Increased levels of HIF-1α are potentially associated with more aggressive tumors
Schindl et al., 2002	157/209 (76.2)	0/NA	Shorter overall survival Shorter disease-free survival	HER-2 expression Estrogen receptor density	12.1% of patients showed a combination of strong/moderate HIF-1α expression with p53 overexpression as analyzed by confocal laser scanning microscopy. 60% of them developed recurrent disease and 52% died from the disease
Bos et al., 2003	113/150 (75)		Poor overall and disease-free Survival Poor histological grade Increased mitotic activity index Her-2/neu protein and Gene amplification Proliferation VEGF expression Loss of PR	Tumor size Lymph node status Age Menopausal status	
Gruber et al., 2004	43/77 (56)		PR expression Poor outcome	T stage Grade Positive lymph nodes	HIF-1α expression could be used as a prognostic indicator in node-positive patients with T1/T2 tumors
Okada et al., 2005	20 DCISs 36 IDCs NA	0/20 (0)	Necrosis in DCISs VNPI in DCISs VEGF expression in IDCs		HIF-1α is expressed in the early stage of mammary carcinogenesis
Dales et al., 2005	543/745 (73)		Poor overall survival Early and widespread metastasis Relapse risk		HIF-1α expression could be used as a prognostic indicator
Vleugel et al., 2005	88/200 (44)		Poor histological grade Necrosis CA9 expression GLUT-1 expression		
Bos et al., 2005	25/45 (56)		MVD EGFR expression PDGF-BB expression bFGF expression		HIF-1α expression was predominantly perinecrotic
Schoppmann et al., 2006	91/119 (76.5)	0/NA	Lymphatic MVD VEGF expression	Lymphatic vessel invasion Histological grade	
Kronblad et al., 2006	90/377 (24)		Tumor size Histological grade Proliferation Her2 expression Cyclin E expression	Lymph node status Cyclin D expression ER and PR expression	In lymph node-positive and in NHG1/2 tumors, HIF-1α expression was significantly associated with an impaired recurrence free survival

DCIS, ductal carcinomas in situ; IDCs, invasive ductal carcinomas; NA, not applicable; MVD, microvessel density; ER, estrogen receptor; PR, progesterone receptor; VNPI, Van Nuys prognostic index; VEGF, vascular endothelial growth factor; CA9, carbonic anhydrase 9; GLUT-1, glucose transporter 1; EGFR, epidermal growth factor receptor; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; NHG, Nottingham histological grade.

venous and tumor invasion, liver metastasis, Dukes' stages, VEGF expression, MVD and a tendency for poor prognosis in patients with high HIF-1α-expressing tumors (Kuwai et al., 2003). Another study stated that only combined HIF-1α and HIF-2α expression had an impact on patient prognosis and survival (Yoshimura et al., 2004).

A number of reports studied HIF-1α mRNA expression in colorectal adenocarcinomas and adenomas using in situ hybridization (Jiang et al., 2003a, 2003b, 2004; Fan et al., 2004). Positive rates of HIF-1α mRNA were detected in both colorectal adenocarcinomas and adenomas. Interestingly, HIF-1α mRNA expression, VEGF protein expression and MVD increased with the Dukes' stages and were significantly higher in adenocarcinoma specimens than in adenoma specimens (Jiang et al., 2003a,b, 2004; Fan et al., 2004).

Bladder cancer

Detection of HIF-α proteins (HIF-1α or HIF-2α) expression in bladder adenocarcinoma samples was first reported by Talks et al. (2000) using IHC (Talks et al., 2000). Subsequently, several groups studied the expression of HIF-α proteins in hundreds of bladder cancer specimens (Wykoff et al., 2000; Jones et al., 2001; Xia et al., 2002; Hoskin et al., 2003; Theodoropoulos et al., 2004, 2005; Palit et al., 2005; Deng et al., 2006). The major findings in these studies were that accumulated HIF-α is associated with a poor overall and disease-free survival or with recurrence and chemo-resistance. When HIF-1α expression was combined with p53 (Theodoropoulos et al., 2005) or with HIF-target genes including CA9 and Glut-1 (Hoskin et al., 2003; Palit et al., 2005) the correlation with clinical measures was even stronger. HIF-1α expression was detected as nuclear staining. It was seen in 58-100% of the samples (Theodoropoulos et al., 2004, 2005; Deng et al., 2006). The reactivity was invariably detected in tumor cells that were close to areas of necrosis or away from blood vessels. No expression of HIF-1α protein was demonstrated in normal bladder tissues. Some of the studies found association of HIF-α's with the pathologic grade and clinical stage (Deng et al.,

Table 3. HIF-1α protein expression in lung cancer.

Authors (Ref.)	HIF-1α positive expression No. of total (%)				Positive correlation	No correlation	Comments
	NSCLC	SCLC	LC*	NL			
Zhong et al., 1999	2/2 (100)			0/10 (0)			
Volm and Koomagi, 2000	NA/96				Longer survival Caspase-3, Fas and Fas ligand expression	HIF-1ß, expression Proliferation	
Giatromanolaki et al., 2001	68/108 (63)			0/NA	HIF-2α expression Reduced Bcl-2 expression P53 nuclear accumulation	Histological grade Proliferation N-stage Necrosis	A marginal association with poor prognosis
Fan et al., 2002	NA (21.6)	NA (66.7)	17/60 (28.3)		Clinical stage Increased metastasis Bax expression Reduced Bcl-2 expression Apoptosis	Proliferation	The positive rate of HIF-1α expression was divided to SCLC (66.7%) and to NSCLC (21.6%)
Lee et al., 2003	38/84 (45.2)				Histological type MVD	Overall survival	
Swinson et al., 2004	101/172 (58.7)				T stage Squamous histology Extensive tumor necrosis EGFR, CA9, MMP-9 & P53 expression	N- or overall stage Gender Positive tumor margins Bcl-2 expression	
Zhang et al., 2004a			36/57 (63)		VEGF expression P53 expression		Positive correlation with mutant p53
Kim et al., 2005b	74/74 (100)				CA9 expression Tumor necrosis	Tumor size Nodal involvement Pathologic stage Recurrence Disease-free survival	

NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; LC, lung cancer; NL, normal lung; NA, not applicable; EGFR, epidermal growth factor receptor; CA9, carbonic anhydrase 9; MMP, matrix metalloproteinase; MVD, microvessel density. * without specifying histology.

2006) while others attributed this association to VEGF expression regardless of HIF-α expression (Theodoropoulos et al., 2005).

Ovarian cancer

HIF-1α protein expression in ovarian cancer was first detected using IHC by Zhong et al. (1999). Thereafter, HIF-1α protein overexpression was shown in 54-69% of the specimens tested while the expression of non-cancerous ovarian tissues was only 12.5-31.4% (Birner et al., 2001; Wong et al., 2003). Expression of HIF-1α was increased in tumor cells adjacent to areas of necrosis and with high MVD. All studies found that HIF-1α expression correlated with VEGF expression or MVD and with the apoptotic rate of tumor cells (Birner et al., 2001) and negatively correlated with the level of differentiation (Nakayama et al., 2002). However, the expression level was independent of age, clinical stage, histological subtype, and has no effect on survival of ovarian cancer patients (Nakayama et al., 2002).

Renal cell carcinoma

Renal cell carcinoma (RCC) is the most "HIF-related" malignancy because of the functional inactivation of the tumor suppressor gene VHL mutations, which occurs in up to 70% of clear cell RCC (CCRCC). The majority of these tumors lack functional VHL protein, which leads to HIF-α's stabilization and consequent HIF transcriptional activation (see above). HIF-α proteins expression was found in 40-81% of RCC or CCRCC (Wiesener et al., 2001; Na et al., 2003; Gong et al., 2005; Shao et al., 2005; Zhang et al., 2006) in contrast to no expression in normal kidney tissues (Shao et al., 2005; Zhang et al., 2006). Expression of mRNA of two known HIF-target genes, VEGF and Glut-1 correlated with HIF-1α protein expression in tumor extracts (Wiesener et al., 2001). Tumors with the VHL mutation show higher rates of HIF-1α expression (70.6-93%) compared to those without the mutation (26.7-66%) (Na et al., 2003; Guo et al., 2004; Gong et al., 2005). Patients with high HIF-1α-staining tumors show a trend towards a prolonged survival and better prognosis compared to those with low HIF-1α-staining tumors (Lidgren et al., 2006).

Pancreatic cancer

HIF-1α protein expression was found in 40-100% of pancreatic carcinomas including endocrine tumors (Kitada et al., 2003; Shibaji et al., 2003; Couvelard et al., 2005a,b) in contrast to benign tumors, in which it was found in only 5% (Kitada et al., 2003). HIF-1α expression was predominantly in the nucleus of pancreatic cancer cells while in adjacent, non-cancerous or benign pancreatic tissues weak staining was detected in the nucleus or only in the cytoplasm. HIF-1α expression was significantly associated with poor tumor differentiation (Couvelard et al., 2005a), large tumor size, higher cellular proliferation (Kitada et al., 2003; Couvelard et al., 2005a), presence of necrosis

Table 4. HIF-1α protein expression in colorectal carcinoma.

Authors (Ref.)	HIF-1α positive expression No. of total (%)		Positive correlation	No correlation	Comments
	Colorectal Adenocarcinoma	Normal Mucosa			
Zhong et al., 1999	22/22 (100)	0/24 (0)			Overexpression of HIF-1α can occur very early in carcinogenesis, before histological evidence of angiogenesis or invasion
Talks et al., 2000	4/5 (80)	0/NA			HIF-2α was also strongly expressed by subsets of tumor-associated macrophages, sometimes in the absence of any tumor cell expression
Kuwai et al., 2003	89/149 (60)	0/NA	Tumor invasion Tumor stage Lymphatic invasion Venous invasion Liver metastasis MVD and VEGF expression		Tendency for poor prognosis in patients with high HIF-1α expression tumors
Yoshimura et al., 2004	39/87 (44.8)	0/NA	Prognosis and survival only when combined with HIF-2α expression	Pathological features of tumor aggressiveness	HIF-2α expression was less frequent (29.9%) Tumor aggressiveness was significantly correlated with overexpression of HIF-2α HIF-2α expression was directly correlated with MVD and COX-2 expression

MVD, microvessel density; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase 2; NA, not applicable.

(Couvelard et al., 2005a,b), advanced TNM stage (Kitada et al., 2003) and presence of liver metastasis (Couvelard et al., 2005a).

Hepatocellular carcinoma

HIF-1α protein expression in hepatocellular carcinoma was first studied by Zhong et al. (1999) and Talks et al. (2000) and was detected only in 2 out of 13 samples. Subsequently, HIF-1α protein expression in the hepatocellular carcinoma was studied more extensively and described in 67-94% of the samples compared to only 7% of normal liver tissue (Ding et al., 2004; Huang et al., 2005). A significant positive correlation was found between HIF-1α, VEGF, metastasis and MVD (Huang et al., 2005) while an inverse correlation was shown with differentiation (Ding et al., 2004). No correlation between HIF-1α expression and prognosis, existence of portal tumor emboli or status of HBsAg was found (Ding et al., 2004).

Gastric cancer

Using IHC staining, HIF-1α expression was positive in 32-61% of gastric cancer specimens (Takahashi et al., 2003; Urano et al., 2006). HIF-1α expression correlated significantly with increased expression of p53 (Urano et al., 2006) and VEGF (Takahashi et al., 2003; Chen et al., 2005; Urano et al., 2006), in addition to tumor size, MVD, poor prognosis (Takahashi et al., 2003; Urano et al., 2006) and liver (Takahashi et al., 2003) or other distant metastases (Chen et al., 2005). Moreover, no correlation was found between HIF-1α expression and clinicopathological status and chemosensitivity (Urano et al., 2006). Interestingly, several tumor suppressor genes, which are relevant to the HIF pathway, such as VHL and PTEN, are frequently inactivated in gastric cancer (Griffiths et al., 2005).

Esophageal cancer

In reports on esophageal squamous cell carcinoma (SCC), HIF-1α protein was expressed in both the nuclei and the cytoplasm of the tumor cells. Its protein expression was recognized by IHC in 51-95% (Koukourakis et al., 2001; Katsuta et al., 2005; Matsuyama et al., 2005). Correlation was found with VEGF expression, lymph node invasion, depth of invasion and blood vessel invasion (Katsuta et al., 2005; Matsuyama et al., 2005). Furthermore, it was found that a high level of HIF-1α expression was associated with poor response to photodynamic therapy (Koukourakis et al., 2001). Although the recurrence rate in the HIF-1α positive group was higher than in the HIF-1α negative group, the difference was not significant (Katsuta et al., 2005). In contrast, HIF-1α mRNA, as measured by semi quantitative RT-PCR, was recognized in all esophageal SCC tumor and normal mucosal tissues. No relationship was found between HIF-1α mRNA scores and clinicopathological factors, prognosis, VEGF or p53 oncoprotein expression (Matsuyama et al., 2005).

Brain tumors

Glioblastoma

Glioblastoma multiforme (GBM) is characterized by exuberant angiogenesis, a key event in tumor growth and progression. The pathologic mechanisms driving this biological behavior of gliomas remain unclear. Activation of the HIF-1 pathway is a common feature of gliomas and may explain the intense vascular hyperplasia often seen in GBM (Kaur et al., 2005).

Sondergaard et al. (2002) found that HIF-1α mRNA expression was significantly higher in glioblastomas than in astrocytomas and normal brain tissues. Strong immunoreactivity was observed in tumor cell nuclei in 78% of glioblastomas, particularly in areas surrounding necrosis. Such expression was not observed in normal brain tissues. No correlation was found between HIF-1α protein expression or mRNA expression and p53 immunoreactivity (Sondergaard et al., 2002). In addition, Irie et al. (2004) observed an association between progression-free survival of irradiated patients and HIF-1α expression (Irie et al., 2004). This association could indicate that high expressing HIF-1α tumors are more radioresistance.

Astrocytoma

Sixty-three human astrocytic gliomas were analyzed by IHC for HIF-1α (Giannopoulou et al., 2006). HIF-1α was detected only in grades III and IV astrocytic gliomas, both in the nucleus and in the cytoplasm. When HIF-1α protein expression was correlated with clinical outcomes of 39 patients no correlation with survival was found (Giannopoulou et al., 2006). In contrast, Korkolopoulou et al. (2004) studied 83 astrocytomas and found that HIF-1α protein expression is increased significantly with increasing grade and proliferative potential (Korkolopoulou et al., 2004). HIF-1α levels were associated with shortened survival in the entire cohort and when combined with grade it was a significant prognostic indicator (Korkolopoulou et al., 2004).

Head and neck cancers

Using IHC, expression of HIF-1α and HIF-2α proteins in squamous cell head and neck cancer samples was between 14-87% compared to mucosa from normal individuals that showed no HIF-α immunoreactivity (Beasley et al., 2002; Koukourakis et al., 2002; Kyzas et al., 2005). A significant association was found between HIF-α expression and bone/cartilage involvement, VEGF, thymidine phosphorylase expression, tumor necrosis (Beasley et al., 2002) and incomplete response to chemoradiation (Koukourakis et al., 2002, 2006). In

multivariate analysis, HIF-α's expression were independent prognostic factors and HIF-1α alone was significantly associated with worse disease-specific and disease-free survival (Koukourakis et al., 2006; Winter et al., 2006).

Oral squamous cell carcinoma

Two groups reported on the expression of HIF-1· in oral SCC (Kurokawa et al., 2003; Fillies et al., 2005). HIF-1α overexpression was shown in 63.5-68% and was confined to the nuclei of neoplastic cells. The frequency of high HIF-1α expression increased with tumor stage, depth of tumor invasion, lymph node metastasis, distant metastasis and lymphatic invasion (Kurokawa et al., 2003). No correlation was demonstrated between HIF-1α and Glut-1, CA9, cyclin D1 or ki67. In addition, no correlation was found between HIF-1α and tumor size and differentiation (Fillies et al., 2005). The association between HIF-1α and survival was less clear. Kurokawa et al. (2003) (Kurokawa et al., 2003) observed shorter survival of patients with high HIF-1α expression tumors compared to patients with low expression tumors while Fillies et al. (2005) (Fillies et al., 2005) showed that low HIF-1α expression was significantly associated with poorer disease-free and overall survival.

Nasopharygeal carcinoma

Nasopharygeal tumor cells showed HIF-1α nuclear staining in 58% of cases, whereas 57% of the cases showed CA9 staining (Hui et al., 2002). In contrast to oral SCC, there was no significant association between the expression of HIF-1α or CA9 with gender, histology type, T-, N- or overall stages (HO's stages) and distant metastases (Hui et al., 2002).

Conclusions and future directions

The extensive IHC data in multiple human tumors demonstrate unequivocally that the HIF pathway is a major contributor to tumor development and progression. Clearly, the role of the HIF pathway in cancer might be different during the different stages and among tumor subtypes. In some diseases HIF-1α is an independent prognostic factor and could be used to predict clinical outcome and survival or alternately used to assist in decisions for adjuvant therapies.

Evidence of the importance of HIF as a key factor in tumorigenesis, angiogenesis and tumor progression from the IHC data of human tumors and the growing knowledge of the molecular mechanism of its regulation will ultimately reveal novel anticancer therapies in the near future. Currently, a number of agents that inhibit HIF-1α accumulation are in clinical trials (http://clinicaltrial.gov) including topotecan (Rapisarda et al., 2004a,b), 2-methoxyestradiol (Mabjeesh et al., 2003; Ricker et al., 2004) and the Hsp90 inhibitor 17-AAG (Neckers, 2003, 2006). Today, HIF-1 has become an accepted target for cancer therapy.

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