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Forum Review

The Potential Role of Intrinsic Hypoxia Markers as Prognostic Variables in Cancer

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ABSTRACT

Tumor hypoxia is related to tumor progression and therapy resistance, which leads to poor patient outcome. It has been suggested that measuring the hypoxic status of a tumor helps to predict patient outcome and to select more targeted treatment. However, current methods using needle electrodes or exogenous markers have limitations due to their invasiveness or necessity for preinjection. Recent studies showed that hypoxia-regulated genes could be alternatively used as endogenous hypoxia markers. This is a review of 15 hypoxia-regulated genes, including hypoxia-inducible factor-1 and its targets, and their correlation with tumor hypoxia and patient outcome from 213 studies. Though most of the studies showed significance of these genes in predicting prognosis, there was no definitive prognostic and hypoxia marker. In conclusion, this review suggests the need for further studies with standardized methods to examine gene expression, as well as the use of multiple gene expressions. *Antioxid. Redox Signal.* 9, 1237–1294.

INTRODUCTION

TUMOR HYPOXIA RESULTS from oxygen consumption that exceeds oxygen supply and leads to increased vessel formation and genetic alterations. These adaptive responses promote tumor progression and treatment resistance to both radio- and chemotherapy. Several studies have shown that tumor hypoxia is significantly related to poor patient prognosis in various tumors, including head and neck and cervical cancers (113, 220). Improved knowledge of tumor oxygenation may assist in predicting treatment outcome and ideally help select patients for investigation of new therapies designed to overcome or target these adaptive characteristics.

There are several methods to assess tumor hypoxia, ranging from direct invasive *in vivo* methods to indirect *ex vivo* methods. Direct measurements using microelectrodes and oxygen-sensitive fiberoptic probes are widely used; however, these invasive techniques have their limitations, such as tumor accessibility, sparse sampling, and relative inability to distinguish amongst hypoxia occurring in necrotic versus viable tumor and in adjacent surrounding normal stroma, unless biopsies are examined from the areas of electrode track (114).

Immunohistochemistry using 2-nitroimidazoles can spatially localize tumor hypoxia. 2-Nitroimidazoles are exogenous markers that are metabolized and bind to proteins in oxygen-deficient cells (28, 70, 205). There are two types of 2-nitroimidazoles in clinical use: EF5 and pimonidazole. Recent studies have shown that pimonidazole binding, along with vascular density, can predict treatment outcome in head and neck cancer (136). Comparative studies of exogenous markers and needle electrodes, however, showed no correlation. This lack of correlation may reflect the inability of users to identify and avoid measurement of microscopic necrotic regions with oxygen electrodes (133, 219). A disadvantage of hypoxia marker drugs, on the other hand, is the need to be intravenously injected several hours or even a day before biopsy.

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Assessing intrinsic molecular markers might be a more reliable method for determining the relation between hypoxia and consequential molecular and physiologic changes. Several recent key publications have identified hypoxia-induced genes, using microarray (36, 164) and SAGE analysis (182). These genes are correlated with poor patient treatment outcome (36, 59).

This review will specifically focus on 15 hypoxia-inducible genes in serum and tissue samples of various types of cancers. Their activation under hypoxia and their influence on prognosis will be discussed with summaries from 213 studies. Articles were identified from a MEDLINE search, using a strategy based on the combinations of keywords, tumor, patients, hypoxia, and names of genes.

HYPOXIA-RESPONSIVE FACTORS

Tumor hypoxia upregulates various transcription factors and chaperone proteins, as shown in Fig. 1. Hypoxia-inducible factor-1 (HIF-1), in particular, is the most studied transcription factor since it is highly involved in tumor progression. Hypoxia causes HIF-1 to regulate >70 genes involved in tumor metabolism, angiogenesis, tissue remodeling, apoptosis, and erythropoiesis. Other transcription factors such as nuclear factor-κB (NFκB) and activator protein-1 (AP-1) are also activated by hypoxia, but their induction is less sensitive than that of HIF-1. Stress-induced chaperone proteins, including heat shock proteins (Hsps) and glucose-regulated proteins (GRPs), are also upregulated by hypoxia.

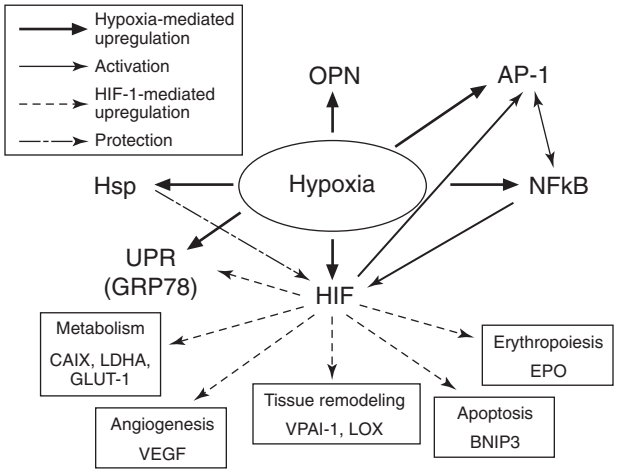

FIG. 1. Hypoxia upregulates various transcription factors (such as HIF, AP-1, and NF-kB) and chaperone proteins (such as Hsp and UPR), as well as OPN. These hypoxia-responsive factors especially interact with HIF by activating and protecting it. HIF also regulates gene transcription that is involved in tumor progression such as metabolism, angiogenesis, tissue remodeling, apoptosis, and erythropoiesis. AP-1, activator protein-1; HIF, hypoxia-inducible factor; Hsp, heat shock protein; NF-kB, nuclear factor-kB; OPN, osteopontin; UPR, unfolded protein response.

Transcription factors

HIF-1. Mechanism of HIF-1 response. Activation of HIF-1 is a primary response to hypoxia. HIF-1 is a heterodimeric transcription factor, and both of its α and β subunits belong to a basic helix–loop–helix (bHLH)-PER-ARNT-SIM (PAS) family (310). Whereas HIF-1β, which is also known as ARNT, is constitutively active, HIF-1α is activated by hypoxia. Hypoxia-induced HIF-1α activation was first identified by its binding to erythropoietin (EPO) promoter (254). Activated HIF-1α binds to the target gene promoter, known as the hypoxia response element (HRE), and regulates transcription with other cofactors, such as CBP/p300 (6). These cofactors bind to the transactivation domain (TAD) to help maintain the proper conformation of HIF-1.

Under normoxia, stability and activation of HIF-1α are disrupted by the hydroxylation-mediated pathways. In this process of destabilization, two highly conserved proline sites (Pro402 and Pro564) in the oxygen-dependent degradation (ODD) domain are hydroxylated by prolyl 4-hydroxylase (PHD). In mammalian cells, three PHDs (PHD1, 2, and 3) exist, with PHD2 being the predominant moiety (16). Hydroxylated prolines facilitate the binding of von Hippel Lindau protein (VHL) (125, 201) to form the E3 ligase complex that leads to ubiquitination-mediated proteasomal degradation of HIF-1α. In addition to PHD, the factor inhibiting HIF-1 (FIH-1) also regulates HIF-1α stability and transcriptional activity. FIH-1 hydroxylates asparaginyl residue (Asn803) in the TAD domain and inhibits the binding of the coactivators, CBP and p300 (194). Both PHDs and FIH require O_2, Fe^{2+}, and 2-oxoglutarate for activation (253), which explains the stabilization of HIF-1α by incubating cells with an iron chelator such as desferrioxamine (DFO).

HIF-1α is also regulated via oxygen independent means. Growth factors, including epidermal growth factor (EGF) and insulin-like growth factor (IGF), and their receptors such as Her2/*neu* upregulate HIF-1α via the PI3K/Akt/mTOR pathway. MAPK, especially p42/44 MAPK, is also reported to phosphorylate HIF-1α and increase its transcriptional activity (242), though its mechanism is not well understood.

HIF-1α is involved in tumorigenesis by the activation of its downstream genes that are associated with angiogenesis, apoptosis, and glycolysis. Overexpression of HIF-1α was found in breast, cervix, lung, brain, ovarian, and prostate cancers, though its relation with poor prognosis seems to be dependent upon tumor type. HIF-1α expression is also associated with resistance to radiotherapy (3, 169). Disruption of HIF-1 activity inhibits tumor growth and increases radiosensitivity (173, 332), and recently, the mechanism of HIF-1α stabilization by tumor reoxygenation following radiotherapy was determined (210). Therefore, HIF-1α has a significant potential as a prognostic marker and therapeutic target.

HIF-1-dependent markers. We have categorized HIF-1 regulated hypoxia markers by their involvement in cancer metabolism, angiogenesis, tissue remodeling, apoptosis, and erythropoiesis.

Metabolism

CAIX

Carbonic anhydrase IX (CAIX) is one of the transmembrane CAs catalyzing the reversible hydration of carbon dioxide that is crucial for normal physiologic function ($H_2O + CO_2 \leftrightarrow H^+ +$

HCO_3^-). It has a conserved zinc binding site and an extracellular catalytic domain. Its N-terminal region has similarity with the helix–loop–helix (HLH) family, which suggests its DNA binding potential.

The expression of CAIX depends on hypoxia. Gene expression studies using SAGE showed the greatest induction of CAIX among other hypoxia-induced genes (182). Studies using VHL-defective renal cell carcinoma (RCC) demonstrated that upregulation of CAIX under hypoxia is regulated by VHL, and ultimately by HIF-1α, since its HRE sequence (5′-TACGTGCA-3′) was identified in the promoter region (319). Immunohistochemical analysis of tumor samples also suggested correlation of CAIX with tumor hypoxia in patients by showing overexpression of CAIX in perinecrotic areas of tumor and its relation to tumor stage and microvessel density (5, 319). However, direct measurement of tumor hypoxia using the microelectrode failed to show consistant correlation with CAIX in different tumor types (183, 202).

Other than its correlation with hypoxia, CAIX plays a role in maintaining an acidic extracellular pH of the tumor (126). It has been known that lactic acid (or lactate), an end product of glycolysis, can cause the low pH, which characterizes tumors. However, several studies using glycolysis- (217) or lactate dehydrogenase (LDH)-deficient (321) cells suggested that CAIX is involved in tumor acidosis by converting CO_2 to carbonic acid and increasing the extracellular proton, which results in decreased pH of tumors (279).

LDHA

Tumor cells, even in the presence of oxygen, produce ATP by glycolysis, a process that converts glucose to pyruvate and produces lactate. Lactate dehydrogenase (LDH) is involved in the conversion of pyruvate to lactate. There are five LDH isoforms, and each of them has different numbers of M and H subunits. As the number of H subunits increases, the efficiency of pyruvate to lactate conversion decreases. LDHA, which is composed of four M subunits, displays the highest efficiency for this reaction, and its expression is related to continued glycolytic flux.

Hypoxia-induced expression of LDHA and its HIF-1 binding site was identified in previous studies (78, 79). Also, a strong correlation of LDHA with HIF-1 expression and poor prognosis was determined in non-small cell lung cancer (NSCLC) (168) and colorectal cancer (167). In addition to HIF-1, the involvement of LDHA in c-myc-mediated cancer cell transformation under hypoxia was studied (260).

GLUT-1

Glucose transporter-1 (GLUT-1) is one of fourteen members of the GLUT family that are involved in glucose supply and homeostasis by transporting glucose across the cell membrane (193). It is ubiquitously expressed in normal tissues and mainly associated with glucose supply in tissues.

GLUT-1 expression is regulated by hypoxia and inhibition of oxidative phosphorylation through two cis-acting elements in GLUT-1 promoter (14). They are involved in the distinct response to hypoxia by HIF-1 binding, and to inhibitors of mitochondria via serum responsive element (62).

Overexpression of GLUT-1 was found in breast (98), cervix (4), and rectal cancers (42), and it was correlated with tumor aggressiveness. Recently, the role of GLUT-1 as a prognostic marker was suggested in lung (208), gastric (143), rectal (42), and bladder (115) cancers. However, its correlation with tumor oxygenation is still disputed in several studies (4, 5, 203).

Angiogenesis

VEGF

Vascular endothelial growth factor (VEGF) is a secreted protein that is involved in the growth and survival of endothelial cells, vascular permeability, and angiogenesis (75). Other than VEGF (or VEGF-A), its family includes VEGF-B, C, D, E, and placental growth factor (PLGF). Whereas VEGF-A is the predominant factor that influences angiogenesis, VEGF-C and D regulate lymphatic angiogenesis. Alternative splicing of VEGF-A forms four isoforms including $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$ (297). $VEGF_{165b}$, a recently identified isoform has an anti-angigenic property (12).

VEGF-A forms a 46-kDa homodimer and binds to three receptor tyrosine kinases: flt-1 (VEGFR-1) (49), Flk-1/KDR (VEGFR-2) (292), and flt-4 (VEGFR-3) (85). Among them, binding of VEGF with Flk-1/KDR mainly induces angiogenesis and a mitogenic effect. Neuropilin (NRP-1), which was initially known to be associated with axonal guidance in nervous system, was also identified as a receptor for $VEGF_{165}$, but its signaling pathway is unknown (267).

VEGF expression is mostly regulated by oxygen. Under hypoxia, HIF-1 upregulates VEGF expression by binding to its HRE region (262) and this activates the tumor angiogenesis. Tumor cells require angiogenesis for growth and metastasis (103, 314), and inhibition of VEGF (149) and its receptor (207) inhibits tumor growth. PI3K/Akt pathway, EGFR, and loss of PTEN also regulates VEGF in an oxygen-independent manner (235). The recent study also suggested hypoxia and a HIF-1-independent mechanism that regulates proangiogenic activity of VEGF by showing induction of tumor angiogenesis before the activation of HIF-1 (31).

Tissue remodeling

PAI-1

In the process of fibrinolysis, plasminogen is converted to plasmin by two serine proteases, tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA). These PAs are inhibited by PA inhibitor (PAI), particularly by PAI-1. PAI-1 is produced by platelets, vascular endothelial cells, vascular smooth muscle cells, and several nonvascular cells such as hepatocytes (56) and associated with various functions including fibrinolysis, fibrosis, extracellar matrix turnover, and inflammation.

Especially in tumor cells, PAI-1 plays a critical role in regulating angiogenesis and cell growth. Several studies using PAI-1 knock-out mice showed delayed tumor growth and inhibition of angiogenesis (11, 101). A recent study suggested that excessive plasmin formation in the absence of PAI-1 disrupts stabilization and assembly of blood vessels (11). This indicates that PAI-1 promotes angiogenesis by inhibiting proteolysis.

The association of low oxygen and PAI-1 expression was first identified in rat hepatocytes (147). This study showed PAI-1 was induced at oxygen concentration $\leq$8%, and this induc-

tion was mediated by HIF-1 binding to HRE-2, a hypoxia-responsive promoter sequence. A later study of PAI-1 further demonstrated its upregulation in human hepatoma cell lines at 1% and 2% oxygen concentration (77). PI3K/Akt and MAPKs are also known to be involved in PAI-1 expression in an HIF-1-dependent way (56).

LOX

Lysyl oxidase (LOX), a copper-dependent amine oxidase, initiates covalent crosslinking of collagen and elastin, which maintains extracelluar membrane (ECM) stability. Since LOX expression is decreased or absent in various tumor cells including human melanoma, head and neck cancer, and fibrosarcoma, it has been known as a tumor suppressor gene. Ras-transfected NIH 3T3 cells showed decreased expression of LOX (41), and inhibition of LOX results in phenotypic transformation and Ras activation (91). A later study identified that LOX regulated Ras-mediated transformation by inhibiting upstream signal pathways of NFκB (131). Thus, decreased LOX expression reduces ECM stability and leads to tumor progression by increasing cell invasion.

On the other hand, several studies showed increased LOX expression in invasive/metastatic breast cancer (158). Comparison of highly invasive/metastatic and poorly invasive/metastatic breast cancer indicated that LOX expression was tumor type dependent, and when it was overexpressed, LOX played a role in cancer cell invasion (157). Recently, it was shown that LOX regulates cell migration via hydrogen peroxide-mediated pathways (231).

Hypoxia-induced LOX expression was first identified from microarray studies in human tumor cell lines (53). Recently, hypoxia-induced expression of LOX and its association with HIF-1 was reported in human breast and head and neck cancers (69). In this study, overexpression of LOX was also related to poor prognosis and tumor metastasis. Thus, LOX can be used as a hypoxia marker and potential target to inhibit hypoxia-induced metastasis.

Apoptosis

BNIP3

Bcl-2/E1B 19 kDa interacting protein (BNIP3), also known as a member of the Bcl-2 homology 3 (BH3)-only family, promotes mitochondrial apoptosis. Though it interacts with anti-apoptotic proteins, Bcl-2 and Bcl-x through BH3 domain, the apoptotic function of BNIP3 is dependent on its transmembrane domain (238). Cell death induced by BNIP3 has characteristics of both apoptosis and necrosis. It shows DNA condensation, characteristic of apoptosis, as well as caspase independence and mitochondrial damage, which are characteristics of necrosis (306).

In most organs, expression of BNIP3 is barely detectable, but it is highly induced under hypoxic conditions via the HIF-1 pathway (24). BNIP3 is also induced by a zinc finger protein, PLAGL2, arsenic trioxide (As_2O_3), and nitric oxide in a HIF-1 independent way.

In breast (270) and NSCLC (94), perinecrotic expression of BNIP3 is significantly increased with tumor malignancy. This indicates that BNIP3 plays a role in the selection of more aggressive forms of tumor. In the selecting pathway, the proapoptotic role of BNIP3 is regulated by various factors. A recent study showed that hypoxia-induced EGF and IGF suppresses BNIP3 (165). This study demonstrated that EGF and IGF protected BNIP3-mediated apoptosis without expressional change of BNIP3. Also, it was reported that hypermethylation of the promoter region caused BNIP3 silencing in pancreatic cancer (222), and colorectal and gastric cancers (213). Therefore, expression of BNIP3 regulates tumor progression by balancing pro- and anti-apoptotic process.

Erythropoiesis

EPO

Erythropoietin (EPO) is a glycoprotein that has an important role in erythropoiesis and O_2 delivery. It regulates survival and proliferation of red blood cell progenitors by inhibiting apoptosis; this process results in increased hemoglobin levels. EPO is mainly produced in the fetal liver and adult kidney, but is also found in the central nervous system, endothelial cells, and uterus. EPO binding induces homdimerization of its cell surface receptors and activates intrinsic signaling pathways such as JAK2, Ras, PI3K, STAT5, and MAPK (132). After activation, an EPO–EPO receptor complex internalizes and undergoes proteasomal degradation that controls EPO signaling.

Semenza and Wang first identified that increased EPO is mediated by HIF-1 (254). This suggests a role of EPO in an adaptive response to hypoxia by inducing cell proliferation and angiogenesis. Recently, elevated expression of EPO and EPO receptor (EPOR) was also found in various human cancers including lung, breast, cervix cancers, and melanoma (73). However, the role of this ligand/receptor in tumor progression has not been extensively examined clinically. In preclinical studies, expression of the EPO receptor confers increased AKT phosphorylation in response to EPO, but this does not seem to translate into significant effects on tumor growth, angiogenesis, or chemotherapy response (108).

HIF-2α and HIF-3α. Two other bHLH-PAS proteins, HIF-2α and HIF-3α, which share sequence identity of HIF-1α, were identified (100, 317). HIF-2α and HIF-3α regulate gene expression through the HREs, when they are stabilized and dimerized with ARNT under hypoxia. Similar to HIF-1α, these transcription factors are also targeted by VHL for ubiquitination-mediated degradation.

HIF-2α, previously termed as endothelial PAS protein-1 (EPAS-1), is expressed in most of tumor cells including bladder, breast, colon, glial, hepatocellular, ovarian, pancreatic, prostate, and renal tumors, and also in tumor-associated macrophages (TAM) (286). HIF-2α also upregulates transcription of HIF-1α target genes such as EPO, VEGF, uPAR, and PAI-1. However, HIF-1α and HIF-2α each have separate, cell-type specific roles in gene regulation. In VHL-deficient renal cell carcinoma, the VEGF expression induced by HIF-2α was higher than HIF-1α (161). In addition, inhibition of HIF-1α or HIF-2α using siRNA showed their distinct gene regulation in breast cancer and renal cell carcinoma (271). To explain these differential roles, a recent study suggested reciprocal relationship of these factors by showing increased HIF-1α-induced VEGF expression following the inhibition of HIF-2α in breast cancer cells (32).

The expression of HIF-3α was identified in human thymus, lung, brain, heart, and kidney (105). However, in contrast to HIF-1α and HIF-2α, HIF-3α, which lacks C-terminal transactivation domain, suppressed HRE-driven gene expression.

NF-kB. Nuclear factor-kB (NF-kB) is a transcription factor that plays a crucial role in inflammatory response, apoptosis, and cell cycle regulation. Its target genes include cell adhesion molecules, cytokines/growth factors, c-myc, and possibly the tumor suppressor gene, p53.

NF-kB forms homo- or heterodimer of NF-kB/Rel family members including p65 (RelA), RelB, c-Rel, v-Rel, p50 (NF-kB1), and p52 (NF-kB2). p65–p50 is the most prominent form of the dimer, and it is ubiquitously expressed. A NF-kB dimer consisting of p65 or RelB, exists in cytoplasm and interacts with the inhibitors of kB (IkB) (293). When NF-kB is activated by signals including hydrogen peroxide and cytokines, it induces IkB phosphorylation, which leads to ubiquitination-mediated degradation of IkB (55). Then, dissociated NF-kB dimer translocates to the nucleus and binds to DNA. DNA binding is mainly mediated by p65 (251).

Activation of NF-kB under hypoxia was first identified by showing increased binding of NF-kB p65 (RelA) to its consensus sequence on the cyclooxygenase 2 (COX-2) promoter in response to hypoxia (250). However, it is not known whether NF-kB is regulated by reoxygenation rather than hypoxia.

Involvement of NF-kB in tumorigenesis was first found from highly oncogenic v-Rel, which is a viral homolog of c-Rel. Overexpression, amplification, and rearrangement of other family members are also found in various cancer types (233). Involvement of NF-kB in oncogenic signaling pathways, apoptosis, and cell adhesion also suggests its role in tumor progression. Recently, it was reported that NF-kB played a role in TNFα-mediated HIF-1 accumulation, which was hypoxia independent (334).

AP-1. Activator protein-1 (AP-1) is a transcription factor, which forms homo- or heterodimer with basic-region leucine Zipper (bZIP) proteins including Jun and Fos. AP-1 complexes bind to TPA response element (TRE) and cAMP response element (CRE) of the target gene promoter and activate gene transcription. AP-1 is activated by growth factors, environmental stress, and cytokines (255). Its activation is associated with cell proliferation, survival, differentiation, tumor invasion, and angiogenesis by regulating target genes such as bFGF, VEGF, and c-fos, and the ERK1/2MAPK pathway (64). Therefore, it is involved in the oncogenic pathway.

Hypoxia-induced activation of AP-1, especially c-jun and JunD, is found in various cancer cells such as hepatoma, colorectal and cervix cancer cells. A recent study reported that AP-1 induction and phosphorylation under hypoxia were mediated by HIF-1 (181). Phosphorylation of c-jun is required for dimerization with c-fos. HIF-1-mediated activation of AP-1 was also associated with hypoxia-induced VEGF transcription (45). AP-1 also interacts with NF-kB and this interaction increased their transactivation (275).

Chaperone proteins

Heat shock protein (Hsp). Heat shock protein (Hsp) is a chaperone protein involved in protein refolding, inhibition of unfolded protein aggregation, and ultimately, cell survival in response to various stimuli including heat, hydrogen peroxide, and toxic chemicals (29). The family of Hsp includes Hsp10, Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp110, which are named according to their molecular weight.

Hsps are involved in tumorigenesis by regulating apoptosis and mitogenesis (273). They are involved in anti-apoptotic pathways by inhibiting cytochrome c release or by regulating anti-apoptotic proteins such as Bcl-2. However, stabilization of p53 or procaspase 3 indicates their pro-apoptotic function. Hsps also play a role in cell survival and proliferation by regulating PI3K/Akt pathway. Recently, activation of host immunity by Hsp60, Hsp70, and Hsp90 was reported (298). These all suggest that Hsps regulate tumorigenesis by balancing pro- or anti-oncogenic pathways.

Hsps are regulated by heat shock factor (HSF) that binds to heat shock element (HSE) on the promoter. Among the four types of HSF, HSF-1 is the main stress-induced transcription factor. HSF-1, when it is not activated, exists as a monomer. Its binding with Hsp90 inhibits its activation. In response to stimuli, HSF-1 dissociates from Hsp90, hyperphosphorylates, and forms a trimer, which is its activated form.

Hypoxia-induced activation of Hsps was first identified in cardiac tissue. Following studies also showed expression of Hsps and HSF under hypoxia in various cell lines (15, 257, 276). Hsps are overexpressed in most cancer cells and are associated with poor prognosis (39). Especially, Hsp27 and 70 expression is involved in therapy resistance and anti-apoptotic pathway (86). Hsp90 also plays a role in tumor progression by regulating Her2 and c-myc. A recent study also showed that Hsp90 interacts with HIF-1α and this binding, which is involved in HIF-1 stabilization, is oxygen and VHL independent (335).

Unfolded protein response (UPR). The endoplasmic reticulum (ER) plays a role in the protein synthesis, post-translational modification, proper folding, and secretion. Under various ER stress including nutrient deprivation, disrupted calcium signaling, hypoxia, redox status, and inhibition of glycosylation, unfolded proteins are accumulated in ER and unfolded protein response (UPR) is induced. As a consequence of UPR, translation of misfolded protein is decreased, while that of ER chaperones such as glucose-regulated protein (GRP) is enhanced. Also, ER-associated degradation of unfolded proteins increases. These mechanisms are highly involved in cell survival and apoptosis.

UPR is initiated by three membrane receptors: PERK, IRE1, and ATF6. Normally, these receptors are inactivated by the association with GRP78, a chaperone protein. GRP78, itself a UPR target gene, and was first identified to be induced in the response of glucose deprivation along with GRP94 (185). Dissociation of GRP78 by ER stress allows PERK, IRE1, and ATF6 to bind to unfolded proteins, to reduce the translation of newly synthesized proteins via phosphorylation of transcription initiation factor eIF2α which inhibits ER stress-induced cell apoptosis. This process also induces the activation of the transcription factor that binds to the ER stress response element (ERSE) as well as an unfolded protein response element (UPRE), and activates the transcription of UPR target genes, GRP78 and GRP94. Recent studies also identified another transcription factor, ATF4, that regulates GRP78 in ERSE-independent manner (192).

Hypoxia-induced upregulation of GRP78 and GRP94 was found in various cancer cells (74, 311, 320) and is associated with tumor aggressiveness and poor differentiation. GRP78 expression is involved in tumor growth (130) and therapy resistance by inhibiting apoptosis (239). A recent study demonstrated the increased GRP78 expression following cisplatin treatment that indicates its role in chemoresistance (197). Expression of GRP94 in colonic cancer is also related to lymph node and remote metastasis and poorly differentiated cancer cells (312). A recent study showed that HIF-1α regulates hypoxia-induced GRP94 expression through HRE sequences binding (230). However, no HRE sequences are found in GRP78 promoter suggesting that its regulation is HIF-1 independent.

Others. *Osteopontin.* Osteopontin (OPN) is a glycoprotein, which is secreted, phosphorylated, and accumulated in body fluids. As a key bone matrix protein, OPN is produced by osteoclasts, macrophages, kidneys, lymphocytes, and vascular smooth muscle cells; it is associated with various roles in bone remodeling, the immune system, and angiogenesis (237).

OPN is also involved in tumorigenesis by interacting with integrin receptors to regulate cell growth, adhesion, degradation of extracellular matrix, and migration (243). Expression of OPN in breast, lung, prostate, gastric, brain, and ovarian cancers suggests OPN as a potential tumor progression marker and therapy target (313).

Hypoxia-induced expression of OPN was assessed in human cervix squamous cell carcinoma (SiHa) along with other hypoxia markers, CAIX, GLUT-1, and LDHA (268). Slow induction of OPN under hypoxia, and its oxygen sensitivity, suggest its potential as a chronic hypoxia marker rather than acute hypoxia. Comparing plasma levels of OPN with pO_2 measurement confirmed the correlation between patient tumor hypoxia and the OPN level which is associated with poor clinical outcome (184). This study also identified the inverse correlation of OPN expression and VHL using microarray though its association with HIF-1 was not determined yet.

CLINICAL APPLICATION OF HYPOXIA MARKERS

The significance of hypoxia markers in predicting cancer patient outcome has been evaluated in a number of studies. Table 1 summarizes 213 studies analyzing the potential of hypoxia-inducible genes as prognostic markers in 19 different tumors. Twenty-five of these studies evaluated more than two genes. To determine the statistical significance, all studies used Kaplan–Meier's survival curve, and 161 of these studies also included multivariate analysis. Cancers were categorized into carcinoma, sarcoma, brain tumor, lymphoid/leukemia, and others. Correlation between hypoxia-inducible genes and patient survival was evaluated mostly using immunohistochemistry or ELISA. Several studies also analyzed expression of mRNA by RT–PCR or Northern blot, and protein was analyzed by Western blot. Table 2 reveals that in most of cases, these genes showed prognostic significance for treatment outcome, and some of them were identified as independent prognostic factors using multivariate analysis.

HIF-1

HIF-1 expression was analyzed in 37 studies with 11 different types of tumors. They include breast (22, 44, 99, 171, 249, 309), head and neck (3, 13, 76, 119, 141, 155, 169, 170, 176, 200), lung (66, 92, 151, 280), ovarian (18), cervix (10, 19, 27, 121, 124, 204), gastric cancers (304), renal cell carcinoma (187), colorectal (325), bladder cancers (216, 227, 294, 295), endometrial carcinoma (265), and brain tumors (18, 236). Among them, 22 studies showed significant correlation with poor patient outcome by using p value from Kaplan–Meier's survival curve. Multivariate analysis was performed in 23 studies, and 12 studies identified HIF-1 as an independent prognostic marker for poor patient outcome. On the other hand, three studies determined HIF-1 expression as a favorable prognostic factor (13, 76, 187). This suggests that HIF-1 is not the most efficient prognostic factor. Its conflicting prediction of poor or better survival of patients might be due to the role of HIF-1 in promoting apoptosis by inducing BNIP3, a pro-apoptotic gene, or by stabilizing p53, a tumor suppressor gene. Also, since conventional RCC is characterized by its high VHL mutation rate, HIF-1 expression could be regulated independently of hypoxia.

In various tumors including breast, head and neck, lung, cervix, gastric cancers, and endometrial carcinoma, HIF-1 expression was also associated with CAIX and VEGF: this indicates its role in tumor hypoxia and angiogenesis. However, the direct measurement of hypoxia was performed only in three studies by measuring hemoglobin concentration (27), pimonidazole staining (121), or using microelectrodes (204), and two of those studies showed significance.

In four studies, though expression of HIF-1 alone was not statistically significant, combined expression of HIF-1/HIF-2, HIF-1/p53, HIF-1/VEGF, and HIF-1/CAIX was correlated with patient outcome (19, 119, 169, 325). These studies indicated that the combined expression of HIF-1 could be the alternate use of endogenous hypoxia markers.

HIF-2

The correlation of HIF-2 expression and patient outcome was analyzed in seven studies with head and neck (13, 166, 169), lung (92), colorectal (325), bladder cancers (159), and endometrial carcinoma (265). Five of those studies showed statistical significance of HIF-2 expression with p value from Kaplan–Meier's survival curve. Among five studies that performed multivariate analysis, four studies in head and neck (92, 169, 325), lung (325), and bladder (166) cancers identified independence of HIF-2 as a prognostic marker.

In head and neck (13, 169) and bladder cancers (159), HIF-2 expression was found in tumor-associated macrophages (TAM). It was also correlated with HIF-1, VEGF, and microvessel density (MVD) (92, 169, 325). This indicates that HIF-2 also plays a role in tumor angiogenesis.

As already mentioned above, combined expression of HIF-1 and HIF-2 was a prognostic factor in colorectal cancer (325). In addition, when HIF-2 was coexpressed with CAIX, it was associated with poorer patient survival in head and neck cancer, though there was no correlation between expression of HIF-2 and CAIX (166).

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types

						Overall survival			*Disease free survival*			*Local control*			
		S	*R*	*C*	*H*	p	*HR*	*Kaplan-Meier's* p	p	*HR*	*Kaplan-Meier's* p	p	*HR*	*Kaplan-Meier's* p	*Additional comments*
Carcinoma															
Breast cancer															
HIF-1	LN +, 206 patients, Schindl (2002), IHC: S/I (249)			X	X	0.003	1.41	0.0454	0.0001	1.4	0.0008				
	150 (81) patients, Bos (2003), IHC: S (I for LN-) (22)	X	X	X	X	0.066 0.021 :LN−	2.155 6.365 :LN−	0.059 0.008 :LN−	0.115 0.008 :LN−	1.672 4.194 :LN−	0.11 0.004 :LN−				Prognostic significance with LN-patients.
	T1/T2 stage, 77 (55) patients, Gruber (2004), IHC: (S/I for T1/2) (99)	X	X	X	X	0.09 :LN+	2.66 :LN+	0.21	0.01 :T1/2 0.3 :LN+	7.04 :T1/2 1.68 :LN+	0.004 0.028 :T1/2	0.01 :T1/2 0.18 :LN+ metastasis free	7.12 :T1/2 1.94 :LN+ metastasis free	0.04 0.016 :T1/2 metastasis free	Prognostic significance in T1/T2 stage and LN+ tumors.
	Invasive, 166 patients, Vleugel (2005), IHC: S (309)	X		X	X						0.01: overall 0.04: diffuse 0.02: necrotic				Perinecrotic HIF-1 expression was associated with CAIX and Glut-1 expression.
	745 patients, Dales (2005), IHC: S (44)	X				0.03		0.019	0.158 0.317 :LN− relapse free		0.015 0.035 :LN− relapse free	0.023 0.0106 :LN− metastasis free		0.008 0.03 :LN− metastasis free	Weak significance with LN-patients.
	564 patients, Kronblad (2006), IHC: S (I for NHG I/II) (171)	X	X	X	X			0.11	0.18 0.033 NHG I/II	1.4 2.3 NHG I/II	0.048 0.59 :LN− 0.014 :LN+ recurrence free 0.028 cancer specific				No significant association with :LN− patients, no predictive value to tamoxifen response.
	Invasive, 103 patients, Chia (2001), IHC, S/I (37)	X	X	X	X	0.05	2.61	0.001 (+/−) <0.001 (level)	0.06 relapse free	2.13 relapse free	0.004 (+/−) relapse free				Strong association with tumor necrosis, higher grade, and Er.

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			Disease free survival			Local control			
						Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Additional comments
		S	R	C	H	p	HR	p	p	HR	p	p	HR	p	
CAIX	253 patients, Span (2003), RT-PCR NS (but S/I for therapy response) (272)	X	X	X	X					0.018 0.624 :radio <0.001 :chemo :endocrine therapy relapse free	0.77 0.89 :radio 1.31 :chemo 1.41 :endodrine therapy relapse free	0.926 relapse free			Significant correlation with resistance to adjuvant chemo- and endocrine therapy
Glut-1	141 patients, Stackhouse (2005), IHC (tissue microarray), NS (274)		X	X	X			0.063							
VEG-F	LN−, 525 patients, Linderholm (1998), ELISA: VEGF165, S/I (189)	X	X	X		0.0199	2.72 3.09 :patients w/o treatment	0.0012 0.0128 :patients w/o treatment							Higher VEGF expression in ER positive patients.
	LN−, 362 patients, Linderholm (2000), ELISA: VEGF165, S/I for OS (188)	X	X	X	X	0.017 0.042: endocrine treatment	1.82 1.9: endocrine treatment	0.0004 0.0263 ER + 0.121 endocrine treatment 0.0235 chemo-	0.2222 0.142: endocrine treatment	1.29 1.48: endocrine treatment	0.0289 0.0346 ER + 0.0238 endocrine treatment 0.6129 chemo- relapse free				Independent predictor for LN− patients patients with ER after endocrine treatment.
	LN−, 242 patients De Paloa (2002), IHC, NS (46)	X	X					0.242			0.398				No correlation with survival, but strong correlation with MVD.
	LN−, 489 patients, Manders (2003), breast-conserving therapy, ELISA, S (195)	X	X			0.004 :local treatment	29.22 :local treatment	0.028	0.012 :local treatment	2.96 :local treatment	0.004 :relapse free				

	193 (99) patients, Desruisseau (2004), ELISA, S/I (53)	X	X	X	X	0.03 :LN+	3.89 :LN+	0.06 0.03 :LN+				Strong correlation with amphiregulin (AR), more expression in ductal carcinoma rather than lobular.
	611 patients, Konecny (2004), ELISA, VEGF121 & 165, S/I for LN+ patients (163)	X	X	X	X	0.1475: 121 0.1483: 165 0.0103: 121 0.0150: 165 :LN+	1.05: 121 1.08: 165 1.12: 121 1.18: 165 :LN+	0.0068: 121 0.0046: 165 0.0003: 121 0.0038: 165 :LN+	0.9287: 121 0.9754: 165	1.00: 121 1.00: 165		Strong correlation with Her2 expression, combination of Her2 & VEGF showed additional prognostic information (p = 0.0133, 0.0092).
PAI-1	657 patients, Foekens (1994), ELISA, S/I (80)	X	X	X	X	0.02	1.5	<0.001	<0.001 <0.001 LN− <0.001 LN+	2.03 3.1 LN− 1.8 LN+	<0.001 relapse free	uPA also showed significant correlation with patient survival.
	LN+, 100 (premenopausal)/ 1/50 (postmenopausal) patients, Grondahl-Hansen (1997), ELISA, S/I for postmenopausal women (97)		X	X	X	0.98 pre- 0.001 post-	− pre- 2.27 post-	0.51 :pre- 0.0001 :post-	0.76 pre- 0.16 post- recurrence free		0.62 pre- 0.03 post- recurrence free	Negative correlation with ER and PgR, uPA was also an independent marker for recurrence free survival of postmenopausal women.
	LN−, 130 patients, Kim (1998), ELISA, S (153)	X	X	X	X				0.141	3.03	0.032	uPA was an independent marker, while tPA showed inverse correlation with patient outcome.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

					Overall survival			*Disease free survival*			*Local control*			
					Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
	S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
LN−, 90 patients, Kute (1998), ELISA, S/I for disease free survival (177)	X	X			0.82		0.93	0.0001		0.25				Significant correlation with uPA and uPAR, but not with cathepsinD, combined expression with cathepsinD is an independent marker.
316 patients (139: LN−, 120: relapsed), Harbeck (1999), ELISA, S/I (107)	X	X	X	X	<0.001 <0.001 :LN− 0.006 relapsed	2.8 6.6 :LN− 1.9 :relapsed	<0.001 <0.001 :LN− <0.001 :relapsed	<0.001 <0.001 :LN−	3.1 4.1 :LN−	<0.001 <0.001 :LN−				Combined expression with uPA showed better prognostic prediction.
892 patients, de Witte (1999), ELISA, S/I (51)	X	X	X	X	<0.001	2.41	<0.001	<0.001 relapse free	1.84 relapse free	<0.001 relapse free				Strong correlation with uPA, coexpression with uPA showed poorer outcome.
865 patients,de Witte (1999), ELISA, S/I (50)	X	X	X	X	<0.001 cytosol <0.001 pellet	2.01 cytosol 1.7 pellet	<0.001 cytosol <0.001 pellet	0.002 cytosol <0.001 pellet	1.43 cytosol 1.45 pellet					Cytostolic tPA and PAI-1 complex was an significant predictor. tPA was correlated with favorable prognosis.
342 patients, Pedersen (2000), ELISA, S/I (232)	X	X			<0.001	3.5	<0.001	<0.001 recurrence free	3.8 recurrence free	<0.001 recurrence free				uPA-PAI-1 complex is highly expressed in LN− tumors, and significantly associated with OS ($p = 0.005$) and RFS ($p = 0.03$).

	276 patients (130 LN−), Harbeck (2001), ELISA, S/I (106)	X	X	X	X	<0.01 0.004 :LN−	1.9 3.7 :LN−		0.027 0.004 :LN−	1.7 3.7 :LN−	<0.001 without treatment		Strong correlation with uPA, no significance in predicting outcome after adjuvant therapy.
	228 patients, Hansen (2003), ELISA, S/I for DFS (104)	X	X	X	X	0.28	1.3	0.0004	0.4	1.7	0.0001		Positively correlated with LN metastasis.
	LN−, 576 patients, Manders (2004), ELISA, S/I for RFS (196)	X	X					0.025	0.007 recurrence free	2.37 recurrence free	<0.001 recurrence free		uPA and uPA-PAI-1 complex were independent predictors of overall survival.
	193 (99 LN+) patients, Desruisseau (2004), ELISA, S/I for OS (54)	X	X	X	X	0.05 LN+	3.10 LN+						Strong correlation with VEGF.
LOX	ER−, Erler (2006), microarray, S (69)	X	X	X	X			0.015				0.009 distant metastasis free	Significant correlation with hypoxia ($p < 0.0001$).
NFkB	82 patients, Buchholz (2005), IHC, NS (25)	X		X				0.979			0.359	0.951 local regional recurrence free	Strong correlation with Bcl-2, Bax, and NFkB.
AP-1	85 patients, Bland (1995), IHC: c-fos, S (20)	X	X	X	X	0.0252	4.214						Coexpression with other oncogenes (H-ras, c-myc, and p53) showed poor survival and recurrence ($p < 0.0001$).
	78 patients, Gee (2000), IHC: phosphorylated c-Jun, S trend (87)	X			X			0.061 (all) 0.14 (ER+) 0.055 (ER−) 0.176 (all) 0.018 (ER+) : endocrine response					Correlation with MAPK elements (TGFα, 1/2 MAP kinase) but not with c-fos, correlation with distant metastasis.

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			Disease free survival			Local control			
						Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	
		S	R	C	H	p	HR	p	p	HR	p	p	HR	p	Additional comments
	Invasive tumor patients, Vleugel (2006), IHC phosphorylated c-Jun, NS (308)	X	X	X	X			0.49 nuclear 0.09 mitotic 0.1 stromal							Expression in nuclei and invasive front of mitotic cell, correlation with MVD, pRB.
Hsp	Axillary LN, 425 patients (western blot) and 788 patients (IHC)< Oesterreich (1996): Hsp27, NS (221)	X	X	X	X			0.46 :western blot 0.13 :IHC			0.58 western blot 0.44 :IHC				Nuclear: VEGF, Mitotic: metastasis, Stromal: grade Correlation with ER, PgR, and aneuploidy. Significant trend of OS and DFS in ER+ or Dox untreated patients.
	Estrogen receptor positive, 205 patients, Ciocca (1998), IHC, Hsp27 and Hsp70, cyto-plasmic (c) vs. nuclear (n), NS (40)			X				0.57 hsp27c 0.23 hsp27n 0.57 hsp70c 0.1 hsp70n							Correlation between hsp27 and hsp70 expression, no significant relation of response to tamoxifen treatment.
	35 patients, Vargas-Roig (1998), IHC, S for hsp27 and 70 (307)	X	X	X	X			NS			0.027 hsp27 0.045 hsp70n NS hsp90, hsc70				Increased expression of hsp27 and nuclear hsp70 after chemotherapy.
	243 patients, Mestiri (2001), PCR: hsp70-2, S for longer overall survival (206)	X	X	X	X			<0.04			NS				

OPN	Metastatic, 70 patients, Singhal (1997), ELISA, S/I (264)					<0.0001		<0.0001							Higher expression of OPN in tumor patients with multiple metastasis, association with other metastatic indicators.
	LN−, 154 patients Tuck (1998), IHC, S/I (299)				0.0138	2.971	0.0294	0.3217	1.634	0.0025					OPN expression in macrophages and lymphocytes.
	333 patients, Rudland (2002), IHC, S/I (245)	X						<0.0001 cancer related	<0.0001 cancer related	12.9					Expression in macrophages and lymphocytes, correlation with c-erbB3, p53, and pS2.
	Metastatic, 109 patients, Bramwell (2006), ELISA, S/I (23)	X	X	X	X	0.038 baseline 0.0003: OPN >250 ng	1.001 baseline 3.261 OPN >250 ng	0.0012 baseline (>123 ng/ml)							Increases in OPN levels have strong association with poor survival.
	Invasive, 312 patients, de Silva Rudland (2006), IHC, S (48)	X	X	X	X	0.067		0.005							Correlation with ERα staining.
Head and neck squamous cell carcinoma (HNSCC)															
HIF-1	Orsopharyngeal cancer, 98 patients, Aebersold (2001), IHC: S/I (3)		X	X		0.0009	2.35	0.001	0.004	2.2	0.008	0.002	2.43	0.006	
	Early esophageal cancer, 37 patients, Koukourakis (2001), IHC: NS (170)	X						0.08						0.14: local relapse free	Inverse correlation with Bcl-2.
	79 patients, Beasley (2002), IHC: S/I for favorable prognosis (13)	X	X			0.035	0.292	0.027	0.028	0.364	0.016				

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

					Overall survival			*Disease free survival*			*Local control*			
					Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
	S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Nasopharyngeal carcinoma, 90 patients, Hui (2002), IHC: NS (119)			X	X			0.06			0.12 progression free				HIF-1 expression was correlated with CAIX and VEGF, co-expression: significant with PFS (0.04).
75 patients Koukourakis (2002), IHC: NS (169)		X	X		0.88		0.05	0.3		0.003 relapse free				Combined expression of HIF1 and HIF2 significance.
Oesophageal cancer, 130 patients, Kurokawa (2003), IHC: S (176)	X	X	X		0.1669	1.539	0.007							Correlation with LN metastasis.
Oesophageal squamous cell carcinoma, 82 patients (47), Kimura (2004), IHC: S (155)	X	X	X				0.078 0.044 without chemotherapy							HIF-1 expression was associated with VEGF.
Esophageal squamous cell carcinoma, 170 patients, Matsuyama (2005), IHC: S (200)	X						0.112	0.142	1.574	0.027				HIF-1 expression was correlated with VEGF expression, significant correlation with DFS not with OS.
T1/T2 stage squamous cell carcinoma of oral floor, 85 patients, Fillies (2005), IHC: S/I for favorable outcome (76)	X	X			0.001	0.2	0.05	0.01	0.03	0.02				HIF-1 expression predicted favorable prognosis.

	Esophageal cancer, 48 patients, Katsuta (2005), IHC: NS (141)	X					0.3408						HIF-1 expression was correlated with VEGF-C and node metastasis.
	79 patients, Beasley (2002), IHC, NS (13)	X	X				0.583		0.188				Strong expression of HIF-2 in tumor associated macrophages (TAM).
HIF-2	Locally advanced, 75 patients, Koukourakis (2002, IHC, S/I (169)		X	X	0.009		0.001			0.004		0.003 local relapse free	Strong correlation with HIF-1. MVD and VEGF, high expression in tumor infiltrating macrophages.
	198 patients, Koukourakis (2006), IHC, S/I (166)		X		0.0014	2.07	0.0004			0.006	1.89	0.002 locoregional control	No correlation with CAIX, but combined expression of HIF-2 and CAIX showed poorer OS and loco-regional control.
CAIX	Nasopharyngeal carcinoma, 90 patients, Hui (2002), IHC, NS (119)		X	X			0.4		0.12 :progression free				Strong correlation with HIF-1 but not with VEGF, combined expression of HIF-1 + CAIX ($p = 0.04$) and VEGF + HIF-1 ($p = 0.0095$) showed worse PFS.
	58 patients, Jonathan (2006), IHC, s for favorable outcome (134)		X									0.04 locoregional control 0.02 metastasis free	Weak association with MVD.
	198 patients, Koukourakis (2006), IHC, S/I (166)		X		0.012	1.73	0.002			0.006	1.88	0.004	Significant correlation with HIF-2, combination with HIF-2 showed poorer outcome.

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	Oral squamous carcinoma, 118 patients, Kunkel (2003), IHC, S (174)	X	X			0.010 all the patients	2.65 all the patients	0.045 stage I/II 0.029 stage III/IV							High standardized uptake value of FDG-PET was also correlated with shorter survival.
Glut-1	Oral squamous cell carcinoma, 54 patients, Oliver (2004), IHC, S, (223)	X									0.002 :disease specific 0.0032 :recurrence 0.005 LN recurrence				
	58 patients, Jonathan (2006), IHC, S (134)		X					0.001						0.0005 metastasis free	
VEGF	Oral and oropharyngeal squamous cell carcinoma, 56 patients, Smith (2001), IHC, S/I for OS (266)	X	X	X		<0.01	3.53	<0.001				NS NS	NS NS	0.09 :locoregional recurrence 0.006 :distant recurrence	
	Advanced laryngeal carcinoma, 183 patients, Teknos (2002), ELISA, S/I for pretreatment VEGF (290)		X	X	X	0.065 0.31: surgery 0.199: chemo/ radiotherapy	1.474 1.373: surgery 1.480: chemo/ radiotherapy	0.018: surgery 0.9185: 0.9185: chemo/ radiotherapy							
	Nasopharyngeal carcinoma, 90 patients, Hui (2002), IHC, NS (119)		X	X				0.78			0.24 progression free				Strong correlation with HIF-1 but not with CAIX, expression, combination of HIF-1 and CAIX showed worse PFS ($p = 0.0095$).

Oesophageal squamous cell carcinoma, 82 (47), patients, Kimura (2004) IHC, S (155)	X	X	X			0.002 0.012 without chemo-therapy				Strong correlation with HIF-1 expression, absence of both HIF-1 and VEGF showed longer survival.
69 patients, Kyzas (2005), IHC, S (179)	X					0.006 HNSCC 0.005 oral cancer				Not statistically significant, but VEGF expression showed trend of correlation with MVD.
Oral squamous cell carcinoma, 220 patients, Arora (2005), IHC, S/I (7)	X						0.004	4.47	0.0041	
Meta-analysis 1002 patients, Kyzas (2005), IHC, S/I (178)					1.56					Metal analysis of 12 studies showed increased mortality with VEGF expression, but no relation with LN metastasis.
Esophageal adenocarcinoma, 75 patients, Saad (2005), IHC, S (246)	X		X	<0.001		0.005				Correlation with angiolymphatic invasion, LN status, distant metastasis, and tumor grade.
29 patients, Druzgal (2005), ELISA, S (60)	X	X	X			0.09				Elevated levels of VEGF along with IL-6, HGF, GRO-1, and IL-8 in tumor samples.
Esophageal cancer, 48 patients, Katsuta (2005), IHC: VEGF-C, NS (141)						0.4659				Correlation with expression of HIF-1.
Oral tongue cancer, 38 patients, Kim (2006), IHC, S (150)		X							0.019	Strong correlation with MVD and LN metastasis.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	Sinonasal carcinoma, 105 patients (squamous cell carcinoma (SCC), 34 patients), Valente (2006), IHC, S/I for SCC (305)	X	X	X						1.10 3.02 (SCC)	0.07 (SCC)		marker.		MVD was an independent
	54 patients, Onesto (2006), ELISA: VEGF-A, S/I (224)	X	X	X		0.0004	3.61	0.003	0.001 progression free	3.16	0.01 progression free			0.02 :metastasis free 0.37 :local recurrence free	
PAI-1	Esophageal squamous cell carcinoma, 49 patients, Sakakibara (2004), Q-PCR, S (247)	X						0.002							Correlation with LN metastasis and TNM stage.
LOX	91 patients, Erler (2006), IHC, S (69)	X	X	X				0.046						0.02 metastasis free	Correlation with CAIX expression ($p = 0.006$).
EPO	151 patients, Winter (2005), RT-PCR, NS (318)	X						0.59			0.88				Cytoplasmic expression, significant correlation with HIF-1 and CAIX.
OPN	54 patients, Le (2003), ELISA, S/I (184)	X	X	X		0.02	6.3	0.0006	0.01	3.0	0.005 relapse free				Strong correlation with pO2 (eppendorf measurement), and inverse association with VHL.

NFkB	Esophageal adenocarcinoma, 97 patients, Abdel-Latif (2004), IHC: NFkBp65, S/I (1)	X	X	X		0.07	1.65	<0.05					Higher expression in stage IIb/III, high expression is associated with lower IkB expression and poor response to chemo- and radiotherapy.
	Squamous cell carcinoma of the tonsil, 45 patients (29 with high-grade dysplasia), Zhang (2005), IHC, p-NFkB, S (331)	X				0.006: high-grade dysplasia		0.047	high-grade dysplasia recurrence free		0.049:		pakt and EGFR also showed significant relation with tumor dysplasia.
	Esophageal carcinoma, 43 patients, Izzo (2006), IHC, S/I (128)	X	X	X			0.016	0.079 (+)	0.0013	0.011	0.069 (+)	0.006	Strong association with perineural, vascular, and lymphatic invasion along with LN metastasis and poor pCR.
Hsp	Oral squamous cell carcinoma, 47 patients, Kaur (1998), IHC, hsp70, S (142)	X										0.0246	Significantly associated with dysplasia, histological grade, size of tumor, loss of differentiation, and tobacco consumption.
	Squamous cell carcinoma of the esophagus, 102 patients, Kawanishi (1999), IHC: S for hsp70, S/I for hsp27 → better prognosis (144)	X			hsp27-NS hsp70-	hsp27-1.827 hsp70-	0.0017 hsp27-<0.0001 hsp70-	3.908	<0.0001				Strong correlation between Hsp27 and 70, negative association with LN metastasis.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	Esophageal squamous cell carcinoma, 62 patients, Nakajima (2002), IHC, S, better prognosis with hsp70 expression (215)	X					NS hsp27-0.046 hsp70-	NS	0.329						Significant correlation between hsp27 and 70, inverse correlation with depth of invasion and pathologic stage, and positive with lymphocyte infiltration.
	Oesophageal squamous cell carcinoma, 123 patients, Faried (2004), IHC, S, better prognosis with hsp60 expression (72)	X							0.0371 hsp60-0.7965 hsp90+						Strong correlation of hsp60 with apoptotic index (AI), but not with hsp90, high AI/Hsp60 showed better survival ($p = 0.0281$).
CAIX + Glut-1	67 patients, De Schutter (2005), IHC, S/I (47)		X	X			0.33	1.53		0.04	2.06	0.02 local control 0.06 regional control	2.33 local control 2.24 regional control		II-6 serum level was also an independent marker.
Lung cancer															
HIF-1	NSCLC, 108 patients, Giatromolaki (2001), IHC: NS (93)	X				0.57	0.55	0.08							Strong correlation with VEGF, bFGF, Bcl-2, and p53.
	NSCLC, 172 patients, Swinson (2004), IHC: S/I (281)	X	X			0.006	2.05	0.034							Correlation with EGFR, p53, MMP9, and CAIX.

	NSCLC, 74 patients, Kim (2005), IHC: S (151)	X	X	X				0.6182	0.6779	0.048	Correlated with CAIX and VEGF, but not with MMP9.
	Small-sized adenocarcinoma of lung, 78 patients, Enatsu (2006), IHC: S (66)	X					0.05				Associated with VEGF-A expression.
HIF-2	NSCLC, 108 patients, Giatromanolaki (2001), IHC, S/I (93)	X			0.04	2.01	0.008				Strong association with HIF-1, VEGF, and Bcl-2.
CAIX	NSCLC, 107 patients, Giatromanolaki (2001), IHC, S/I (92)	X			0.02		0.02				Correlation with PD-ECGF, bFGFr, MVD, and HIF-1.
	NSCLC, 175 patients, Swinson (2003), IHC, S/I for perinuclear CAIX (282)	X			0.004 perinuclear	1.84 perinuclear	0.035 perinuclear 0.11 membranous 0.13 cytoplasmic				Strong correlation with tumor necrosis and distance from blood vessel.
	NSCLC, 172 patients, Swinson (2004), IHC, S/I for perinuclear CAIX (281)	X	X		0.002	1.96					Correlation with HIF-1.
	NSCLC, 177 patients, Swinson (2004), IHC, CAIX, S (280)	X					0.03				Positive association with EGFR and MMP9, co-expression with EFR showed poorer prognosis.
	Early stage NSCLC, 75 patients, Kim (2004), IHC, S/I for DFS (152)	X	X	X	0.05			<0.01		<0.01 0.029 stageI 0.003	Strongly correlated with tumor necrosis and cell proliferation, but weakly with MVD.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	Stage I/II NSCLC, 74 patients, Kim (2005), IHC, S/I (151)	X	X	X					0.0052	3.1715	0.004				Correlation with HIF-1 and tumor necrosis.
	Early stage NSCLC, 20 patients, Le (2006), IHC, NS (183)	X	X	X				0.37			0.47 relapse free				Significantly correlated with tumor/normal pO2 (Eppendorf).
	NSCLC, 90 patients, Simi (2006), RT-PCR, S/I (263)	X						0.001	0.000 cancer specific	3.478 cancer specific	0.0042 disease free				Positive correlation with VEGF and MMP-9, highly predictive in advanced and squamous or adenosquamous NSCLC.
LDHA	NSCLC, 76 patients, Koukourakis (2003), IHC, S (168)	X						0.02							Strong correlation with HIF-1/ HIF-2, VGEF, and bFGF, but not with CAIX, serum LDH level was significantly associated with LDH expression.
Glut-1	Stage I, 47 patients, Minami (2002), IHC, S/I (208)	X				0.0261	0.173	<0.0001							Strong correlation with Ki-67 and MVD, but not p53 and VEGF.
VEGF	NSCLC, 72 patients Yuan (2000), RT-PCR & IHC, S/I for IHC analysis (326)	X				<0.0001 IHC		<0.0001 :stage I/II <0.0001 :stage IIIA/B mRNA <0.0001 <0.0001 IHC	<0.0001 IHC		<0.0001 mRNA <0.0001 IHC relapse free				Strong correlation of VEGF mRNA and protein expression, and its association with MVD, LN metastasis, and tumor stage.

NSCLC, 57 patients, Yuan (2001), RT-PCR & IHC: VEGF189, S/I (327)	X	0.001 RT-PCR		0.0001 RT-PCR 0.0197 IHC	0.0283 RT-PCR relapse free	0.0086 RT-PCR 0.0491 IHC relapse free	No correlation of VEGF 121, 165, and 206 with clinicopathologic variables. VEGF 189 was associated with tumor angiogenesis, survival and early relapse.
NSCLC, 132 patients, Inoshima (2002), IHC, S/I (123)	X	0.0069	2.327	<0.0001			VEGF was correlated with low dendritic cell infiltration and high MVD.
Stage I, 47 patients, Minami (2002), IHC, NS (208)	X		0.834	NS			Strong correlation with MVD, but not with Glut-1.
NSCLC, 75 patients, Kaya (2004), ELISA, S (145)				<0.0001			
NSCLC, 71 patients, Iwasaki (2004), ELISA, S/I (127)	X	0.0428	2.06	0.0134			Combined expression with bFGF showed poorer prognosis ($p < 0.0001$).
NSCLC IB/IIA stage, 51 patients, Mineo (2004), IHC, S/I (209)	X	0.042	3.617	0.0029			Correlation with vessel invasion.
NSCLC, 194 patients, Laack (2005), ELISA, S/I (180)	X	0.01	1.03	0.04			
NSCLC, 21 patients, Dudek (2005), ELISA, S/I (61)	X	0.0113	1.003	<0.03			

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

					Overall survival			Disease free survival			Local control			
					Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	
	S	R	C	H	p	HR	p	p	HR	p	p	HR	p	Additional comments
NSCLC, 173 patients, Huang (2005), IHC, VEGF-A & C S/I for stage I (118)	X	X	X		0.03: VEGF-A 0.03: VEGF-C Stage I	2.37: VEGF-A 2.1: VEGF-C Stage I	0.01: VEGF-A 0.01 VEGF-C Stage I							No significance in stage II/III cancer.
T1, 129 patients, Kojima (2005), IHC, VEGF & VEGF-C, S (160)	X				0.0710 (I) VEGF 0.2059 VEGF-C	2.011 VEGF 1.646 VEGF-C	0.0018 VEGF 0.0031 VEGF-C							Weak correlation of VEGF and VEGF-C, combination of VEGF-C + VEGFR-3 was an independent marker.
NSCLC, 74 patients, Kim (2005), IHC, nearly S (151)	X	X	X					0.1057	2.1467	0.070				Weak correlation with HIF-1 expression (0.07).
NSCLC, 63 patients, Shimanuki (2005), ELISA, S (261)							0.008			Significant				Correlated with MVD, but not intratumoral VEGF expression (IHC).
NSCLC, 70 patients, Yuan (2006), RT-PCR, S (328)	X	X	X				0.0046						0.0033 relapse free	Correlation with Cox2.
NSCLC, 79 patients, Takenaka (2006), RT-PCR, S/I with VEGF/Flt-1 for better prognosis (284)	X				0.043	0.446	0.037							High Flt1/VEGF patients showed increased survival.

PAI-1	Pulmonary adenocarcinoma, 99 patients, Pappot (2006), ELISA, S/I (229)	X			0.03	1.75	0.04				Low PAI-1 and high uPA-PAI-1 complex showed better survival.
Bnip3	NSCLC, 105 patients, Giatromanolaki (2004), IHC: S/I (94)	X			0.035	2.13	0.004				Significant correlation with expression of HIF-1, CAIX, and LDH5, but not with VEGF.
GRP	132 patients, Uramoto (2005), IHC: S/I for better prognosis (303)	X		X	<0.001	2.35	0.11				
	NSCLC, 82 patients, Schneider (2004), RT-PCR, S (252)	X					0.014				High OPN + low SPARC showed poorer survival.
OPN	NSCLC, 207 patients (136 for stage I), Donati (2005), IHC, S/I for stage I cancer patient (58)	X			0.037 stage I	1.88 stage I	0.14 0.034 stage I	0.049 0.013 stage I	1.491 2.08 stage I	0.074 0.011 stage I	
	Early stage NSCLC, 20 patients, Le (2006), IHC, S (183)	X	X	X			0.009			0.001	Significantly correlated with tumor/normal pO2 (Eppendorf).
VEGF + OPN	Stage I lung adenocarcinoma, 87 patients, Shijubo (1999), IHC, VEGF+OPN, S (259)	X					0.005			0.005 postoperataive relapse.	

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Ovarian tumor															
HIF-1	102 patients, Birner (2001), IHC: NS (18)	X		X		0.182		0.3959	0.353		0.6848				Strong correlation with p53 expression (<0.001, 7). Combined expression of HIF-1 + p53 was an independent prognostic marker.
Glut-1	113 patients, Cantuaria (2001), IHC, S (30)	X		X							0.024				Cytoplasmic expression, strong correlation with pCR to chemotherapy ($p = 0.028$).
VEGF	60 patients, Tempfer (1998), ELISA, S/I (291)	X		X		0.008	2.7	0.007	0.02	1.8	0.003				
	56 (40 with residual disease less than 2 cm) patients, Chen (1999), ELISA, S/I (34)	X	X			<0.001 <0.001 less than 2 cm	4.47 5.37 less than 2 cm	<0.001 0.0035 less than 2 cm	0.002 <0.001 less than 2 cm	3.34 6.62 less than 2 cm	0.001 0.001 less than 2 cm after surgery				
	94 patients, Shen (2000), IHC, S/I (256)	X				0.006		0.0004							No correlation with MVD.
	80 patients, Nishida (2004), IHC, VEGF-A & C, NS (281)	X						0.46 VEGF-A 0.0018 VEGF-C	0.0070 VEGFA 0.36 VEGFC disease free 0.24 VEGF-A 0.2 VEGF-C cancer specific	7.16 VEGFA 1.76 VEGF-C disease free 2.83 VEGFA 0.29 VEGF-C cancer specific	0.47 VEGF-A 0.25 VEGF-C cancer specific				Association of VEGF-C with LN metastasis and peritoneal metastasis.

	44 patients, Kassim (2004), ELISA, S/I (140)	X			0.02	5.6	0.004				Significant association with IL-8 mRNA.
	73 patients, Ueda (2005), IHC: VEGF-C S (300)	X					0.0241				Strong correlation with clinical stage, LN metastasis, MMP-2, and lymphangio-genesis, combination of strong VEGF expression and low apoptotic index showed poorer survival ($p = 0.0195$).
	67 patients, Ino (2006), IHC, S/I (122)	X			0.018	12.18	0.046	0.008	7.49	0.036 progression free	Correlation with type I angio-tensin II and MVD.
	314 patients (FIGO stage I, 56 patients), Hefler (2006), ELISA, S/I (111)	X		X	0.03 0.001 FIGO I	1.8 1.1 FIGO I	<0.001 <0.001 FIGO I				Independent prognostic information for low-risk group.
	Stage I, 77 patients, Goodheart (2005), IHC, borderline S (96)			X				NS		0.02 disease specific 0.078 recurrence	
PAI-1	131 patients with stage III/IV), Chambers (1998), IHC,s S/I for OSs (33)	X	X	X	0.003 III/IV	1.5 III/IV	0.04 0.011 III/IV			0.076	Combined expression of PAI-1 + uPA, PAI-1 + uPAR, PAI-1 + CSF, or PAI-1 + loss of PAI-2 was also an independent marker.
	FIGO IIIc, 86 patients, Kuhn (1999), ELISA, S/I for OS (172)	X		X	<0.001	3.1	0.012				uPA showed significant correlation with poor OS.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	103 patients, Konecny (2001), ELISA, S (162)	X		X		0.582		0.007	0.31		0.039 progression free				uPA was an independent predictors for OS and PFS.
	51 patients, Kimura (1993), Northern blot for Hsp60 mRNA, S/I (154)	X		X		0.012	4.59	0.0018 0.0013 FIGO stage III 0.044 grade 2 or 3							Significantly reduced OS before, but not after chemotherapy.
Hsp	99 patients, Geisler (1998), IHC: Hsp27, S (89)	X		X		0.041									
	77 patients (60 with stage III/IV), patients, Arts (1999), IHC: Hsp27, S (8)	X		X		0.15		<0.06	0.14		<0.05 progression free				
	52 patients, Elpek (2003), IHC: Hsp27, 70, 90, S/I only for Hsp27 (65)	X				0.0424 hsp27	2.1015 hsp27	0.0063 hsp27 0.2855 hsp70 0.1131 hsp90							
Cervix cancer															
HIF-1	Early stage, 91 patients, Birner (2000), IHC: S/I (19)	X	X			0.0129	2.89	0.0307	0.0002	5.04	<0.0001				No association with p53.
	91 patients, Burri (2003), IHC: S/I (27)	X	X	X		0.02	1.57	0.01 0.01 LN+ 0.33 LN−			0.15			0.04 local progression free	Correlation of hemoglobin concentration and HIF-1.

	67 patients, Bachtiary (2003), IHC: S/I (10)		X					0.04 cancer specific 0.036 progression free	2.1 cancer specific 2.1 progression free	0.006 cancer specific 0.011 progression free					Perinecrotic expression, Not correlated with HPV infection.
	Locally advanced 38 patients, Mayer (2004), IHC:NS (204)	X	X	X			0.11			0.19 recurrence free					No correlation with pO2 measurement using Eppendorf.
	FIGO stage IIIB, 38 patients, Ishikawa (2004), IHC: S (124)		X				NS			0.04 recurrence free NS cause specific			0.03 metastasis free 0.17 local recurrence free		Significant difference in MFS and LRS, no correlation with apoptotic genes (p53, Bax, Bcl-2) and HPV infection.
	Locally advanced 99 patients, Hutchison (2004), IHC: NS (121)		X							0.56			0.54: metastasis free 0.27 local recurrence free		Weak correlation with pimo staining, weak but significant with CAIX ($p = 0.001$), associated with tumor size: good outcome in large tumor, poor outcome in small tumor.
	Locally advanced, 68 patients, Loncster (2001), RT (IHC), S/I (191)		X					0.05		0.018 (level) 0.0041 (−/+) cancer specific	0.021 metastasis free		0.73 (level) 0.72 (−/+) local control 0.022 (level) 0.0049 (−/+) metastasis free		Expression on the plasma membrane, correlation with tumor hypoxia (Eppendorf).
CAIX	Locally advanced, 110 patients, Hedley (2003), Immuno-fluorescence, NS (110)		X					0.5		0.76					Not correlated with pO_2 measurement using Eppendorf: due to the intratumoral heterogeneity.
	56 patients, Kim (2006), RT-PCR, S/I for metastasis (148)		X		0.98	1.52	0.83	0.74 recurrence free	0.78 recurrence free	0.98 recurrence free	0.008 metastasis free	34.8 metastasis free	0.07: metastasis free		Correlation with VEGF expression.

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Glut-1	Locally advanced, 54 patients, Airley (2001), IHC, S for metastasis free survival (4)		X							0.29 disease free 0.022 metastasis free 0.24 recurrence free					Weak correlation with pO_2 (Eppendorf).
	Locally advanced, 80 patients, Mayer (2005), IHC, S (203)	X	X					0.004		0.007 recurrence free					Correlation with FIGO and high pT stage. No association with pO_2 (Eppendorf).
VEGF	Locally advanced, 100 patients, Loncaster (2000), IHC, S/I (190)		X			0.001	2.3	0.0008				NS Local control 0.021 metastasis free	— Local control 2.2 metastasis free	0.23 Local control 0.0062 metastasis free	VEGF was an independent marker for OS and MFS, but not for local control.
	52 patients, Ueda (2002), IHC: VEGF-C, S/I (301)	X				0.0232	3.305	0.132							Correlation with MMP-2, invasion, and LN metastasis.
	59 patients, Kim (2006), RT-PCR, NS (148)		X			0.44	0.56	0.99	0.49	0.60	0.89 recurrence	0.28	0.41	0.40 metastasis	Correlation with CAIX expression.
PAI-1	108 patients, Hazelbag (2004), IHC, S/I (109)	X				0.04	8.9	0.01	0.02	4.68	0.01				Correlation with FIGO stage, distant metastasis, recurrent disease, and TGF-β expression.
Gastric cancer															
HIF-1	141 patients Urano (2006), IHC: NS (304)	X		X				0.6215							Strong association with VEGF and p53 expression, increased metastasis without HIF-1 ($p = 0.0143$).

Glut-1	667 patients, Kawamura (2001), IHC, S/I (143)	X		<0.0001	1.410	0.0001				Strong association with depth of invasion, lymphatic permeation, venous invasion, LN metastasis, hepatic metastasis, and carcinoma stage.
VEGF	56 patients, Kido (2001), ELISA, S (146)	X	X	NS		<0.05				Associated with depth of invasion and LN metastasis.
	58 patients, Karayiannakis (2002), ELISA, S/I (138)	X		0.007	2.91	<0.0001				Correlation with invasion depth and distant metastasis, but not with LN metastasis.
	156 patients, Fondevila (2004), IHC, S/I (81)	X	X	<0.01	2.99	0.01	<0.02 disease free <0.005 recurrence	— disease free 5.88 recurrence	<0.02 disease free <0.02 recurrence	p53 was also an independent marker.
	55 patients, Shida (2005), IHC: VEGF-D, S/I (258)	X		0.0426	2.16		0.0017 relapse free	3.31 relapse free	0.0017 pTNM stage	Strong correlation with LN metastasis and peritoneum recurrence.
	91 patients, Juttner (2006), IHC, VEGF-C & D (S/I only for VEGF-D) (135)	X	X				0.485 (C) 0.023 (D) disease free 0.675 (C) cancer specific	1.26 (C) 2.65 (D) disease free 0.86 (C) cancer specific	0.044 (C) 0.036 (D) disease free 0.114 (C) cancer specific	Correlated with lymphatic metastasis.
	146 patients, Urano (2006), IHC, NS (304)	X	X			0.1168				Correlation with HIF-1.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
	189 patients (139 patients curatively resected) Heiss (1995), ELISA, S/I for resected patients (112)	X	X	X		0.005 0.001 resected	1.465 2.251 resected	0.0019 0.0043 curatively resected	0.001 resected	2.117 resected	0.0003 resected				Inverse correlation of shorter survival with PAI-1, uPA, uPAR but not with PAI-2.
PAI-1	104 patients,	X	X	X		0.003 resection	1.619 resection	<0.001 all <0.001 curative resection	0.001 resection	1.1812 resection	<0.001 resection				uPA was an independent marker for DFS, less prognostic significance of uPAR, strong correlation with *H. pylori*.
NFkB	290 patients, Lee (2005), IHC: NFkBp65 → better prognosis, S (186)	X						0.0228							Negative correlation of nuclear NFkB with lymphatic invasion and LN metastasis and positive with pAkt, p16, APC, Smad4, and KAI1.
	stage IV, 42 patients, Takeno (2001), IHC: Hsp27, S (285)	X		X		0.22	8.27	0.0191							
Hsp	86 patients, Kapranos (2002), IHC: Hsp27, S (137)	X				NS		0.04							Correlation with metastatic LN.

Renal cell carcinoma (RCC)

HIF-1	66 patients Lidgren (2005), Western blot: S/I for favorable prognosis (187)	X		0.024 coventional RCC	0.413 conventional RCC	0.024 conventional RCC							Significant correlation only in conventional RCC.
CAIX	RT-PCR, S for favorable outcome (214)	X							0.0254: cancer specific				High expression in clear cell RCC.
	321 patients w/ clear cell RCC, Bui (2003), IHC, S/I for favorable outcome (26)	X					<0.001 metastatic	3.10 metastatic	0.25 non-metastatic <0.001 metastatic ;disease specific				Low expression in metastatic region: involved in progression.
	66 patients w/ clear cell RCC, Atkins (2005), IHC, S for favorable outcome (9)		X			0.03							Favorable response to IL-2 treatment ($p < 0.01$).
	Cortical tumor, 41 patients, Gilbert (2006), RT-PCR, S for DFS (95)	X				0.93			0.048 disease free 0.57 disease specific				Expression of epithelial cells in peripheral blood.
	229 patients, Jacobsen (2004), IHC, S (129)	X			0.859	0.011 :microarray 0.055 :tissue section							Correlation with tumor stage, no difference in expression among different RCC types.
VEGF	54 patients w/ clear cell RCC, Fukata (2004), IHC, S (83)	X								0.826 metastasis free	1.188 metastasis free	0.039 metastasis free	MVD and MPP to E-cadherin ratio were independent markers.
	48 patients, Yildiz (2004), radical nephrectomy, IHC, S/I (322)	X					<0.01		0.15 cancer specific				

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Additional comments*
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	
Hsp	43 patients, Santarosa (1997), IHC: Hsp72, S/I for favorable outcome (248)	X			X			NS	<0.01		0.002				High expression in relapsed patients.
	76 patients Erkizan (2004), IHC: Hsp27, NS (68)	X									NS (0.05) :progression free, cause-specific survival				High expression in RCC. Correlated with tumor stage.
Colorectal cancer															
HIF-1	87 patients Yoshimura (2004), IHC: NS (S/I with HIF-1 + HIF2) (325)	X						>0.05							Significant correlation in eary tumor stage, combined HIF-1 & HIF-2 expression was an independent marker ($p < 0.055$).
HIF-2	87 patients, Yoshimura (2004), IHC, S (325)	X						<0.05							Correlation with MVD and Cox-2. Combined expression of HIF-1 & HIF-2 was an independent marker ($p < 0.055$).
Glut-1	Rectal carcinoma, 43 patients, Cooper (2003), IHC, S/I (42)	X	X	X		0.013		0.17 (−/+) 0.041 (level)						0.69 (−/+) 0.21 (level) local recurrence free 0.16 (−/+) 0.42 (level) metastasis free	Perinecrotic expression.

VEGF	84 patients, White (2002), IHC: VEGF-D, S/I (316)	X			0.037	3.811	0.0072	0.026	4.133	0.0041				No correlation with MVD.
	139 patients, Onogawa (2004), IHC, VEGF C & D, S (VEGF-C is I for LN metastasis) (225)	X					<0.05 VEGF-C <0.05 VEGF-D			<0.05 VEGF-C <0.05 VEGF-D LN metastasis	0.0104 VEGF-C 0.0561 VEGF-D LN metastasis	2.272 VEGF-C 2.742 VEGF-D LN metastasis		Association with MVD, LN metastasis, lymphatic invasion, and tumor invasion.
	104 patients, Galizia (2004), IHC, S/I (84)	X						0.008 disease free 0.023 disease specific	3.73 disease free 3.623 disease specific					No correlation with MVC. p27 and p53 were also independent markers.
	Stage II/III, 72 patients, Ottaiano (2006), IHC, S/I (226)	X		X			0.1613	3.23	0.0399					Significant association with tumor invasion and LN involvement.
	150 patients, Soumaoro (2006), IHC, VEGF-C, S (269)	X			0.6198	1.235	0.0282							Significant association with COX-2, LN metastasis, and invasion.
Hsp	256 patients, Sun (1997), IHC: Hsp72/73, NS (S for rectal) (278)	X			0.97 colonic 0.08 rectal		0.13 colorectal 0.03 rectal							Association with cytoplasmic p53, but not with nuclear p53, combination of Hsp negative/ p53 negative expression showed longer survval.
Bladder cancer														
	93 patients, Theodoropoulos (2004), IHC, S/I (294)	X	X	X	0.02 grade/ stage	6.01 grade/ stage	0.009 0.078 T1	0.04 grade/ stage	1.76 grade/ stage	0.03			0.037 T1 recurrence	Not significant when VEGF and MVD are included for multivariate anaysis.

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			Disease free survival			Local control			
						Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	
		S	R	C	H	p	HR	p	p	HR	p	p	HR	p	Additional comments
HIF-1	Superficial, 140 patients, Theodoropoulos (2005), IHC, NS (S/I HIF-1+ p53) (295)	X							NS	NS	0.284 recurrence free 0.058 progression free				When combined with p53 expression, independent prognostic factor ($p = 0.042$, HR = 2.64).
	63 patients, Palit (2005), IHC: S for superficial tumor (227)	X	X	X				0.0783 (superficial) 0.5176 (invasive)			0.0254 (superficial) recurrence free				Significant correlation with superficial bladder cancer, but not with invasive one, no correlation with Glut-1 expression.
	127 patients, Nakanishi (2005), IHC & in situ: S/I (216)	X				0.0013 0.0011 (invasive tumor)	4.87 7.42 (invasive tumor)	0.0002	0.0009 0.001 (invasive tumor)	6.08 11.44 (invasive tumor)	0.0002				Associated with p53 expression.
HIF-2	69 patients, Koga (2004), IHC, S/I for TAM associated HIF-2 in the invasive front (159)	X							0.046 invasive front	1.3 invasive front	0.038 :invasive front cancer specific				Expression is limited in the small subset of TAM, but TAM associated HIF-2 expression was a significant marker.
CAIX	21 patients, Hoskin (2003), IHC, S/I (115)	X	X			0.02	3.21	0.0021	Similar with OS	Similar with OS	0.041 :cause specific			0.36	Colocalization with Glut-1 and pimo staining
	57 patients, Hussain (2004), IHC, NS (120)		X	X			0.21								More expression in superficial tumor than invasive one.
	21 patients, Hoskin (2003), IHC, S/I (115)	X	X			0.03	3.14	0.012	Similar with OS	Similar with OS	0.016 cause specific			0.21	Significant correlation with CAIX and pimo staining.

Glut-1	37 patients, Palit (2005), IHC, S for invasive tumor (227)	X	X	X				0.8286 (superficial) 0.0012 (invasive)			0.3285 (superficial) :recurrence	Significant association of HIF-1 in superficial cancer.
VEGF	93 patients, Theodoropoulos (2004), IHC, S (294)	X	X	X		0.71	1.46	0.04	0.46	1.27	0.03	Strong correlation with HIF-1 expression.
Hsp	91 patients, Syrigos (2003), IHC: Hsp70, S (283)					NS		0.05				Correlation with grade and stage.
Prostate cancer												
VEGF	390 patients, Bok (2001), ELISA, S/I (21)				X	0.02	1.72	0.024				No significance of bFGF expression.
	1390 patients, George (2001), ELISA: cut points of VEGF level, S/I (90)				X	0.006	2.42	0.002				Significant at various cut points.
NFkB	136 patients, Ross (2004), IHC: NFkBp65, S/I (244)	X							0.006		0.001 recurrence free	Correlation with increased tumor stage, high prostate specific antigen (PSA), and DNA ploidy status.
	86 patients, Domingo-Domenech (2005), IHC for nuclear NFkBp65, S/I (57)	X							0.002 nuclear 0.86 cytoplasmic recurrence free	5.00 nuclear 0.93 cytoplasmic recurrence free	0.0009 nuclear 0.74 cytoplasmic recurrence free	Nuclear NFkB staining had a significant prognostic value.
AP-1	Androgen independent, 51 patients, Edwards (2004), IHC: phosphor-ylated c-Jun, S (63)			X				0.023				No correlation with PKC, but combined expression showed poorer survival.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Hsp	85 patients, Cornford (2000), IHC: Hsp27 S/I (43)	X				0.0143 hsp27	2.496 hsp27	0.0001 hsp27							Hsp60 and 70 were not significantly related to clinical outcome.
OPN	100 patients, Hotte (2002), ELISA, S/I (116)			X		<0.0001	2.38	<0.001							Correlation with bone metastasis.
	116 patients, Forootan (2006), IHC, S (82)							0.03							Highly expressed in malignant regions.
Endometrial carcinoma															
HIF-1	81 patients Sivridis (2002), IHC: S/I (265)	X	X			0.01 without angiogenic factors		0.03							Strong association with VEGF expression.
HIF-2	81 patients, Sivridis (2002), IHC, NS (265)	X	X			0.54		0.36							Associated with thymidine phosphorylase (TP).
VEGF	86 patients, Yokoyama (2000), IHC, S (324)	X				0.47		<0.05							Flt-4 (VEGFR-3) was an independent marker.
PAI-1	92 patients, Tecimer (2001), ELISA, S/I for OS (289)	X				<0.05		0.0003				0.005			uPA was associated with other prognostic factors but not with patient survival.
EPO	107 patients, Acs (2004), IHC, S/I for disease related survival (2)	X							0.002 disease related		0.037 0.008: endometrioid tumor disease related 0.605 0.231 endometrioid tumor : recurrence free				Negative correlation with ER and PR. High nuclear expression of HIF1.

AP-1	63 patients, Yokoyama (1998), IHC: c-Jun, c-fos, S for c-Jun (323)	X	X				<0.05 c-Jun NS c-fos						Correlation with pelvic LN and paraaortic LN metastasis.
Hsp	153 patients Geisler (1999), IHC: Hsp27, S/I (88)	X	X								0.02 recurrence		Association with myometrial invasion.
Hepatocellular carcinoma													
VEGF	108 patients, Poon (2004), ELISA, S/I (234)	X		0.032	1.86	0.012				0.022			Associated with venous invasion and advanced tumor stage.
Hsp	58 patients, King (2000), IHC: Hsp27, S/I (156)	X		0.015	2.72	0.0009	0.034	2.25		<0.001			
OPN	240 patients, Pan (2003), Northern blot, S (228)	X		0.039	1.32	0.00013							Strong association with p53 mutation, tumor grade II-IV, and portal vein tumor invasion.
Pancreatic cancer													
VEGF	58 patients, Kurahara (2004), IHC, VEGF-C & D, S (175)	X				0.075 VEGF-C 0.055 VEGF-D 0.017 C + D							Association with LN metastasis.
	76 patients, Chung (2006), IHC w/tissue microarray S (38)	X	X	X		0.0207							VEGF receptor FLT-1 was highly ass w/OS (0.0044, 9.872).
Bnip3	70 patients Erkan (2005), IHC, S: favorable prognosis (67)	X				0.013							

(*continued*)

Table 1. Gene Expression and Patient Prognosis in Different Tumor Types (Cont'd)

						Overall survival			Disease free survival			Local control			
						Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	Multivariate		Kaplan-Meier's	
		S	R	C	H	p	HR	p	p	HR	p	p	HR	p	Additional comments
Sarcoma															
Soft tissue sarcoma															
CAIX	47 patients, Maseide (2004), IHC, S (199)	X	X	X				0.044			0.2 relapse free 0.033 disease specific				CAIX expression in necrotic area.
VEGF	115 patients, Yudoh (2001), ELISA, S/I (329)	X	X	X		0.025	1.94	<0.05				0.001 local recurrence <0.001 metastasis free	1.32	<0.001	No correlation with MVD.
Osteosarcoma															
VEGF	57 patients, Sulzbacher (2002), IHC, S (277)	X		X				0.0841			NS				
Hsp	60 patients, Uozaki (1997) IHC: Hsp27, S/I (302)	X		X		0.014	3.26	0.001 biopsy 0.011 surgery							Glutathione-S-transferase (GST) and lung resistance-related protein (LRP) were also independent markers.
OPN	57 patients, Sulzbacher (2002), IHC, NS (277)	X		X			NS								Correlation with VEGF.
Brain tumor															
	Oligodendroglioma, 51 patients, Birner (2001), IHC: S/I (18)	X	X	X		0.0187	6.82	0.0434							Not correlated with p53.

HIF-1	Ependyoma, 100 patients, Preusser (2005), IHC: NS (236)	X	X	X	X			0.3584	Significantly correlated with necrotic area. Patients with high hypoxia score showed shorter survival ($p = 0.0402$).
CAIX	Ependyoma, 100 patients, Preusser (2005), IHC: NS (236)	X	X	X	X			0.1065	Significantly correlated with necrotic and. perivascular areas. Patients with high hypoxia score showed shorter survival ($p = 0.0402$).
	Astrocytic tumor, 284 patients, Haapasaio (2006), IHC, S/I (102)	X				0.038	1.188	0.0011	Expression in perinecrotic area, no association with p53 and EGFR.
VEGF	Ependyoma, 100 patients, Preusser (2005), in situ: NS (236)	X	X	X	X			0.0604	Patients with high hypoxia score showed shorter survival ($p = 0.0402$).
PAI-1	Gioma, 59 patients, Muracciole (2002), ELISA, S/I (212)	X	X	X		0.007	1.9	<0.0001	Correlation with high grade and necrosis, EGFR was also significantly correlated to patient outcome.
GRP	Neuroblastoma (NB), 68 patients, Hsu (2005), IHC: for better prognosis (117)	X		X		0.005	3.097	<0.0001	Better prognosis with GRP78 expression both in differentiated and undifferentiated NB.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Lymphoid/Leukemia															
	Multiple myeloma, 50 patients, Ribas (2004), IHC, S (240)		X			0.13		0.043	0.08 event free		0.024 event free				Correlation with MVD. Serum LDH level was also an prognostic marker.
VEGF	Childhood acute lymphoblastic leukemia, 96 patients, Wellmann (2004), RT-PCR, S/I (315)	X	X						0.023 event free <0.001 relapse free		0.003 event free				High HIF-1 expression. Correlation with HIF-1 (IHC).
NFkB	Acute lymphoblastic leukemia, 95 patients, Faderl (2005); ELISA, S/I for favorable prognosis (71)		X			0.001	8.01	0.004							VEGFR and bFGF are poor prognostic markers.
	Peripheral T-cell lymphoma, 62 patients (7 anaplastic), Martinez-Delgado (2005), microarray, S/I for favorable prognosis (198)		X			0.022	2.984	0.032							Significantly different expression of NFkB target CFLAR and MMP9 in NFkB +/− cells.
Hsp	Acute myeloid leukemia, 124 patients, Kasimir-Bauer (2002), western blot, hsp27, NS (139)		X					0.1364			0.1854 remission				

	Acute myeloid leukemia, 98 patients (grouped by chromosomal analysis: favorable, intermediate, unfavorable karyotypes), Thomas (2005), IHC: Hsp27, 60, 70, 110, S/I (296)	X	<0.001 hsp110 intermediate 0.01 hsp27 0.05 hsp60 unfavorable karyotype	3.24 hsp110 intermediate 3.25 hsp27 2.97 hsp60		Correlation with drug resistance and apoptosis (Bcl-2), and negative association with pCR, Hsp27 and 60 were independent prognostic markers in patients with unfavorable karyotype.
Miscellaneous						
Mesothelioma						
VEGF	Malignant mesothelioma, 40 patients, Demirag (2006), IHC, S/I (52)		0.001	39.789	0.0002	Significant correlation with tumor necrosis (TN). TN and mitotic activity index (MAI) showed association with poor prognosis.
Adenoid cystic carcinoma of salivary gland						
VEGF	80 patients, Zhang (2005), IHC, S/I (330)	X	0.035	9.96	0.003	Correlation with iNOS, NFkB expression, and MVD, clinical stage, tumor size, vascular invasion, recurrence, and metastasis. iNOS was also an independent marker.
NFkB	80 patients, Zhang (2005), IHC, NFkB-65, S/I (330)	X	0.049	6.24	0.000	Correlation with iNOS, VEGF expression, and MVD, clinical stage, tumor size, vascular invasion, recurrence, and metastasis. iNOS was also an independent marker.

(*continued*)

TABLE 1. GENE EXPRESSION AND PATIENT PROGNOSIS IN DIFFERENT TUMOR TYPES (CONT'D)

						Overall survival			*Disease free survival*			*Local control*			
						Multivariate		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	*Multivariate*		*Kaplan-Meier's*	
		S	*R*	*C*	*H*	p	*HR*	p	p	*HR*	p	p	*HR*	p	*Additional comments*
Melanoma															
Hsp	40 patients, Ricaniadis (2001), IHC: Hsp70 expression S for better prognosis (241)							0.0159							

S, significant; S/I, significant and independent; NS, not sitnificant; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; LN−, lymph node negative; LN+, lymph node positive; RT, radiotherapy; S, surgery; C, chemotherapy; R, radiotherapy; H, hormone therapy; p, p value; HR, hazard ratio; OS, overall survival; DFS, disease free survival; PFS, progression free survival; RFS, recurrence free survival; MFS, metastasis free survival; LRS, local recurrence free survival; MVD, microvessel density; MVC, microvessel count; ER, estrogen receptor; PR, progesterone receptor; pCR, patient complete response; Pimo, pimonidazole.

No highlight, gene expression showed significant correlation with patient outcome.

Lighter gray highlight, gene expression showed correlation with favorable patient outcome.

*Darker gray highlight,*gene expression showed no significant correlation with patient outcome.

Underline, hypoxia measurement.

Table 2. Correlation Between the Expression of Hypoxia Markers and Patient Outcome

Marker	*Total number of studies*	*Studies correlated with hypoxia (correlation/ number of studies)*	*Prognostic importance (poor + favorable outcome)*	*Multivariate analysis: significance (poor + favorable outcome)/number of studies*
HIF-1	37	2/3	22 + 3	12 + 3/23
HIF-2	7	0	5	4/5
Regulated by HIF-1				
CAIX	26	2/3	16 + 4	13 + 2/15
LDHA	1	0	1	0
Glut-1	13	2/3	12	5/5
VEGF	76	0	68 + 1	41 + 1/54
PAI-1	22	0	22	19/21
LOX	1	1	1	0
BNIP3	2	0	1 + 1	1/1
EPO	2	0	1	1/1
NFkB	9	0	6 + 2	5 + 1/6
AP-1	5	0	4	1/1
Hsp	25	0	15 + 5	8 + 1/15
UPR (GRP78)	2	0	0 + 2	0 + 2/2
OPN	14	2/2	13	7/9

Hypoxia measurement: Eppendorf electrodes measurement, pimonidazole staining, hemoglobin concentration, hypoxia score.
Prognostic importance: p value from Kaplan-Meier's survival curve multivariate analysis.
Poor outcome: correlation of high gene expression with poor patient survival and prognosis.
Favorable outcome: correlation of high gene expression with better patient survival and prognosis.

CAIX

CAIX expression was analyzed in 26 studies with eight different types of tumors: breast (37, 272), head and neck (119, 134, 166), lung (92, 151, 152, 183, 263, 280-282), cervix (110, 148, 191), bladder cancers (115, 120), RCC (9, 26, 95, 214), soft tissue sarcoma (199), and brain tumors (102, 236). Among them, significant association with poor patient outcome was determined in 16 studies by using *p* value from Kaplan–Meier's survival curve. Multivariate analysis was done in 15 studies, and 13 of them identified CAIX as an independent prognostic marker for poor prognosis. However, in head and neck cancer (134) and RCC studies (9, 26, 214) CAIX expression was significantly correlated with favorable prognosis, and one of these studes identified CAIX as a favorable prognostic marker (26). High expression of CAIX in RCC might be due to HIF-1 overexpression since mutation of VHL is commonly found.

The correlation between CAIX expression and hypoxia was evaluated in four studies with lung (183), cervix (110, 191), and bladder (115) cancer patients. Tumor oxygenation was measured by using Eppendorf oxygen probes or pimonidazole staining. Three of these studies demonstrated correlation while the other study (110) failed to show significance of CAIX expression with either tumor hypoxia and patient outcome.

As discussed above, though CAIX expression alone was not statistically correlated with patient outcome, the combined expression of CAIX and HIF-1, or CAIX and Glut-1 was significantly associated with poorer survival in head and neck cancer patients (47, 119).

LDHA

The relation between high LDH serum level and poor prognosis has been shown in pancreatic carcinoma (288), NSCLC (287), RCC (211), and nasopharyngeal carcinoma (35). However, only one study has been done to determine LDHA isoform specific correlation with patient outcome in lung cancer (168).

This study, though it lacked multivariate analysis, showed significant correlation of LDHA expression with patient overall survival. By comparing LDHA expression and LDH serum level in patients, it also determined the correlation between them. LDHA expression was associated with expression of HIF-1, HIF-2, VEGF, and bFGF, which indicates HIF-1-mediated upregulation of LDHA and its role in tumor angiogenesis. However, it was not coexpressed with CAIX which suggests they might be regulated under different hypoxic threshold.

GLUT-1

The potential of GLUT-1 as a prognostic marker was evaluated in 13 studies with eight different tumor types including breast (274), head and neck (47, 134, 174, 223), lung (208), ovarian (30), cervix (4, 203), gastric (143), colorectal (42), and bladder (115, 227) cancers. Twelve out of these 13 studies showed significant relation with patient survival. Multivariate analysis was performed in five studies and all of them identified GLUT-1 expression as an independent prognostic factor. As mentioned above, they include the study that analyzed the

combined expression of GLUT-1 with CAIX in head and neck cancer (47).

In bladder cancer, GLUT-1 expression was strongly associated with CAIX, suggesting its potential role as a hypoxia marker. Also, the correlation between GLUT-1 and tumor hypoxia was measured in three studies with cervix (4, 203) and bladder (115) cancers by using Eppendorf oxygen probes or pimonidazole staining. However, since one of those studies failed to show significance, further studies are needed. Other than hypoxia, Glut-1 might be involved in tumor progression since it was highly correlated with tumor invasion and metastasis in gastric cancer (301).

VEGF

VEGF was analyzed in 76 studies, which is the greatest number compared to other hypoxia markers, with 19 tumor types. Among them, 68 studies showed the significant correlation of VEGF serum level or expression with poor patient outcome, using *p* value from Kaplan–Meier's survival curve. By multivariate analysis, 41 out of 54 studies identified it as an independent prognostic marker. Though in acute lymphoblastic leukemia VEGF serum level was an independent factor for favorable prognosis, VEGFR and bFGF were identified as poor prognostic markers (71).

VEGF studies also include VEGF-A isoform or other VEGF family specific studies. In breast (163, 188, 189) and lung (327) cancers, $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ were identified as independent prognostic markers. VEGF-C and D, which are involved in lymphangiogenesis, also showed significant relation with patient outcome in lung (118, 160), ovarian (300), cervix (301), gastric (135, 258), colorectal (225, 269, 316), and pancreatic (175) cancers. VEGF-C and D was also associated with lymph node metastasis and tumor invasion that suggests their possible inovolvement in tumor metastasis and angiogenesis.

VEGF expression was also associated with HIF-1 (119, 141, 151, 155, 294, 304, 315) and, as mentioned above, their combined expression showed worse patient survival (119). However, VEGF expression showed significant relation with poor patient outcome not only in conventional RCC with high VHL mutation (83, 322), but also in other types of RCC (129). These studies suggest that VEGF might be upregulated by both HIF-1-dependent and independent pathways. Therefore, though VEGF is mainly involved in tumor angiogenesis, it is not an efficient hypoxia and prognostic marker.

PAI-1

The correlation between PAI-1 and patient outcome was assessed in 22 studies with eight types of tumors. They include breast (50, 51, 54, 80, 97, 104, 106, 107, 153, 177, 196, 232), head and neck (247), lung (229), ovarian (33, 162, 172), cervix (109), gastric cancers (17, 112), endometrial carcinoma (289), and brain tumor (212). Except for four studies that evaluated PAI-1 expression using quantitative PCR (247) and immunohistochemistry (17, 33, 109), the rest of studies analyzed PAI-1 serum levels. All PAI-1 studies showed its significant correlation with patient outcome by using *p* value from Kaplan–Meier's survival curve. Multivariate analysis was performed in 21 studies, and 19 of them identified PAI-1 as an independent prognostic marker. This suggests the use of PAI-1 as an efficient prognostic factor. However, since none of the PAI-1 studies evaluated the correlation of PAI-1 with tumor hypoxia, its role as a hypoxia marker is still in doubt.

Regardless of its role in uPA inhibition, PAI-1 was strongly correlated with uPA, and combined expression of them showed poorer patient outcome (33, 51, 107, 196, 229, 232). PAI-1 was also correlated with lymph node metastasis (104, 247) and tumor stage or grade (109, 289) that suggests its association with tumor progression.

LOX

Since hypoxia and HIF-1-regulated LOX expression was recently reported, there is only one study evaluating the correlation of LOX with patient outcome in ER negative breast and head and neck cancers (69). Though multivariate analysis was not performed, significant association of LOX with poorer overall survival and metastasis free survival was determined in both of these tumors. In this study, LOX expression in breast cancer was significantly correlated with hypoxia. Hypoxia was particularly analyzed by using a hypoxia score. Hypoxia score was obtained from the expression value of unique gene clusters that have hypoxia gene signature. LOX expression in head and neck cancer was also associated with CAIX expression. Though more studies are needed, its known functions in cell invasion and migration suggests its future use as a prognostic marker.

BNIP3

The correlation of BNIP3 with patient prognosis was analyzed in two studies with NSCLC (94) and pancreatic cancer (67). Whereas expression of BNIP3 was an independent prognostic factor in NSCLC, loss of BNIP3 showed significant relation with poor patient outcome in pancreatic cancer. In NSCLC BNIP3 was shown to be associated with HIF-1, LDHA, and CAIX. This suggests that this pro-apoptotic gene functions in tumorigenesis by selecting out more aggressive form of tumor cells. On the other hand, loss of BNIP3 was detected in the late stage of pancreatic cancer. This suggests that BNIP3 might be silenced by hypermethylation (222) when the tumor has already transformed to a malignant stage. Since there is only small number of studies reporting the role of BNIP3 under hypoxia, further studies are needed to use BNIP3 as a prognostic marker.

EPO

There are two studies analyzing the correlation of EPO and patient outcome. EPO expression was evaluated in head and neck cancer (318) and endometrial carcinoma (2). In head and neck cancer, though expression of EPO was not significantly associated with patient survival, it showed strong correlation with HIF-1 and CAIX. Expression of EPO was an independent prognostic factor in endometrial carcinoma. These studies suggest that in tumors EPO might be upregulated in response to hypoxia via HIF-1 mediated pathway, and could be used as a marker for prognosis, though it needs to be studied further.

NF-kB

Nine studies evaluated the correlation of NF-kB and patient prognosis in six different tumor types, including breast (25), head and neck (1, 128, 331), gastric (186), prostate cancers (57, 244), peripheral T cell lymphoma (198), and adenoid cystic carcinoma of salivary gland (330). Six out of nine studies showed significant relation of NF-kB using p value from Kaplan–Meier's survival curve, and in five out of six multivariate analyses it was identified as an independent prognostic marker of poor patient outcome. On the other hand, NF-kB expression was significantly associated with favorable prognosis in gastric cancer and T cell lymphoma. In T cell lymphoma, NF-kB was also determined as an independent factor for better patient outcome.

NF-kB expression was also strongly associated with anti-apoptotic genes, Bcl-2 and Bax (25), VEGF and iNOS (330), and vascular invasion and metastasis (128, 330). These studies suggest the role of NF-kB in tumor progression such as anti-apotosis, angiogenesis, and metastasis. However, no study analyzed the correlation of NF-kB with hypoxia and HIF-1 expression to determine NF-kB as a hypoxia marker.

In gastric cancer, NF-kB was mostly activated in the early stage of tumor and negatively associated with tumor invasion and metastasis. On the other hand, NF-kB was strongly correlated with phosphorylated Akt and tumor suppressor genes such as Smad4 and APC. Since NF-kB is also required for T cell survival (333), the role of NF-kB in cell proliferation might cause better patient outcome in both gastric cancer and T cell lymphoma. Therefore, anti- or pro-oncogenic role of NF-kB in various tumor stages needs to be further studied before determining its expression as a prognostic marker.

AP-1

In five studies with three different tumor types, AP-1 was analyzed by evaluating expression of c-fos, c-jun, or phosphorylated c-jun. Tumors included breast (20, 87, 308) and prostate (63) cancers and endometrial carcinoma (323), and four of those studies determined significant correlation with poor patient outcome. However, none of them performed multivariate analysis.

In breast cancer c-fos was correlated with oncogenic genes such as H-ras, c-myc, TGFα, and MAP kinase (20). c-Jun was also associated with metastasis (87, 323), VEGF and MVD (308) in breast cancers and endometrial carcinoma. These studies suggest the role of AP-1 in tumorigenesis and angiogenesis. Since expression of AP-1 does not necessarily means its activation, c-jun, especially the phosphorylated form of c-jun, might have a potential as a more efficient prognostic marker.

Hsp

The correlation between Hsp and patient outcome is analyzed in 25 studies with 13 different types of tumors. Tumors include breast (40, 206, 221, 307), head and neck (72, 142, 144, 215), ovarian (8, 65, 89, 154), gastric (137, 285) cancers, RCC (68, 248), colorectal (278), bladder (283), prostate (43) cancers, endometrial carcinoma (88), hepatocellular carcinoma (156), osteosarcoma (302), acute myeloid leukemia (AML) (139, 296), and melanoma (241). Twenty studies showed significant relation of Hsp but five of them were associated with better prognosis. Also, eight studies identified it as an independent prognostic marker from 15 multivariate analyses, while it was an independent factor for favorable patient outcome in one of those studies.

Most studies evaluated the expression of Hsp27 and Hsp70. Among 17 studies regarding Hsp27 expression, ten studies showed significant association with poor patient outcome and six studies determined it as an independent prognostic marker (43, 65, 88, 156, 296, 302). One study identified Hsp27 as an independent factor for favorable prognosis (144). Hsp70 was analyzed in 12 studies and four of them showed its significant correlation with poor survival (142, 283, 296, 307). However, in five studies, Hsp70 was correlated with better patient outcome (144, 206, 215, 241, 248). Thus, since Hsp70 has ambiguous correlation with poor or better prognosis, Hsp27 is a better candidate for a prognostic marker of poor patient outcome. Expression of Hsp60, Hsp90, and Hsp110 were also evaluated in several studies. Whereas Hsp60 was associated with both poor (154, 296) and better (72) prognosis, expression of Hsp90 (65) and Hsp110 (296) was only correlated with poor patient outcome.

The conflicting correlation of Hsp, especially Hsp70, with patient survival might be due to its dual role in tumorigenesis. As mentioned above, Hsps promote both anti- and pro-apoptotic pathways. Also, they increase not only the survival and proliferation of tumor cells, but also the host immunity. Therefore, Hsps might exhibit different functions according to tumor type and stage and its potential as a prognostic marker needs to be further studied.

UPR (GRP78)

Regarding the correlation of UPR and patient outcome, only two studies have been done with GRP78. No studies are done with GRP94 yet. In those two studies with lung cancer (303) and neuroblastoma (117), GRP78 was identified as a favorable prognostic factor. Since these studies included patients who received chemotherapy, especially cisplatin-based treatment in lung cancer patients, overexpression of GRP78 might be due to the chemoresistant response which was reported before (197). However, to determine GRP78 as a prognostic marker, further studies are needed with various types of tumors.

OPN

The correlation of OPN and patient prognosis was evlauted in 14 studies. These studies included six different tumor types, including breast (23, 48, 245, 264, 299), head and neck (184), lung (58, 183, 252, 259), prostate cancers (82, 116), hepatocellular carcinoma (228), and osteosarcoma (277). Among them, 13 studies showed significant association of OPN with patient outcome, and seven out of nine multivariate analyses identified it as an independent prognostic marker.

OPN was also associated with bone metastasis (116), tumor invasion (228), and VEGF (277). In additon, in lung cancer, coexpression of OPN and VEGF showed significant relation with patient outcome (259). These studies suggest a potential role of OPN in tumor angiogenesis and metastasis. Though correlation of OPN and HIF-1 was not determined, OPN was in-

versely associated with VHL expression in head and neck cancer (184). Also, the strong correlation of OPN and hypoxia was determined in two studies with head and neck cancer (184) and lung cancer (183) by using Eppendorf oxygen probes that suggests the possible use of OPN as a hypoxia and prognostic marker.

DISCUSSION

From our summaries of 213 papers, correlation of 15 genes and patient outcome was analyzed by using *p* values from Kaplan–Meier analyses and *p* value and hazard ratios from multivariate analysis. Whereas Glut-1, PAI-1, and OPN showed the most consistent correlations with patient outcome in univariate analysis, multivariate analysis suggested that CAIX and PAI-1 were the most consistent prognostic markers. Glut-1 was identified as an independent predictor in 5/5 multivariate analyses, but more studies are needed to determine its reliability as a hypoxia and prognostic marker. The majority of 22 reported PAI-1 studies have been done in breast cancer, and direct comparison of tumor hypoxia with serum PAI-1 has not been performed. Additional validation of this marker is required in other histologic types, particularly where cross validation is done vs. other established methods for assessing hypoxia. CAIX expression, on the other hand, has been positively correlated with other measurements of hypoxia in four studies and has been shown in several studies to be an independent prognostic factor. There are some negative reports as well, however (203).

There might be additional benefit gained by using more than one hypoxia-dependent protein as a hypoxia marker. Of the 213 studies in this review, only 25 compared more than one marker. In six reports, combining two genes was found to be prognostically important (19, 47, 119, 166, 169, 325). However, there has been no systematic attempt to compare one vs. multiple reporter proteins. This is an important area for future investigation.

In conclusion, although the majority of reports have shown correlations between putative hypoxia marker proteins and prognosis, none of the 15 genes reviewed in this report stand out as a clear winner in the search for tissue-based reporter of hypoxia. Methodological differences between reports, such as use of different antibodies and methods of quantification may have contributed to some of the variability. Standardization of evaluation methods may be required to go to the next step. Combinations of reporter proteins may be superior to use of one.

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ABBREVIATIONS

AML, acute myeloid leukemia; AP-1, activator protein-1; bHLH-PAS, basic helix–loop–helix-PER-ARNT-SIM; BNIP3, Bcl-2/E1B 19 kDa interacting protein; bZIP, basic-region leucine Zipper; CAIX, carbonic anhydrase IX; COX-2, cyclooxygenase-2; CRE, cAMP response element; DFO, desferrioxamine; ECM, extracellular matrix; EGF, epidermal growth factor; EPAS-1, endothelial PAS protein-1; EPO, erythropoietin; EPR, EPO receptor; ER, endoplasmic reticulum; ERSE, ER stress response element; FIH-1, factor inhibiting HIF-1; GLUT-1, glucose transporter-1; GRP, glucose-regulated protein; HIF-1, hypoxia-inducible factor-1; HRE, hypoxia response element; HSE, heat shock element; HSF, heat shock factor; Hsp, heat shock protein; IGF, insulin growth factor; IkB, inhibitors of kB; LDH, lactate dehydrogenase; LOX, lysyl oxidase; MVD, microvessel density; NF-kB, nuclear factor-kB; NSCLC, non-small cell lung carcinoma; ODD, oxygen-dependent domain; OPN, osteopontin; PAI-1, plasminogen activator inhibitor-1; PHD, prolyl 4-hydroxylase; RCC, renal cell carcinoma; TAD, transactivation domain; TAM, tumor associated macrophages; tPA, tissue-type plasminogen activator; TRE, TPA response element; uPA, urokinase plasminogen activator; UPR, unfolded protein response; UPRE, unfolded protein response element; VEGF, vascular endothelial factor; VHL, von Hippel Lindau.

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