Angiogenesis in Gliomas: Biology and Molecular Pathophysiology

Ingeborg Fischer[1,2,3]; Jean-Pierre Gagner[1,2,3]; Meng Law[4,5,6]; Elizabeth W. Newcomb[2,6]; David Zagzag[1,2,3,5,6]

[1]Microvascular and Molecular Neuro-oncology Laboratory, [2]Department of Pathology, [3]Division of Neuropathology, [4]Department of Radiology, [5]Department of Neurosurgery, [6]New York University Cancer Institute, New York University School of Medicine.

Corresponding author:
David Zagzag, MD, PhD, Department of Pathology, Division of Neuropathology, New York University Medical Center, 550 First Avenue, New York, NY 10016 (E-mail: *dz4@nyu.edu*)

Glioblastoma multiforme (GBM) is characterized by exuberant angiogenesis, a key event in tumor growth and progression. The pathologic mechanisms driving this change and the biological behavior of gliomas remain unclear. One mechanism may involve cooption of native blood vessels by glioma cells inducing expression of angiopoietin-2 by endothelial cells. Subsequently, vascular apoptosis and involution leads to necrosis and hypoxia. This in turn induces angiogenesis that is associated with expression of hypoxia-inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF) in perinecrotic pseudopalisading glioma cells. Here we review the molecular and cellular mechanisms implicated in HIF-1-dependent and HIF-1-independent glioma-associated angiogenesis. In GBMs, both tumor hypoxia and genetic alterations commonly occur and act together to induce the expression of HIF-1. The angiogenic response of the tumor to HIF-1 is mediated by HIF-1-regulated target genes leading to the upregulation of several proangiogenic factors such as VEGF and other adaptive response molecules. Understanding the roles of these regulatory processes in tumor neovascularization, tumor growth and progression, and resistance to therapy will ultimately lead to the development of improved antiangiogenic therapies for GBMs.

Brain Pathol 2005;15:297-310.

INTRODUCTION

Angiogenesis is recognized as a key event in the natural progression of gliomas (15, 40, 197). Of all solid tumors, those of the brain show the highest degree of vascular proliferation (28). Newly formed brain tumor blood vessels possess a defective blood-brain barrier that contributes to the pathogenesis of tumor-associated edema (47, 182, 203, 207). They are associated with increased risk of intratumoral hemorrhage (41, 105) and are responsible for contrast enhancement (39, 45, 53, 179, 203). Unlike tumors in other locations, intracranial tumors rarely metastasize to distant organs and their malignant behavior and prognosis are determined by their histological grade. The WHO classification distinguishes low grade from high grade diffuse astrocytomas by the presence of microvascular proliferation as a diagnostic criterion and an independent prognostic parameter (1, 32, 45, 94, 169). Neovascularization in brain tumors correlates directly with their biological aggressiveness, degree of malignancy and clinical recurrence and inversely with the post-operative survival of patients with gliomas (32, 45, 94). Diffuse astrocytomas tend to progress from grade II to grade III tumors with a time interval of several years, whereas, progression of grade III to grade IV is more rapid, typically 2 years. The time intervals for tumor progression are variable and the pathologic mechanisms driving the change in biologic behavior between different grades remain unclear. GBMs that arise from a low-grade glioma lesion are called "secondary glioblastoma" (94). However, in most cases, GBMs appear "de novo" and are thus termed "primary glioblastoma" (95). Regardless of their mode of progression, primary and secondary GBMs are morphologically indistinguishable and show their histologic hallmarks, ie, "glomeruloid" microvascular tufts and necrosis (Figure 1A, B).

The characteristic vascular morphology in the GBMs has led to the hypothesis that the formation of new blood vessels or angiogenesis is crucial to their growth. GBMs were first recognized to be angiogenic, eg, capable of inducing vascular sprouting, when implanted in the rabbit cornea, a well-characterized angiogenesis assay (27). In addition, glioma cells were found to have angiogenic activities, eg, capillary morphogenesis and endothelial mitogenic activities in vitro (2, 170). Subsequently, vascular endothelial growth factor (VEGF), characterized as an endothelial-cell mitogen (60) and a permeability factor (52, 148), was found to be present in pseudopalisading tumor cells adjacent to necrotic zones and hyperplastic vessels, implicating its role in glioma angiogenesis (140, 163). The discovery of hypoxia inducible factor-1 (HIF-1) (161) and the observation that hypoxia-induced HIF-1α expression in pseudopalisading cells (Figure 1C) (209) was concomitant with the expression of one of its target genes, VEGF (140, 163), established a biological link between hypoxia and angiogenesis (161). Since then, the field of angiogenesis research in gliomas has evolved rapidly (15, 22, 46, 121).

The formation of new blood vessels occurs physiologically during embryogenesis (34, 194). In adult life it is observed in the female reproductive system and during wound healing (175) and in a wide range of pathologic settings, such as ischemic diseases, chronic inflammatory reactions, and neoplasia (35, 65). During embryonic development, blood vessels are newly formed from endothelial precursors and hematopoietic stem cells, a process known as vasculogenesis (194). In contrast, angiogenesis, the sprouting of new blood vessels from pre-existing ones (65, 74, 194) results from an altered balance of proangiogenic factors (Table 1) and antiangiogenic factors (Table 2). The role of endothelial cell progenitors in angiogenesis is unclear and will be discussed below.

For the most part, cells need access to the vascular system to provide a source of nutrients and oxygen as well as to eliminate metabolic waste products. To a limited

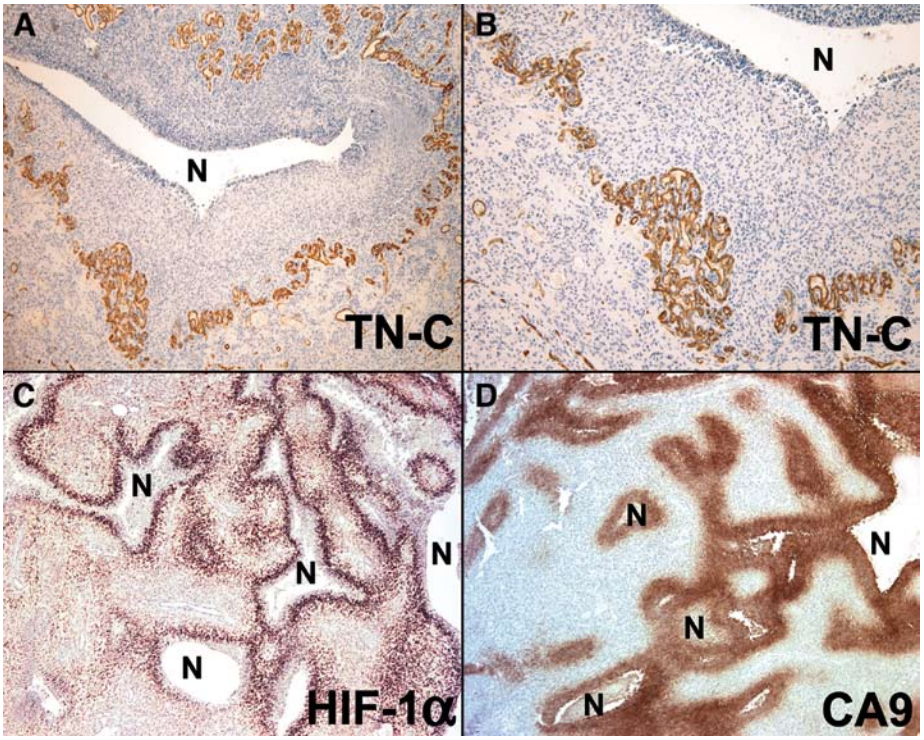

Figure 1. *Imunohistochemistry for tenascin-C (TN-C), HIF-1α and carbonic anhydrase (CA9) expression in a GBM.* **A, B.** TN-C immunoreactivity decorates the hyperplastic vessels seen adjacent to a necrotic zone (N); **C, D.** HIF-1α and CA9: Pseudopalisading cells around areas of necrosis (N) show intense immunoreactivity for HIF-1α (**C**) and CA9 (**D**); (immunoperoxidase, **A** ×100; **B** ×200; **C, D** ×50).

extent, this can be accomplished by diffusion if the cells are situated within ~100 µm of the nearest blood vessel (64, 147). In several experimental tumor systems, the balance between proliferation and apoptotic rates is characteristic of the dormant state (64, 74). It is now well accepted that a change in this balance is associated with malignant transformation and that the recruitment of a new blood supply is critical for tumor growth (74). Angiogenesis results in exponential growth of the tumor beyond the limit of a few millimeters seen in the absence of angiogenesis (64). The sequence of events leading to the formation of new blood vessels is well characterized and involves an initial VEGF-mediated increase of vascular permeability leading to extravasation of plasma proteins associated with dilatation of native vessels and reduction in their pericyte coverage. Subsequently, endothelial cells migrate and proliferate. For this cascade to occur, deposition of a proangiogenic matrix for the newly sprouting vessel is essential. This involves breakdown of the vascular basement membrane and extracellular matrix (ECM) through the action of cathepsin B, matrix metalloproteases (MMPs) and other enzymes as well as the expression of matrix proteins such as fibronectin, laminin, tenascin-C and vitronectin (68, 106, 122, 200, 201). Several of these ECM molecules enhance phosphorylation of focal adhesion kinase, a critical step in glioma angiogenesis (75, 202, 208). Finally, the angiogenic process culminates in the assembly of endothelial cells to form a vascular lumen followed by the elaboration of a new basement membrane and the recruitment of pericytes (52, 65, 74). The important roles that ECM and proteases play in mediating angiogenesis in gliomas is reviewed in the accompanying articles (see Wang et al and Lakka et al; this issue).

As described above, early models of tumor growth presumed that tumors showed angiogenic activity only when they had grown to a size beyond that which the tumor cells could no longer be nourished by mere diffusion (64). However, recent in vivo experiments performed with experimental gliomas provided evidence contrary to this notion (80, 198). In contrast to the accepted dogma that tumor development occurs in 2 phases (avascular and vascular), we observed that tumor growth in the brain follows 2 vascular phases. In the first vascular phase, the vessels are native cerebral vessels, which are coopted by tumor cells, while in the second phase, there is true neovascularization arising from existing vessels. During the transition period between these two phases, hypoxia driven HIF-1 expression occurs which results in VEGF secretion and the induction of neovascularization. As illustrated in Figure 2, glioma cells first accumulate around existing vasculature (Stage I). This is associated with a mechanical disruption of the normal contact between endothelial cells and the basement membrane by the insinuating glioma cells lifting off the astrocytic foot processes (198). In response, the endothelial cells of the coopted blood vessels express angiopoietin-2 (Ang-2) (80, 198, 204). This leads to the destabilization of the blood vessel wall associated with decreased pericyte coverage (80, 198, 204). In Stage II, perivascular proliferation takes place. In Stage III, these blood vessels become apoptotic and undergo involution (80, 198). This vascular collapse results in the loss of neighbouring tumor cells. In Stage IV, angiogenesis adjacent to the necrotic area is triggered in response to increased expression of HIF-1α and VEGF, a process that rescues the remaining tumor cells (80, 198). Thus, our experimental evidence suggests four sequential steps in glioma progression (Figure 2): *i)* perivascular organization, *ii)* proliferation, *iii)* vascular regression followed by necrosis, and *iv)* angiogenesis (198).

In addition to the vascular collapse described above, it has been suggested that a procoagulative state combined with the inherent genetic instability of the tumor could elicit the necrosis typically observed in GBMs (142). Necrosis has been consistently used as a grading criterion in gliomas (32, 94, 127) and reported to predict poor patient outcome (14, 98). For example, tissue factor, the catalyst of the extrinsic pathway of hemostasis, is overexpressed under hypoxic conditions and could be involved in vascular thrombosis (26, 150). Thus, vascular regression and necrosis constitute necessary events for the subsequent development of angiogenesis. As mentioned earlier, this angiogenic response results from an altered balance of a large number of proangiogenic (Table 1) and antiangiogenic factors (Table 2). Among the proangiogenic factors described in gliomas, the best characterized and the most important in glioma progression include VEGF and the angiopoietins.

Factors	Descriptions	Ligand Chromosomal Locations*	Cognate Receptors	Receptor Chromosomal Locations*	References
Tyrosine Kinase Receptor Ligands					
Angiopoietin-1, -4	Tie2 agonists	8q22, 20p13	Tie2	9q21	121
EGF	Epidermal Growth Factor	4q25	EGFR/HER1	**7p12.3-p12.1**	51
aFGF	acidic Fibroblast Growth Factor, FGF-1	5q31	FGFR-1/-2	8p11.2-p11.1, **10q26**	51
bFGF	basic Fibroblast Growth Factor, FGF-2	4q25-q27	FGFR-1/-2	8p11.2-p11.1, **10q26**	51, 121
HGF/SF	Hepatocyte Growth Factor/Scatter Factor	7q21.1	c-Met	7q31	46
PDGFAA	Platelet-Derived Growth Factor, A homodimer	7p22	PDGFRα	4q12	51, 121
PDGFBB	Platelet-Derived Growth Factor, B homodimer	**22q12.3-q13.1**	PDGFRα/β	4q12, **5q31-q32**	51, 121
PLGF-2	Placental Growth Factor-2	14q24-q31	VEGFR-1, NRP-1	13q12, **10p12**	6, 78
TGFα	Transforming Growth Factor, Alpha	2p13	EGFR/HER1	**7p12.3-p12.1**	51
TGFβ	Transforming Growth Factor, Beta	**19q13.1**	TGFBR-1/-2	9q33-q34, 3p22	51, 121
VEGF-A, -B	Vascular Endothelial Growth Factor	6p12, 11q13	VEGFR-1/-2, NRP-1	13q12, 4q12, **10p12**	30, 70, 78, 121
Other Receptor Type Ligands					
Adrenomedullin	calcitonin-like peptide	11p15.4	CRLR/RAMP2/3	17q12-q21.1, 7p13-p12	46
Endothelin-1	survival/antiapoptotic peptide	6p24-p23	EDRNA/B	4q31.2, 13q22	54
IGF-1, -2	Insulin-like Growth Factor-1, -2	12q22-q24.1, 11p15.5	IGF1R	15q25-q26	79, 108
IGFBP-2, -3	IGF Binding Protein-2, -3	2q33-q34, 7p14-p12			69, 103
Interleukin-6	cytokine	7p21	IL6R	1q21.3	107
Interleukin-8	CXCL8 chemokine	4q12-q13	IL8RA/B	2q35, 2q35	25
Pleiotrophin	angiogenic/mitogenic heparin-binding protein	7q33	RPTPzeta	7q31.3	121
SDF-1	Stromal cell-Derived Factor-1, CXCL12	**10q11.1**	CXCR4	2p21	144, 155
PGE2	prostaglandin E2	n.a.	EPs	many	31, 128, 178

Table 1. *Endogenous proangiogenic factors in gliomas.* Abbreviations: CRLR/RAMP2/3, calcitonin receptor-like receptor/receptor activity-modifying protein-2/-3; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; EDRNA/B, endothelin receptor type A or B; EP, prostaglandin E2 receptor; HER1, human epidermal growth factor receptor 1; n.a., not applicable; NRP-1, neuropilin-1; RPTPzeta, receptor protein tyrosine phosphatase zeta; Tie2, Tyrosine kinase with Immunoglobulin and Epidermal growth factor homology domain 2. *Chromosomal locations with known gene overexpression, mutation or loss of heterozygosity are indicated in bold (See accompanying article by Gagner et al; this issue). Chromosomal location information was obtained from the Online Mendelian Inheritance in Man website.

THE VEGF FAMILY

The VEGF family includes 6 secreted glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PLGF) (11, 12, 60, 78). VEGF-A, the prototype of the VEGF family, is simply referred to as VEGF in this review. VEGF mRNA undergoes alternative splicing to yield several mature isoforms, with $VEGF_{165}$ being the predominant isoform expressed in a variety of human solid tumors (60, 78). VEGF is required for normal development of blood vessels, and loss of a single allele during embryonic development is lethal (34). In postnatal life, VEGF expression levels are minimal, except at sites of physiologically active angiogenesis, such as the uterus or during wound healing (175). In tumors, overexpression of VEGF leads to increased vascularity and vascular permeability. In fact, VEGF was first described as "vascular permeability factor," since it was observed to render both existing and newly formed tumor blood vessels leaky (52, 148).

The migration and proliferation of endothelial cells occur along the proangiogenic ECM (201, 208) following chemotactic signals mediated by growth factors, such as VEGF (212). Using a murine glioma model, overexpression of VEGF was shown to produce formation of hyperplastic microvascular proliferations known as "glomeruloid bodies" which share structural similarities with the glomeruloid proliferation seen in GBMs (171). The microvascular glomeruloid proliferations in GBMs are composed of hyperplastic endothelial cells surrounded by a basement membrane of irregular thickness and a discontinuous layer of smooth-muscle actin positive pericytes and vascular smooth muscle cells. Astrocytic foot processes are typically absent (189). These microvascular proliferations regress upon VEGF withdrawal (171).

The VEGF isoforms exert their function through three cognate VEGF receptor tyrosine kinases: VEGFR-1/Flt-1, VEGFR-2 Flk-1/KDR, and VEGFR-3 and 2 neuropilins receptors, NRP-1 and NRP-2 (48, 49, 61, 123, 183). NRP-1 and NRP-2 are VEGFR co-receptors. The VEGF receptors are primarily expressed on tumor endothelium, but are absent in normal surrounding tissue (139). VEGFR signaling triggers increased vascular permeability and proliferation, differentiation, survival and migration of endothelial cells (97, 183, 211). The effect of VEGF signaling depends on the receptor subtype (61, 77, 211). For example, VEGFR-2 is primarily responsible for proliferation (77, 78).

VEGF mRNA is highly expressed in pseudopalisading cells around necrotic zones (17, 140, 163). The expression of VEGF and VEGFR-l and VEGFR-2 correlates with the grade in diffuse astrocytomas, is crucial for glioma growth, and displays a temporal and spatial correlation with the angiogenesis seen in human gliomas (1, 17, 40, 100, 139, 140, 156, 165, 177, 199).

Factors	Descriptions	Chromosomal Location† of Parent Molecules	Cognate Receptors or Target Mechanisms	Protein or Receptor Chromosomal Locations†	References
Matrix and Plasma-Derived Proteins					
Angiostatin	internal fragment of plasminogen	6q26	αvβ3 integrin, others	many	132, 143
Endostatin	C-terminal fragment of collagen type XVIII	21q22.3	α5β1 integrin, HSPG, others	many	132, 143
PEX	C-terminal fragment of MMP-2	16q13	αvβ3 integrin, MMP-2	many	132, 143
TFPI-2	Tissue Factor Pathway Inhibitor-2	7q22	TF - FVIIa - FXa	many	193
TIMP-1	Tissue Inhibitor of Metalloprotease-1	Xp11.3-p11.2	MMPs, VEGF, others	many	132, 143
TIMP-3, -4	Tissue Inhibitor of Metalloprotease-3, -4	**22q12.1-q13.2**, 3p25	MMPs, VEGF, others	many	126, 132, 143
TSP-1, -2, -3	Thrombospondin-1, -2, -3	15q15, 6q27, 1q21	CD36, MMPs, others	7q11.2, many	132, 143
Factors and Receptors					
Angiopoietin-2*	antagonist to angiopoietin-1	8p23	Tie2	9q21	121
BAI-1, -2, -3	Brain-specific Angiogenesis Inhibitor-1, -2, -3	8q24, **1p35**, 6q12	n.d.	n.d.	92
Vasculostatin	proteolytic extracellular domain of BAI-1	8q24	n.d.	n.d.	89
ING4	Inhibitor of Growth 4	12p13.3	n.d.	n.d.	66
Interferon-α, -β	cytokine	9p22, 9p21	IFNAR-1	21q22.1	132
Interleukin-4	cytokine	5q31.1	IL4RA	16p12.1-p11.2	132, 152
PEDF	Pigment Endothelial-Derived Factor, SERPINF1	17p13.3	TSP-1, VEGF, others	many	132, 143
Platelet factor-4	CXCL4 chemokine	4q12-q13	CXCR3, HSPG, others	8p12-p11.2, many	121, 143
16KDa PRL	16 KDa fragment of prolactin	6p22.2-p21.3	16KDa PRL receptor	n.d.	42, 44
PRP	Proliferin-Related Peptide	n.d.	n.d.	n.d.	16, 44
Somatostatin	peptide hormone	3q28	somatostatin receptor-2, -3	17q24, **22q13.1**	38, 120
VEGI	Vascular Endothelial Growth Inhibitor, TNFSF15	9q33	n.d.	n.d.	191
sVEGFR-1/Flt-1	soluble VEGFR-1/Flt-1	13q12	acts as decoy receptor	13q12	100, 132

Table 2. *Endogenous antiangiogenic factors in gliomas.* Abbreviations: CXCL, CXC chemokine ligand; CXCR3, CXC chemokine receptor 3; HSPG, heparan sulfate proteoglycan; IFNAR-1, interferon alpha, beta, and omega receptor 1; MMP, matrix metalloprotease; n.d., not determined; SERPINF1, serine protease inhibitor F1; TF - FVIIa - FXa, tissue factor-factor VIIa-factor Xa complex; Tie2, Tyrosine kinase with Immunoglobulin and Epidermal growth factor homology domain 2; TNFSF15, tumor necrosis factor ligand superfamily 15. *Isolated expression of Angiopoietin-2 not followed by VEGF signaling leads to endothelial cell apoptosis (80).

† Chromosomal locations with known gene overexpression, mutation or loss of heterozygosity are indicated in bold (See accompanying article by Gagner et

Similarly, PLGF and NRP-1 are upregulated in GBMs and their expression correlate with tumor grade (30, 49, 130, 135).

PLGF deficient mice showed normal vascular development, but impaired tumor vascularization (36). PLGF acts synergistically with VEGF in increasing tumor vascularization (6, 11, 12, 36, 110) and recruits hematopoietic stem cells to the tumor vasculature (76). However, such contribution of endothelial progenitor cells and hematopoietic stem cells to tumor vasculature has not been entirely elucidated. Specifically, the capacity to recruit these precursors to the vascular bed may depend on the tumor type and production by the tumor of mobilization signals for endothelial precursor cells (71, 86, 111, 112, 114).

Regulation of VEGF expression. The transcriptional activation of VEGF is enhanced largely through the transcription factor HIF-1 (161). Other transcription factors capable of inducing VEGF transcription, include ETS-1 proto-oncogene and STAT-3 (107, 181). Through binding to specific ETS response element DNA sequences, ETS proteins activate genes such as VEGFR-1 and VEGFR-2, integrin β_3, some MMPs and urokinase plasminogen activator (uPA) (151). VEGF itself, induces ETS-1 in adjacent endothelial cells, therefore enhancing VEGFR signaling (181). Accordingly, ETS-1 expression is increased in GBMs compared to low grade astrocytomas. Its distribution correlates with vascular proliferation, with the most prominent expression observed in the glomeruloid tufts of GBMs (181). Inactivation of ETS-1 reduced rat C6 glioma cell proliferation (151). In addition to transcription factors, cytokines and growth factors, including TGF-β, EGF, PDGF-B, basic FGF, upregulate VEGF (43, 62, 73, 107, 138, 176, 185). Genetic alterations seen in gliomas, such as EGFR activation and PTEN mutation (13, 50, 102, 109, 133, 134, 141, 167, 187), lead to enhanced VEGF expression thereby promoting angiogenic activity as described in more detail below.

THE ANGIOPOIETIN FAMILY

The angiopoietins are important endothelial growth factors which signal via the Tie2 receptor tyrosine kinase expressed on endothelial cells. In particular, Ang-1 and -2, have been implicated in glioma angiogenesis (80, 168, 196, 198, 202, 204). Ang-1 mediated activation of Tie2 is required for stabilization, remodelling and maturation of blood vessels (115). Overexpression of Ang-1 in a rat glioma model promotes angiogenesis and tumor growth and is associated with an increased number of highly branched vessels covered by pericytes (113). While VEGF and Ang-1 may act in concert (proliferation and maturation), Ang-2 has been implicated in further remodeling of the initial microvasculature (80, 168, 198, 204). Increased expression of Ang-2 on GBM microvasculature appears early during glioma angiogenesis (168, 198, 204). However, binding of Ang-

2 to the Tie2 receptor on endothelial cells antagonizes this receptor's phosphorylation, thereby disrupting contacts between endothelial and periendothelial support cells and disengaging pericytes from the tumor vessels during initiation of vessel sprouting or regression (80, 115, 204). Examination of the expression patterns of angiopoietins and their receptors suggest a role in GBM vasculature and malignant transformation (168, 177, 196, 198, 204). For example, increased Tie2 expression has been observed with increasing grade of human astrocytoma (196). Ang-2 and Tie2 expression are absent in the normal brain vasculature but are induced in tumor endothelium of coopted tumor vessels prior to their regression (168, 198, 204). Of particular importance, treatment of glioma cell derived mouse xenografts with a dominant negative form of Tie2 results in a significant decrease in tumor growth (196). The role of angiopoietins in glioma angiogenesis is reviewed in an accompanying article (see Reiss et al; this issue).

OTHER ANGIOGENIC FACTORS

A number of other factors modulate the neovascularization seen in GBMs (197) as shown in Table 1. These include fibroblast growth factor (FGF), platelet derived growth factor (PDGF), hepatocyte growth factor (HGF) and tumor necrosis factor (TNF)-α, (29, 51, 121, 197). The FGF family includes several proteins that share structural properties. Both acidic FGF (FGF1) and basic FGF (FGF2) are upregulated in GBMs (158, 206) and are responsible for resistance of endothelial cells to apoptosis (7). The presence of basic FGF in cerebrospinal fluids from children and adults with brain tumors has been correlated with tumor microvessel formation (101). Furthermore, in vivo tumor growth of C6 glioma cells is inhibited when the cells are transfected with dominant negative FGF receptor (FGFR1 or FGFR2), an effect associated in part with inhibition of angiogenesis (10). PDGF-B and PDGFR-β mRNA are upregulated in GBMs (162, 164). PDGF's effects on angiogenesis are mediated, at least in part, by VEGF (73, 185). In gliomas, the expression of HGF, also known as Scatter Factor (HGF/SF), correlates with microvessel density (124, 158). In addition, c-met, the HGF receptor is upregulated by hypoxia (137). The exact role of TNF-α in glioma's angiogenesis remains unclear. Although enhanced VEGF expression has been reported in TNF-α-treated glioma cells (125), treatment of brain microvascular endothelial cells with TNF-α inhibits their proliferation (129). Among the other factors that have been linked to angiogenesis in GBMs are interleukins (IL)-6 and -8, adrenomedullin, insulin-like growth factor binding proteins (IGFBPs), and stromal cell derived factor (SDF-1) (Table 1). Many of these factors are overexpressed with increasing tumor grade in gliomas (9, 23, 25, 55, 79, 144, 149, 154, 155) and predict adverse clinical outcome (9, 149).

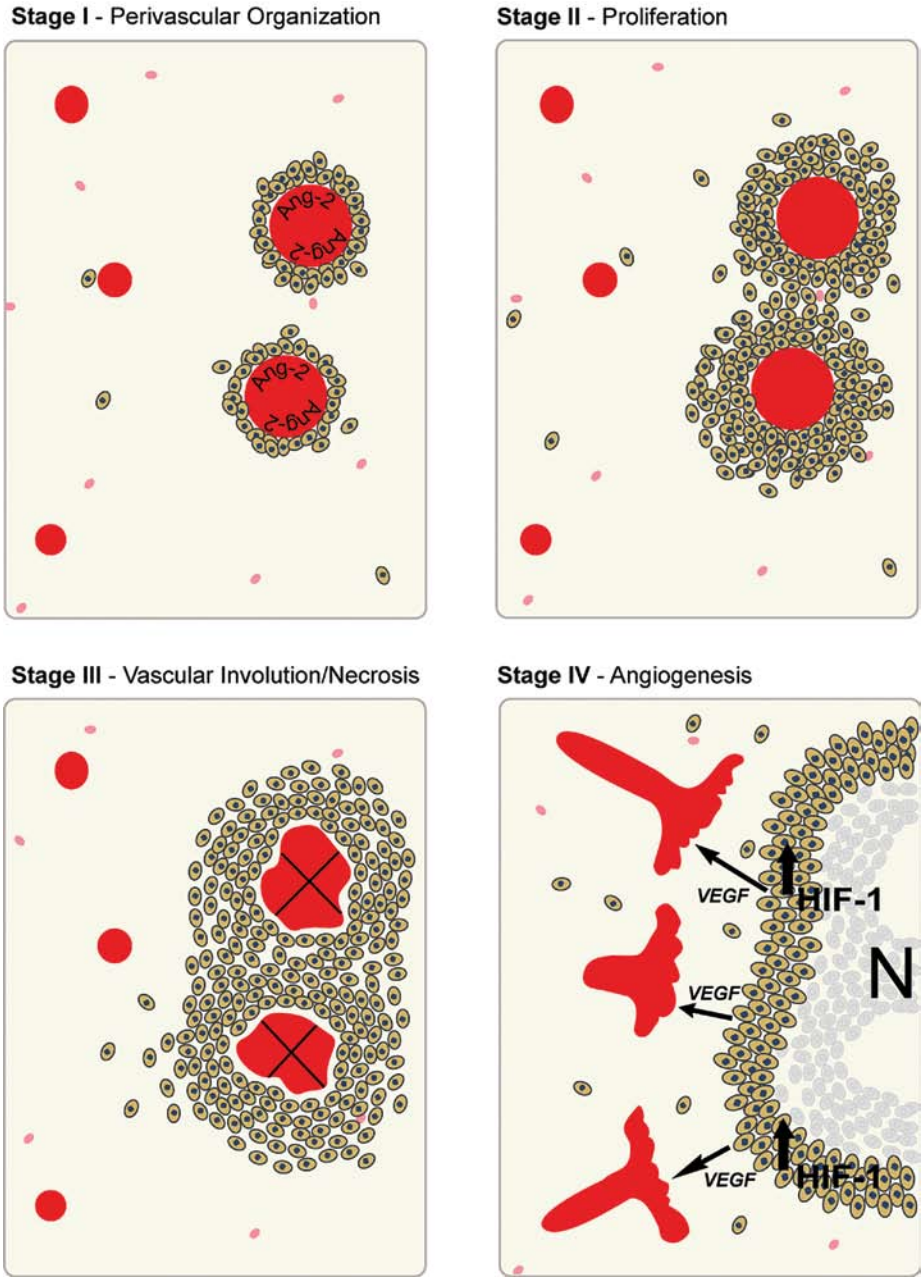

Figure 2. *Schematic representation of distinct stages of glioma growth.* **Stage I:** Perivascular organization in which tumor cells are concentrated around some native vessels. Endothelial cells express Ang-2. **Stage II:** Proliferation phase when tumor cells actively proliferate around existing viable blood vessels. **Stage III:** Vascular involution of the host vascular cells resulting in degeneration of blood vessels. This leads to necrosis and hypoxia, which in turn promotes tumor-derived VEGF expression and angiogenesis. **Stage IV:** Angiogenesis occurs as blood vessels grow towards and vascularize the now necrosing tumor (N). The overexpression of HIF-1α and VEGF mRNA in pseudopalisading cells leads to the release of VEGF and induction of angiogenesis in neighboring blood vessels.

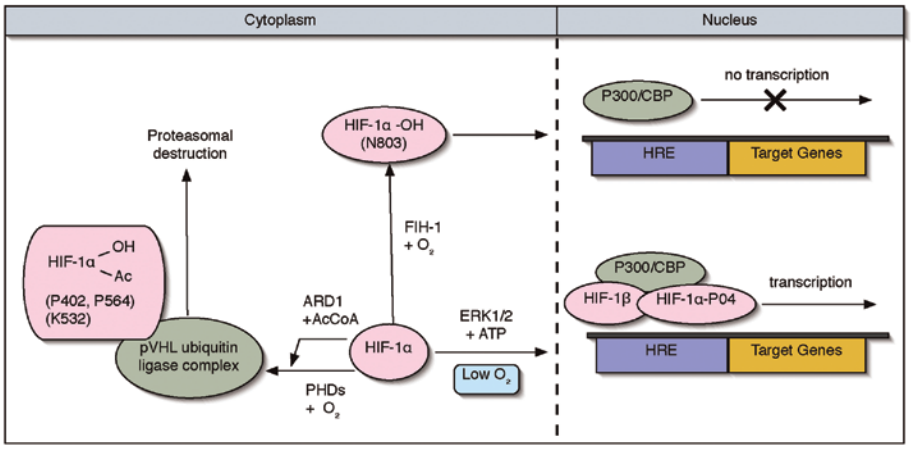

Figure 3. *Oxygen-dependent and -independent posttranslational regulation of HIF-1α.* In tissues, O_2 is a limiting substrate and the enzymes Factor Inhibiting HIF (FIH-1) and Prolyl Hydroxylase Domain proteins (PHDs) serve as oxygen sensors because of their utilization of O_2. Under normoxic conditions, hydroxylation of HIF-1α at asparagine 803 catalyzed by FIH-1 prevents the recruitment of coactivator p300/CBP (cAMP-response element binding protein (CREB)-Binding Protein) and inhibits the transcriptional response. Also, hydroxylation of HIF-1α at prolines 402 and 564 by PHD isozymes targets the HIF-1α subunit to the von Hippel-Lindau (pVHL) ubiquitin ligase complex that mediates its proteasomal degradation. However, under hypoxic conditions, these hydroxylases are inactive, resulting in accumulation of the constitutively expressed HIF-1α protein. This subunit is phosphorylated by Extracellular signal-Related protein Kinase (ERK)-1/2 and translocated to the nucleus, where it dimerizes with the HIF-1β subunit, recruits p300/CBP, binds to hypoxia response elements (HRE) upstream of HIF-1-regulated target genes and initiates their transcription. Other oxygen-independent posttranslational modifications of HIF-1α include acetylation of lysine 532 by ARrest Defective-1 (ARD1) acetyltransferase and acetyl-coenzyme A (AcCoA), which promotes HIF-1α binding to the pVHL ubiquitin ligase complex. The interdependence among these different modifications (eg, ARD1 and PHDs) has been simplified for illustration purposes and is still being investigated (24).

NATURALLY-OCCURRING ANGIOGENESIS INHIBITORS

A number of endogenous inhibitors of angiogenesis have been identified in gliomas as shown in Table 2. They exert their effects through multiple protein-protein or protein-proteoglycan interactions (eg, angiostatin, endostatin) or through specific receptor binding (eg, interferons). In many but not all studies, the expression level of some of these inhibitors was actually increased in GBM tumor biopsies compared to low grade gliomas, which suggests the existence of a host antiangiogenic response that partially antagonizes the tumor-driven angiogenesis (143). For example, the levels of angiostatin, endostatin, thrombospondin (TSP)-1 and –2, and tissue inhibitor of metalloprotease (TIMP)-1 were predominantly expressed in the hyperplastic vessels of GBMs. On the other hand, the expression of other endogenous inhibitors correlated inversely with glioma histologic grade in some studies (90, 143; Wang et al; this issue). Some examples are pigment endothelial-derived factor (PEDF) and TIMP-4. These factors mediate some of their antiangiogenic effects through inhibition or downregulation of VEGF or other proangiogenic molecules (132, 143). Therefore, in addition to a potential host antiangiogenic response, stimulation of angiogenesis as a result of reduced expression of endogenous inhibitors also occurs during glioma progression.

HYPOXIA, HIF-1 AND GLIOMA PROGRESSION

Eukaryotic cells require oxygen for their metabolism and survival. The condition of low oxygen or hypoxia results in growth arrest or apoptosis. Cells can respond acutely to hypoxia, at the cellular level, by a variety of molecular mechanisms. For example, upregulation of glucose transporters on the cell membrane and switching from aerobic to anaerobic metabolism, ensure continued ATP supply. This adaptation is mediated by the upregulation of a wide variety of hypoxia-inducible genes induced by several transcription factors including HIF-1, NFκB, and Activator Protein-1 (AP-1) (96, 99, 159, 161, 195). Of these, HIF-1 is of special interest, since it is regulated by a unique oxygen-sensing mechanism (160). HIF-1 overexpression has been consistently linked with malignant progression and unfavourable outcome in various tumor types (173, 214).

Hypoxia-inducible factor-1. The transcription factor HIF-1 and its target genes play a critical role in glioma-induced angiogenesis. The HIF-1 transcriptional complex is a heterodimer composed of two bHLH (basic helix loop helix)-PAS subunits, HIF-1α and HIF-1β (also termed aryl hydrocarbon nuclear translocator (ARNT) (160, 161). To date, three HIF-α isoforms have been described, with the best characterized being HIF-1α and HIF-2α. HIF-2α was first designated HRF/EPAS-1/HLF (HIF-related factor/endothelial PAS protein-1/HIF-like factor) (57, 63, 188). In GBMs, HIF-1α is overexpressed in pseudopalisading cells around necrotic foci (Figure 1C; 209), a pattern similar to that of VEGF mRNA. Moreover, the expression of HIF-2α is upregulated in GBMs both at the mRNA and at the protein level (4, 91).

HIF-1 regulation. Whereas the β-subunit of HIF-1 is constitutively expressed, the α-subunit is subject to complex regulatory mechanisms at the transcriptional and posttranslational levels. The stability of the HIF-1α protein is tightly regulated by an oxygen-sensing as well as other mechanisms as shown in Figure 3. Under normoxic conditions, HIF-1 activity is inhibited following hydroxylation of prolyl residues by prolyl hydroxylase (PHD) enzymes leading to rapid degradation of the HIF-1α subunit (160). The oxygen-, 2-oxoglutarate-, iron-, and ascorbate dependent prolyl hydroxylase PHD1-4 isozymes mediate the hydroxylation of the HIF-1α subunit at proline residues 402 and 564. This hydroxylation allows for binding of the von-Hippel-Lindau protein (pVHL), which mediates ubiquitination of HIF-1α and subsequent proteasomal degradation (81, 84, 104, 116-118). The prolyl hydroxylases themselves are also subject to proteasomal degradation. Physiologically, the VHL E3 ubiquitin ligase plays a similar role in the regulation of both HIF-1α and HIF-2α. Since the prolyl hydroxylation requires oxygen for catalysis, HIF-1α protein accumulates only under hypoxic conditions. Subsequently, HIF-1α translocates to the nucleus where it dimerizes with the β-subunit and binds to the hypoxia-responsive element (HRE) of a wide array of hypoxia inducible target genes, activating

their transcription (Figure 4). The coactivator proteins p300/CBP, Ref-1, SRC-1, and thioredoxin activate the transcriptional complex (37, 56). In addition, HIF-1 is hydroxylated at asparagine residue 803 by factor inhibiting HIF-1 (FIH-1), which prevents the binding of the coactivator p300/CBP thereby interfering with HIF-1 mediated target gene transcription. Additional posttranslational modifications of HIF-1α also occur, including acetylation, phosphorylation, and SUMOylation (24). Finally, the recently characterized 35-kDa protein CITED2/p35srj is upregulated by hypoxia and by HIF-1 and competes with HIF-1 for the coactivator p300/CBP, thus representing a negative feedback mechanism (18).

HIF-1 is further regulated at the transcriptional level through intracellular signaling pathways induced by growth factors. These growth factors include EGF, TGF-α, PDGF-A, IGF-1 and -2, insulin, angiotensin, and thrombin (5, 82, 146, 210), many of which have proangiogenic properties (Table 1) and also upregulate VEGF (62, 73, 176, 185, 192). Growth factor induced transcription of HIF-1 is part of several positive feedback-loops. For example, binding of TGF-α to EGFR activates the intracellular PI3K pathway and induces HIF-1α transcription. HIF-1 itself activates TGF-α transcription through binding to an HRE in the promoter region of the TGF-α gene (161). In a similar fashion, activation of IGFR-1 induces HIF-1, which activates the transcription of HRE-regulated genes encoding the ligand for the IGFR-1 receptor and for the IGFBPs (59, 161). GBMs often express high levels of IGFs, IGF receptors and IGFBPs compared with normal brain tissue (55, 213).

Effects of HIF-1 transcriptional activity. HIF-1 activity in gliomas induces a wide variety of genes as shown in Figure 4 (99, 159, 161, 195). The protein products of these genes play crucial roles in angiogenesis and vascular tone, pH regulation, apoptosis, iron metabolism, cell proliferation and survival, transcriptional regulation, and extracellular matrix metabolism and invasion, which confers increased resistance of the tumor cells to the hostile tumor microenvironment. Hypoxia through HIF-1 is one of the most potent stimulators of VEGF expression in vitro and in vivo (161). HIF-1

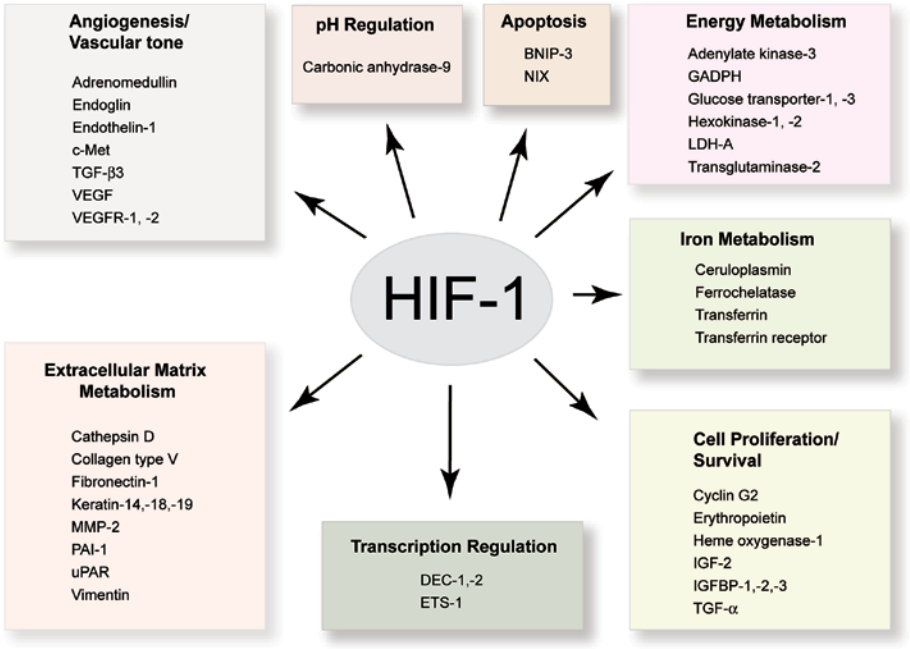

Figure 4. *Examples of genes transcriptionally activated by HIF-1.*

binds to the HRE in the VEGF promoter region leading to increased VEGF transcription (161). To control energy metabolism glucose transporters, such as GLUT-1 and GLUT-3, and enzymes of the glycolytic pathways are induced to ensure an adequate supply of ATP for the cells under hypoxic conditions. Lactate, one of the endproducts of the glycolytic pathways, contributes to the typically acidic microenvironment of gliomas and the transcriptional activation of the carbonic anhydrase CA9 by HIF-1, which enables glioma cells to regulate their intracellular pH (85, 161, 190). CA9 expression is increased in pseudopalisading cells of GBMs showing colocalization with HIF-1α (Figure 1D). HIF-1 target genes include growth factors such as adrenomedullin that sustains glioma growth (93). In addition, HIF-1-induced genes include uPAR and plasminogen activator inhibitor-1 (PAI-1) (161) known to be upregulated in gliomas (8). These genes act in synergy with the MMPs to aid in the breakdown of the ECM, facilitating endothelial cell migration during the angiogenic process (8).

More recently, HIF-1 activity, in relation to apoptosis, has been investigated (33, 83). Upregulation of HIF-1 has been associated with both proapoptotic (20, 33, 136) and antiapoptotic (83) effects. For example, HIF-1α activates the proapoptotic proteins BNIP3 and NIX (20, 166). Similarly, overexpression of HIF-2α in rat gliomas enhances angiogenesis but reduces growth of these tumors, in part by increasing tumor cell apoptosis (4).

GENETIC ALTERATIONS AND HYPOXIA IN GLIOMA PROGRESSION

During progression from low grade to high grade, gliomas accumulate genetic alterations in tumor suppressor genes and oncogenes (109, 184). These include loss of function of p53, amplification of EGFR and chromosome 10 alterations, including inactivation of PTEN through mutations and loss of heterozygosity (13, 50, 102, 133, 134, 167). Also, loss of heterozygosity of chromosomes 1, 10, 19, and/or 22 would be associated with loss of gene loci encoding proangiogenic (Table 1) and/or antiangiogenic (Table 2) factors or their receptors in patients with gliomas harboring these chromosomal abnormalities (reviewed in Gagner et al; this issue). These genetic alterations combine with hypoxia to drive chromosome instability (145, 186) promoting continual selective growth advantage for the tumor cells (Figure 5). In support of this concept that the hypoxic microenvironment of GBMs drives genomic instability, comparative genome hybridization (CGH) analysis of GBM xenograft specimens showed increased number of genetic alterations in poorly vascularized and hypoxic xenografts compared with well-vascularized and less-hypoxic ones (67). Some of these genetic alterations also affect HIF-1 activity and thus angiogenesis.

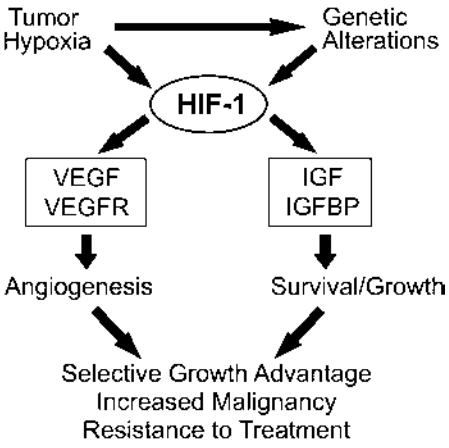

Figure 5. *Molecular mechanisms by which tumor hypoxia and genetic alterations promote angiogenesis and tumor growth or survival.* Tumor cells respond to decreased O_2 concentration by increasing HIF-1 transcriptional activity that induces adaptive changes in gene expression patterns (eg, increased VEGF, VEGFR, IGF and IGFBP) that result in enhanced tumor angiogenesis, growth and survival. Alternatively, genetic alterations, such as gain-of-function mutations in oncogenes (eg, EGFR), loss-of-function mutations in tumor-suppressor genes (eg, PTEN, p53) and chromosomal alterations, accumulate in tumor cells that lead to O_2-independent increase in HIF-1 activity associated with similar changes in gene expression patterns. In addition, severe hypoxia itself can induce genetic instability that can result in genetic and chromosomal alterations (for review, see 145, 186). These adaptive responses have been associated with tumor growth advantage, increased malignancy as well as treatment resistance and failure (161).

Several studies looking at gene expression profiling of gliomas have demonstrated clusters of genes highly linked to the angiogenic phenotype, eg, VEGF, VEGFR-1, IGFBP2, pleiotrophin, AP-1 (69, 103, 119, 131, 153). These results support the crucial role of genetic changes, hypoxia and angiogenesis in glioma growth and progression.

Tumor suppressor genes. In addition to growth factors, tumor suppressor genes and oncogenes modulate HIF-1 activity and angiogenesis in gliomas. For example, wild type PTEN downregulates HIF-1 mediated gene expression (215). In addition, induced expression of PTEN in glioma cells has been shown to decrease accumulation of HIF-1α in vitro and induce TSP-1 expression and reduce angiogenesis in vivo (3, 187, 215). The tumor suppressor gene p53 competes with HIF-1 for its coactivator p300 under hypoxic conditions (157). Cells with mutant p53 upregulate basic FGF expression (172) that could be suppressed when the mutant cells are transfected with wild-type p53 (180). VHL mutations are rare in gliomas (88). As described above, VHL protein is essential for proteasomal degradation of the HIF-1α subunit. Therefore, loss of function of pVHL leads to a constitutive activation of HIF-1 and consequently to the upregulation of HIF-1 target genes such as VEGF, erythropoietin, CXCR4 and CA9 (161, 205).

Oncogenes. Both Ras and v-src have been shown to modulate angiogenesis in gliomas (72, 174). Genetic alterations in EGFR, EGFRvIII and PDGFR in GBMs activate the Ras signaling pathways (72, 82, 133). Chronically active Ras and PI3K pathway signaling enhanced angiogenesis in GBMs whereas Ras inhibition in vitro, achieved by trans-farnesylthiosalicylic acid, downregulates HIF-1α and its target genes (21). V-src expression in gliomas has been shown to modulate angiogenesis (174) and v-src induces HIF-1 expression and its transcriptional activity (87).

Thus, independently of hypoxia-induced HIF-1 expression, genetic alterations can affect HIF-1 expression at the transcriptional or at the proteasomal degradation level leading to the upregulation of proangiogenic factors to promote angiogenesis (Table 1). This "pseudohypoxic" HIF-1 activation, ie, the involvement of an oncogenic stimulus rather than a physiologic stimulus as a source for HIF-1α expression is highly relevant for GBM progression (19, 209).

CONCLUSION

GBMs are amongst the most angiogenic tumors. Both proangiogenic and antiangiogenic mechanisms control tumor neovascularization. The observation that angiogenic inhibitors decrease tumor growth in vivo suggests that angiogenesis is not merely an epiphenomenon of malignancy. For example, the VEGFR-1, PDGF-B, and basic FGF tyrosine kinase inhibitor SU6668 decreased tumor growth in a rat glioma model, which is associated with decreased vascularization and peritumoral edema (58). Because diffusely infiltrating gliomas are mostly refractory to current surgical and adjuvant treatments, the topic of angiogenesis and its relation to tumor growth, progression, and resistance to therapy is of special interest. A variety of antiangiogenic approaches are currently undergoing preclinical or clinical trials (22). In the future, these experimental strategies may develop and find wide clinical applications equivalent to "traditional" radiotherapy and chemotherapy. Progress made in imaging angiogenesis and hypoxia in gliomas and in developing antiangiogenic therapeutics are reviewed in an accompanying article (see Gagner et al; this issue).

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health grant R01 CA100426 (DZ). We thank Julio Garcia for his art work and Dr Yevgeniy Lukyanov for his help in the preparation of the manuscript.

REFERENCES

1. Abdulrauf SI, Edvardsen K, Ho KL, Yang XY, Rock JP, Rosenblum ML (1998) Vascular endothelial growth factor expression and vascular density as prognostic markers of survival in patients with low-grade astrocytoma. *J Neurosurg* 88:513-520.

2. Abe T, Okamura K, Ono M, Kohno K, Mori T, Hori S, Kuwano M (1993) Induction of vascular endothelial tubular morphogenesis by human glioma cells. A model system for tumor angiogenesis. *J Clin Invest* 92:54-61.

3. Abe T, Terada K, Wakimoto H, Inoue R, Tyminski E, Bookstein R, Basilion JP, Chiocca EA (2003) PTEN decreases in vivo vascularization of experimental gliomas in spite of proangiogenic stimuli. *Cancer Res* 63:2300-2305.

4. Acker T, Diez-Juan A, Aragones J, Tjwa M, Brusselmans K, Moons L, Fukumura D, Moreno-Murciano MP, Herbert JM, Burger A, Riedel J, Elvert G, Flamme I, Maxwell PH, Collen D, Dewerchin M, Jain RK, Plate KH, Carmeliet P (2005) Genetic evidence for a tumor suppressor role of HIF-2alpha. *Cancer Cell* 8:131-141.

5. Acker T, Plate KH (2002) A role for hypoxia and hypoxia-inducible transcription factors in tumor physiology. *J Mol Med* 80:562-575.

6. Adini A, Kornaga T, Firoozbakht F, Benjamin LE (2002) Placental growth factor is a survival factor for tumor endothelial cells and macrophages. *Cancer Res* 62:2749-2752.

7. Alavi A, Hood JD, Frausto R, Stupack DG, Cheresh DA (2003) Role of Raf in vascular protection from distinct apoptotic stimuli. *Science* 301:94-96.

8. Arai Y, Kubota T, Nakagawa T, Kabuto M, Sato K, Kobayashi H (1998) Production of urokinase-type plasminogen activator (u-PA) and plasminogen activator inhibitor-1 (PAI-1) in human brain tumours. *Acta Neurochir (Wien)* 140:377-385.

9. Arrieta O, Gracia E, Guevara P, Garcia-Navarrete R, Ondarza R, Rembao D, Sotelo J (2002) Hepatocyte growth factor is associated with poor prognosis of malignant gliomas and is a predictor

for recurrence of meningioma. *Cancer* 94:3210-3218.

10. Auguste P, Gursel DB, Lemiere S, Reimers D, Cuevas P, Carceller F, Di Santo JP, Bikfalvi A (2001) Inhibition of fibroblast growth factor/fibroblast growth factor receptor activity in glioma cells impedes tumor growth by both angiogenesis-dependent and -independent mechanisms. *Cancer Res* 61:1717-1726.

11. Autiero M, Luttun A, Tjwa M, Carmeliet P (2003) Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. *J Thromb Haemost* 1:1356-1370.

12. Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, Kroll J, Plaisance S, De Mol M, Bono F et al(2003) Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med* 9:936-943.

13. Baeza N, Weller M, Yonekawa Y, Kleihues P, Ohgaki H (2003) PTEN methylation and expression in glioblastomas. *Acta Neuropathol (Berl)* 106:479-485.

14. Barker FG 2nd, Davis RL, Chang SM, Prados MD (1996) Necrosis as a prognostic factor in glioblastoma multiforme. *Cancer* 77:1161-1166.

15. Bello L, Giussani C, Carrabba G, Pluderi M, Costa F, Bikfalvi A (2004) Angiogenesis and invasion in gliomas. *Cancer Treat Res* 117:263-284.

16. Bengtson NW, Linzer DI (2000) Inhibition of tumor growth by the antiangiogenic placental hormone, proliferin-related peptide. *Mol Endocrinol* 14:1934-1943.

17. Berkman RA, Merrill MJ, Reinhold WC, Monacci WT, Saxena A, Clark WC, Robertson JT, Ali IU, Oldfield EH (1993) Expression of the vascular permeability factor/vascular endothelial growth factor gene in central nervous system neoplasms. *J Clin Invest* 91:153-159.

18. Bhattacharya S, Michels CL, Leung MK, Arany ZP, Kung AL, Livingston DM (1999) Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. *Genes Dev* 13:64-75.

19. Birner P, Gatterbauer B, Oberhuber G, Schindl M, Rossler K, Prodinger A, Budka H, Hainfellner JA (2001) Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. *Cancer* 92:165-171.

20. Birse-Archbold JL, Kerr LE, Jones PA, McCulloch J, Sharkey J (2005) Differential profile of Nix upregulation and translocation during hypoxia/ischaemia in vivo versus in vitro. *J Cereb Blood Flow Metab* 25:1356-1365.

21. Blum R, Jacob-Hirsch J, Amariglio N, Rechavi G, Kloog Y (2005) Ras inhibition in glioblastoma down-regulates hypoxia-inducible factor-1α, causing glycolysis shutdown and cell death. *Cancer Res* 65:999-1006.

22. Bogler O, Mikkelsen T (2003) Angiogenesis in glioma: molecular mechanisms and roadblocks to translation. *Cancer J* 9:205-213.

23. Boudouresque F, Berthois Y, Martin PM, Figarella-Branger D, Chinot O, Ouafik L (2005) Role of adrenomedullin in glioblastomas growth. *Bull Cancer* 92:317-326.

24. Brahimi-Horn C, Mazure N, Pouysségur J (2005) Signalling via the hypoxia-inducible factor-1α requires multiple posttranslational modifications. *Cell Signal* 17:1-9.

25. Brat DJ, Bellail AC, Van Meir EG (2005) The role of interleukin-8 and its receptor in gliomatosis and tumoral angiogenesis. *Neuro-Oncology* 7:122-133.

26. Brat DJ, Van Meir EG (2004) Vaso-occlusive and prothrombotic mechanisms associated with tumor hypoxia, necrosis, and accelerated growth in glioblastoma. *Lab Invest* 84:397-405.

27. Brem S (1976) The role of vascular proliferation in the growth of brain tumors. *Clin Neurosurg* 23:440-453.

28. Brem S, Cotran R, Folkman J (1972) Tumor angiogenesis: a quantitative method for histologic grading. *J Natl Cancer Inst* 48:347-356.

29. Brockmann MA, Ulbricht U, Gruner K, Fillbrandt R, Westphal M, Lamszus K (2003) Glioblastoma and cerebral microvascular endothelial cell migration in response to tumor-associated growth factors. *Neurosurgery* 52:1391-1399.

30. Broholm H, Laursen H (2004) Vascular endothelial growth factor (VEGF) receptor neuropilin-1's distribution in astrocytic tumors. *APMIS* 112:257-263.

31. Buccoliero AM, Caldarella A, Arganini L, Mennonna P, Gallina P, Taddei A, Taddei GL (2004) Cyclooxygenase-2 in oligodendroglioma: possible prognostic significance. *Neuropathology* 24:201-207.

32. Burger PC, Vogel FS, Green SB, Strike TA (1985) Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. *Cancer* 56:1106-1111.

33. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshet E (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* 394:485-490.

34. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 380:435-439.

35. Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. *Nature* 407: 249-257.

36. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H et al (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 7:575-583.

37. Carrero P, Okamoto K, Coumailleau P, O'Brien S, Tanaka H, Poellinger L (2000) Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. *Mol Cell Biol* 20:402-415.

38. Cervera P, Videau C, Viollet C, Petrucci C, Lacombe J, Winsky-Sommerer R, Csaba Z, Helboe L, Daumas-Duport C, Reubi JC, Epelbaum J (2002) Comparison of somatostatin receptor expression in human gliomas and medulloblastomas *J Neuroendocrinol* 14:458-471.

39. Cha S, Johnson G, Wadghiri YZ, Jin O, Babb J, Zagzag D, Turnbull DH (2003) Dynamic, contrast-enhanced perfusion MRI in mouse gliomas: correlation with histopathology. *Magn Reson Med* 49:848-855.

40. Cheng SY, Huang HJ, Nagane M, Ji XD, Wang D, Shih CC, Arap W, Huang CM, Cavenee WK (1996) Suppression of glioblastoma angiogenicity and tumorigenicity by inhibition of endogenous expression of vascular endothelial growth factor. *Proc Natl Acad Sci U S A* 93:8502-8507.

41. Cheng SY, Nagane M, Huang HS, Cavenee WK (1997) Intracerebral tumor-associated hemorrhage caused by overexpression of the vascular endothelial growth factor isoforms VEGF121 and VEGF165 but not VEGF189. *Proc Natl Acad Sci U S A* 94:12081-12087.

42. Clapp C, Weiner RI (1992) A specific, high affinity, saturable binding site for the 16-kilodalton fragment of prolactin on capillary endothelial cells. *Endocrinology* 130:1380-1386.

43. Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ (1996) Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biol Chem* 271:736-741.

44. Corbacho AM, Martinez de la Escalera G, Clapp C (2002) Roles of prolactin and related members of the prolactin/growth hormone/placental lactogen family in angiogenesis. *J Endocrinol* 173:219-238.

45. Daumas-Duport C, Tucker ML, Kolles H, Cervera P, Beuvon F, Varlet P, Udo N, Koziak M, Chodkiewicz JP (1997) Oligodendrogliomas. Part II: A new grading system based on morphological and imaging criteria. *J Neurooncol* 34:61-78.

46. De Boüard S, Guillamo JS (2005) [Angiogenesis and anti-angiogenic strategies for glioblastomas]. *Bull Cancer* 92:360-372.

47. Del Maestro RF, Megyesi JF, Farrell CL (1990) Mechanisms of tumor-associated edema: a review. *Can J Neurol Sci* 17:177-183.

48. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT (1992) The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science* 255:989-991.

49. Ding H, Wu X, Roncari L, Lau N, Shannon P, Nagy A, Guha A (2000) Expression and regulation of neuropilin-1 in human astrocytomas. *Int J Cancer* 88:584-592.

50. Duerr EM, Rollbrocker B, Hayashi Y, Peters N, Meyer-Puttlitz B, Louis DN, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A (1998) PTEN mutations in gliomas and glioneuronal tumors. *Oncogene* 16:2259-2264.

51. Dunn IF, Heese O, Black PM (2000) Growth factors in glioma angiogenesis: FGFs, PDGF, EGF, and TGFs. *J Neuro-Oncology* 50:121-137.

52. Dvorak HF (2005) Angiogenesis: update 2005. *J Thromb Haemost* 3:1835-1842.

53. Earnest F 4th, Kelly PJ, Scheithauer BW, Kall BA, Cascino TL, Ehman RL, Forbes GS, Axley PL (1988) Cerebral astrocytomas: histopathologic correlation of MR and CT contrast enhancement with stereotactic biopsy. *Radiology* 166:823-827.

54. Egidy G, Eberl LP, Valdenaire O, Irmler M, Majdi R, Diserens AC, Fontana A, Janzer RC, Pinet F, Juillerat-Jeanneret L (2000) The endothelin system in human glioblastoma. *Lab Invest* 80:1681-1689.

55. Elmlinger MW, Deininger MH, Schuett BS, Meyermann R, Duffner F, Grote EH, Ranke MB (2001) In vivo expression of insulin-like growth factor-binding protein-2 in human gliomas increases with the tumor grade. *Endocrinology* 142:1652-1658.

56. Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, Poellinger L, Fujii-Kuriyama Y (1999) Molecular mechanisms of transcription activation by HLF and HIF-1α in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J* 18:1905-1914.

57. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y (1997) A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1α regulates the VEGF expression, and is potentially involved in lung and vascular development. *Proc Natl Acad Sci U S A* 94:4273-4278.

58. Farhadi MR, Capelle HH, Erber R, Ullrich A, Vajkoczy P (2005) Combined inhibition of vascular endothelial growth factor and platelet-derived growth factor signaling: effects on the angiogenesis, microcirculation, and growth of orthotopic malignant gliomas. *J Neurosurg* 102:363-370.

59. Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL (1999) Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res* 59:3915-3918.

60. Ferrara N (2004) Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 25:581-611.

61. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. *Nat Med* 9:669-676.

62. Finkenzeller G, Marme D, Weich HA, Hug H (1992) Platelet-derived growth factor-induced transcription of the vascular endothelial growth factor gene is mediated by protein kinase C. *Cancer Res* 52:4821-4823.

63. Flamme I, Froehlich T, von Reutern M, Kappel A, Damert A, Risau W, Plate KH (1997) HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1α and developmentally expressed in blood vessels. *Mech Dev* 63:51-60.

64. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 82:4-6.

65. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1:27-31.

66. Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, Brown E, Barnett GH, Jain RK (2004) The candidate tumor suppressor protein ING4 regulates brain tumor growth and angiogenesis. *Nature* 428:328-332.

67. Gilhuis HJ, Bernse HJ, Jeuken JW, Wesselin P, Sprenger SH, Kerstens HM, Wiegant J, Boerman RH (2001) The relationship between genetic aberrations as detected by comparative genomic hybridization and vascularization in glioblastoma xenografts. *J Neurooncol* 51:121-127.

68. Gladson CL (1999) The extracellular matrix of gliomas: modulation of cell function. *J Neuropathol Exp Neurol* 58:1029-1040.

69. Godard S, Getz G, Delorenzi M, Farmer P, Kobayashi H, Desbaillets I, Nozaki M, Diserens AC, Hamou MF, Dietrich PY et al (2003) Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer Res* 63:6613-6625.

70. Gollmer JC, Ladoux A, Gioanni J, Paquis P, Dubreuil A, Chatel M, Frelin C (2000) Expression of vascular endothelial growth factor-b in human astrocytoma. *Neuro-oncology* 2:80-86.

71. Gothert JR, Gustin SE, van Eekelen JA, Schmidt U, Hall MA, Jane SM, Green AR, Gottgens B, Izon DJ, Begley CG (2004) Genetically tagging endothelial cells in vivo: bone marrow-derived cells do not contribute to tumor endothelium. *Blood* 104:1769-1777.

72. Guha A (1998) Ras activation in astrocytomas and neurofibromas. *Can J Neurol Sci* 25:267-281.

73. Guo P, Hu B, Gu W, Xu L, Wang D, Huang HJ, Cavenee WK, Cheng SY (2003) Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. *Am J Pathol* 162:1083-1093.

74. Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86:353-364.

75. Haskell H, Natarajan M, Hecker TP, Ding Q, Stewart J Jr, Grammer JR, Gladson CL (2003) Focal adhesion kinase is expressed in the angiogenic blood vessels of malignant astrocytic tumors in vivo and promotes capillary tube formation of brain microvascular endothelial cells. *Clin Cancer Res* 9:2157-2165.

76. Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, Hicklin DJ, Zhu Z, Bohlen P, Witte L, Hendrikx J, Hackett NR, Crystal RG, Moore MA, Werb Z, Lyden D, Rafii S (2002) Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med* 8:841-849.

77. Heidenreich R, Machein M, Nicolaus A, Hilbig A, Wild C, Clauss M, Plate KH, Breier G (2005) Inhibition of solid tumor growth by gene transfer of VEGF receptor-1 mutants. *Int J Cancer* 111:348-357.

78. Hicklin DJ, Ellis LM (2005) Role of vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 23:1011-1027.

79. Hirano H, Lopes MB, Laws ER Jr, Asakura T, Goto M, Carpenter JE, Karns LR, VandenBerg SR (1999) Insulin-like growth factor-1 content and pattern of expression correlates with histopathologic grade in diffusely infiltrating astrocytomas. *Neuro-oncology* 1:109-119.

80. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* 284:1994-1998.

81. Hon WC, Wilson MI, Harlos K, Claridge TD, Schofield CJ, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DI, Jones EY (2002) Structural basis for the recognition of hydroxyproline in HIF-1 alpha by pVHL. *Nature* 417:975-978.

82. Hu P, Margolis B, Skolnik EY, Lammers R, Ullrich A, Schlessinger J (1992) Interaction of phosphatidylinositol 3-kinase-associated p85 with epidermal growth factor and platelet-derived growth factor receptors. *Mol Cell Biol* 12:981-990.

83. Iida T, Mine S, Fujimoto H, Suzuki K, Minami Y, Tanaka Y (2002) Hypoxia-inducible factor-1alpha induces cell cycle arrest of endothelial cells. *Genes Cells* 7: 143-149.

84. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr (2001) HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* 292:464-468.

85. Ivanov S, Liao SY, Ivanova A, Danilkovitch-Miagkova A, Tarasova N, Weirich G, Merrill MJ, Proescholdt MA, Oldfield EH, Lee J, Zavada J, Waheed A, Sly W, Lerman MI, Stanbridge EJ (2001) Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. *Am J Pathol* 158:905-919.

86. Jain RK, Duda DG (2003) Role of bone marrow-derived cells in tumor angiogenesis and treatment. *Cancer Cell* 3:515-516.

87. Jiang BH, Agani F, Passaniti A, Semenza GL (1997) V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. *Cancer Res* 57:5328-5335.

88. Kanno H, Shuin T, Kondo K, Yamamoto I, Ito S, Shinonaga M, Yoshida M, Yao M (1997) Somatic mutations of the von Hippel-Lindau tumor suppressor gene and loss of heterozygosity on chromosome 3p in human glial tumors. *Cancer Res* 57:1035-1038.

89. Kaur B, Brat DJ, Devi NS, Van Meir EG (2005) Vasculostatin, a proteolytic fragment of brain angiogenesis inhibitor 1, is an antiangiogenic and antitumorigenic factor. *Oncogene* 24:3632-3642.

90. Kazuno M, Tokunaga T, Oshika Y, Tanaka Y, Tsugane R, Kijima H, Yamazaki H, Ueyama Y, Nakamura M (1999) Thrombospondin-2 (TSP2) expression is inversely correlated with vascularity in glioma. *Eur J Cancer* 35:502-506.

91. Khatua S, Peterson KM, Brown KM, Lawlor C, Santi MR, LaFleur B, Dressman D, Stephan DA, MacDonald TJ (2003) Overexpression of the EGFR/FKBP12/HIF-2alpha pathway identified in childhood astrocytomas by angiogenesis gene profiling. *Cancer Res* 63:1865-1870.

92. Kee HJ, Ahn KY, Choi KC, Won Song J, Heo T, Jung S, Kim JK, Bae CS, Kim KK (2004) Expression of brain-specific angiogenesis inhibitor 3 (BAI3) in normal brain and implications for BAI3 in ischemia-induced brain angiogenesis and malignant glioma. *FEBS Lett* 569:307-316.

93. Kitamuro T, Takahashi K, Nakayama M, Murakami O, Hida W, Shirato K, Shibahara S (2000) Induction of adrenomedullin during hypoxia in cultured human glioblastoma cells. *J Neurochem* 75:1826-1833.

94. Kleihues P, Burger PC, Collins V, Newcomb EW, Ohgaki H, Cavenee WK (2000) Glioblastoma. In: *Pathology and Genetics of Tumours of the Nervous System*, Kleihues P, Cavenee WK (eds.), pp. 29-39, IARC Press: Lyon.

95. Kleihues P, Ohgaki H (1999) Primary and secondary glioblastomas: from concept to clinical diagnosis. *Neuro-oncology* 1:44-51.

96. Koong AC, Chen EY, Giaccia AJ (1994) Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. *Cancer Res* 54:1425-1430.

97. Kremer C, Breier G, Risau W, Plate KH (1997) Up-regulation of flk-1/vascular endothelial growth factor receptor 2 by its ligand in a cerebral slice culture system. *Cancer Res* 57:3852-3859.

98. Lacroix M, Abi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, Lang FF, McCutcheon IE, Hassenbusch SJ, Holland E, Hess K, Michael C, Miller D, Sawaya R (2001) A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg* 95:190-198.

99. Lal A, Peters H, St Croix B, Haroon ZA, Dewhirst MW, Strausberg RL, Kaanders JH, van der Kogel AJ, Riggins GJ (2001) Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst* 93:1337-1343.

100. Lamszus K, Ulbricht U, Matschke J, Brockmann MA, Fillbrandt R, Westphal M (2003) Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. *Clin Cancer Res* 9:1399-1405.

101. Li VW, Folkerth RD, Watanabe H, Yu C, Rupnick M, Barnes P, Scott RM, Black PM, Sallan SE, Folkman J (1994) Microvessel count and cerebrospinal fluid basic fibroblast growth factor in children with brain tumours. *Lancet* 344:82-86.

102. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943-1947.

103. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA (2005) Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A* 102:5814-5819.

104. Lisztwan J, Imbert G, Wirbelauer C, Gstaiger M, Krek W (1999) The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. *Genes Dev* 13:1822-1833.

105. Liwnicz BH, Wu SZ, Tew JM (1987) The relationship between the capillary structure and hemorrhage in gliomas. *J Neurosurg*, 66:536-541.

106. Ljubimova JY, Fujita M, Khazenzon NM, Ljubimov AV, Black KL (2006) Changes in laminin isoforms associated with brain tumor invasion and angiogenesis. *Front Biosci* 11:81-88.

107. Loeffler S, Fayard B, Weis J, Weissenberger J (2005) Interleukin-6 induces transcriptional activation of vascular endothelial growth factor (VEGF) in astrocytes in vivo and regulates VEGF promoter activity in glioblastoma cells via direct interaction between STAT3 and Sp1. *Int J Cancer* 115:202-213.

108. Lopez-Lopez C, LeRoith D, Torres-Aleman I (2004) Insulin-like growth factor I is required for vessel remodeling in the adult brain. *Proc Natl Acad Sci U S A* 101:9833-9838.

109. Louis DN, Holland EC, Cairncross JG (2001) Glioma classification: a molecular reappraisal. *Am J Pathol* 159:779-786.

110. Luttun A, Autiero M, Tjwa M, Carmeliet P (2004) Genetic dissection of tumor angiogenesis: are PlGF and VEGFR-1 novel anti-cancer targets? *Biochim Biophys Acta* 1654:79-94.

111. Luttun A, Carmeliet G, Carmeliet P (2002) Vascular progenitors: from biology to treatment. *Trends Cardiovasc Med* 12:88-96.

112. Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* 7:1194-1201.

113. Machein MR, Knedla A, Knoth R, Wagner S, Neuschl E, Plate KH (2004) Angiopoietin-1 promotes tumor angiogenesis in a rat glioma model. *Am J Pathol* 165:1557-1570.

114. Machein MR, Renniger S, de Lima-Hahn E, Plate KH (2003) Minor contribution of bone marrow-derived endothelial progenitors to the vascularization of murine gliomas. *Brain Pathol* 13:582-597.

115. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 277:55-60.

116. Maxwell PH (2004) HIF-1's relationship to oxygen: simple yet sophisticated. *Cell Cycle* 3:156-159.

117. Maxwell PH, Pugh CW, Ratcliffe PJ (2001) The pVHL-HIF-1 system. A key mediator of oxygen homeostasis. *Adv Exp Med Biol* 502:365-376.

118. Maxwell P, Salnikow K (2004) HIF-1: an oxygen and metal responsive transcription factor. *Cancer Biol Ther* 3:29-35.

119. Mehrian Shai R, Reichardt JK, Ya-Hsuan H, Kremen TJ, Liau LM, Cloughesy TF, Mischel PS, Nelson SF (2005) Robustness of gene expression profiling in glioma specimen samplings and derived cell lines. *Mol Brain Res* 136:99-103.

120. Mentlein R, Eichler O, Forstreuter F, Held-Feindt J (2001) Somatostatin inhibits the production of vascular endothelial growth factor in human glioma cells. *Int J Cancer* 92:545-550.

121. Mentlein R, Held-Feindt J (2003) Angiogenesis factors in gliomas: a new key to tumour therapy. *Naturissenschaften* 90:385-394.

122. Mikkelsen T, Yan PS, Ho KL, Sameni M, Sloane BF, Rosenblum ML (1995) Immunolocalization of cathepsin B in human glioma: implications for tumor invasion and angiogenesis. *J Neurosurg* 83:285-290.

123. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A (1993) High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* 72:835-846.

124. Moriyama T, Kataoka H, Koono M, Wakisaka S (1999) Expression of hepatocyte growth factor/scatter factor and its receptor c-Met in brain tumors: evidence for a role in progression of astrocytic tumors. *Int J Mol Med* 3:531-536.

125. Nabors LB, Suswam E, Huang Y, Yang X, Johnson MJ, King PH (2003) Tumor necrosis factor alpha induces angiogenic factor upregulation in malignant glioma cells: a role for RNA stabilization and HuR. *Cancer Res* 63:4181-4187.

126. Nakamura M, Ishida E, Shimada K, Kishi M, Nakase H, Sakaki T, Konishi N (2005) Frequent LOH on 22q12.3 and TIMP-3 inactivation occur in the progression to secondary glioblastomas. *Lab Invest* 85:165-175.

127. Nelson JS, Tsukada Y, Schoenfeld D, Fulling K, Lamarche J, Peress N (1983) Necrosis as a prognostic criterion in malignant supratentorial, astrocytic gliomas. *Cancer* 52:550-554.

128. Nie D, Honn KV (2004) Eicosanoid regulation of angiogenesis in tumors. *Semin Thromb Hemost* 30:119-125.

129. Niida H, Takeuchi S, Tanaka R, Minakawa T (1995) Angiogenesis in microvascular endothelial cells induced by glioma cells and inhibited by tumor necrosis factor in vitro. *Neurol Med Chir (Tokyo)* 35:209-214.

130. Nomura M, Yamagishi S, Harada S, Yamashima T, Yamashita J, Yamamoto H (1998) Placenta growth factor (PlGF) mRNA expression in brain tumors. *J Neurooncol* 40:123-130.

131. Nutt CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, Pohl U, Hartmann C, McLaughlin ME, Batchelor TT, Black PM, Von Deimling A, Pomeroy SL, Golub TR, Louis DN (2003) Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res* 63:1602-1607.

132. Nyberg P, Xie L, Kalluri R (2005) Endogenous inhibitors of angiogenesis. *Cancer Res* 65:3967-3979.

133. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schuler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lutolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. *Cancer Res* 64:6892-6899.

134. Okamoto Y, Di Patre PL, Burkhard C, Horstmann S, Jourde B, Fahey M, Schuler D, Probst-Hensch NM, Yasargil MG, Yonekawa Y, Lutolf UM, Kleihues P, Ohgaki H (2004) Population-based study on incidence, survival rates, and genetic alterations of low-grade diffuse astrocytomas and oligodendrogliomas. *Acta Neuropathol (Berl)* 108:49-56.

135. Osada H, Tokunaga T, Nishi M, Hatanaka H, Abe Y, Tsugu A, Kijima H, Yamazaki H, Ueyama Y, Nakamura M (2004) Overexpression of the neuropilin 1 (NRP1) gene correlated with poor prognosis in human glioma. *AntiCancer Res* 24:547-552.

136. Ozawa T, Hu JL, Hu LJ, Kong EL, Bollen AW, Lamborn KR, Deen DF (2005) Functionality of hypoxia-induced BAX expression in a human glioblastoma xenograft model. *Cancer Gene Ther* 12: 449-455.

137. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM (2003) Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 3:347-361.

138. Pertovaara L, Kaipainen A, Mustonen T, Orpana A, Ferrara N, Saksela O, Alitalo K (1994) Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *J Biol Chem* 269:6271-6274.

139. Plate KH, Breier G, Weich HA, Mennel HD, Risau W (1994) Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. *Int J Cancer* 59:520-529.

140. Plate KH, Breier G, Weich HA, Risau W (1992) Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature* 359:845-848.

141. Pore N, Liu S, Haas-Kogan DA, O'Rourke DM, Maity A (2003) PTEN mutation and epidermal growth factor receptor activation regulate vascular endothelial growth factor (VEGF) mRNA expression in human glioblastoma cells by transactivating the proximal VEGF promoter. *Cancer Res* 63:236-241.

142. Raza SM, Lang FF, Aggarwal BB, Fuller GN, Wildrick DM, Sawaya R (2002) Necrosis and glioblastoma: a friend or a foe? A review and a hypothesis. *Neurosurgery* 51:2-12; discussion 12-13.

143. Rege TA, Fears CY, Gladson CL (2005) Endogenous inhibitors of angiogenesis in malignant gliomas: Nature's antiangiogenic therapy. *Neuro-Oncology* 7:106-121.

144. Rempel SA, Dudas S, Ge S, Gutierrez JA. (2000) Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma. *Clin Cancer Res* 6:102-111.

145. Reynolds TY, Rockwell S, Glazer PM (1996) Genetic instability induced by the tumor microenvironment. *Cancer Res* 56:5754-5757.

146. Richard DE, Berra E, Pouysségur J (2000) Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1 α in vascular smooth muscle cells. *J Biol Chem* 275:26765-26771.

147. Rijken PFJW, Bernsen HJJA, Peters JPW, Hodgkiss RJ, Raleigh JA, van der Kogel AJ (2000) Spatial relationship between hypoxia and the (perfused) vascular network in a human glioma xenograft: a quantitative multi-parameter analysis. *Int J Rad Oncol Biol Phys* 48:571-582.

148. Roberts WG, Palade GE (1997) Neovasculature induced by vascular endothelial growth factor is fenestrated. *Cancer Res* 57:765-772.

149. Rolhion C, Penault-Llorca F, Kemeny JL, Lemaire JJ, Jullien C, Labit-Bouvier C, Finat-Duclos F, Verrelle P (2001) Interleukin-6 overexpression as a marker of malignancy in human gliomas. *J Neurosurg* 94:97-101.

150. Rong Y, Post DE, Pieper RO, Durden DL, Van Meir EG, Brat DJ (2005) PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. *Cancer Res* 65:1406-1413.

151. Sahin A, Velten M, Pietsch T, Knuefermann P, Okuducu AF, Hahne JC, Wernert N (2005) Inactivation of Ets 1 transcription factor by a specific decoy strategy reduces rat C6 glioma cell proliferation and MMP-9 expression. *Int J Mol Med* 15:771-776.

152. Saleh M, Davis ID, Wilks AF (1997) The paracrine role of tumour-derived mIL-4 on tumour-associated endothelium. *Int J Cancer* 72:664-672.

153. Sallinen SL, Sallinen PK, Haapasalo HK, Helin HJ, Helen PT, Schraml P, Kallioniemi OP, Kononen J (2000) Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques. *Cancer Res* 60:6617-6622.

154. Salmaggi A, Eoli M, Frigerio S, Silvani A, Gelati M, Corsini E, Broggi G, Boiardi A (2003) Intracavitary VEGF, bFGF, IL-8, IL-12 levels in primary and recurrent malignant glioma. *J Neurooncol* 62:297-303.

155. Salmaggi A, Gelati M, Pollo B, Frigerio S, Eoli M, Silvani A, Broggi G, Ciusani E, Croci D, Boiardi A, De Rossi M (2004) CXCL12 in malignant glial tumors: a possible role in angiogenesis and cross-talk between endothelial and tumoral cells. *J Neurooncol* 67:305-317.

156. Samoto K, Ikezaki K, Ono M, Shono T, Kohno K, Kuwano M, Fukui M (1995) Expression of vascular endothelial growth factor and its possible relation with neovascularization in human brain tumors. *Cancer Res* 55:1189-1193.

157. Schmid T, Zhou J, Brune B (2004) HIF-1 and p53: communication of transcription factors under hypoxia. *J Cell Mol Med* 8:423-431.

158. Schmidt NO, Westphal M, Hagel C, Ergun S, Stavrou D, Rosen EM, Lamszus K (1999) Levels of vascular endothelial growth factor, hepatocyte growth factor/scatter factor and basic fibroblast growth factor in human gliomas and their relation to angiogenesis. *Int J Cancer* 84:10-18.

159. Schofield CJ, Ratcliffe PJ (2004) Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* 5:343-354.

160. Semenza GL (2001) HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 107:1-3.

161. Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3:721-732.

162. Shih AH, Holland EC (2005) Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett*, *http://www.sciencedirect.com/*

163. Shweiki D, Itin A, Soffer D, Keshet E (1992) Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 359:843-845.

164. Smits A, Funa K (1998) Platelet-derived growth factor (PDGF) in primary brain tumours of neuroglial origin. *Histol Histopathol* 13:511-520.

165. Sonoda Y, Kanamori M, Deen DF, Cheng SY, Berger MS, Pieper RO (2003) Overexpression of vascular endothelial growth factor isoforms drives oxygenation and growth but not progression to glioblastoma multiforme in a human model of gliomagenesis. *Cancer Res* 63:1962-1968.

166. Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL (2001) HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. *Cancer Res* 2001 61:6669-6673.

167. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 15:356-362.

168. Stratmann A, Risau W, Plate KH (1998) Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis. *Am J Pathol* 153:1459-1466.

169. Straume O, Chappuis PO, Salvesen HB, Halvorsen OJ, Haukaas SA, Goffin JR, Begin LR, Foulkes WD, Akslen LA (2002) Prognostic importance of glomeruloid microvascular proliferation indicates an aggressive angiogenic phenotype in human cancers. *Cancer Res* 62:6808-6811.

170. Suddith RL, Kelly PJ, Hutchison HT, Murray EA, Haber B (1975) In vitro demonstration of an endothelial proliferative factor produced by neural cell lines. *Science* 190:682-684.

171. Sundberg C, Nagy JA, Brown LF, Feng D, Eckelhoefer IA, Manseau EJ, Dvorak AM, Dvorak HF (2001) Glomeruloid microvascular proliferation follows adenoviral vascular permeability factor/vascular endothelial growth factor-164 gene delivery. *Am J Pathol* 158:1145-1160.

172. Takahashi JA, Mori H, Fukumoto M, Igarashi K, Jaye M, Oda Y, Kikuchi H, Hatanaka M (1990) Gene expression of fibroblast growth factors in human gliomas and meningiomas: demonstration of cellular source of basic fibroblast growth

factor mRNA and peptide in tumor tissues. *Proc Natl Acad Sci U S A* 87:5710-5714.

173. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL (2000) The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* 157:411-421.

174. Theurillat JP, Hainfellner J, Maddalena A, Weissenberger J, Aguzzi A (1999) Early induction of angiogenetic signals in gliomas of GFAP-v-src transgenic mice. *Am J Pathol* 154:581-590.

175. Tonnesen MG, Feng X, Clark RA (2000) Angiogenesis in wound healing. *J Investig Dermatol Symp Proc* 5:40-46.

176. Tsai JC, Goldman CK, Gillespie GY (1995) Vascular endothelial growth factor in human glioma cell lines: induced secretion by EGF, PDGF-BB, and bFGF. *J Neurosurg* 82: 864-873.

177. Tse V, Xu L, Yung YC, Santarelli JG, Juan D, Fabel K, Silverberg G, Harsh G (2003) The temporal-spatial expression of VEGF, angiopoietins-1 and 2, and Tie-2 during tumor angiogenesis and their functional correlation with tumor neovascular architecture. *Neurol Res* 25:729-738.

178. Tuettenberg J, Grobbolz R, Korn T, Wenz F, Erber R, Vajkoczy P (2005) Continuous low-dose chemotherapy plus inhibition of cyclooxygenase-2 as an antiangiogenic therapy of glioblastoma multiforme. *J Cancer Res Clin Oncol* 131:31-40.

179. Tynninen O, Aronen HJ, Ruhala M, Paetau A, Von Boguslawski K, Salonen O, Jaaskelainen J, Paavonen T. (1999) MRI enhancement and microvascular density in gliomas. Correlation with tumor cell proliferation. *Invest Radiol* 34:427-434.

180. Ueba T, Nosaka T, Takahashi JA, Shibata F, Florkiewicz RZ, Vogelstein B, Oda Y, Kikuchi H, Hatanaka M (1994) Transcriptional regulation of basic fibroblast growth factor gene by p53 in human glioblastoma and hepatocellular carcinoma cells. *Proc Natl Acad Sci U S A* 91:9009-9013.

181. Valter MM, Hugel A, Huang HJ, Cavenee WK, Wiestler OD, Pietsch T, Wernert N (1999) Expression of the Ets-1 transcription factor in human astrocytomas is associated with Fms-like tyrosine kinase-1 (Flt-1)/vascular endothelial growth factor receptor-1 synthesis and neoangiogenesis. *Cancer Res* 59:5608-5614.

182. Vaquero J, Zurita M, Morales C, Cincu R, Oya S (2000) Expression of vascular permeability factor in glioblastoma specimens: correlation with tumor vascular endothelial surface and peritumoral edema. *J Neurooncol* 49:49-55.

183. Veikkola T, Karkkainen M, Claesson-Welsh L, Alitalo K (2000) Regulation of angiogenesis via vascular endothelial growth factor receptors. *Cancer Res* 60:203-212.

184. von Deimling A, Louis DN, Wiestler OD (1995) Molecular pathways in the formation of gliomas. *Glia* 15:328-338.

185. Wang D, Huang HJ, Kazlauskas A, Cavenee WK (1999) Induction of vascular endothelial growth factor expression in endothelial cells by platelet-derived growth factor through the activation of phosphatidylinositol 3-kinase. *Cancer Res* 59:1464-1472.

186. Weinmann M, Belka C, Plasswilm L (2004) Tumour hypoxia: impact on biology, prognosis and treatment of solid malignant tumours. *Onkologie* 27:83-90.

187. Wen S, Stolarov J, Myers MP, Su JD, Wigler MH, Tonks NK, Durden DL (2001) PTEN controls tumor-induced angiogenesis. *Proc Natl Acad Sci U S A* 98:4622-4627.

188. Wenger RH, Gassmann M (1997) Oxygen(es) and the hypoxia-inducible factor-1. *Biol Chem* 378:609-616.

189. Wesseling P, Schlingemann RO, Rietveld FJ, Link M, Burger PC, Ruiter DJ (1995) Early and extensive contribution of pericytes/vascular smooth muscle cells to microvascular proliferation in glioblastoma multiforme: an immunolight and immuno-electron microscopic study. *J Neuropathol Exp Neurol* 54:304-310.

190. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* 60:7075-7083.

191. Xiao Q, Hsu CY, Chen H, Ma X, Xu J, Lee JM (2005) Characterization of cis-regulatory elements of the vascular endothelial growth inhibitor gene promoter. *Biochem J* 388:913-920.

192. Yamahata H, Takeshima H, Kuratsu J, Sarker KP, Tanioka K, Wakimaru N, Nakata M, Kitajima I, Maruyama I (2002) The role of thrombin in the neo-vascularization of malignant gliomas: an intrinsic modulator for the up-regulation of vascular endothelial growth factor. *Int J Oncol* 20:921-928.

193. Yanamandra N, Kondraganti S, Gondi CS, Gujrati M, Olivero WC, Dinh DH, Rao JS (2005) Recombinant adeno-associated virus (rAAV) expressing TFPI-2 inhibits invasion, angiogenesis and tumor growth in a human glioblastoma cell line. *Int J Cancer* 115:998-1005.

194. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J (2000) Vascular-specific growth factors and blood vessel formation. *Nature* 407:242-248.

195. Yeo EJ, Chun YS, Park JW (2004) New anticancer strategies targeting HIF-1. *Biochem Pharmacol* 68:1061-1069.

196. Zadeh G, Qian B, Okhowat A, Sabha N, Kontos CD, Guha A (2004) Targeting the Tie2/Tek receptor in astrocytomas. *Am J Pathol* 164:467-476.

197. Zagzag D (1995). Angiogenic growth factors in neural embryogenesis and neoplasia. *Am J Pathol* 146:293-309.

198. Zagzag D, Amirnovin R, Greco MA, Yee H, Holash J, Wiegand SJ, Zabski S, Yancopoulos GD, Grumet M (2000) Vascular apoptosis and involution in gliomas precede neovascularization: a novel concept for glioma growth and angiogenesis. *Lab Invest* 80:837-849.

199. Zagzag D, Capo V (2002) Angiogenesis in the central nervous system: a role for vascular endothelial growth factor/vascular permeability factor and tenascin-C. Common molecular effectors in cerebral neoplastic and non-neoplastic "angiogenic diseases". *Histol Histopathol* 17:301-321.

200. Zagzag D, Friedlander DR, Miller DC, Dosik J, Cangiarella J, Kostianovsky M, Cohen H, Grumet M, Greco MA (1995) Tenascin expression in astrocytomas correlates with angiogenesis. *Cancer Res* 55:907-914.

201. Zagzag D, Friedlander DR, Dosik J, Chikramane S, Chan W, Greco MA, Allen JC, Dorovini-Zis K, Grumet M (1996) Tenascin-C expression by angiogenic vessels in human astrocytomas and by human brain endothelial cells in vitro. *Cancer Res* 56:182-189.

202. Zagzag D, Friedlander DR, Margolis B, Grumet M, Semenza GL, Zhong H, Simons JW, Holash J, Wiegand SJ, Yancopoulos GD (2000) Molecular events implicated in brain tumor angiogenesis and invasion. *Pediatr Neurosurg* 33:49-55.

203. Zagzag D, Goldenberg M, Brem S (1989) Angiogenesis and blood-brain barrier breakdown modulate CT contrast enhancement: an experimental study in a rabbit brain-tumor model. *Am J Roentgenol* 153:141-146.

204. Zagzag D, Hooper A, Friedlander DR, Chan W, Holash J, Wiegand SJ, Yancopoulos GD, Grumet M (1999) In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. *Exp Neurol* 159:391-400.

205. Zagzag D, Krishnamachary B, Yee H, Okuyama H, Chiriboga L, Ali MA, Melamed J, Semenza GL (2005) Stromal cell-derived factor-1alpha and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: von Hippel-Lindau loss-of-function induces expression of a ligand and its receptor. *Cancer Res* 65:6178-6188.

206. Zagzag D, Miller DC, Sato Y, Rifkin DB, Burstein DE (1990) Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. *Cancer Res* 50:7393-8.

207. Zagzag D, Robert F, Brem S (1988) Neovascularization and tumor growth in the rabbit brain: A model for experimental studies of angiogenesis and blood-brain barrier. *Am J Pathol* 131:361-372.

208. Zagzag D, Shiff B, Jallo GI, Greco MA, Blanco C, Cohen H, Hukin J, Allen JC, Friedlander DR (2002) Tenascin-C promotes microvascular cell migration and phosphorylation of focal adhesion kinase. *Cancer Res* 62:2660-2668.

209. Zagzag D, Zhong H, Scalzitti JM, Laughner E, Simons JW, Semenza GL (2000) Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. *Cancer* 88:2606-2618.

210. Zelzer E, Levy Y, Kahana C, Shilo BZ, Rubinstein M, Cohen B (1998) Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *EMBO J* 17:5085-5094.

211. Zeng H, Dvorak HF, Mukhopadhyay D (2001) Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) receptor-1 down-modulates VPF/VEGF receptor-2-mediated endothelial cell proliferation, but not migration,

through phosphatidylinositol 3-kinase-dependent pathways. *J Biol Chem* 276:26969-26979.

212. Zhang X, Groopman JE, Wang JF (2005) Extracellular matrix regulates endothelial functions through interaction of VEGFR-3 and integrin alpha5beta1. *J Cell Physiol* 202:205-214.

213. Zhang W, Wang H, Song SW, Fuller GN (2002) Insulin-like growth factor binding protein 2: gene expression microarrays and the hypothesis-generation paradigm. *Brain Pathol* 12:87-94.

214. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW (1999) Overexpression of hypoxia-inducible factor-1alpha in common human cancers and their metastases. *Cancer Res* 59:5830-5835.

215. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D, Giaccia AJ (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* 14:391-396.