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Growth factors in glioma angiogenesis: FGFs, PDGF, EGF, and TGFs

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Summary

It has become well accepted that solid tumors must create a vascular system for nutrient delivery and waste removal in order to grow appreciably. This process, angiogenesis, is critical to the progression of gliomas, with vascular changes accompanying the advancement of these tumors. The cascade of events in this process of blood vessel formation involves a complex interplay between tumor cells, endothelial cells, and their surrounding basement membranes in which enzymatic degradation of surrounding ground substance and subsequent endothelial cell migration, proliferation, and tube formation occurs. It is likely that a host of growth factors is responsible for mediating these key events. To date, a role for Vascular Endothelial Growth Factor (VEGF) in glioma angiogenesis has been convincingly demonstrated. This review explores the contribution of other growth factors—Fibroblast Growth Factors (FGFs), Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), and Transforming Growth Factors (TGFs)—to glioma angiogenesis. These growth factors may influence glioma angiogenesis by directly stimulating endothelial cell proliferation, by mediating the expression of key proteases on endothelial cells necessary for angiogenesis, or by regulating the expression of VEGF and of each other.

Introduction

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a central component in the development, progression, and metastasis of a number of human tumors including gliomas [1,2]. Gliomas appear to obey the central tenet of angiogenesis: that to grow rapidly beyond 1–2 mm^3, they must create a system of blood vessels for nutrient delivery and waste removal [3]. Consistent with this observation, vascular changes accompany the malignant progression of gliomas and are included in the criteria used for glioma grading [4–8]. Noted vascular alterations include endothelial cell proliferation and loss of vessel structural integrity [9,10]. Moreover, neovascularization is correlated with biological aggressiveness, degree of malignancy, and clinical recurrence of gliomas and is inversely correlated with postoperative survival of patients [7,11].

Angiogenesis in gliomas is both similar and distinct from neovascularization in extracranial solid tumors. Gliomas require neovascularization for growth and continued progression, as do extracranial solid tumors. However, additional consequences of the recruitment of structurally compromised vessels to the glioma tumor bed – namely, ischemic steal from hyperemia, peritumoral edema, and increased likelihood of spontaneous hemorrhage – are of great concern in an organ of exquisite oxygen-dependence and sensitivity to increased intracranial pressure [12–14]. Hence the particular context of oxygen and pressure sensitivity in which glioma angiogenesis takes place compounds the morbidity of the tumor growth and invasion fueled by new blood vessel growth. A thorough understanding of angiogenesis in gliomas is thus necessary for therapeutic targeting of this process.

During angiogenesis, new blood vessels grow by sprouting from established blood vessels; this process is characterized by a cascade of events including enzymatic degradation of basement membrane and endothelial cell migration, proliferation, and tube formation [15]. Vascular endothelial growth factor

(VEGF) appears to be the most potent of the myriad angiogenic growth factors described to date. (It is discussed in a separate chapter.) Identical to vascular permeability factor (VPF), tumor- or endothelial-derived VEGF exerts its effects in an autocrine or paracrine manner in gliomas through binding to high affinity tyrosine kinase receptors KDR/Flk-1 and Flt-1 on endothelial cells [16,17]. A precise role for the neuropilins, the most recent addition to the VEGF receptor family [18], has yet to be described in glioma angiogenesis. In addition to stimulating normal and tumor angiogenesis and vasculogenesis, VEGF also induces blood vessel permeability and protein extravasation; hence it is responsible for both new blood vessel formation and for the accompanying vasogenic edema observed in tumor angiogenesis [19–21].

The role of VEGF in glioma angiogenesis has been convincingly demonstrated [22], and while it may be the principal angiogenic factor in this process, a number of other growth factors like Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), and the Transforming Growth Factors-α and -β (TGF-α and -β) and their corresponding receptors may also play roles in regulating and modifying tumor angiogenesis in malignant gliomas. These tumor-secreted growth factors have both direct and indirect effects on glioma angiogenesis: they can directly stimulate endothelial cell proliferation, mediate the expression of key proteases on endothelial cells necessary for angiogenesis, and regulate the expression of VEGF and of each other. They can act in a paracrine fashion, in which ligand is produced by cells distinct from the cells expressing receptor, or they may act in an autocrine manner in which the same cell type expresses ligand and receptor. In this review, we will discuss FGFs, PDGF, EGF, and TGF-α and -β in turn, highlighting the basic properties of these growth factors and their cognate receptors, their expression in gliomas, and their roles in angiogenesis in these tumors.

FGF

Ligand and receptor

The FGF growth factor family now comprises 19 polypeptides: FGF-1 (acidic FGF) [23], FGF-2 (basic FGF) [24,25], FGF-3 (int-2) [26], FGF-4 (hst-l/kaposi-FGF) [27,28], FGF-5 [29], FGF-6 (hst-2) [30], FGF-7 (keratinocyte growth factor) [31], FGF-8 (androgen induced growth factor) [32], FGF-9 (glia-activating factor) [32] and FGFs 10 and 12-19 [34–42] which as new members still have incompletely characterized biological functions. The FGFs are involved in a wide range of biological activities and have been shown to be involved in mitogenesis, differentiation, chemotaxis, and angiogenesis; as such they have been implicated in the development of skeletal, nervous, and vascular systems, tissue integrity and repair, and wound healing, as well as a host of pathophysiologic processes [43,44]. Here, we will focus on those FGFs that have been reported to have roles in glioma angiogenesis: FGF-1 and FGF-2. Additionally, a potential role for FGF-4 in this process will also be discussed.

Of the 19 proteins in the FGF family, FGF-1 and FGF-2 have been studied in greatest depth. Both have been shown to be potent inducers of endothelial cell migration, proliferation, and tube formation *in vitro* and are highly angiogenic in a number of tissues *in vivo* [43,45]. FGF-1, or acidic FGF (aFGF), is encoded on chromosome 5q [23] and is a 154-amino acid protein (with N-terminal truncation forms of 140 and 134 amino acids) with a molecular mass of 18 kDa [43,46] (Table 1). FGF-2, encoded on chromosome 4q [47,48], is expressed in 4 forms of varying molecular masses: 18, 22, 22.5, and 24 kDa [48,49]. The 18 kDa form has 55% sequence homology to aFGF [46]. An intriguing structural feature of FGF-1 and FGF-2 is the absence of a classical hydrophobic signal sequence for secretion despite their extracellular activities. That these proteins may be secreted during inhibition of classical secretory routes suggests that secretion occurs through an unconventional non-ER/Golgi pathway [51,52]. The molecular weights and chromosomal locations of all growth factors and receptors discussed in this review are listed in Table 1.

While the targets of these factors are many, each exerts its effects through one or more members of four well-characterized FGF receptor families: FGF receptor-1 (flg) [53], FGF receptor-2 (bek) [54], FGF receptor-3 (FGFR-3) [55], and FGF receptor-4 (FGFR-4) [56–58]; these are located on chromosomes 8p, l0q, 4p, and 5q, respectively [59–62]. Once secreted, acidic and basic FGF exert their effects by binding to FGFRs 1–4. These proteins are receptor tyrosine kinases. FGF molecules – perhaps bound as dimers [63] – bind to FGFRs to induce receptor dimerization, which is required to initiate the protein tyrosine kinase activity and subsequent

Table 1. Angiogenic growth factors and receptors: size and genetic location

Factor	Molecular weight (kDa)	Chromosome
FGF-l	18[24]	5q[24]
FGF-2	18, 22, 22.5, 24[50,51]	4q[46,47]
FGF-4	18[27]	11q[28]
FGFR-1	150[53]	8p[59]
FGFR-2	135[54]	lOq [60]
FGFR-3	125[55]	4p [61]
FGFR-4	95, 110[56]	5q[62]
PDGF-A	15[97]	7p[98,99,100]
PDGF-B	15[97]	22q[9,103]
PDGFR-α	170[109]	4q[109]
PDGFR-β	180[107,108,109]	5q[107,108,109]
TGF-α	5–20137	2p[143]
EGFR	170-180[132]	7p[148]
TGF-βl	12–15[184,190]	19q[187]
TGF-β2	12–15[182,188]	1q[188]
TGF-β3	12–15[182,188]	14q[189]
TβR-I	55[191]	9q[195]
TβR-II	75[191]	3p[195]
TβR-III	250–350 [193]	1p[197]
Endoglin	68 [194]	9q[198]

trans-autophosphorylation events and activation (Figure 1).

It has been shown that heparan sulfate proteoglycans (HSPGs) such as heparin may increase the binding affinity of FGFs to their receptors [64]. Proteins such as phospholipase-C γ (PLC-γ) and Ras GTPase Activating Protein (GAP), for instance, then bind the phosphorylated regions, engaging the receptor tyrosine kinase signaling cassette en route to mediating gene transcription events.

Expression in gliomas and roles in angiogenesis

FGF-l and FGF-2, two of the first angiogenic factors isolated [65], have been implicated in glioma angiogenesis. Early *in vitro* studies demonstrated high levels of expression of FGF-2 mRNA and protein in U87MG glioma cell lines [66]. Robust expression of FGF-1 in glioma as compared to normal brain has also been shown; an early study showing FGF-1 overexpression in gliomas by Northern blot analysis and *in situ* hybridization reported that 93% of tumors studied showed elevated FGF-1 mRNA expression as compared to nonmalignant control brain tissue [67]. FGF-2 overexpression has been linked to increased endothelial activity, as well. Strong expression of FGF-2 has been reported in the perivascular space of more malignant tumors as compared to normal brain [67]. Peritumoral endothelial cell FGF-2 immunoreactivity has also been shown to correlate with tumor grade; capillaries in glioblastoma and anaplastic astrocytoma are immunoreactive for FGF-2, in contrast to those of low-grade astrocytomas [68]. Higher levels of FGF receptors have also been demonstrated in gliomas; indeed, it has been suggested that glioma grade is marked by the differential expression of FGFR molecules, with the malignant progression from glial cell to glioblastoma accompanied by diminishing levels of FGFR-2 and increasing levels of FGFR-1 [69,70]. These results suggest that alterations in FGFR signal transduction pathways may play a critical role in the malignant progression of astrocytic tumors. While these changes have been reported in glioma cells, there is conflicting evidence regarding the status of FGFR expression on intratumoral endothelial cells [69–71].

It is thought that FGF-1 and FGF-2 may participate in angiogenesis in two primary ways: by modulating endothelial cell activity and by regulating VEGF expression in tumor cells. Both factors are well-established mitogens and chemoattractants for endothelial cells [65,72,73]. In order to invade extracellular matrix to vascularize new tissues, endothelial cells must enhance their expression of molecules that activate key proteases such as plasmin [74–76]. One of such molecules, urokinase-type plasminogen activator (uPA), acts to convert the inactive zymogen plasminogen to the active proteolytic enzyme plasmin [77]. Plasmin, in turn, is able to degrade multiple extracellular matrix (ECM) components including fibronectin and laminin, permitting endothelial cell migration into new tissue space [78]. FGF-2 has been shown to upregulate uPA and collagenase expression on endothelial cells [79] and has also been shown to induce expression of the receptor for uPA [80], thus modulating endothelial cell migration in a feed-forward fashion. Hence one way in which FGF-2 may participate in angiogenesis is by mediating the proteolytic digestion of ECM by invading endothelial cells. Furthermore, FGF-2 is chemotactic for endothelial cells [81] and has been shown to induce capillary endothelial cells to migrate into three-dimensional collagen matrices to form capillary-like tubes [82].

A second way in which FGF-2 may participate in angiogenesis is by inducing expression of VEGF, an endothelial cell mitogen [83–86] which has been shown to be a potent angiogenesis factor in human gliomas *in vivo* [86]. FGF-2 has been shown to stimulate VEGF

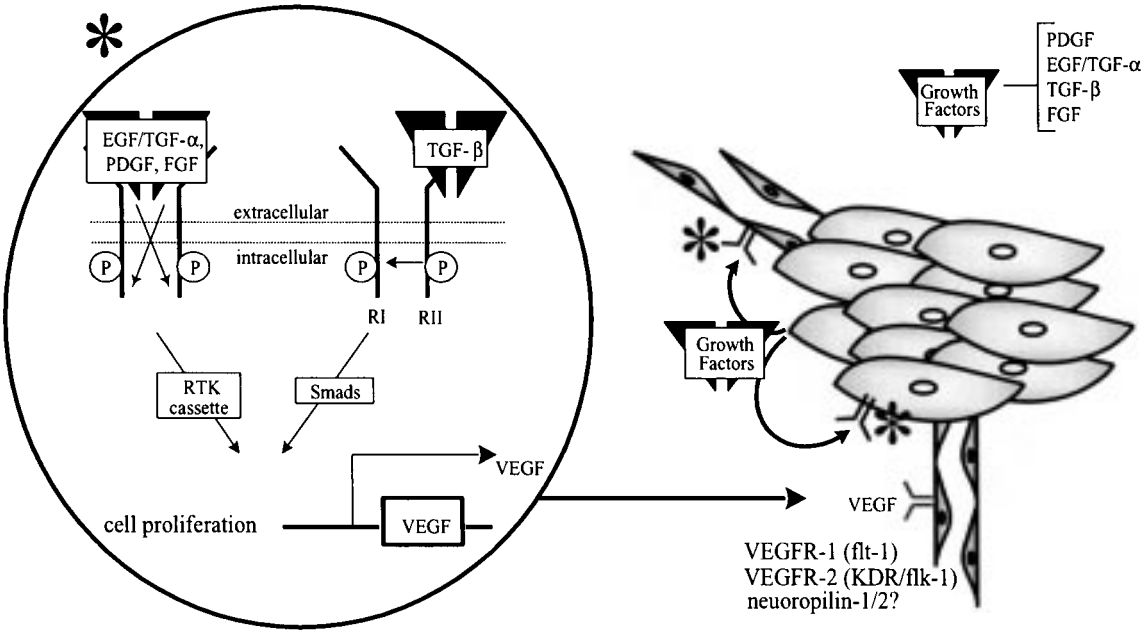

Figure 1. Growth factor-mediated angiogenesis in gliomas. In this scheme, tumor cells secrete indicated growth factors represented by dimerized triangles which bind their respective receptors either on the same or adjacent tumor cells or on surrounding tumor endothelial cells. Growth factor receptors are marked by asterisks and are shown in greater detail in the inset. Growth factor binding may lead to cell proliferation, upregulation of key proteases, and/or initiation of signaling leading to VEGF transcription in tumor cells or endothelial cells. VEGF secreted from tumor cells and/or endothelial cells after growth factor-mediated signaling may then bind to one of its receptors, flt-1 or KDR/flk-1. Neuropilins 1 and 2 were recently shown to be the newest members of the VEGF receptor family and may be involved in glioma angiogenesis. *Inset:* Details of signaling by growth factor receptors (marked by asterisks in figure). Proposed growth factor-mediated signaling pathways leading to VEGF transcription in tumor cells or endothelial cells. EGF, TGF-α, the FGFs, and PDGF signal through receptor tyrosine kinases (RTKS); the ligands, which are dimerized, bind to tyrosine transreceptor subunits and induce dimerization. This in turn leads to *trans*-autophosphorylation by the intracellular receptor tyrosine domains, and subsequent signaling through molecules such as Raf/Ras and others, represented by 'RTK cassette' above. The details of TGF-β signaling differ. First, TGF-β – again binding as a dimer – binds to only the RII subunit, and binding induces recruitment of the RI subunit and *trans*-phosphorylation of RI by the constitutively phosphorylated RII. Downstream signaling is initiated by activated RI and is mediated by Smad proteins.

secretion in a dose-dependent fashion using U-105MG and D-54MG glioma cell lines [87]. Thus FGF-2 secreted by tumor cells may induce angiogenesis in a paracrine fashion, acting on endothelial cells directly as well as stimulating tumor cells in an autocrine and paracrine fashion to synthesize VEGF.

Studies such as these have established that FGF-1 and FGF-2 may modulate endothelial cell activity directly and indirectly. While high levels of these growth factors have been detected in gliomas, functional roles for these proteins in glioma angiogenesis have also been investigated directly. Direct evidence for the role of FGF-2 in glioma angiogenesis is given by *in vivo* studies in which intracranially implanted U87MG tumors showed a significantly lower degree of neovascularization after treatment with an anti-FGF-2 antibody as compared to untreated tumors [88]. Additionally, endothelial tube formation stimulated by the presence of glioma cells in a three-dimensional collagen was markedly inhibited after incubation with an anti-FGF-2 antibody [89].

In addition to reports establishing both the presence and functional involvement of FGFs in glioma angiogenesis, studies have also correlated FGF expression with clinical tumor progression and patient prognosis. Increased neovascularization accompanies the mitotic and pleiomorphic cellular changes in the malignant progression of gliomas. Immunohistochemical studies on primary glioma specimens showing that FGF-2 is expressed in human gliomas also showed that FGF-2 levels are increased in high grade as compared to low-grade gliomas [68]. Additional studies have also reported increasing FGF-2 levels with glioma grade, noting that FGF-2 expression correlates with glioma

vascularity [90]. Moreover, Li *et al.* detected FGF-2 in the CSF of 62% of patients with brain tumors (16/26), while no FGF-2 was detected in controls [91]. Endothelial cells from CSF that contained FGF-2 were more proliferative when compared with controls; the degree of endothelial cell stimulation correlated with FGF-2 levels; and the level of FGF-2 correlated with microvessel density. Most strikingly, patients with CSF containing FGF-2 recurred earlier, with earlier tumor recurrence correlating with increased microvessel density. Interestingly, while a number of different types of brain tumors were included in this study, the highest level of FGF-2 was detected in a pilocytic astrocytoma. Studies from our laboratory have supported the importance of microvessel density as a prognostic indicator of postoperative survival of patients with astroglial brain tumors [92].

Research to date has focused on the involvement of FGF-1 and FGF-2. A recent study provided evidence that FGF-4 may also participate in glioma angiogenesis [93]. In 18 glioblastomas, 17 anaplastic astrocytomas, and 19 low-grade gliomas, Western blot and immunohistochemical analysis demonstrated the presence of FGF-4 and its related receptors, FGFRs-1, 2, and 4, in tumor and tumor-associated endothelial cells. The expression of these proteins was undetectable in control non-neoplastic tissue. Moreover, the expression of FGF-4 and its receptors correlated with histological grade, tumor type, microvessel count, and cell density. These observations were corroborated by *in vitro* studies in which U87MG, U373MG, and U118MG glioma cell lines were shown to secrete FGF-4. Additional studies showed that FGF-4 could regulate VEGF expression and the formation of new blood vessels. These data suggest that other FGF family members such as FGF-4, in addition to FGF-1/2, may be important in blood vessel growth in gliomas.

PDGF

Ligand and receptor

Platelet-derived growth factor (PDGF) has also been implicated in glioma angiogenesis. PDGF was initially discovered as a mitogen for fibroblasts contained in human serum and localized in the alpha granules of platelets [94,95]. While initially described as a platelet alpha-granule release product, subsequent studies have shown that PDGF is produced by a variety of cell types and that among its many targets of action are capillary endothelial cells, vascular smooth muscle cells, osteoblasts, glia, and neurons [96–98]. PDGF has pleiotropic effects and has roles in embryonic development, CNS development, the vascular system, tissue homeostasis, and wound healing. An angiogenic role has also been demonstrated, albeit weaker than the angiogenic effects of VEGF and the FGFs [98].

PDGF is a 30 kDa protein consisting of disulfide-bonded dimers of A and/or B chains. The A and B subunits are approximately 100 amino acids in length and share a 60% sequence homology; the isoforms are functionally active when dimerized as either PDGF-AA, PDGF-AB, or PDGF-BB [97,99]. PDGF-A is encoded by a gene on chromosome 7p [100–102]; PDGF-B, the cellular homolog of the retroviral oncogene v-sis carried by the Simian Sarcoma Virus (SSV) [103,104], is encoded by the c-sis/PDGF-2 gene on chromosome 22q [101,105].

The PDGF receptors are members of the protein tyrosine kinase family of receptors [106] and as such are activated by ligand-induced dimerization of receptor subunits. Two receptor subunits have been described: PDGF receptor-α (PDGFR-α) and PDGF receptor-β (PDGFR-β). PDGFR-α, a 170 kDa protein, is encoded on chromosome 4q, while PDGFR-β, a 180 kDa protein, is encoded on chromosome 5q [107–109]. Ligand binding and subsequent dimerization leads to juxtaposition of the two tyrosine kinase domains and *trans*-autophosphorylation, and to the activation of signal transduction pathways and downstream gene transcription events (Figure 1).

PDGF dimer combinations bind to dimeric combinations of PDGF receptor subunits. PDGF-A binds only to PDGFR-α, whereas PDGF-B binds to the β receptor with higher affinity but can bind both subtypes. Consequently, PDGF-AA can only activate the $\alpha\alpha$ receptor complex; PDGF-AB can activate $\alpha\alpha$ or $\alpha\beta$; and PDGF-BB can activate $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ [110–112]. PDGF-BB is able to activate all three receptor complexes and has been shown to be the most mitogenic of the PDGF dimer combinations [113–117].

Expression in gliomas and roles in angiogenesis

Human astrocytomas express high levels of both the PDGF ligand and corresponding receptor subtypes. *In situ* hybridization and immunocytochemistry studies have demonstrated that PDGF-A is expressed in low-grade and anaplastic astrocytomas as well as in glioblastomas. and that expression increases with

tumor grade [118]. PDGFR-α was also expressed at higher levels in gliomas as compared to control gliosis cases. PDGF-B, while still expressed, was found at lower levels than PDGF-A in cell-rich areas; PDGFR-β was not detected in glioma cells. Later studies have corroborated these results, and studies in oligodendrogliomas have demonstrated similar ligand and receptor expression [123,125]. The overexpression of PDGFR-α in all tumor grades and the increasing expression of PDGF-A and PDGF-B with tumor grade are consistent with the concept that the establishment of a functional autocrine/paracrine loop may be important in glioma pathogenesis [118,120–124]. Interestingly, robust expression of both PDGF-B and PDGFR-β was reported in hyperplastic tumor endothelial cells in glioblastoma [119]. Further *in situ* hybridization and immunocytochemistry studies in normal brain, low-grade astrocytoma, anaplastic oligo-astrocytoma, and glioblastoma have demonstrated that PDGFR-β mRNA is expressed in the vasculature of glioma tissue, most notably in areas of endothelial cell proliferation in glioblastoma tissue [126]. No mRNA message was detected in normal brain and expression in tumors appeared to be confined to tumor endothelial cells. The detection of increased levels of PDGF-B and PDGFR-β on high-grade astrocytoma endothelial cells suggests a role for PDGF in glioma angiogenesis. Subsequent *in vitro* studies have taken steps towards addressing the biological significance of these observations. PDGF was shown to be a chemotactic factor in rat brain capillary endothelial cells, with PDGF-BB having a more potent effect than PDGF-AA [127]. Earlier studies by Westermark and co-investigators provide a possible molecular basis for this effect by showing that PDGFR-β is able to mediate the actin reorganization necessary for a chemotactic motility response [128].

Most recently, the observed overexpression of PDGFR-β on tumor endothelial cells was studied by transfecting aortic endothelial cells with wild-type PDGFR-β. Incubation with PDGF-B led to increased transcription and secretion of VEGF by endothelial cells expressing the β receptor. Furthermore, it was shown that VEGF regulation by PDGF was mediated by phosphoinositol-3 kinase (PI-3 kinase) as PI-3 kinase-specific inhibitors abrogated the PDGF-induced VEGF expression [129]. Interestingly, Tsai *et al.* demonstrated that physiological concentrations of PDGF-BB and not PDGF-AA can also induce VEGF secretion in a variety of human glioma cell lines [87]. Moreover, it was demonstrated that activation of convergent signaling pathways of EGF, PDGF-BB, and FGF-2 led to increased VEGF secretion.

The emerging picture is that inappropriate expression of PDGF by astrocytes and the upregulation of the PDGF receptor on the surrounding endothelial cells may represent an important step in the development, proliferation and maintenance of malignant astrocytomas [126,130,131]. An increase in PDGF activity *in vivo* may stimulate VEGF expression in endothelial cells and in tumor cells, establishing both paracrine and autocrine loops for endothelial cell activation and proliferation. PDGF-induced VEGF expression may contribute not only to expansion of an established tumor but also to the regulation of the angiogenic switch for initial tumor development as induction of PDGFR-β on endothelial cells may occur in early stages of glioma formation.

EGF/TGF-α/EGFR

Ligand and receptor

EGF, TGF-α, and their receptor, epidermal growth factor receptor (EGFR), are thought to be important in the mediation of proliferative and transforming response in a variety of cancers via autocrine or paracrine mechanisms [132–134]. A considerable body of work has demonstrated its importance in glioma transformation. In these tumors, overexpression of EGFR is a late event, and may be one of the last molecular event to occur in the pathogenesis of gliomas [135]. Ligands which bind EGFR include EGF, TGF-α, heparin-binding epidermal growth factor-like growth factor (HB-EGF), amphiregulin, and epiregulin [136–140]. Of these, EGF and TGF-α have been implicated in glioma angiogenesis. EGF – one of the earliest peptide growth factors purified – is a 53-amino acid protein of molecular weight 6 kDa whose gene has been mapped to chromosome 4q [132,141,142]; it is involved in the regulation of cell proliferation and differentiation in a number of physiological systems. TGF-α, which in active form is 50 amino acids long and is 5–20 kDa depending on extent of glycosylation, maps to chromosome 2p [137,143]. First found in the medium of retrovirally transformed fibroblasts [133,144], TGF-α shares 42% homology with EGF [145] and is widely expressed in developing embryos and in a number of normal adult tissues. It is thought to have roles in wound healing and homeostatsis in number of tissues, and to serve as

a broad regulator of normal growth and development [146]. It is structurally unrelated to TGF-β and binds to an entirely different set of receptors.

EGF and TGF-α both exert their effects through EGFR, the cellular homolog of the viral oncogene erbB from the avian erythroblastosis virus [147]. EGFR (ErbB1), encoded on chromosome 7p [148], is a receptor tyrosine kinase with a molecular weight of 170–180 kDa [132,137,145]. A classic receptor tyrosine kinase, two molecules of EGFR are cross-linked and brought together by dimerized ligand binding, which can occur among two EGFR molecules or among other members of the EGF receptor protein tyrosine kinase family, such as ErbB2, ErbB3, ErbB4 [149–151]. It has recently been demonstrated that EGF most likely expedites receptor subunit dimerization by itself binding as a dimer, and the same may be true for TGF-α [152]. Receptor dimerization induces *trans*-autophosphorylation and recruitment and activation of various signal transduction pathways (Figure 1); the Ras pathway has been implicated in EGFR-activated glioma pathogenesis [153].

A number of altered forms of EGFR – the result of gene rearrangements, alternative splicing, deletions, and/or translational alterations – have been detected in human glioma tissue and cell lines. The most common mutant form, termed $p140^{EGFR}$, EGFRvIII, or ΔEGFR, lacks a significant portion of the extracellular binding site of the wild-type protein and is constitutively phosphorylated and hence activated [154,155]. This truncated mutant has been shown to confer a considerable growth advantage when stably expressed *in vivo* when stably expressed in U87MG astrocytoma cells in nude mice [156]. Other mutant EGFR molecules that have been detected in gliomas lack cytoplasmic regions necessary for receptor downregulation [157,158].

Expression in gliomas and roles in angiogenesis

EGFR is rarely present in normal glial cells but is expressed in human gliomas, certain neurons, and in reactive astrocytes [159]. Studies have demonstrated the expression of EGFR in gliomas in the cell membrane, cytoplasm, and nucleus of positive cells by EGF binding and by immunohistochemistry [160,161].

Increased levels of EGFR in glioma are due to amplification of the erbB-1 gene, the first major molecular genetic alteration identified in human gliomas [162]. Amplification has been detected in 3% of low-grade astrocytomas, 7% of anaplastics, 40%–50% glioblastomas [160, 162–164], and in a number of glioma cell lines. Moreover, in a large number of glioblastomas with EGFR gene amplification, the EGFR gene undergoes rearrangements and/or deletions; 50% of glioblastomas in which EGFR amplification occurred expressed EGFR in its preponderant mutant form, EGFRvIII [154,158]. In addition to amplification of mutant EGFR, wild-type EGFR, as well as EGF and TGF-α mRNA, appear to be upregulated in gliomas. Hence not only is the mutant receptor able to induce mitogenesis and activation, but the wild-type ligand and receptor system is also induced [165]. The overexpression of EGFR has been inversely correlated with the length of survival for patients with malignant glioma [166], but others have not shown a significant correlation [167,183]. Interestingly, Bello has shown that urinary EGF levels correlated with tumor anaplasia, and that immunohistochemistry on tumor specimen correlated with urinary EGF levels [168]. Additionally, recent studies have reported expression of other members of the EGF receptor family members such as erbB-2 in gliomas [145,169].

TGF-α is secreted by a number solid tumors [170], including gliomas; its expression has been reported to correlate not only with tumor grade but also with EGFR and Ki-67 expression [171–173]. Like EGFR, it has been suggested that increased TGF-α levels are due to gene amplification [174], but this has not been supported by subsequent studies [175]. TGF-α is a potent mitogen and its functional effects on glioma cell proliferation were demonstrated by growth inhibition of U251 cells by antisense TGF-α [176]. Direct consequences of increased expression in tumor cells of a TGF-α/EGFR autocrine or paracrine loop include increased cell proliferation and survival. Increased EGFR activity, whether through mutation or by TGF-α binding, increases cell proliferation and survival and has been shown to confer a growth advantage on cells *in vitro* [177].

The TGF-α/EGFR pathway is also purported to play a role in glioma angiogenesis. Tumor endothelial cells are immunoreactive for TGF-α and EGFR, and both TGF-α and EGF are potent mitogens *in vitro* for endothelial cells. Both molecules have been shown to be angiogenic *in vivo*, with TGF-α having a more profound effect [178]. In addition to its mitogenic effects on endothelial cells, EGFR in glioma may mediate its angiogenic effects by regulating VEGF expression. Engagement of the EGFR by EGF and

TGF-α has been shown to stimulate VEGF expression by glioma cells *in vitro* [87,180,181]; this secretion was blocked by incubation with anti-EGFR [182]. Moreover, Feldkamp and investigators recently showed that the EGFRvIII – the truncated and constitutively activated EGFR – is able to increase VEGF expression in glioma cells [183]. Hence as in the other growth factor systems discussed, the TGF-α/EGFR system may mediate glioma angiogenesis through both direct and indirect effects on endothelial cells.

TGF-β

Ligand and receptor

The TGF-β proteins are members of a large group of closely related growth factors including Bone Morphogenic Proteins (BMPs), Mullerian Inhibiting Substance (MIS), inhibin, and the activins [184,185]. The TGF-β proteins affect cell fate by regulating proliferation, differentiation, motility, adhesion, and apoptosis [186], but are most notable for inhibiting proliferation in a variety of systems.

At least three genes encode TGF-β precursors in humans: TGF-βl, on chromosome 19q; TGF-β2, on lq; and TGF-β3, on 14q [187,188,189]. The corresponding TGF proteins share 64%–82% sequence homology and interestingly have nearly identical effects in some biological systems and opposite effects in others [190]. Like the other growth factors discussed in this review, TGF-β proteins act functionally as disulfide-linked dimers, each subunit of which is about 112 amino acids in length and 12–15 kDa in molecular weight [184,190]. The proteins are initially inactive and are activated by enzymatic cleavage of the latent form.

The TGF-β proteins bind with high affinity to a set of receptors distinct from others discussed in this review. TGF-β receptors I and II (TβR-I/II) are transmembrane serine/threonine receptor kinases of 55 and 75 kDa [190,191], respectively, which together mediate the intracellular signals induced by TGF-β binding. The current view is that these subunits work in tandem, with the TGF-β dimer first binding to the constitutively phosphorylated RII subunit. The RI subunit is then recruited and *trans*-phosphorylated by RII, subsequently transmitting the signal to the cell by phosphorylation of Smad proteins which may operate independently or in conjunction with other signaling pathways to produce their responses (Figure 1) [186,192]. TGF-β is known to bind other cell surface proteins such as betaglycan (TβR-III), which is expressed on a variety of cell types including microvascular endothelial cells [185,193], and endoglin (CD105), which is expressed primarily on endothelial cells [194]. These have no apparent role in directly transducing cellular signals but may present TGF-β proteins to TβR-II [192]. TβR-I has been mapped to chromosome 9q [195]; TβR-II, to 3p [196]; betaglycan, to 1p [197]; and endoglin, to 9q [198].

Expression in gliomas and roles in angiogenesis

To date, immunohistochemical studies have revealed that the TGF-β proteins, as well as their functional signaling receptors TβR-I/RII, are expressed in glioblastoma and anaplastic astrocytoma, but are barely detectable, if at all, in low-grade gliomas, gliosis, and normal brain [199–202]. In these studies, levels of TGF-β1 and of TβR-I/II were high in higher grade glioma tissue, while low-grade astrocytomas and gliosis cases had moderate expression of TβR-I and weak immunopositivity for TGF-β proteins and TβR-II [199,201]. TGF-βII/III were also upregulated, but not significantly. Interestingly, immunohistochemical studies of TGF-β and TβR have demonstrated that areas of neovascularization in gliomas are strongly immunoreactive [201,203]. In normal endothelial cells, TGF-β is expressed primarily as an inactive precursor; TβR-I/II, betaglycan, and endoglin are also expressed, though TβR-I/II have been most easily detected *in vitro* [193,204,205]. Moreover, increasing levels of TGF-β expression has been correlated inversely with survival among malignant glioma patients [206].

The functional significance of increased TGF-β activity in glioma has also been investigated. Despite its central physiologic role as a growth inhibitor, TGF-β has been shown to be mitogenic for a number of glioma cell lines [201,206,207]. The switch from TGF-β's inhibitory to proliferative effects may be explained by a selective resistance to TGF-β binding to TβR through mutation of the receptor, or to a downregulation of the receptors [202]. However, while TβR-I/II are considerably downregulated in other extracranial neoplasms such as colorectal cancer [208], studies to date indicate that receptor expression is upregulated in glioma, suggesting that TGF-β's switch from a growth inhibitor to a mitogen may be due to other growth factor mediators such as PDGF which are upregulated by TGF-β, or to a

dysregulation in the TGF-β signaling pathway. Indeed, TGF-β proteins have been shown to induce expression of the proto-oncogene c-sis (PDGF-B), PDGFR-β, and PDGF-A in glioma cells [209,210], suggesting that TGF-β's conversion from inhibitor to mitogen may be due in part to modulation of expression of other growth factor systems.

TGF-β's pleiotropic effects include roles in angiogenesis, but the exact nature of its participation in this process is unclear. TGF-β has been described as angiogenic or anti-angiogenic, depending on the nature of the assay. *In vivo* disc angiogenesis studies suggest a role in angiogenesis [211], but *in vitro* studies on endothelial cells have demonstrated an inhibitory effect on endothelial cell proliferation [212–214], as well as decreased expression of molecules necessary for endothelial cell migration such as plasminogen activators and a correspondent increase in Plasminogen Activator Inhibitors (PAIs) [215,216]. TGF-β has also been shown to increase synthesis and secretion of specific ECM proteins including fibronectin and collagen. These effects, coupled with its induction of endothelial cell quiescence, have led some to speculate that TGF-β takes part in the resolution phase of angiogenesis in which endothelial cells cease proliferating and functional basement membranes and ECM complexes are laid down [215,216].

TGF-β may also participate in angiogenesis in glioma by influencing the activity and/or expression of proteins in other growth factor systems. When added at low concentrations to endothelial cells in a three-dimensional collagen gel model, TGF-β1 potentiates angiogenic effects of VEGF and bFGF [217] such as cord formation *in vitro*. TGF-β1 has also been shown to induce PDGF-A and -B chain synthesis in endothelial cells [218,219] and to increase PDGFR-β expression in vascular smooth muscle cells [220]. In addition to possible regulation of the FGF and PDGF systems, TGF-β has also been shown to induce EGFR [221]. Interestingly, Koochekpour and co-investigators have demonstrated that TGF-β isoforms differentially stimulate VEGF production in glioma cells [222]. TGF-β1 has also been observed to upregulate expression of VEGF in bone angiogenesis and breast cancer cells [223,224], further suggesting that TGF-β may act as in indirect angiogenic factor.

Other evidence suggesting a role for the TGF-β system in glioma angiogenesis includes the high immunopositivity for endoglin in childhood gliomas [225]. Endoglin, a TGF-β binding protein, is expressed primarily on endothelial cells and appears to be essential for angiogenesis, recently demonstrated by the defective vascular development in mice lacking the gene [226]. Consistent with a possible role in angiogenesis, endoglin binds the TGF-β proteins TGF-β1 and TGF-β3 that have been shown to be most angiogenic in other studies [227].

Thus, studies to date suggest that TGF-β proteins may participate in glioma angiogenesis in three main ways. First, TGF-β may mediate its effects through the regulation of expression and activity of other growth factors and/or growth factor receptors such as FGF-2, PDGF, PDGFR, and EGFR. Secondly, like other growth factors discussed in this review, it may stimulate secretion of VEGF from the same or surrounding tumor cells in an autocrine or paracrine fashion. Third, it may interact directly with endothelial cells in two ways: by acting as a mitogen in advanced glioma malignancy despite its physiologic role as an inhibitor of proliferation [200f] or by participating in the resolution phase of angiogenesis in gliomas by inducing endothelial cell quiescence and by increasing levels of secretion of basement membrane proteins.

Conclusion

We have reviewed the potential involvement of FGFs, PDGF, EGF, and TGF-α/β in glioma angiogenesis. The immunohistochemical studies, mRNA expression, and *in vitro* and *in vivo* studies discussed herein suggest a pattern of involvement of these growth factors in the progression of malignant gliomas in which increased levels of these tumor-derived growth factors and/or their cognate receptors mediate their angiogenic effects in three primary ways: by direct endothelial action and tube formation; by indirect endothelial stimulation through increasing VEGF or other growth factor expression from tumor or endothelial cells, or both; and by upregulation of key proteases on endothelial cells to remodel surrounding ECM, permitting endothelial cell migration. Acting as such, glioma-derived angiogenic factors such as FGFs, PDGF, EGF, and TGF-α/β may bridge the interdependent processes of tumor cell and endothelial cell proliferation in gliomas. Taken together, the process of glioma angiogenesis appears to depend on an array of growth factors. While VEGF is perhaps the most critical to the process of neovascularization in these tumors and likely provides the

strongest therapeutic target to limiting this process, an understanding of the roles of supporting growth factors such as FGFs, PDGF, EGF, and TGFs may also prove beneficial.

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References

1. Cavallo T, Sade R, Folkman J, Cotran RS: Tumor angiogenesis. Rapid induction of endothelial mitoses demonstrated by autoradiography. J Cell Biol 54: 408–20, 1972
2. Folkman J, Klagsbrun M: Angiogenic factors. Science 235: 442–7, 1987
3. Folkman J: What is the evidence that tumors are angiogenesis dependent? J Nat Cancer Inst 82: 4–6, 1990
4. Gough J: The structure of the blood vessels in cerebral tumors. J Pathol Bacteriol 51: 23–8, 1940
5. Feigin I, Allen LB, Gross SW: The endothelial hyperplasia of the cerebral blood vessels with brain tumors and its sarcomatous transformation. Cancer 11: 265–77, 1958
6. Nystron S: Pathological changes in blood vessels of human glioblastoma multiforme. Comparative study using plastic coating angiography with reference to some other brain tumors. Acta Pathol Microbiol Scan 49: 1083, 1960
7. Burger PC, Vogel FS, Green SB, Strike TA: Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. Cancer 56: 1106–11, 1985
8. Daumas-Duport C, Scheithauer BW, O'Fallon J, Kelly PJ: Grading of astrocytomas: a simple and reproducible method. Cancer 62: 2152–65, 1988
9. Long DM: Capillary ultrastructure and the blood–brain barrier in human malignant brain tumors. J Neurosurg 32: 127–44, 1970
10. Weller RO, Foy M, Cox S: The development and ultrastructure of the microvasculature in malignant gliomas. Neuropathol Appl Neurobiol 3: 307–22, 1977
11. Leon SP, Folkerth RD, Black PM: Microvessel density is a prognostic indicator for patients with astroglial brain tumors. Cancer 77: 362–72, 1996
12. Ito M, Lammertsma AA, Wise RJ, Bernardi S, Frackowiak RS, Heather JD, McKenzie CG, Thomas DG, Jones T: Measurement of regional cerebral blood flow and oxygen utilisation in patients with cerebral tumours using 15O and positron emission tomography: analytical techniques and preliminary results. Neuroradiology 23: 63–74, 1982
13. Kaye A, Laws E (ed) Brain tumors: an encyclopedic approach. New York, Churchill Livingstone, 1995
14. Liwnicz BH, Wu SZ, Tew JM: The relationship between the capillary structure and hemorrhage in gliomas. J Neurosurg 66: 536–41, 1987
15. Klagsbrun M, Folkman J: Angiogenesis. In: Sporn MB, Roberts AB (eds) Peptide growth factors and their receptors. New York, Springer, 1990, pp 549–86
16. De Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT: The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255: 989–91, 1992
17. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Bohlen P: Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun 187: 1579–86, 1992
18. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M: Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 92: 735–45, 1998
19. Senger DR, Galli SJ, Dvorak AM, Peruzzi CA, Harvey VS, Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219: 983–5, 1983
20. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT: Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 246: 1309–12, 1989
21. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J: Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 84: 1470–8, 1989
22. Plate KH, Breier G, Weich HA, Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. Nature 359: 845–8, 1992
23. Jaye M, Howk R, Burgess W, Ricca GA, Chiu I-M, Ravera MW, O'Brien SJ, Modi WS, Maciag T, Drohan WN: Human endothelial cell growth factor: cloning, nucleotide sequence, and chromosome localization. Science 233: 541–5, 1986
24. Abraham JA, Whang JL, Tumolo A, Mergia A, Fiddes JC: Human basic fibroblast growth factor: nucleotide sequence, genomic organization, and expression in mammalian cells. Cold Spring Harbor Symp Quant Biol 51: 657–68, 1986a
25. Abraham JA, Mergia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC: Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science 233: 545–8, 1986b
26. Dickson C, Smith R, Brookes S, Peters G: Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region int-2. Cell 37: 529–36, 1984
27. Sakamoto H, Mori M, Taira M, Yoshida T, Matsukawa S, Shimizu K, Sekiguchi M, Terada M, Sugimura T: Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. Proc Natl Acad Sci USA 83: 3997–4001, 1986
28. Yoshida MC, Wada M, Satoh H, Yoshida T, Sakamoto H, Miyagawa K, Yokota J, Koda T, Kakinuma M, Sugimura

T: Human HST1 (HSTF1) gene maps to chromosome band 11q13 and coamplifies with the INT2 gene in human cancer. Proc Natl Acad Sci USA 85: 4861–4, 1988
29. Zhan X, Bates B, Hu XG, Goldfarb M: The human FGF-5 oncogene encodes a novel protein related to fibroblast growth factors. Mol Cell Biol 8: 3487–95, 1988
30. Marics I, Adelaide J, Raybaud F, Matteio MG, Coulier F, Planche J, de Lapeyriere O, Birnbaum D: Characterization of the HST-related FGF-6 gene, a new member of the fibroblast growth factor family. Oncogene 4: 335–40, 1989
31. Rubin JS, Osada H, Finch PW, Taylor WG, Rudikoff S, Aaronson SA: Purification and characterization of a newly identified growth factor specific for epithelial cells. Proc Nati Acad Sci USA 86: 802–6, 1989
32. Tanaka A, Miyamoto K, Minamino HN, Takeda M, Sato B, Matsuo H, Matsumoto K: Cloning and characterization of an androgen-induced growth factor essential for the androgen-dependent growth of mouse mammary carcinoma cells. Proc Natl Acad Sci USA 89: 8928–32, 1992
33. Miyamoto M, Nauro K, Seko C, Matsukmoto S, Kondo T, Kurokawa T: Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion property. Mol Cell Biol 13: 4251–9, 1993
34. Yamasaki M, Miyake A, Tagashira S, Itoh N: Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family. J Biol Chem 271: 15918–21, 1996
35. Hartung H, Feldman B, Lovec H, Coulier F, Birnbaum D, Goldfarb M: Murine FGF-12 and FGF-13: expression in embryonic nervous system, connective tissue, and heart. Mech Dev 64: 31–9, 1997
36. Liu Y, Chiu IM: Assignment of FGF12, the human FGF homologous factor 1 gene, to chromosome 3q29–3qter by fluorescence *in situ* hybridization. Cytogenet Cell Genet 78: 48–9, 1997
37. Greene HM, Li YL, Yourey PA, Gruber J, Carter KC, Shell BK, Dillon PA, Florence C, Duan DR, Blunt A, Ornitz DM, Ruben SM, Alderson RF: Identification and characterization of a novel member of the fibroblast growth factor family. Eur J Neurosci 10: 1911–25, 1998
38. McWhirter JR, Goulding M, Weiner JA, Chun J, Murre C: A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. Development 124: 3221–32, 1997
39. Miyake A, Konishe M, Martin FH, Hernday NA, Ozaki K, Yamamoto S, Mikami T, Arakawa T, Itoh N: Structure and expression of a novel member, FGF-16, on the fibroblast growth factor family. Biochem Biophys Res Commun 243: 148–52, 1998
40. Hoshikawa M, Ohbayashi N, Yonamine A, Konishi M, Ozaki K, Fukui S, Itoh N: Structure and expression of a novel fibroblast growth factor, FGF- 17, preferentially expressed in the embryonic brain. Biochem Biophys Res Commun 244: 187–91, 1998
41. Ohbayashi N, Hoshikawa M, Kimura S, Yamasaki M, Fukui S, Itoh N: Structure and expression of the mRNA encoding a novel fibroblast growth factor, FGF-18. J Biol Chem 273: 18161–4, 1998
42. Nishimura T, Utsunomiya Y, Hoshikawa M, Ohuchi H, Itoh N: Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. Biochim Biophys Acta 1444: 148–51, 1999
43. Klagsbrun M: The fibroblast growth factor family: structural and biological properties. Prog Growth Factor Res 1: 207–35, 1989
44. Klagsbrun M, D'Amore P: Regulators of angiogenesis. Annu Rev Physiol 53: 217-39, 1991
45. Klein S, Roghani M, Rifkin DB: Fibroblast growth factors as angiogenesis factors: new insights into their mechanism of action. EXS 79: 159–92, 1997
46. Bikfalvi A, Klein S, Pintucci G, Rifkin DB: Biological roles of fibroblast growth factor-2. Endocr Rev 18: 26–45, 1997
47. Lafage-Pochitaloff M, Galland F, Simonetti J, Prats H, Mattei MG, Birnbaum D: The human basic fibroblast growth factor gene is located on the long arm of chromosome 4 at bands q26–q27. Oncogene Res 5: 241–4, 1990
48. Fukushima Y, Byers MG, Fiddes JC, Shows TB: The human fibroblast growth factor gene (FGFB) is assigned to chromosome 4q25. Cytogenet Cell Genet 54: 159–60, 1990
49. Florkiewicz RZ, Baird A: Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. Proc Natl Acad Sci USA 86: 3978–81, 1989
50. Prats H, Kaghad M, Prats AC, Klagsbrun M, Lelias JM, Liauzun P, Chalon P, Tauber JP, Amalric F, Smith JA, Caput D: High molecular mass forms of basic fibroblast growth factor are initiated by alternative CUG codons. Proc Natl Acad Sci USA 86: 1836–40, 1989
51. Florkiewicz RZ, Majack RA, Buechler RD, Florkiewicz E: Quantitative export of FGF-2 occurs through an alternative, energy-dependent, non-ER/Golgi pathway. J Cell Physiol 162: 388–99, 1995
52. Mignatti P, Morimoto T, Rifkin DB: Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-Golgi complex. J Cell Physiol 151: 81–93, 1992
53. Lee PL, Johnson DE, Cousens LS, Fried VA, Williams LT: Purification and complementary DNA of a receptor for basic fibroblast growth factor. Science 245: 57–60, 1989
54. Dionne CA, Crumley G, Bellot F, Kaplow JM, Searfoss G, Ruta M, Burgess WH, Jaye M, Schlessinger J: Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. EMBO J 9: 2685–92, 1990
55. Keegan K, Johnson DE, Williams LT, Hayman JF: Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. Proc Natl Acad Sci USA 88: 1095–9, 1991
56. Partanen J, Makela TP, Eerola E, Korhonen J, Hirvonen H, Claesson-Welsh L, Alitalo K: FGFR-4, a novel acidic

fibroblast growth factor receptor with a distinct expression pattern. EMBO J 10: 1347–54, 1991
57. Basilico C, Moscatelli D: The FGF family of growth factors and oncogenes. Adv Cancer Res 59: 115–65, 1992
58. Jaye M, Schlessinger J, Dionne C: Fibroblast growth factor receptor for acidic and basic fibroblast growth factors. Biochim Biophys Acta 1135: 185–99, 1992
59. Lafage M, Pedeutour F, Marchetto S, Simonetti J, Prosperi MT, Gaudray P, Birnbaum D: Fusion and amplification of two originally non-syntenic chromosomal regions in a mammary carcinoma cell line. Genes Chromosomes Cancer 5: 40–9, 1992
60. Mattei MG, Moreau A, Gesnel MC, Houssaint E, Breathnach R: Assignment by *in situ* hybridization of a fibroblast growth factor receptor gene to human chromosome band 10q26. Hum Genet 87: 84–6, 1991
61. Keegan K, Rooke L, Hayman M, Spurr NK: The fibroblast growth factor receptor 3 gene (FGFR3) is assigned to human chromosome 4. Cytogenet Cell Genet 62: 172–5, 1993
62. Armstrong E, Partanten J, Cannizzaro L, Huebner K, Alitalo K: Localization of the fibroblast growth factor receptor-4 gene to chromosome region 5q33-ter. Genes Chromosomes Cancer 4: 94–8, 1992
63. Plotnikov AN, Schlessinger J, Hubbard SR, Mohammadi M: Structural basis for FGF receptor dimerization and activation. Cell 98: 641–50, 1999
64. Ornitz DM, Yayon A, Flanagan JG, Svahn CM, Levi E, Leder P: Heparin is required for cell-free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells. Mol Cell Biol 12: 240–7, 1992
65. Folkman J, Klagsbrun M: Angiogenic factors. Science 235: 442–7, 1987
66. Murphy PR, Sato R, Sato Y, Friesen HG: Fibroblast growth factor messenger ribonucleic acid expression in a human astrocytoma cell line: regulation by serum and cell density. Mol Endocrinol 2: 591–8, 1988
67. Stefanik DF, Rizkalla LR, Soi A, Goldblatt SA, Rizkalla WM: Acidic and basic fibroblast growth factors are present in glioblastoma multiforme. Cancer Res 51: 5760–5, 1991
68. Zagzag D, Miller DC, Sato Y, Rifkin DB, Burstein DE: Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. Cancer Res 50: 7393–8, 1990
69. Morrison RS, Yamaguchi F, Saya H, Bruner JM, Yahanda AM, Donehower LA, Berger M: Basic fibroblast growth factor and fibroblast growth factor receptor I are implicated in the growth of human astrocytomas. J Neuro-Oncol 18: 207–16, 1994a
70. Morrison RS, Yamaguchi F, Bruner JM, Tang M, McKeehan W, Berger MS: Fibroblast growth factor receptor gene expression and immunoreactivity are elevated in human glioblastoma multiforme. Cancer Res 54: 2794–9, 1994b
71. Ueba T, Takahashi JA, Fukumoto M, Ohta M, Ito N, Oda Y, Kikuchi H, Hatanaka M: Expression of fibroblast growth factor receptor-1 in human glioma and meningioma tissues. Neurosurgery 221–6, 1994
72. Thomas KA, Gimenez-Gallego G: Fibroblast growth factors: broad spectrum mitogens with potent angiogenic activity. Trends Biochem Sci 11: 81–4, 1986
73. Thomas KA: Fibroblast growth factors. FASEB J 1: 434–40, 1987
74. VassaHi JD, Sappino AP, Belin D: The plasminogen activator/plasmin system. J Clin Invest 88: 1067–72, 1991
75. Mignatti P, Rifkin DB: Biology and biochemistry of proteinases in tumor invasion. Physiol Rev 73: 161–95, 1993
76. Saksela 0, Rifkin DB: Release of basic fibroblast growth factor-heparan sulfate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. J Cell Biol 110: 767–75, 1990
77. Blasi F: Urokinase and urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness. Bioessays 15: 105–11, 1993
78. Werb Z, Banda MJ, Jones PA: Degradation of connective tissue matrices by macrophages. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. J Exp Med 152: 1340–57, 1980
79. Moscatelli D, Presta M, Rifkin DB: . Purification of a factor from human placenta that stimuary endothelial cell protease production, DNA synthesis, and migration. Proc Natl Acad Sci USA 83: 2091–5, 1986
80. Mignatti P, Mazzieri R, Rilkin DB: Expression of the urokinase receptor in vascular endothelial cells is stimulated by basic fibroblast growth factor. J Cell Biol 113: 1193–201, 1991
81. Connolly DT, Stoddard BL, Harakas NK, Feder J: Human fibroblast-derived growth factor is a mitogen and chemoattractant for endothelial cells. Biochem Biophys Res Commun 144: 705–12, 1987
82. Montesano R, Vassalli JD, Baird A, Guillemin R, Orci L: Basic fibroblast growth factor induces angiogenesis *in vitro*. Proc Natl Acad Sci USA 83: 7297–301, 1986
83. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z: Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 13: 9–22, 1999
84. Dvorak HF, Nagy JA, Feng D, Brown LF, Dvorak AM: Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. Curr Top Microbiol Immunol 237: 97–132, 1999
85. Ferrara N: Vascular endothelial growth factor: molecular and biological aspects. Curr Top Microbiol Immunol 237: 1–30, 1999
86. Plate KH, Breier G, Weich HA, Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo.* Nature 359: 845–8 l992
87. Tsai JC, Goldman CK, Gillespie GY: Vascular endothelial growth factor in human glioma cell lines: induced secretion by EGF, PDGF-BB, and bFGF. J Neurosurg 82: 864–73, 1995
88. Stan AC, Nemati MN, Pietsch T, Walter GF, Dietz H: *In vivo* inhibition of angiogenesis and growth of the human U-87 malignant glial tumor by treatment with an antibody against basic fibroblast growth factor. J Neurosurg 82: 1044–52, 1995

89. Abe T, Okamura K, Ono M, Kohno K, Mori T, Hori S, Kuwano M: Induction of vascular endothelial tubular morphogenesis by human glioma cells. A model system for tumor angiogenesis. J Clin Invest 92: 54–61, 1993
90. Takahashi JA, Fukumoto M, Igarashi K, Oda Y, Kikuchi H, Hatanaka M: Correlation of basic fibroblast growth factor expression levels with the degree of malignancy and vascularity in human gliomas. J Neurosurg 76: 792–8, 1992
91. Li VW, Folkerth RD, Watanabe H, Yu C, Rupnick M, Barnes P, Scott RM, Black PM, Sallan SE, Folkman J: Microvessel count and cerebrospinal fluid basic fibroblast growth factor in children with brain tumors. Lancet 344: 82–6, 1994
92. Leon SP, Folkerth RD, Black PM: Microvessel density is a prognostic indicator for patients with astroglial brain tumors. Cancer 77: 367–72, 1996
93. Bello L, Zhang J, Nikas D, Strasser J, Carroll R, Villani RM, Bikfalvi A, Black PM: FGF-4 as an angiogenesis factor in gliomas. (Abstract) Congress of Neurological Surgeons meeting. Boston Massachusetts October 30–November 4, 1999
94. Kaplan DR, Chao FC, Stiles CD, Antoniades HN, Scher CD: Platelet alpha granules contain a growth factor for fibroblasts. Blood 53: 1043–52, 1979
95. Heldin CH, Wasteson A, Westermark B: Platelet-derived growth factor. Mol Cell Endocrinol 39: 169–87, 1985
96. Antoniades HN: PDGF: a multifunctional growth factor. Baillieres Clin Endocrinol Metab 5: 595–613, 1991
97. Antoniades HN, Scher CD, Stiles CD: Purification of human platelet-derived growth factor. Proc Natl Acad Sci USA 76: 1809–13, 1979
98. Heldin CH, Westermark B: Mechanism of action and *in vivo* role of platelet-derived growth factor. Physiol Rev 79: 1283–316, 1999
99. Bowen-Pope DF, Hart CE, Seifert RA: Sera and conditioned media contain different isoforms of platelet derived growth factor (PDGF) which bind to different classes of PDGF receptor. J Biol Chem 264: 2502–8, 1989
100. Betsholtz C, Johnsson A, Heldin CH, Westermark B, Lin d P, Urdea MS, Eddy R, Shows TB, Philpott K, Mellor AL, Knott TJ, Scott J: cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. Nature 320: 695–9, 1986
101. Dalla-Favera R, Gallo RC, Giallonga A, Croce CM: Chromosomal location of the human homolog (c-sis) of the simian sarcoma virus oncogene. Science 218: 686–8, 1982
102. Bonthron DT, Morton CC, Orkin SH, Collins T: Platelet-derived growth factor A chain: gene structure, chromosomal location, and basis for alternative mRNA splicing. Proc Natl Acad Sci USA 85: 1492–6, 1988
103. Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades HN: Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221: 275–7, 1983
104. Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin CH, Huang JS, Deuel TF: Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus. Nature 304: 35–9, 1983
105. Swan DC, McBride OW, Robbins KC, Keithley DA, Reddy EP, Aaronson SA: Chromosomal mapping of the simian sarcoma virus onc gene analogue in human cells. Proc Natl Acad Sci USA 79: 4691–5, 1982
106. Claesson-Welsh L: Signal transduction by the PDGF receptors. Prog Growth Factor Res 5: 37–54, 1994
107. Gronwald RGK, Grand FJ, Haldeman BA, Hart CE, O'Hara PJ, Hagen FS, Ross R, Bowen-Pope DF, Murray MJ: Cloning and expression of a cDNA cloning for the human platelet-derived growth factor receptor: evidence of more than one receptor class. Proc Natl Acad Sci USA 85: 3435–9, 1988
108. Hart CE, Forstrom JW, Kelly JD, Seifert RA, Smith RA, Ross R, Murray MJ, Bowen-Pope DF: Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240: 1529–31, 1988
109. Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M, Pierce J, Aaronson S: Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. Science 243: 800–4, 1989
110. Hammacher A, Mellstrom K, Heldin CH, Westermark B: Isoform-specific induction of actin reorganization by platelet-derived growth factor suggests that the functionally active receptor is a dimer. EMBO J 8: 2489–95, 1989
111. Kanakaraj P, Raj S, Khan SA, Bishayee S: Ligand-induced interaction between alpha- and beta-type platelet derived growth factor (PDGF) receptors: role of receptor heterodimers in kinase activation. Biochemistry 30: 1761–7, 1991
112. Seifert RA, Hart CE, Phillips PE, Forstrom JW, Ross R, Murray M, Bowen-Pope DF: Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. J Biol Chem 264: 8771–8, 1989
113. Chiu IM, Reddy EP, Givol D, Robbins KC, Tronic SR, Aaronson SA: Nucleotide sequence analysis identifies the human c-sis proto-oncogene as a structural gene for platelet-derivied growth factor. Cell 37: 123–9, 1984
114. Betsholtz CH, Johnsson A, Heldin C, Westermark B: Efficient reversion of simian sarcoma virus-transformation and inhibition of growth factor induced mitogenesis by suramin. Proc Natl Acad Sci USA 83: 6440–4, 1986
115. Rao CD, Igarashi H, Chin IM, Robbins KC, Aaronson SA: Structure and sequence of the human c-sis platelet-derived growth factor 2 (sis/PDGF2) transcriptional unit. Proc Natl Acad Sci USA 83: 2392–6, 1986
116. Beckmann MP, Betsholtz C, Heldin CH, Westermark B, DiMarco E, DiFiore PP, Robbins KC, Aaronson SA: Comparison of the biological properties and transforming potential of human PDGF-A and PDGF-B chains. Science 241: 1346–9, 1988
117. La Rochelle WJ, Giese N, May-Siroff M, Robbins KC, Aaronson SA: Molecular localisation of the transforming and secretory properties of PDGF-A and PDGF-B. Science 248: 1541–4, 1990
118. Hermanson M, Funa K, Hartman M, Claesson-Weish L, Heldin CH, Westermark B, Nister M: Platelet-derived

growth factor and its receptors in human glioma tissue: Expression of mRNA and protein suggests the presence of autocrine and paracrine loops. Cancer Res 52: 3212–9, 1992
119. Hermansson M, Nister M, Betsholtz C, Heldin CH, Westermark B, Funa K: Endothelial cell hyperplasia in human glioblastoma: coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. Proc Natl Acad Sci 85: 7748–52, 1988
120. Nister M, Liebermann TA, Betsholtz C, Petterson M, Claesson-Welsh L, Heldin CH, Schlessinger J, Westermark B: Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor-alpha and their receptors in human malignant glioma cell lines. Cancer Res 48: 3910–18, 1988
121. Mercola M, Deininger PL, Shamah SM, Porter J, Wang CY, Stiles CD: Dominant-negative mutants of a platelet-derived growth factor gene. Genes Dev 4: 2333–41. 1990
122. Guha A: Platelet derived growth factor: A general review with emphasis on astrocytomas. Pediatr Neurosurg 17: 14–20, 1991–1992
123. Shamah SM, Stiles CD, Guha A: Dominant-negative mutants of platelet-derived growth factor revert the transformed phenotype of human astrocytoma cells. Mol Cell Biol 13: 7203–12, 1993
124. Guha A, Dashner K, Black, PM, Wagner JA, Stiles CD: Expression of PDGR and PDGF receptors in human astrocytoma operative specimens supports the existence of an autocrine loop. Int J Cancer 60: 168–73, 1995
125. Di Rocco F, Carroll RS, Zhang J, Black PM: Platelet-derived growth factor and its receptor expression in human oligodendrogliomas. Neurosurgery 42: 341–6, 1998
126. Plate KH, Breier G, Farrell C, Risau W: Platelet-derived growth factor-B is induced during tumor development and upregulated during tumor progression in endothelial cell in human gliomas. Lab Invest 67: 529–34, 1992
127. Risau W, Drexler H, Mironov V, Smits A, Siegbahn A, Funa K, Heldin C: Platelet-derived growth factor is angiogenic *in vivo*. Growth Factors 7: 261–6, 1992
128. Westermark B, Siegbahn A, Heldin CH, Claesson-Welsh L: B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein tyrosine kinase. Proc Natl Acad Sci USA 87: 128–32, 1990
129. Wang D, Su Huang HJ, Kazlauskas A, Cavanee WK: 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: 1464–72, 1999
130. Maxwell M, Galanopoulos T, Hedley-Whyte ET, Black PM, Antoniades HN: Human meningiomas co-express platelet-derived growth factor (PDGF) and PDGF-receptor genes and their protein products. Int J Cancer 46: 16–21, 1990
131. Mapstone T, McMichael M, Goldthwait D: Expression of platelet-derived growth factors, transforming growth factors, and the ras gene is a variety of primary human brain tumors. Neurosurgery 28: 216–22, 1991
132. Carpenter G, Cohen S: Epidermal growth factor. Annu Rev Biochem 48: 193–216, 1979
133. Todaro GH, Fryling C, Delakco JE: Transforming growth factors produced by certain human tumor cells: polypeptides that interact with human EGF-receptors. Proc Natl Acad Sci USA 77: 5258–62, 1980
134. Alroy I, Yarden Y: The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions. FEBS Lett 410: 83–6, 1997
135. Feldkamp MM, Lau N, Guha A: Signal transduction pathways and their relevance in human astrocytomas. J Neuro-Oncol 35: 223–48, 1997
136. Carpenter G: Epidermal growth factor: biology and receptor metabolism. J Cell Sci 3: 1–9, 1985
137. Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV: Human transforming growth factor-alpha: precursor structure and expression in *E. coli*. Cell 38: 287–97, 1984
138. Higashiyama S, Abraham JA, Miller J, Fiddes JC, Klabsbrun M: A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. Science 251: 936–9, 1991
139. Plowman GD, Green JM, McDonald VL, Neubauer MG, Disteche CM, Todaro GJ, Shoyab M: The amphiregulin gene encodes a novel epidermal growth factor-related protein with tumor-inhibitory activity. Mol Cell Biol 10: 1969–81, 1990
140. Komurasaki T, Toyoda H, Uchida D, Morimoto S: Epiregulin binds to epidermal growth factor receptor and ErbB-4 and induces tyrosine phosphorylation of epidermal growth factor receptor, ErbB-2, ErbB-3 and ErbB-4. Oncogene 4: 2841–8, 1997
141. Zabel BU, Eddy RL, Lalley PA, Scott J, Bell GI, Shows TB: Chromosomal locations of the human and mouse genes for precursors of epidermal growth factor and the beta subunit of nerve growth factor. Proc Natl Acad Sci USA 82: 469–73, 1985
142. Boonstra J, Rijken P, Humbel B, Cremers F, Verkleij A, van Bergen en Henegouwen P: The epidermal growth factor. Cell Biol Int 19: 413–30, 1995
143. Tricoli JV, Nakai H, Byers MG, Rall LB, Bell GI, Shows TB: The gene for human transforming growth factor alpha is on the short arm of chromosome 2. Cytogenet Cell Genet 42: 94–8, 1986
144. DeLarco J, Todaro GJ: Membrane receptors for murine leukemia viruses: characterization using the purified viral envelope glycoprotein, gp71. Cell 8: 365–71, 1976
145. Von Bossanyi P, Sallaba J, Dietzmann K, Warich-Kirches M, Kirches E: Correlation of TGF-alpha and EGF-Receptor with proliferative activity in human astrocytic gliomas. Pathol Res Pract 194: 141–8, 1998
146. Kumar V, Bustin SA, McKay IA: Transforming growth factor alpha. Cell Biol Int 19: 373–88, 1995
147. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD: Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 307: 521–7, 1984

148. Kondo I, Shimizu N: Mapping of the human gene for the epidermal growth receptor (EGFR) gene on the p13–q22 region on chromosome 7. Cytogenet Cell Genet 35: 9–14, 1983
149. Heldin CH: Dimerization of cell surface receptors in signal transduction. Cell 80: 213–23, 1995
150. Soltoff SP, Carraway III KL, Prigent SA, Gullick WG, Cantley LC: ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. Mol Cell Biol 14: 3550–8, 1994
151. Murali R, Brennan PJ, Kieber-Emmons T, Greene MI: Structural analysis of p185($c_n$eu) and epidermal growth factor receptor tyrosine kinases: Oligomerization of kinase domains. Proc Natl Acad Sci USA 93: 6252–7, 1996
152. Earp HS, Dawson TL, Li X, Yu H: Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer research. Breast Cancer Res Treat 35: 115–32, 1995
153. Lemmon MA, Bu Z, Ladbury JE, Zhou M, Pinchasi D, Lax I. Engelman DM, Schlessinger J: Two EGF molecules contribute additively to stabilization of the EGFR dimer. EMBO 16: 281–94, 1997
154. Guha A: Ras activation in astrocytomas and neurofibromas. Can J Neurol Sci 25: 267–81, 1998
155. Steck PA, Lee P, Hung MC, Yung WK: Expression of an altered epidermal growth factor receptor by human glioblastoma cells. Cancer Res 48: 5433–9, 1988
156. Ekstrand AJ, Longo N, Hamid ML, Olson JJ, Liu L, Collins VP, James CD: Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification. Oncogene 9: 2315–20, 1994
157. Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, Huang HJ: A mutant epidermal growth factor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci USA 91: 7727–31, 1994
158. Goike HM, Asplund AC, Pettersson EH, Liu L, Sanodou D, Collins VP: Acquired rearrangement of an amplified epidermal growth factor receptor (EGFR) in a human glioblastoma xenograft. J Neuropathol Exp Neurol 58: 697–701, 1999
159. Ekstrand AJ, Sugawa N, James CD, Collins VP: Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci USA 89: 4309–13, 1992
160. Torp SH, Helseth E, Dalen A, Unsgaard G: Epidermal growth factor receptor expression in human gliomas. Cancer Immunol Immunother 33: 61–4, 1991
161. Libermann TA, Razon N, Bantal AD, Yarden Y, Schlessinger J, Soreq H: Expression of epidermal growth factor receptors in human brain tumors. Cancer Res 44: 753–60, 1984
162. Arita N, Hayakawa T, Izumoto S, Taki T, Ohnishi T, Yamamoto H, Bitoh S, Mogami H: Epidermal growth factor in human glioma. J Neurosurg 70: 916–19, 1989
163. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield M, Ullrich A, Schlessinger J: Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human tumors of glial origin. Nature 313: 144–7, 1985
164. Collins VP: Gene amplification in human gliomas. Glia 15: 289–96, 1995
165. Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B: Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc Natl Acad Sci USA 84: 6899–903, 1987
166. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Petterson RF, Collins VP: Genes for epidermal growth factor and their expression in human gliomas *in vivo*. Cancer Res 51: 2164–72, 1991
167. Hurtt MR, Moosy J, Donovan-Peluso M, Locker J: Amplification of epidermal growth factor in gliomas: histopathology and prognosis. J Neuropathol Exp Neurol 51: 84–90, 1992
168. Newcomb EW, Cohen H, Lee SR, Bhalla SK, Bloom J, Hayes RL, Miller DC: Survival of patients with glioblastoma multiforme is not influenced by altered expression of p16, p53, EGFR, MDM2 or Bcl-2 genes. Brain Pathol 8: 655–67, 1998
169. Bello L: Urinary epidermal growth factor (EGF) levels and epidermal growth factor receptor (EGF-R) expression in human cerebral tumors. J Neurosurg Sci 40: 167-82, 1996
170. Haapasalo H, Hyytinen E, Sallinen P, Helm H, Kallioniemi OP, Isola J: c-erbB-2 in astrocytomas: infrequent overexpression by immunohistochemistry and absence of gene amplification by fluorescence *in situ* hybridization. Br J Cancer 73: 620–3, 1996
171. Nickell KA, Halper J, Moses HL: Transforming growth factors in solid human malignant neoplasms. Cancer Res 43: 1966–71, 1983
172. Tang P, Steck PA, Yung WKA: The autocrine loop of TGF-a/EGFR and brain tumors. J Neuro-Oncol 35: 303–14, 1997
173. Maruno M, Kovach JS, Kelly PJ, Yanagihara T: Transforming growth factor-a, epidermal growth factor receptor, and proliferating potential in benign and malignant gliomas. J Neurosurg 75: 97–102, 1991
174. Schlegel U, Moots PL, Rosenblum MK, Thaler HT, Furneaux HM: Expression of transforming growth factor alpha in human gliomas. Oncogene 5: 1839–42, 1990
175. Yung WK, Zhang X, Steck PA, Hung MC: Differential amplification of the TGF-alpha gene in human gliomas. Cancer Comm 2: 201–5, 1990
176. Diedrich U, Lucius J, Baron E, Behnke J, Pabst B, Zoll B: Distribution of epidermal growth factor receptor gene amplification in brain tumours and correlation to prognosis. J Neurol 242: 683–8, 1995
177. Tang P, Jasser SA, Sung JC, Shi Y, Steck PA, Yung WKA: Transforming growth factor-alpha antisense vectors can inhibit glioma cell growth. J Neuro-Oncol 43: 127–35, 1999
178. U HS, Expiritu OD, Kelley PY, Klauber MR, Hatton JD: The role of the epidermal growth factor receptor in human gliomas: I. The control of cell growth. J Neurosurg 82: 841–6, 1995

179. Schreiber AB, Windler ME, Derynck R: Transforming growth factor-alpha: a more potent angiogenic mediator than epidermal growth factor. Science 232: 1250–3, 1986
180. Okamura K, Morimoto A, Hamanaka R, Ono M, Kohno K, Uchida Y, Kuwano M: A model system for tumor angiogenesis: involvement of transforming growth factor-alpha in tube formation of human microvascular endothelial cells induced by esophageal cancer cells. Biochem Biophys Res Commun 186: 1471–9, 1992
181. Goldman CK, Kim J, Wong WL, King V, Brock T, Gillespie GY: Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: a model of glioblastoma multiforme pathophysiology. Mol Biol Cell 4: 121–33, 1993
182. Valter MM, Wiestler OD, Pietsche T: Differential control of VEGF synthesis and secretion in human glioma cells by IL-1 and EGF. Int J Devl Neuroscience 17: 565–77, 1999
183. Feldkamp MM, Lau N, Rak J, Kerbel RS, Guha A: Normoxic and hypoxic regulation of vascular endothelial growth factor (VEGF) by astrocytoma cells is mediated by ras. Int J Cancer 81: 118–24, 1999
184. Dorwald NL, Hawkins RA, Whittle IR: Epidermal growth factor receptor activity and clinical outcome in glioblastoma multiforme. Br J Neurosurg 7: 197–200, 1993
185. Attisano L, Wrana JL, Lopez-Casillas F, Massague J: TGF-beta receptors and actions. Biochim Biophys Acta 1222: 71–80, 1994
186. Massague J: The transforming growth factor-beta family. Annu Rev Cell Biol 6: 597–641, 1990
187. Engel ME, Datta PK, Moses HL: Signal transduction by transforming growth factor-beta: a cooperative paradigm with extensive negative regulation. J Cell Biochem (Suppl 30–31): 111–22, 1998
188. Fujii D, Brissenden JE, Derynck R, Francke U: Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. Somat Cell Mol Genet 12: 281–8, 1986
189. Barton DE, Foellmer BE, Du J, Tamm J, Derynck R, Francke U: Chromosomal mapping of genes for transforming growth factors beta 2 and beta 3 in man and mouse: dispersion of TGF-beta gene family. Oncogene Res 3: 323–31, 1988
190. ten Dijke P, Geurts van Kessel AH, Foulkes JG, Le Beau MM: Transforming growth factor type beta 3 maps to human chromosome 14, region q23-q24. Oncogene 3: 721–4, 1988
191. Massague J, Cheifetz S, Laiho M, Ralph DA, Weis FMB, Zentella A: Transforming Growth Factor-beta. In: Cancer Surveys Vol.12: Tumour Suppressor Genes, the Cell Cycle, and Cancer. Imperial Cancer Research Fund, 1992
192. Cheifetz S, Like B, Massague J: Cellular distribution of type I and type II receptors for transforming growth factor-beta. J Biol Chem 261: 9972–78, 1986
193. Massague J, Andres J, Attisano L, Cheifetz S, Lopez-Casillas F, Ohtsuki M, Wrana JL: TGF-beta receptors. Mol Reprod Dev 32: 99–104, 1992
194. Morello JP, Plamondon J, Meyrick B, Hoover R, O'Connor-McCourt MD: Transforming growth factor-beta receptor expression on endothelial cells: heterogeneity of type III receptor expression. J Cell Physiol 165: 201–11, 1995
195. Gougos A, Letarte M: Primary structure of endoglin, an RGD-containing glycoprotein of human endothelial cells. J Biol Chem 265: 8361–4, 1990
196. Pasche B, Luo Y, Rao PH, Nimer SD, Dmitrovsky E, Caron P, Luzzatto L, Offit K, Cordon-Cardo C, Renault B, Satagopan JM, Murty VV, Massague J: Type I transforming growth factor beta receptor maps to 9q22 and exhibits a polymorphism and a rare variant within a polyalanine tract. Cancer Res 58: 2727–32, 1998
197. Mathew S, Murty VV, Cheifetz S, George D, Massague J, Chaganti RS: Transforming growth factor receptor gene TGFbetaR2 maps to human chromosome band 3p22. Genomics 20: 114–5, 1994
198. Johnson DW, Qumsiyeh M, Benkhalifa M, Marchuk DA: Assignment of human transforming growth factor-beta type I and type III receptor genes (TGFbetaR1 and TGFbetaR3) to 9q33-q34 and 1p32-p33, respectively. Genomics 28: 356–7, 1995
199. Fernandez-Ruiz E, St-Jacques S, Bellon T, Letarte M, Bernabeu C: Assignment of the human endoglin gene (END) to 9q34–⟩qter. Cytogenet Cell Genet 64: 204–7, 1993
200. Samuels V, Barrett JM, Bockman S, Pantazis, Allen MG Jr: Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. Am J Pathol 134: 895–902, 1989
201. Horst HA, Kelly PJ, Scheithauer BW, Kovach JS: Immunohistological localization of transforming growth factor-beta in human astrocytomas. Hum Pathol 23: 1284–8, 1992
202. Yamada N, Kato M, Yamashita H, Nister N, Miyazono K, Heldin CH, Funa K: Enhanced expression of transforming growth factor-beta and its type-I and type-Il receptors in human glioblastoma. mt J Cancer 62: 386–92, 1995
203. Jennings M, Maciunas R, Carver R, Bascom C, Juneau P, Misulus K, Moses H: TGFbeta1 and TGFbeta2 are potential growth regulators for low-grade and malignant gliomas *in vitro* evidence in support of an autocrine hypothesis. Int J Cancer 49: 129-39, 1991
204. Stiles JD, Ostrow PT, Balos LL, Greenberg SJ, Plunkett R, Grand W, Heffner RR Jr: Correlation of endothelin-1 and transforming growth factor beta1 with malignancy and vascularity in human gliomas. J Neuropathol Exp Neurol 56: 435–9, 1997
205. Segarini PR, Rosen DM, Seydin SM: Binding of transforming growth factor-beta to cell surface proteins varies with cell type. Molec Endocr 3: 261–72, 1989
206. Hirai R, Kaji K: Transforming growth factor beta1-specific binding proteins on human vascular endothelial cells. Exp Cell Res 201: 119–25, 1992
207. Majewski C, Blisard K, Ballard E, Blair P, Qadir K, Bokhari S, Raza A, Lampkin B: Prognostic significance of immunohistochemical detection of transforming growth factor beta (TGF-beta) in central nervous system (CNS) neoplasia. (Abstract) Growth Control in Central Nervous System International workshop. Boston Massachusetts April 30–May, 1993

208. Merzak A, McCrea S, Koocheckpoor S, Pilkington G: Control of human glioma cell growth, migration and invasion *in vitro* by transforming growth factor beta1. Br J Cancer 70: 199–203, 1994
209. Matsushita M, Matsuzaki K, Date M, Watanabe T, Shibano K, Nakagawa T, Yanagitani S, Amoh Y, Takemoto H, Ogata N, Yamamoto C, Kubota Y, Seki T, Inokuchi H, Nishizawa M, Takada H, Sawamura T, Okamura A, Inoue K: Down-regulation of TGF-beta receptors in human colorectal cancer: implications for cancer development. Br J Cancer 80: 194–205, 1999
210. Jennings MT, Hart CE, Commers PA, Whitlock JA, Martincic D, Maciunas RJ, Moots PL, Shehab TM: Transforming growth factor beta as a potential tumor progression factor among hyperdiploid glioblastoma cultures: evidence for the role of platelet-derived growth factor. J Neuro-Oncol 31: 233–54, 1997
211. Press RD, Misra A, Gillaspy G, Samols D, Goldthwait DA: Control of the expression of c-sis mRNA in human glioblastoma cells by phorbol ester and transforming growth factor beta 1. Cancer Res 49: 2914–20, 1989
212. Fajardo LF, Prionas SD, Kwan HH, Kowalski J, Allison AC: Transforming Growth Factor beta 1 induces angiogenesis *in vivo* with a threshold pattern. Lab Invest 74: 600-8, 1996
213. Baird A, Durkin T: Inhibition of endothelial cell proliferation by type beta-transforming growth factor: interactions with acidic and basic fibroblast growth factors. Biochem Biophys Res Commun 138: 476–82, 1986
214. Frater-Schroder M, Muller G, Birchmeier W, Bohlen P: Transforming growth factor-beta inhibits endothelial cell proliferation. Biochem Biophys Res Commun 137: 295–302, 1986
215. Heimark RL, Twardzik DR, Schwartz SM: Inhibition of endothelial regeneration by type-beta transforming growth factor from platelets. Science 233: 1078–1080, 1986
216. RayChaudhury A, D'Amore PA: Endothelial cell regulation by transforming growth factor-beta. J Cell Biochem 47: 224–9, 1991
217. Pepper MS: Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity. Cytokine Growth Factor Rev 8: 21–43, 1997
218. Pepper MS, Vassalli J-D, Orci L, Montesano R: Biphasic effect of transforming growth factor-beta 1 on *in vitro* angiogenesis. Exp Cell Res 204: 356–63, 1993
219. Starksen NF, Harsh GR, Gibbs VC, Williams LT: Regulated expression of the platelet-derived growth factor A chain gene in microvascular endothelial cells. J Biol Chem 262: 14381–4, 1987
220. Kavanaugh WM, Harsh GR, Starksen NF, Rocco CM, Williams LT: Transcriptional regulation of the A and B chains of platelet-derived growth factor in microvascular endothelial cells. J Biol Chem 263: 8470–2, 1988
221. Battegay EJ, Raines EW, Seifert RA, Bowen-Pope DF, Ross R: TGF-beta induces bimodal proliferation of connective tissue cells via complex control of an autocrine PDGF loop. Cell 63: 515–24, 1990
222. Helseth E, Unsgaard G, Dalen A, Vik R: The effects of type beta transforming growth factor on proliferation and epidermal growth factor receptor expression in a human glioblastoma line. J Neuro-Oncol 6: 269–76, 1988
223. Koochekpour S, Merzak A, Pilkington GJ: Vascular endothelial growth factor production is stimulated by gangliosides and TGF-beta isoforms in human glioma cells *in vitro*. Cancer Lett 102: 209–15, 1996
224. Saadeh PB, Mehrara BJ, Steinbrech DS, Dudziak ME, Greenwald JA, Luchs JS, Spector JA, Ueno H, Gittes GK, Longaker MT: Transforming growth factor-beta1 modulates the expression of vascular endothelial growth factor by osteoblasts. Am J Physiol 277: C628–37, 1999
225. Donovan D, Harmey JH, Toomey D, Osborne DH, Redmond HP, Bouchier-Hayes DJ: TGF beta-1 regulation of VEGF production by breast cancer cells. Ann Surg Oncol 4: 621–7, 1997
226. Bodey B, Bodey B Jr, Siegel SE, Kaiser HE: Upregulation of endoglin (CD105) expression during childhood brain tumor-related angiogenesis: Anti-angiogenic therapy. Anticancer Res 18: 1485–1500, 1998
227. Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP: Defective angiogenesis in mice lacking endoglin. Science 284: 1534–7, 1999
228. Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J, Letarte M: Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. J Biol Chem 267: 19027–30, 1992

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