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Review

Angiogenic Growth Factors in Neural Embryogenesis and Neoplasia

David Zagzag

From the Department of Pathology, Division of Neuropathology, the Kaplan Comprehensive Cancer Center, New York University Medical Center, New York, New York

"Blood vessels have the power to increase within themselves which is according to the necessity whether natural or diseased. As a further proof that this is a general principle, we find that all growing parts are much more vascular than those that are come to their full growth; because growth is an operation beyond the simple support of the part. This is the reason why young animals are more vascular than those that are full grown. This is not peculiar to the natural operation of growth, but applies also to disease and restoration." (John Hunter, 1728–1793)

Angiogenesis, the growth of new blood vessels from those preexisting in tissue, is crucial to both human[1] and *in vivo* experimental brain tumor growth.[2,3] It is also an integral process of central nervous system embryogenesis.[4] The angiogenic cascade begins with enzymatic degradation of the basement membrane, which permits migration and proliferation of endothelial cells and finally culminates in capillary morphogenesis.[5,6] Angiogenic factors (AFs) stimulate *in vivo* neovascularization and are called "direct" when they stimulate endothelial cell division or migration *in vivo* and *in vitro*. "Indirect" AFs have no direct mitogenic effect on endothelial cells *in vitro* but are able to promote angiogenesis *in vivo*, probably in part by stimulation of target cells to release direct AFs.[7,8] Determining events leading to angiogenesis include release of AFs, secretion of proteases that release AFs stored in the extracellular matrix, chemotaxis for macrophages which subsequently release AFs, and the release of endothelial cells from inhibitory control. Once stimulated, endothelial cells ostensibly are programmed to release protease, migrate, divide, form a lumen, and secrete a basal lamina.[5,6]

This review will 1) discuss some of the structural, biochemical, and biological characteristics of AFs, 2) summarize the evidence for their implication in neural embryogenesis and neoplasia, and 3) emphasize that identical AFs are involved in both of these processes. These AFs include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factors (TGFs) α and β, and platelet-derived growth factor (PDGF).

Angiogenic Growth Factors

FGF

The FGF family[9] is composed of at least nine related mitogens that affect a variety of cells of neuroectodermal or mesenchymal origin. FGF-1, or acidic FGF, and FGF-2 or basic FGF, share 53% sequence homology. While both are potent angiogenic factors,[7] basic FGF is more effective than acidic FGF. The FGF family consists of structurally related proteins ranging from 16 to 35 kd. They all have a strong affinity for heparin and are often associated with the heparan sulfate proteoglycans (HSPGs) present in the basement membrane and extracellular matrix. The two classes of FGF receptors consist of high-affinity membrane tyrosine kinase receptors and low-affinity cell surface receptors which are HSPGs. The high-affinity

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Address reprint requests to Dr. David Zagzag, Department of Pathology, Division of Neuropathology, New York University Medical Center, 550 First Avenue, New York, NY 10016.

FGF receptor has at least four members: FGF-R1 or flg, FGF-R2 or bek, FGF-R3, and FGF-R4.[9]

Basic FGF, first described as a fibroblast mitogen isolated from bovine pituitary gland and brain,[10] is synthesized by a variety of tumor and endothelial cells.[9] It is found in reactive astrocytes[11] and neurons and supports the maintenance and differentiation of neurons in culture.[12] Basic FGF is a potent mitogen for astroglial cells[13] and oligodendrocytes.[14] Human basic FGF is expressed in four forms, one of 18 kd (155 amino acids) generated at an AUG codon, and three of 22, 22.5, and 24 kd (196, 201, and 210 amino acids) arising from CUG codons.[15] Basic FGF is a direct AF which promotes every phase of the angiogenic process. It induces *in vitro* synthesis of plasminogen activator, a serine protease that plays a critical role in the angiogenic process,[16] and other proteases.[17] Basic FGF stimulates migration and DNA synthesis of endothelial cells *in vitro*[18] and promotes formation of differentiated capillary tubes *in vitro*.[19] However, basic FGF decreases the *in vitro* expression in microvascular endothelial cells of $\alpha_v\beta_3$ integrin,[20] which has recently been shown to be required for angiogenesis.[21] FGF binds to copper,[22] an important angiogenic cofactor.[23]

Basic FGF lacks a signal peptide[24] and the mechanism for its cellular release is unknown. Cell death could result in its release into the extracellular milieu. In addition, it has been proposed that heparinases[25] or plasmin[26] may release extracellular stores of basic FGF from HSPGs, enabling it to reach its receptor on endothelial cells.[27]

VEGF

VEGF,[28] also known as vascular permeability factor,[29] is a ~45 kd heparin binding glycoprotein dimer. It contains two subunits of equivalent mass and has some structural homology to PDGF.[30,31] Of the four different isoforms arising from alternative mRNA splicing ($VEGF_{121,165,189,206}$), $VEGF_{165}$ is predominantly expressed.[32] The shorter forms are diffusible, whereas the longer ones are bound to the extracellular matrix.[32] VEGF which is secreted after processing through the glycosylation pathway,[33] is a specific endothelial cell mitogen and an angiogenic factor *in vivo*.[31,33,34] This direct AF also increases vascular permeability.[29,35,36] VEGF mRNA levels *in vitro* are up-regulated in hypoxic conditions even in the absence of cell death.[37] The two human VEGF receptors flt-1/VEGFR-1[38] and KDR/VEGFR-2[39] are widely distributed on endothelial cells.[40] Flk-1, the mouse[41] and rat[42] VEGFR-2, is also expressed on endothelial cells. VEGF induces the synthesis of plasminogen activator[43] and collagenase[44] by endothelial cells *in vitro*. VEGF currently appears to be the central mediator of angiogenesis.[45]

EGF

EGF, another direct AF, is a 6045 Da protein with angiogenic properties *in vivo*[46] composed of 53 amino acids.[47] *In vitro*, EGF stimulates proliferation[48] and motility[49] of bovine capillary endothelial cells. The mature EGF receptor (EGF-R) is a transmembrane protein composed of 1186 amino acids and has a molecular weight of 170 kd.[50,51] The binding of EGF to the extracellular domain of EGF-R stimulates the receptor's tyrosine kinase activity, resulting in autophosphorylation of the terminal cytoplasmic segment of the receptor and in a number of intracytoplasmic phosphorylation events.[52] Activation of the EGF-R gene is frequently associated with tumor induction or progression.[53]

TGFs

TGFs are categorized into two functionally distinct classes: TGF-α and TGF-β.

TGF-α

TGF-α has potent angiogenic activity.[54] It is an acid and heat stable single chain polypeptide composed of 50 amino acids and shares about 40% sequence homology with EGF,[55] competes with EGF for binding to its receptors,[56] and therefore produces the same biological signal as EGF in target cells.[57,58] TGF-α and EGF bind to EGF receptors on cultured endothelial cells with the same affinity.[58] As a direct AF, TGF-α is thus functionally similar to EGF.

TGF-β

The TGF-β proteins are multifunctional, acid stable, 25-kd dimers composed of two disulfide-linked polypeptide chains, each with a molecular weight of about 12 kd.[59] Three different isoforms of TGF-β that share 70 to 80% amino acid sequence homology have been found in mammalian cells, ie, TGF-β1, -β2, and -β3.[60] TGF-β1 is secreted as a large precleaved latent 390-amino-acid precursor[61] termed the latent complex. Plasmin cleavage of the N-terminal latency-associated peptide causes a conformational alteration that releases the active homodimer.[62] The secretion of TGF-β as latent molecules is an important step in its functional regulation.[63] The wide range of

cells that synthesize TGF-β includes endothelium.[64] Pericytes have been shown to activate the latent TGF-β molecules produced by endothelial cells[63] and are believed to negatively regulate endothelial cell proliferation, in part, by paracrine production of TGF-β.[65]

Depending on tissue source, cell type and conditions, TGF-βs either stimulate or inhibit growth and differentiation.[66] However TGF-βs are direct inhibitors of cell growth, arresting cell growth in the G_1/S phase of the cell cycle.[67] The stimulating effects are indirect through the synthesis of other proteins or the recruitment of cells which release factors with direct stimulatory effect on cell growth. For example, TGF-β inhibits endothelial cell proliferation[68] and migration[69] *in vitro* but stimulates vessel formation *in vivo*[70] and thus is an "indirect" AF. Since TGF-β1 is a potent chemotactic agent for monocytes and macrophages,[71,72] neutrophils[73], and fibroblasts,[74] it can recruit all of these cell types, which are then capable of releasing direct angiogenic factors.

Although TGF-β isoforms demonstrate similar *in vitro* biological activities, more recently certain isoform-specific functions have been demonstrated. For example, in two-dimensional cultures, TGF-β1 inhibits proliferation of both bovine aortic endothelial cells (BAECs) and microvascular endothelial cells (MVECs), whereas TGF-β2 has no effect on BAECs and only slightly inhibits MVECs.[75] TGF-β1 is also more potent as an inhibitor of migration of BAECs than TGF-β2[75] and appears to be responsible for the *in vitro* TGF-β-induced inhibition of DNA synthesis in aortic endothelial cells.[76] The variation in different biological activities of each isoform could be related to their receptors' binding specificities and the presence of signal transducing receptors on a cell type.[77,78] For example, BAECs have an equal amount of receptors that bind both TGF-β1 and TGF-β2, whereas receptors on MVECs preferentially bind to TGF-β1.[75]

TGF-β prevents matrix breakdown and contributes to the maintenance of the integrity of the ECM by decreasing the synthesis of proteases, increasing the synthesis of protease inhibitors, and inducing the synthesis of matrix molecules. For example, TGF-β decreases the expression of proteolytic enzymes in capillary endothelial cells both by inhibiting the synthesis of plasminogen activator and stimulating the synthesis of plasminogen activator inhibitor-1.[79] TGF-β also increases the secretion of tissue inhibitor of metalloproteinase (TIMP),[80] an efficient inhibitor of collagenase.[81] Endothelial cells produce both matrix metalloproteinases and TIMP.[81–83] TGF-β stimulates the expression of a variety of extracellular matrix molecules including proteoglycans, fibronectin, and collagen and increases their incorporation into the extracellular matrix.[84] These matrix proteins play crucial roles in the angiogenic process.[85] TGF-β also stimulates tenascin expression,[86] which correlates with angiogenesis in astrocytomas (D. Zagzag et al, Cancer Research 1995, in press). Endothelial cells can attach and spread on tenascin *in vitro*.[87] The attachment is mediated by integrins such as $\alpha_v\beta_3$,[87] which is required for angiogenesis.[21] The chemotactic effects of TGF-β outweigh its inhibitory effects on cultured endothelial cells and its down-regulation of proteolytic enzymes and are responsible for TGF-β angiogenic activity *in vivo*.

PDGF

PDGF is angiogenic *in vivo*[88] and enhances capillary network formation *in vitro*[89] and is therefore a direct AF. However, it has been suggested that PDGF may exert its angiogenic activity through activation of connective tissue cells in the vicinity of endothelial cells.[89] PDGF has a molecular weight of 28 to 35 kd and is composed of disulfide-bonded heterodimers or homodimers of A and B chains. The A and B chains share about 60% homology. The molecular weight of the A chain is 14 kd and that of the B chain is 17 kd. PDGF-BB homodimer is a more potent angiogenic factor and chemotactic agent for brain capillary endothelial cells than PDGF-AA[88] and appears to be able to modulate endothelial cell proliferation and angiogenesis *in vitro* via PDGF-β receptors.[90] Proliferating endothelial cells express the c-*sis* gene, the homologous oncogene of PDGF,[91] and produce PDGF-AA and PDGF-BB.[92–94] PDGF receptors have been demonstrated on capillary endothelial cells.[95] Their absence on macrovascular endothelial cells, however, could account for the unresponsiveness of these cells to PDGF.[96]

In addition to the previously described AFs, there are others that have not yet been implicated as AFs in brain tumors or central nervous system embryogenesis. These include platelet-derived endothelial cell growth factor (PD-ECGF),[97] which, like FGF, lacks a signal peptide, and like VEGF is a specific endothelial cell mitogen;[97] angiogenin;[98] angiotropin;[99] interleukin-8[100]; and tumor necrosis factor (TNF-α). TNF-α is secreted by macrophages and tumor cells.[101] Like TGF-β, it inhibits endothelial cell proliferation *in vitro* but is angiogenic *in vivo*[102] and thus can be classified as an "indirect " AF. Angiotropin,[103] angiogenin,[99] and TNF-α,[104] like FGF, all bind copper, a crucial angiogenic cofactor.[23]

AFs and Their Interactions

A number of *in vitro* synergistic or inhibitory interactions have been demonstrated between AFs. VEGF and basic FGF synergistically increase the rate of proliferation, sprout formation, and the number of cord-like structures of bovine capillary endothelial cells *in vitro*.[105] The induction of plasminogen activator by VEGF[43] could be responsible for the release of basic FGF-heparan sulfate complexes and could account for this synergy.[105] Basic FGF is synergistic to PDGF in stimulating DNA synthesis of smooth muscle cells[106] and induces activation of latent TGF-β in endothelial cells.[64] Both EGF[107] and TGF-β[108] up-regulate VEGF. TGF-α participates in the induction of the release of other AFs, eg, FGF.[109] TGF-β1 enhances the mitogenic effect of FGF on corneal endothelial cells[110] but inhibits the basic FGF-induced formation of tube-like structures, resulting in the formation of solid endothelial cell cords in three-dimensional cultures.[111] The results of the interactions between two AFs is variable and is in part dependent upon the nature and type of the target cells. For example, TGF-β inhibits the proliferative activities of both acidic and basic FGF on vascular and capillary endothelial cells[112] but is synergistic with the mitogenic effects of acidic FGF on C6 glioma cells.[113] TGF-β acts in synergy with EGF or TGF-α[57] and has a biphasic effect on EGF binding to its receptors, inducing first a decrease in EGF binding, followed by an increase in EGF binding.[114] PDGF inhibits EGF activity.[50] TGF-β stimulates the production of basic FGF-binding proteoglycans.[115]

AFs also interact with extracellular matrix molecules, which play an important role in angiogenesis. Some of these interactions are thought to mediate some of the biological effects of the AFs. For example, some of the biological effects of TGF-β are mediated by its interactions with extracellular matrix molecules. TGF-β binds to fibronectin.[116] It has been proposed that the growth inhibitory effects of TGF-β on endothelial cells are mediated by its ability to increase cellular fibronectin secretion.[117] PDGF regulates thrombospondin synthesis and deposition.[118] Thrombospondin is a high molecular weight multifunctional glycoprotein that modulates endothelial cell adhesion, motility, and growth[119] and inhibits the mitogenic effect of bFGF on capillary endothelial cells.[119] Thrombospondin has been shown to activate the latent form of TGF-β.[120] AFs also interact with proteolytic enzymes, which play a role in both angiogenesis and cancer cell invasion and metastasis.[121] For example, both basic FGF[16,17] and VEGF[43,44] enhance the expression of a variety of proteases. By contrast, TGF-β decreases the synthesis of proteases[79] and increases the synthesis of protease inhibitors.[80] These modulations of effects between AFs and between AFs and extracellular matrix molecules and proteolytic enzymes result in a complex *in vivo* interplay, stressing the need for caution when interpreting data from *in vitro* experiments as they relate to possible *in vivo* scenarios.

AFs and Central Nervous System Embryogenesis and Maturation

Central nervous system tissue development is, to a large extent, controlled by regulatory growth factors and is influenced by a balance between stimulatory and inhibitory variables, including physical parameters, extracellular matrix components, and cell adhesion molecules. Key roles are played by soluble growth factors, many of which are angiogenic. At least two different processes leading to blood vessel formation have been described in the embryo. "Vasculogenesis"[122] is defined as the development of blood vessels from *in situ* differentiation of endothelial cells, whereas "angiogenesis" is, as described above, the sprouting of capillaries from preexisting vessels. Angiogenesis is essential for the development and differentiation of the nervous system. Nevertheless, during neural embryogenesis, several AFs also control proliferation, survival, migration, and differentiation of a variety of neural cell types.

FGFs

Multipotential FGFs play a central role in neural development. Acidic FGF gene expression is detectable in differentiating and mature neurons during chick neural development[123] and both acidic and basic FGF-related angiogenic activity is present in chick embryo brain.[4,124] However, FGR-1 gene expression[123] and protein[4] are not down-regulated in the adult chick brain when angiogenesis has ceased. Basic FGF receptor mRNA is expressed in the developing mouse brain.[125] Basic FGF immunoreactivity can also be detected in the cranial nerve nuclei of the developing rat brain stem.[126] Levels of both acidic FGF and basic FGF increase in the developing rat[127] and mouse[128] brain. Extracts derived from fetal mesencephalic and telencephalic structures have angiogenic properties which are in part mediated by FGF.[129] Human fetal microglial cells produce basic FGF and have high and low affinity basic FGF receptors.[130]

VEGF

VEGF also plays a crucial role in neural embryogenesis. The temporal and spatial expression of VEGF suggests that VEGF is synthesized and released by the ventricular neuroectoderm and induces ingrowth of capillaries from the perineural vascular plexus.[131] *In situ* hybridization on fetal rat brain sections at day 14 reveals widespread high levels of VEGF mRNA.[132] VEGF mRNA expression is related to blood vessel proliferation occurring in developing tissues.[41] The mouse VEGF-receptor flk-1 tyrosine kinase mRNA is expressed in the proliferating endothelial cells of the first vascular sprouts invading the telencephalon of the developing murine brain. By day 14.5, when vascular proliferation and sprouting are at their highest, strong expression of flk-1 mRNA is observed. By contrast, in the adult, when angiogenesis has ceased, flk-1 expression is very low.[41]

EGF

EGF is present in the developing nervous system[133] and may induce multipotential CNS precursor cells to differentiate into astrocytes and neurons *in vitro*.[134] EGF induces proliferation[135] and differentiation[136] of glial cells in culture. EGF stimulates proliferation and survival of 17- to 18-day-old embryonic rat neurons.[137] In the developing rat brain, EGF-R are concentrated in the forebrain[138] and have also been shown on glial cells.[139] In the rat, EGF-R first appear on astroglial cells on postnatal day 16 and reach a maximum on day 19.[140] Despite its angiogenic properties,[45,48,49] EGF is not able to induce blood island formation,[141] an important step in vasculogenesis.[122]

TGF-α

TGF-α has been shown to play a role in mammalian embryogenesis.[142] TGF-α mimics many of the actions of EGF. For example, TGF-α, like EGF, is able to induce proliferation and migration of progenitor cells.[134] Both TGF-α and EGF act on progenitor cells to induce them to differentiate into neurons and astrocytes.[134] Like EGF, TGF-α is not able to induce blood island formation.[141] TGF-α has been immunolocalized to glial cells of the developing rat brain.[143]

TGF-β

TGF-β plays a multifunctional role in neural embryogenesis.[144] TGF-β1–3 immunostaining has been demonstrated in murine and human brain during development.[145] *In vivo* the differential spatial and temporal expression of TGF-β isoforms seems to be unique throughout embryogenesis.[145] Embryonic expression of TGF-β in murine and human brain is detected in leptomeninges, neurons, and radial glia. TGF-β1 is predominantly expressed in leptomeninges[145,146] whereas TGF-β2 and -β3 immunostaining predominates in differentiating neurons and associated radial glia.[146]

PDGF

PDGF isoforms and receptors are found in neurons of the developing mouse[147,148] and rat[149] brain, suggesting their potential neurotrophic role during embryogenesis. In the rodent central nervous system, myelin-forming oligodendrocytes reach the white matter from the germinal matrix shortly after birth.[150] *In vitro* the germinal matrix O-2A progenitor cell can either form oligodendrocytes (O) or type 2 astrocytes (2A).[151] Both PDGF and FGF are mitogenic for O-2A progenitors but they demonstrate different properties on cell differentiation. FGF inhibits differentiation of the progenitor cells while upon removal of the FGF the cells quickly differentiate.[152] PDGF lacks this property.[153] Nevertheless, PDGF inhibits the premature differentiation of the progenitor cells, induces their division and migration, and appears to induce their bipolar shape.[154] It has been suggested that PDGF may be the most important factor for brain myelination.

AFs and Brain Tumors

The endothelial cell proliferation associated with the neovascularization of gliomas is well recognized[155–158] and is one of the criteria used for their grading.[159,160] Brain tumors, of all solid tumors, show the highest degree of vascular proliferation.[158] Neovascularization often correlates with biological aggressiveness and degree of malignancy of brain tumor as well as clinical recurrence, and inversely, with postoperative survival of patients with anaplastic astrocytomas.[159–162] Infiltration of malignant tumors in the brain occurs along vascular channels.[3,163] The vascular component represents as much as 40 to 50% of the volume of certain meningiomas.[164] Brain tumors such as gliomas[165] or meningiomas[166] *in vitro* produce endothelial cell mitogens. Angiogenic activity and vascular density[167] of some brain tumors correlate with their biological behavior. Newly formed blood vessels of brain tumors which have a defective blood-brain barrier[3,168–170] are responsible for the

contrast enhancement of brain tumors,[170,171] are associated with an increased risk of intratumoral hemorrhage,[172,173] and contribute to the pathogenesis of tumor-associated edema.[3,174] Because the morbidity and mortality associated with malignant central nervous system tumors are related to their vascularity and to the extent of peritumoral edema, which, in part, is attributable to neovascularization, the study of AFs, some of which also increase vascular permeability, is of great interest.

FGF

In addition to its angiogenic effects, FGF is a potent mitogen for glioma cells.[175] Enhanced expression of both acidic FGF and basic FGF occurs in gliomas and other tumors of the brain.[176–182] The expression of basic FGF directly correlates with the degree of malignancy of human gliomas.[176,178,183] Immunocytochemically, basic FGF can be demonstrated in nuclei and cytoplasm of tumor cells and endothelium of such tumors.[176–178] Acidic FGF and basic FGF genes are overexpressed in human glioma xenografts, especially at the tumor periphery,[184] where tumor vascularity is very prominent.[3] Human astrocytoma cell lines express basic FGF[185–188] and have FGF receptors.[187–189] When compared with other human solid tumor cell lines (colon carcinoma and melanoma), gliomas express the highest levels of basic FGF protein and of high affinity receptors for basic FGF.[187] The *flg* gene, which encodes one of the human basic FGF receptors, is expressed at high levels in human glioblastomas.[190] Both FGF-R1 (flg) and FGF-R2 (bek) receptors are expressed in human gliomas, cerebral metastatic carcinomas, and meningiomas.[191–193] There are conflicting reports as to the presence[192] or absence[193] of FGFR expression in endothelial cells of gliomas. If confirmed, the lack of FGFR on endothelial cells makes less certain the *in vivo* angiogenic role of basic FGF. Human gliomas, meningiomas and acoustic schwannomas have elevated expression of basic FGF mRNA.[194,195] Plasminogen activator activity, which is demonstrable in human glioma cell lines,[196] correlates with the invasive potential of high grade brain tumors[197,198] and is increased by basic FGF.[17,18] Basic FGF stimulates a variety of proteolytic enzymes[16,17] including collagenases, which have increased expression in high grade primary brain tumors and cerebral metastatic tumors.[199]

How might FGF, which is not secreted, contribute to the pathobiology of astrocytomas? It is not detected in conditioned medium from glioma cell lines,[188] but is present in the cerebrospinal fluid of patients with brain tumors[167] and in cysts of brain tumors.[200] Necrosis is an invariant feature of glioblastomas. FGF may be released following cell death and then contributes to the marked vascular hyperplasia, astrocytic proliferation, and pseudopalisading, which are characteristic of this tumor. Other possibilities are 1) basic FGF in transformed cells may aberrantly fuse to a secretory signal sequence and thus acquire the ability to be released[201]; 2) an intracellular autocrine mechanism could be active even in the absence of basic FGF release; and 3) basic FGF might be released from HSPGs which have an altered and increased expression in high grade gliomas.[202] Glioma cell growth can be inhibited by suppressing basic FGF expression using antisense deoxynucleotide probes[203] or using neutralizing antibodies to basic FGF.[204]

VEGF

VEGF gene, mRNA, and protein are expressed by animal and human glioma cell lines.[42,205–207] VEGF is immunolocalized in astrocytomas.[37,182,208] Immunoreactivity is detected around necrotic areas in the pseudopalisading cells of glioblastomas,[37,208] in tumor cells along capillaries,[37] and in clusters of tumor cells.[208] *In situ* hybridization of glioblastoma multiforme (GBM) shows the highest levels of VEGF in pseudopalisading cells around necrotic areas, where the highest degree of hypoxia is present. VEGF mRNA is up-regulated in glioma cells cultured in hypoxic conditions.[37] Furthermore, the expression of VEGF receptor (flt) mRNA is up-regulated in endothelial cells adjacent to brain tumor.[208] The same pattern of expression of VEGF receptors is observed in experimental brain tumors. For example, in the rat C6 glioma and 9L gliosarcoma, the VEGF receptors flt-1 and flk-1 are specifically expressed in endothelial cells within the tumor and at its border but are absent from endothelial cells in the normal adjacent brain.[42] VEGF gene expression is significantly elevated in high grade gliomas in comparison with low grade tumors.[206] Messenger RNAs of $VEGF_{165}$, $VEGF_{121}$, and $VEGF_{189}$ are synthesized by human gliomas and high VEGF-like activity can be detected in cyst fluids of brain tumors.[206] GBMs, meningiomas, and cerebral metastases frequently exhibit clinically significant peritumoral edema.[164,209] This may be related to their elevated VEGF expression.[206,210] The G55 glioblastoma multiforme, a cell line tumorigenic in nude mice, expresses VEGF mRNA and releases VEGF in culture medium.[207] Like basic FGF, VEGF increases the secretion of proteolytic enzymes[43,44] which have increased activity in high grade brain tumors.[196–198]

Mutation of the p53 tumor suppressor gene, a likely integral step in the formation of some astrocytomas,[211,212] enhances VEGF expression.[213] Antibodies directed against VEGF result in significant inhibition, up to 80%, of the *in vivo* growth of the G55 GBM.[207] The same antibodies have no effect on the *in vitro* growth of tumor cells suggesting that the *in vivo* inhibition is related to suppression of angiogenesis.[207] Infection of endothelial cells with retrovirus encoding a dominant negative mutant of the flk-1/VEGF receptor prevents the growth of C6 glioma in nude mice.[214]

EGF

There is abundant evidence that EGF and EGF-R are implicated in the biology of brain tumors. Increased EGF expression has been observed in GBM.[215] The EGF-R gene is amplified and the EGF-R is overexpressed in human gliomas[216–220] and the overexpression correlates with tumor grade.[212] The presence of such amplification can occur in association with other genetic changes, eg, loss of heterozygosity of chromosome 10.[221] Rearranged or amplified EGF-R genes in gliomas may provide a growth advantage *in vivo*, presumably resulting in aggressive clinical behavior.[222]

TGF-α

Glioma cell lines express TGF-α.[223,224] TGF-α gene[220] and mRNA[181] are expressed by human gliomas. The expression of TGF-α seems to correlate with high tumor grade. Highest levels of TGF-α are detectable in urine of patients with high grade astrocytomas; levels are lower in patients with low grade astrocytomas, oligodendrogliomas, and meningiomas.[225] TGF-α immunostaining is most prominent in high grade gliomas.[226]

TGF-β

Low grade and, to a lesser degree, high grade gliomas synthesize TGF-β1 and TGF-β2 mRNA.[227,228] Both of these isoforms inhibit the growth of low grade and anaplastic glioma cells *in vitro*.[228] However, TGF-β1 stimulates DNA synthesis of glioblastomas *in vitro*.[228] Multiple isoforms of TGF-β (β1, β2, β3) are synthesized and secreted by glioma cell lines,[223,229,230] and TGF-β1 is secreted in both active and latent forms by a glioblastoma cell line,[231] in which it inhibits its growth. TGF-β1 immunostaining is present in tumor cells and tumor vessels in both low and high grade astrocytomas,[228] predominantly in the hyperplastic vessels of glioblastomas.[232] TGF-β suppresses the urokinase-type plasminogen activator activity in glioblastoma cell lines.[233] Meningioma cells appear to bear type I and II receptors[234] and synthesize TGF-β isoforms *in vitro*.[234,235] Depending on its concentration, TGF-β inhibits[234] or stimulates[235] proliferation of meningioma cells *in vitro*. TGF-βs are potent immunosuppressants and therefore may augment tumor growth by decreased immunosurveillance[236,237] and switching to a mitogenic rather than inhibitory effect on growth.

PDGF

Gliomas express PDGF mRNA, PDGF, and the homologous oncogene c-*sis* and PDGF receptors[224,227,238–240]. Expression of both PDGF A and B chains is higher in glioblastomas than in low grade astrocytomas, whereas PDGF-α receptor mRNA is distributed in all grades.[241] PDGF-B mRNA and PDGFR-β mRNA are up-regulated in the hyperplastic vasculature of high grade gliomas.[242,243] The up-regulation of PDGF-BB in hyperplastic vessels of gliomas is consistent with the *in vitro* and *in vivo* potent angiogenic activity of PDGF-BB.[88] It has been suggested that the vascular hyperplasia of glioblastomas is due to an autocrine loop based on the co-expression of PDGF-B and PDGF receptor.[242] Meningiomas also co-express PDGF and PDGF receptors genes and proteins.[244]

Summary

A number of angiogenic growth factors have biochemical and biological properties in common. Several are members of a family of related polypeptides, and many have receptors on multiple cell types including endothelial cells, suggesting the existence of autocrine and paracrine loops. Some AFs have differential biological effects on subtypes of endothelial cells (capillary endothelial cells versus large caliber vessel endothelial cells), in part explained by the differential distribution of their receptors. Their target cells are not restricted to endothelial cells, and many have, in addition to their stimulatory or inhibitory effect on endothelial cells, other biological effects. For example, some are able to induce the differentiation of precursor cells, are neurotrophic, or are mitogenic for a variety of mesenchymal cells. VEGF is perhaps the only AF which seems to be a specific endothelial cell mitogen and is able to directly induce increased vascular permeability. Newly formed vascular channels

are immature and are permeable, in part, because they lack a well formed basal lamina. Thus, AFs leading to neovascularization also increase vascular permeability. However, VEGF appears able to increase permeability of existing mature vascular channels, perhaps via the mechanism required for dissolution of the basement membrane that is a prerequisite for angiogenesis to occur. Several AFs enhance the expression of proteolytic enzymes, which play an important role in both endothelial cell and cancer cell migration, which results in tumor growth and invasion. The interactions of some AFs with extracellular matrix molecules are responsible for their biological effects. Many, but not all, AFs are secreted through the glycosylation pathway. Some are released in the latent form and rendered bioactive through enzymatic splitting from a latent peptide. Trigger mechanisms for the release of AFs are only partially understood. Hypoxia seems to play a crucial role. AFs are synthesized and secreted by a variety of cell types; some are then bound to the extracellular matrix from which they may be released by a variety of processes.

Endothelial cells proliferate during brain development. They are quiescent in normal adult brain but proliferate again under pathological conditions such as brain injury (stroke, trauma, infection) or neoplasms. Degradation of basal lamina together with the migration and proliferation of endothelial cells are processes common to both central nervous system embryogenesis and neoplasia. The "redundancy in angiogenic regulation"[245] is, in part, related to the interaction and multiplicity of AFs. Yet novel AFs are being identified. For example, placenta growth factor, a growth factor closely related to VEGF, has been recently detected in human gliomas.[206] It is apparent that similar AFs are implicated as important mediators in both neural embryogenesis and neoplasia, suggesting that the angiogenesis occurring in brain tumors recapitulates some aspects of embryonic angiogenesis. In central nervous system embryogenesis, the involvement of each of these AFs is subject to a well-orchestrated regulation resulting in their well-defined spatial and sequential expression. By contrast, their involvement in the angiogenesis of neoplastic diseases of the brain is more unpredictable. For example, patients may carry a "low grade" astrocytoma for many years which then, for yet unknown reasons, "transforms" into a highly malignant glioblastoma, which grows in a rapid exponential manner. This growth is accompanied by vascular hyperplasia which is a triggering or permissive process. Several attempts have been made to prevent angiogenesis in brain tumors and therefore prevent their growth. Neutralizing antibodies against basic FGF or VEGF have resulted in significant reduction in tumor size in treated animals as compared with controls. Suppression of the expression of basic FGF or manipulation of the VEGF receptors have also yielded promising results.

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