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Brain Endothelial Cells as Pharmacological Targets in Brain Tumors

Michel Demeule,[†,1] Anthony Régina,[†,1] Borhane Annabi,[1] Yanick Bertrand,[1] Michel W. Bojanowski,[2] and Richard Béliveau[*,1]

[1]Laboratoire de Médecine Moléculaire, Centre d'Hémato-Oncologie, Hôpital Ste-Justine – Université du Québec à Montréal, Montréal, Québec, Canada, [2]Departement de Neurochirurgie, Hôpital Notre-Dame-Université de Montréal, Montréal, Québec, Canada

Abstract

The blood–brain barrier contributes to brain homeostasis by controlling the access of nutrients and toxic substances to the central nervous system (CNS). The acquired brain endothelial cells phenotype results from their sustained interactions with their microenvironment. The endothelial component is involved in the development and progression of most CNS diseases such as brain tumors, Alzheimer's disease, or stroke, for which efficient treatments remain to be discovered. The endothelium constitutes an attractive therapeutical target, particularly in the case of brain tumors, because of the high level of angiogenesis associated with this disease. Drug development based on targeting differential protein expression in the vasculature associated with normal tissues or with disease states holds great potential. This article highlights some of the growing body of evidence showing molecular differences between the vascular bed phenotype of normal and pathological endothelium, with a particular focus on brain tumor endothelium targets, which may play crucial roles in the development of brain cancers. Finally, an overview is presented of the emerging therapies for brain tumors that take the endothelial component into consideration.

Index Entries: Blood–brain barrier; endothelial cells; brain tumors; antiangiogenesis; irradiation; drug delivery; bone marrow stromal cells.

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[†] Equal first authors
* Author to whom correspondence and reprint requests should be addressed. E-mail: oncomol@nobel.si.uqam.ca

Introduction: The Brain Endothelium as a Barrier

The Blood–Brain Barrier

Endothelial cells (ECs) comprise a heterogeneous population covering the entire inner surface of blood vessels *(1,2).* The structure and function of ECs are differentially regulated in space and time both by various systemic signals (coming through the bloodstream) and those produced locally within the irrigated tissue (paracrine regulation). For a long time, the endothelium was seen merely as a semipermeable barrier between blood and tissue. The endothelium is now considered as an association of smaller EC enterprises located within blood vessels of different tissues *(3).* Although united in certain functions, each association of ECs is uniquely adapted to meet the demands of the underlying tissue. For the brain, the blood–brain barrier (BBB) formed by the brain capillary endothelial cells (BCECs) is considered the major route for the uptake of endogenous and exogenous ligands into the brain parenchyma *(4–6).* ECs of brain capillaries are closely sealed by tight junctions and constitute a continuous endothelium. In addition, brain capillaries possess few fenestrae or endocytic vesicles compared to the capillaries of other organs *(4).* BCECs are surrounded by astrocytes, pericytes, microglial cells, and an extracellular matrix. The close association of BCECs with the astrocyte foot processes and the basement membrane of capillaries is important for the development and maintenance of the BBB properties that permit tight control of the blood–brain exchange of molecules *(4–7).*

The restrictive nature of the BBB results in part, from the tight junctions that prevent significant passive movement of small hydrophilic molecules between blood and brain. However, specialized transport systems mediate the entry of essential substances such as glucose, amino acids, choline, monocarboxylic acids, amines, thyroid hormones, purine bases, and nucleosides *(5–7).* Larger hydrophilic molecules do not cross the BBB to any significant extent, with the exception of specific proteins such as transferrin, lactoferrin and low-density lipoprotein, which are taken up by receptor-mediated endocytosis *(8,9).* Thus, the BBB is considered as a rate-limiting step for the penetration of drugs into the brain. Various factors are crucial for the passive entry of drugs into the central nervous system (CNS) *(10).* Among these, lipid solubility is the predominant element in passive BBB permeability because of the lipidic nature of cell membranes. The overall hydrophilic/lipophilic balance of a molecule appears to be a better predictor of BBB permeability than the octanol/buffer partition coefficient. Molecular size, to which the rate of solute diffusion is inversely related, also appears to be relevant for hydrophilic compounds but does not significantly influence the BBB permeability of lipophilic compounds. Binding to plasma proteins, ionization at physiological pH (pKa), affinity and capacity for transport systems, and potential BBB/cerebral metabolism are also important. The activity of the efflux transporter P-glycoprotein in the BBB prevents significant accumulation of many hydrophobic molecules or drugs in the CNS *(11,12).* A wide range of CNS disorders include events that perturb the BBB *(13).* Mechanisms by which the brain ECs respond to pathological stimuli are numerous. Angiogenic stimuli arising from tumor cells induce major phenotypical modifications of the brain ECs.

This article describes evidence highlighting biochemical differences in the vascular bed phenotype of normal brain and brain tumors, which may play a crucial role in the development of brain tumors. The identification of potential targets in ECs of brain tumors may contribute to design new therapeutical approaches for this type of brain disease. In addition, we discuss the ability of bone marrow-derived stromal cells (BMSCs) to acquire a histology coherent with ECs, which may enable them to contribute to tumor angiogenesis. Finally, general perspectives on the application of antiangiogenesis approaches are also presented.

Table 1
Strategies for Drug Delivery

Strategies	Approaches	Advantages	Limits
Invasive	Surgical techniques		
	Intraventricular injection *(14)*	Control of drugs concentration; target accessibility	Not a widespread technique
			Low diffusion in adjacent parenchyma
	BBB disruption Bradikinin analog RMP-7 *(15,16)*	In clinical phase II for brain tumors	Mainly used in glioma therapy
Pharmacological	Encapsulation techniques Liposomes *(17)* Nanoparticles *(18)*	BBB integrity is preserved Drugs without modification Hydrophilic and lipophilic drugs	Low diffusion in adjacent parenchyma
Target-based	Chemical modifications *(19)* Cationization Increased hydrophobicity Pseudonutrients	Brain endothelium-specific targeting	Limited number of drugs
	Vectorization Chimeric peptides Receptor-mediated transcytosis *(20–22)*	High-affinity transport systems	

Crossing the Barriers

It has been widely accepted that a large number of hydrophilic molecules, such as peptides and proteins, fail to reach their targets within the brain after their peripheral administration. Different approaches have been used to increase CNS penetration to drugs normally shut out by the BBB. These strategies are summarized in Table 1. At least three strategies have been developed for increasing BBB penetration.

1. Invasive strategies aim to bypass the BBB. These strategies include intra-arterial, high-dose intravenous, intracavitary, or interstitial chemotherapy using different approaches, among which the release of highly concentrated agents impregnate on biodegradable polymers and cause BBB disruption by osmotic agents. In this kind of approach, a new bradykinin analog (RMP-7) was shown to selectively increase the permeability of tumor capillaries to methotrexate but leave normal capillaries intact in rats. However, it has been reported that RMP-7 could also increase the passage of pharmacological agents across the normal BBB.
2. Pharmacology-based strategies include modifications of a drug to improve its ability to diffuse across the BBB. Conjugation of a therapeutic protein, to cationic peptides or proteins, such as the R-rich sequence from the third helix of Antennapedia protein *(22)*, the K-rich transportan peptide *(23)*, the Rrich SynB1 *(24)*, and the R-rich sequence of the tat peptide of the human immunodeficiency virus (HIV)-1 *(25,26)*, are under investigation.
3. Target-based strategies for crossing the BBB are currently under development. Strategies using specific transport mechanisms at the BBB to deliver a drug into the brain compartment at a therapeutic concentration are being developed. There are several transport systems at the BBB for nutrients and endogenous compounds. One

of the most advanced delivery systems, the OX-26 antibody against the rat transferrin receptor, uses the receptor-mediated endocytosis pathway *(27,28).* Other classes of large molecular drugs have been described for brain drug targeting and include antisense pharmaceuticals and gene medicines *(29).*

Pathologies Associated With Vascular Changes

Under normal conditions, the BBB is capable of rapid modulation in response to physiological stimuli. This enables it to protect the brain parenchyma and maintain a homeostatic environment. By "loosening" the tight junctions, which is reflected by an increase in paracellular permeability, the BBB is able to "bend without breaking," thereby maintaining structural integrity. In some pathological conditions, BBB dysregulation occurs and contributes to neuroinflammation and brain tissue damage. Indeed, disruption of the tight junctions of the BBB is a hallmark of many CNS pathologies, including stroke, HIV encephalitis, Alzheimer's disease, multiple sclerosis, and bacterial meningitis *(30).* Therefore, vascular leakage and angiogenesis are the two major vascular abnormalities associated with most of these pathological conditions and where the brain endothelium contributes to the focal nature of vasculopathic disease states.

As an example, Alzheimer's disease is a progressive neurodegenerative disease with complex histopathology involving neuronal, glial, and vascular changes *(31,32).* Permeability of the BBB has been suggested to be altered in Alzheimer's disease *(33).* Moreover, the β-amyloid$_{1–42}$ peptide, which is associated with the development of Alzheimer's disease, is reported to impair BBB function by altering BBB permeability after intracarotid infusion in rats *(32).* The β-amyloid peptide was also demonstrated to produce an excess of superoxide radicals that led to alterations in structure and function of brain ECs *(34).* Thus, alterations of the brain microvasculature functions by β-amyloid peptide may subsequently contribute to the neurodegenerative development of this pathology.

Diabetes mellitus is a metabolic disorder associated with alterations in various organs, including the CNS. One major contributor is related to changes in the BBB that affect the physicochemical properties and functions of ECs lining the cerebral microvasculature. Alterations in histology as well as biochemical and neurotransmitter activity have been reported *(35).* Some of the common disease symptoms associated with diabetes, including transient cerebral ischemia, hypertension, and hyperosmolarity, can disrupt or affect BBB integrity, leading to increased albumin accumulation in the brain parenchyma. Changes in the transport function of the BBB also have been reported, including altered transport of glucose and of other nutrients, metabolites, and specific minerals such as sodium and potassium. In summary, several crucial BBB transport processes are selectively altered in chronic hyperglycemia. It was also recently proposed that BBB dysfunction, with leakage of plasma components into the vessel wall and surrounding brain tissue leading to neuronal damage, may contribute to the development of three overlapping and disabling cerebrovascular conditions: lacunar stroke, leukoaraiosis, and dementia *(36).*

Brain Tumors

Brain Tumor Classification

Brain tumors are one of the CNS diseases in which the EC component plays a crucial role. Although not among the most common neoplasms, brain tumors are among the most devastating. Mental impairment, seizures, and paralysis afflict the very core of the person. In addition to these burdens is the knowledge that for most brain tumors, adequate treatment still is not available and the likelihood for long-term survival is poor *(37).* In children, even if they do survive, the devastating impact of disease and treatment often leave permanent neurological damage when they survive. Currently, brain tumors are the second and fourth leading causes of cancer mortality in children and in young

adults between ages 15 and 34, respectively. The treatment of brain cancer is one of the most challenging areas of oncology, and clinical progress in the treatment of these malignancies has been slow. The most frequent primary brain tumors in adults are gliomas and primary CNS lymphomas. According to the World Health Organization (WHO) classification, the three main tumor types are astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas *(37).* Astrocytomas usually include (in order of anaplasia) pilocytic astrocytomas (grade I), diffuse astrocytomas (grade II), anaplastic astrocytomas (grade III), and glioblastomas (grade IV). This classification relies on four main features: nuclear atypia, mitoses, microvasculare proliferation, and necrosis. Analysis of the most malignant region of the tumors allow their grading from low (I and II) to high (III and IV) malignant grades. Therefore, glioblastomas, which are the most frequent tumor subtype, have the highest malignancy. This current morphological classification remains somewhat tentative, and molecular markers or genetic markers would eventually be helpful to improve both classification and patient diagnosis. Because of their capacity to infiltrate normal brain parenchyma, most low-grade gliomas undergo malignant transformation over time. Genetic alterations in gliomagenesis and tumor progression have been reported that are closely associated with loss of cell cycle control *(37).* Alterations in low-grade astrocytomas overexpression of platelet-derived growth factor (PDGF) and inactivation of the *TP53* gene have been observed. In malignant astrocytomas, other alterations affect the *P16/CDKN2A* gene, amplification of cyclin-dependent kinase 4, overexpression of epidermal growth factor receptor in glioblastomas, and *PTEN* mutation.

Conventional Therapies

Malignant gliomas are among the most challenging of all cancers to treat successfully. They are characterized not only by their aggressive proliferation and expansion but also by inexorable tumor invasion into distant brain tissue. Specific treatment for malignant astrocytomas includes surgery, radiotherapy, and chemotherapy. Surgery maintains a dominant role in the therapeutic approaches to gliomas. Maximum resection should be performed to achieve a quick relief of symptoms and establish diagnosis. However, the benefit of surgical resection to survival remains to be confirmed *(38).* Because of their anatomical localization and infiltrative pattern, problems in properly defining the tumor target remain a major obstacle for the success of surgical procedures, leading to incomplete surgical resection of the tumor. In this regard, useful contributions are expected from advances in molecular neurobiology and functional neuroimaging as shown by preliminary investigations with magnetic resonance (MR) spectroscopy *(39).*

Radiotherapy is limited by low brain tolerance as well as by the infiltration of tumor cells into healthy brain. High-grade astrocytomas (anaplastic and glioblastomas) are the most common gliomas. Glioblastomas are about four times more common than anaplastic astrocytomas *(40).* There is no scientific evidence that radiotherapy using hyper- and hypofractionation leads to longer survival for patients with high-grade malignant glioma than conventional radiotherapy. In astrocytomas, radiotherapy led to a decrease in mass effect and an improvement of neurological symptoms in 50–75% of cases *(41).* However, despite the increased progression-free time associated with early postoperative radiotherapy, overall survival did not change compared with radiotherapy, which was deferred until clinical progression *(42).* The current recommendation is to postpone treatment in asymptomatic patients, and focal irradiation should be administered when the patients develop symptoms that substantially affect their quality of life or when unequivocal tumor progression on MR imaging (MRI) suggests the imminence of clinical manifestations.

Adjuvant chemotherapy using nitrosoureas added a small increase (5–20%) to the proportions of patients who were alive at 18 mo without affecting the median survival of patients with high-grade gliomas *(43).* Approaches

using other cytotoxic agents, neoadjuvant chemotherapy, multiple agents, intra-arterial chemotherapy with intact or disrupted BBB, or high-dose chemotherapy with stem-cell rescue were not superior to standard adjuvant nitrosoureas *(37).* Among the recently approved therapies for brain tumors, Gliadel wafer (Guilford Pharmaceuticals, Baltimore, MD) has received approval from the United States Food and Drug Administration for use in newly diagnosed patients with high-grade malignant glioma as an adjunct to surgery and radiation. A phase III clinical trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel, Baltimore, MD) increased median survival from 11.6 mo in the placebo-treated group to 13.9 mo in the BCNU wafer-treated groups *(44).* γ-knife radiosurgery (GKR) has also played a substantial role in the palliative treatment of patients with small-to-medium size brain metastases *(45).* In a review on GKR treatments, it was proposed that this approach could represent an alternative option to conventional radiochemotherapy for unfavorable low-grade gliomas *(46).*

The adjuvant temozolomide, which is useful in recurrent anaplastic astrocytomas, is currently being tested for glioblastomas in a randomized phase III study. This agent, in combination with 13-*cis*-retinoic acid, significantly increased both the 6-mo progresssion survival rate and the median overall survival in a phase II trial *(47).* Conventional chemotherapeutic approaches, although useful in some cases for reducing radiotherapy doses, still produce modest results regarding response rate and median survival. Because of intrinsic chemoresistance, the benefits of chemotherapy remain small, with a rate of response plus stabilization of 20–50% and overall median survival ranging from 4 to 8 mo (shorter for glioblastomas and generally longer for anaplastic astrocytomas). Thus far, limited clinical success has been associated with immunotherapies and biological modifiers for treating gliomas *(48).* Therefore, it is only through understanding of molecular aspects of the phenomena involved in drug delivery and resistance that more efficient clinical treatments of brain tumors can be envisioned. Despite all these efforts, the median survival of patients with malignant astrocytomas remains poor, at approx 2–3 yr in anaplastic astrocytomas and 1 yr for glioblastomas.

Antiangiogenesis Approaches

Although considerable efforts have been made in the treatment of brain tumors with combinations of surgery, radiotherapy, and chemotherapy, high-grade gliomas remain incurable. According to the National Cancer Institute database, there are currently more than 100 clinical trials underway to find cures for brain tumors in adults. One innovative approach under investigation uses antiangiogenic agents to block the formation of new blood vessel network within a tumor. Among currently active clinical trials for brain tumors, 15 are using antiangiogenic molecules, often in combination with a conventional approach such as radiotherapy or chemotherapy (www.nci.nih.gov/clinical_trials).

Proper formation of blood vessels in angiogenesis is vital for delivery of the oxygen, nutrients, and growth factors essential for development, reproduction, and wound-healing processes. It is also well-established that when deranged, angiogenesis contributes to numerous threatening disorders such as cancer. There is increasing evidence supporting the central role of angiogenesis in tumor growth and metastasis. Therefore, tumor angiogenesis, the formation of new blood vessel networks within a tumor, represents an absolute requirement for the maintenance and progression of most solid tumors *(49,50).* Angiogenesis has become one of the most promising therapeutic targets in cancer medicine. Accordingly, tremendous efforts have been made to identify antiangiogenic molecules with antitumor properties. This has led to the development of a variety of molecules that are directed against critical cellular aspects of angiogenesis such as cell adhesion, extracellular matrix degradation,

and the stimulation of ECs by angiogenic cytokines or growth factors. Extensive studies on the cellular and molecular processes underlying angiogenesis have identified key events associated with tumor-induced neovascularization: (a) stimulation of ECs by tumor-derived angiogenic cytokines such as vascular endothelial growth factor (VEGF), resulting in increased EC proliferation and migration; (b) secretion of matrix-degrading enzymes such as matrix metalloproteinases (MMPs) and plasminogen (Pgn) activators, resulting in digestion of the surrounding extracellular matrix; and (c) formation of a three-dimensional capillary network in the vicinity of the tumor cells, allowing their sustained growth by providing oxygen and essential nutrients. These cellular and molecular steps represent attractive antiangiogenic targets and have led to the identification and development of a variety of compounds targeting vessel formation or EC proliferation or migration. More than 60 antiangiogenic molecules are currently being assessed in clinical trials (www.angio.org).

The potential use of antiangiogenic molecules as inhibitors of tumor progression was first suggested by the identification of angiostatin, a Pgn fragment, in the serum and urine of syngenic mice bearing Lewis lung carcinoma *(51).* The protein contained the first four triple-loop disulfide-linked regions of Pgn known as kringle domains and showed significant inhibitory activity toward EC functions *(52).* Several other endogenous inhibitors of angiogenesis have subsequently been described that are fragments of abundant proteins and that become inhibitory to EC function following proteolytic cleavage. These include the Pgn fragment kringle 5 and the collagen fragments endostatin *(53),* canstatin *(54)* and tumstatin *(55),* as well as fragments derived from fibronectin *(56),* prolactin *(57),* MMP-2 *(58),* and calreticulin *(59),* among others. These molecules inhibit EC proliferation and migration and capillary-like structure formation in vitro.

Green tea polyphenols were also shown to possess antiangiogenic properties by the observation that green tea extracted block neovascularization in the chick embryo neovascularisation assay *(60).* Moreover, lower levels of endostatin were found in human glioblastomas than in WHO grade II astrocytomas by immunohistochemistry, with a stronger detection in perinecrotic areas of the tumors *(61).* In contrast, a positive correlation between levels of tissue endostatin and malignancy grades in gliomas were estimated by immunoblotting *(62).* However, both of these studies suggested that endostatin could be released near the tumor blood vessels to counteract angiogenic stimuli *(62).*

Angiogenesis inhibition represents a promising new therapeutic approach for a wide variety of cancers, including brain tumors. A better comprehension of the complex process of angiogenesis is required for the development of future effective antiangiogenic regimens. As mentioned by McCarty *(63),* appropriate patient selection, relevant biological endpoints, and a careful design of therapeutic intervention also are necessary. However, preclinical data indicate that antiangiogenic treatments, when used as a single therapy, only slow tumor growth. Thus, the combination of antiangiogenic agents with cytotoxic chemotherapy or vascular targetting agents might increase the efficacy of antitumoral therapies *(63).*

Targetting the Brain Endothelial Cells

Disregulation of the BBB in Brain Tumors

The molecular mechanisms of angiogenesis have been elucidated in great detail over the past few years. However, much less is known about the nature and the functional status of the angiogenic vascular bed in tumors. The diversity of the vascular endothelium holds great potential for facilitating site-specific drug delivery. Therefore, the efforts of our group and of others have been aimed at defining tissue-specific and/or tumor-associated angiogenesis-related markers in the vasculature and using these for targeted therapeutics. Novel systems have been developed to enable the molecular

phenotyping of the cells forming blood vessels. The identification of proteins that are differentially expressed between healthy and tumoral endothelium is critical for the elucidation of mechanisms involved in the pathogenesis of diseases such as angiogenesis. Tumor-specific endothelial markers have been identified in vitro in ECs exposed to tumor-conditioned media or angiogenic factors *(64–66).* However, the in vivo characterization of normal ECs is a necessary step in understanding changes that occur in pathologies. The molecular features of normal ECs are beginning to be identified *(2,67,68).* A "vascular proteomics" approach using a polyclonal antiserum against bovine brain microvessel endothelial proteins allowed the identification of brain endothelium-specific proteins *(69).* The first brain endothelium-specific protein identified using this approach, the Lutheran membrane glycoprotein, also was expressed in brain tumoral ECs *(70).*

Purification of pure populations of ECs from solid tissues is another approach used to analyze ECs phenotype in a pathological state. Genes encoding tumor endothelial markers have been identified in ECs isolated from both normal and malignant colorectal tissue *(71).* Similar identification would be of particular use for brain tumors, because they are among the solid tumors with the highest degree of neovascularity.

The growth of most primary brain tumors is associated with brain edema. A disregulation of the BBB junctional complex at the blood–tumor barrier has been associated with this phenomenon *(72).* Some BBB-specific transporters, such as the glucose transporter-1 (GLUT-1) have also been shown to be downregulated in tumoral brain vasculature *(73).* To grow beyond minimal size, tumors must generate a new vascular supply by secreting proangiogenic cytokines. VEGF, which is considered the best proangiogenic agent for promoting tumor growth and angiogenesis, is overexpressed in most glioblastoma multiforme (GBM), and its level of expression is correlated with the grade of glioma invasiveness *(74).* In the same study, both the VEGF(121) and VEGF(165) isoforms contributed to glioma vascularization, oxygenation, and growth, whereas they did not drive the formation of anaplastic astrocytoma to the GBM phenotype *(74).* Other mediators, such as the angiostatic factors angiopoietin (Ang1) and angiopoietin-2 (Ang2) are also involved in the tumor-associated angiogenesis process *(75–77).*

Isolation of Endothelial Cells

A molecular comparison between purified populations of normal brain and glioma remains to be established. We recently demonstrated some phenotypical differences between brain, lung, and kidney ECs using a magnetic cell-sorting approach *(78).* This method was used to compare the phenotype of EC isolated from normal brain to those from orthotopic or ectotopic glioma CNS-1 rat models. The CNS-1 cell line has been reported to grow with an infiltrative pattern similar to that observed in human gliomas *(79)* (Fig. 1A). The high migration capacity of the CNS-1 cell line may explain the considerable ability of intracranially implanted CNS-1 cells to invade adjacent normal brain. In experimental brain tumors, the pseudopalisading pattern and the concomitant development of necrosis have been associated with the presence of an angiogenic switch *(80).* Moreover, EC hyperplasia in tumors has been an important indicator of angiogenesis *(81).* Taken together, these observations show that the CNS-1 model presents anatomical and morphological characteristics, including induced angiogenesis, which validate its use for further investigation of the molecular events associated with brain tumors. We assessed the expression of some important molecular determinants in brain tumor pathology—such as the drug efflux pump P-glycoprotein, which is implicated in brain tumor resistance to chemotherapy, and the MMPs, which are involved in the degradation of a variety of extracellular matrix components—for their important role in tumor progression. The results obtained showed differences in protein expression and activity between intrac-

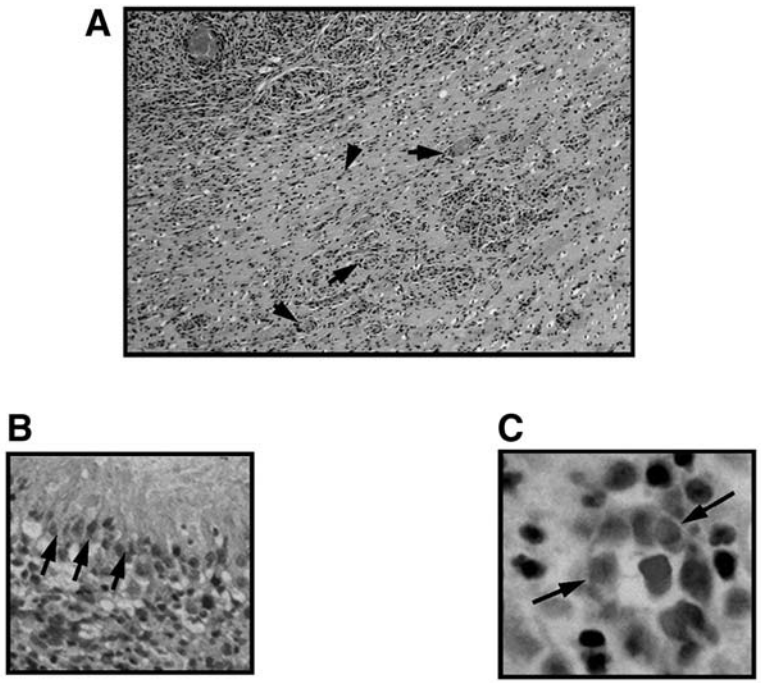


Fig. 1. Major characteristics of glioblastomas. **(A)** Hematoxylin & eosin staining shows perivascular spreading of tumor cells with single-cell permeation of the adjacent parenchyma is found at the tumor border (indicated by arrows). **(B)** Necrosis areas at the tumor center with palisading cells at the borders are also observed (×250). **(C)** Moreover, hyperplasia and tumescent aspect of vascular ECs indicate that they are in a proliferative state and that angiogenesis is occuring. Differences in the expression of some EC proteins are also indicated in Table 2.

erebral glioma-derived ECs and normal brain ECs for the markers studied. Striking differences were also found between experimental-implanted intracerebral and subcutaneous glioma ECs, suggesting that the peritumoral environment is an important determinant for the establishment of the angiogenic phenotype *(82).* Molecular evidence has been reported for phenotypic distinction between tumoral and normal brain vasculature and indicates that the EC phenotype strongly depends on interactions both with tumor cells and with the microenvironment (Fig. 1; Table 2).

Modulation of P-Glycoprotein Isoforms

P-glycoprotein is one of the most important efflux pumps identified at the BBB. P-glycoprotein encoded by *MDR1* in humans and by *mdr1a* and *mdr1b* in rodents is associated with the MDR phenotype *(83).* P-glycoprotein encoded by *MDR2* in humans or *mdr2* in rodents does not play an important role in the transport of drugs *(84).* Mice genetically deficient in the *mdr1b* gene or in both the *mdr1a* and *mdr1b* genes have normal viability. However, they have shown accumulation of various drugs in the brain and other tissues and diminished drug elimination, indicating that P-glycoprotein may act as a guardian by preventing the passage and accumulation of many drugs into the brain *(85,86).* Moreover, it was shown that P-glycoprotein could limit the access of naturally occurring molecules such as the glucocorticoid cortisol to the mouse and human brain, particularly to the hippocampal area

Table 2
Differences Between Endothelial Cells From Normal Brain and Brain Tumors

Class of proteins	Modulation	References
Transporters	Downregulation of GLUT-1	*73*
	Changes in expression of mdr1 isoforms	*82*
Urokinase system	Upregulation of uPA, tPA, and PAI-1	*106*
MMPs	Increased MMP-9 expression and activity	*82*
Extracellular matrix protein receptors	integrins (α_V,β_3,β_1), osteopontin	*181*
Growth factors and their receptors	Upregulation of tissue factor, Ang-2, Tie-2, VEGF, VEGFR-2	*77,181*
Endogenous antiangiogenesis agent	Lower levels of endostatin	*61*
	Positive correlation between endostatin levels and grades in gliomas	*62*
Others	Downregulation of caveolin-1	*119*
	Increased ERK expression	
	Stat 3α (signal transducer and activator)	*182*
	Endothelin system	*116*

Abbreviations: uPA, urokinase plasminogen activator; tPA, tissue-type plasminogen activator; PAI, plasminogene activator-inhibitor; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; ERK, extracellular signal-regulated kinase.

(87,88). It also has been suggested that P-glycoprotein might be involved in the transport of prenylcysteine esthers and cholesterol *(89–91).* In addition, the amphiphatic β-amyloid peptide$_{1–42}$ has been proposed to be transported by P-glycoprotein *(92).* Thus, the pumping out of amphiphatic peptides, proteins lacking signal sequences, or lipid-modified proteins from biological membranes by P-glycoprotein could also contribute to brain secretion (waste disposal) or capillary secretion of molecules *(93).*

Previous immunohistochemical analyses showed that most gliomas and, more specifically, ECs within the gliomas stained positively for MDR1 P-glycoprotein *(94,95).* These studies support the concept that clinical drug resistance may be caused by P-glycoprotein expression not only in cancer cells but also in the capillary ECs of brain tumors. Alterations in the brain capillary ultrastructure have been described that lead to an increase in microvascular permeability in gliomas. Paradoxically, it has been reported that the neovasculature of even high-grade tumors preserves partial BBB permeability properties at the cellular level *(96)* and that the BBB at the tumor periphery is still intact. In addition, P-glycoprotein, one of the best phenotypic markers of the BBB, is expressed at the same levels in all primary tumors as in normal brain, indicating that brain tumors retain an important characteristic of the BBB that allows them to restrict the uptake of chemotherapeutic agents. Thus, BBB, especially at the edge of tumors, remains a formidable obstacle for drug distribution to brain regions that have been infiltrated by neoplastic cells *(97).* Moreover, we observed an upregulation of the *mdr1b* isoform in ECs cultured from brain capillaries and from isolated brain tumor ECs *(82,98,99).* This upregulation has been associated with a dedifferentiation of ECs in culture that are no longer subjected to the paracrine regulation of the surrounding astrocytes. This upregulation of the *mdr1b* gene, concomitant with expression of the brain endothelium-specific *mdr1a* gene, suggests that

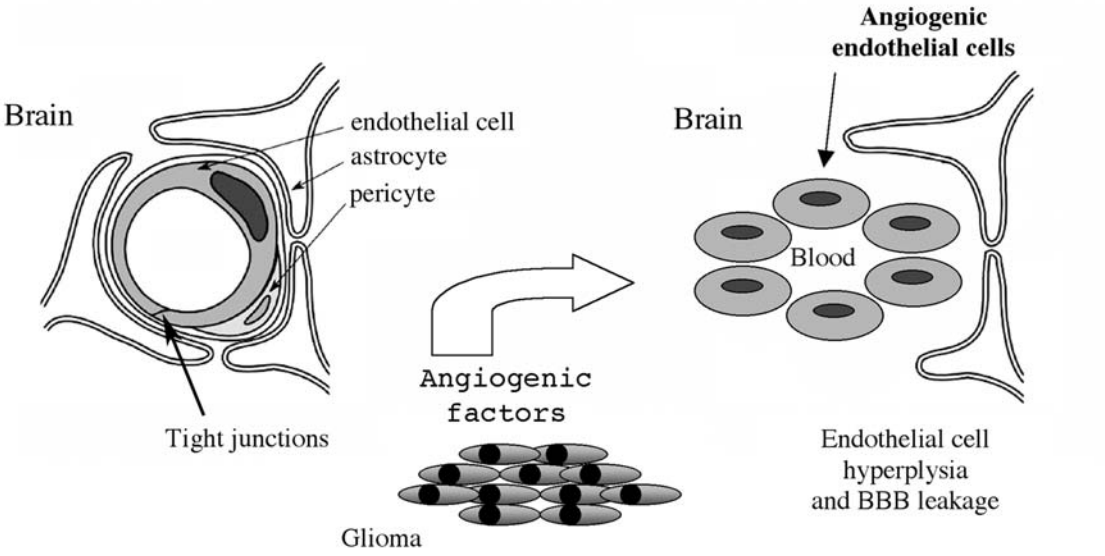
some important barrier properties are maintained in the angiogenic vessels that develop within brain tumors even if EC dedifferentiation occurs. A similar observation was made in studies where the expression of GLUT-1 was found to be completely different in intracerebral vs subcutaneous gliomas *(100).* Thus, the multidrug resistance phenomenon in brain tumors may result from both the ECs and the tumor cells. Because P-glycoprotein expression in brain tumor vasculature might be involved in the high resistance of gliomas to chemotherapy, studies using intracerebral models may be more appropriate as P-glycoprotein disappears from the vasculature of subcutaneous CNS-1 model *(82).*

The efficacy of chemotherapy treatments is limited, probably because of their frequent intrinsic MDR phenotype. Recent data suggest that P-glycoprotein contributes to cellular resistance merely in a small number of glioma cells, whereas multidrug resistance-associated proteins seem to be constitutively expressed in all glioma cell lines *(101,102).* However, P-glycoprotein has been shown to be expressed in human glioma biopsies at the same level as in normal brain, suggesting that P-glycoprotein is expressed at the endothelial blood–tumor barrier *(103).* The development of P-glycoprotein inhibitors to reverse the MDR phenotype was investigated extensively with generally disappointing results. The use of first-generation agents (cyclosporin, verapamil) was limited because of unacceptable toxicity, whereas second-generation agents (valspodar, biricodar) had better tolerability *(104).* However, this second generation of inhibitors has unpredictable pharmacokinetic interactions with coadministered chemotherapy agents and may interact with other transporters. The third-generation inhibitors (including tariquidar [XR9576], zosuquidar [LY335979], laniquidar [R101933], and ONT-093) present a high potency and specificity for P-glycoprotein. They are currently under clinical trials, and further studies are required to establish their contribution to potential therapeutic treatment by reversing P-glycoprotein-mediated MDR.

Upregulation of Proteinases

During the onset of angiogenesis, ECs degrade their basement membrane, migrate into the interstitial matrix, proliferate, and form new microvascular structures. Matrix remodeling proteases of the Pgn activator/ plasmin system and matrix-degrading MMPs, together with their receptors and inhibitors, play pivotal roles in several of these steps. The Pgn system includes several components: (a) Pgn, an inactive proform that is composed of plasmin (the active form) and two inhibitor domains (angiostatin and kringle domain 5); (b) urokinase (uPA) and tissue-type (tPA) Pgn activators, two serine proteinases that convert Pgn into plasmin; (c) the receptors uPAR (a glycosylphosphatidylinositol-linked surface receptor for uPA and tPA), α-enolase, cytokeratin 8, and annexin II for the Pgn receptor *(105);* and (d) Pgn activator inhibitor (PAI) types 1 and 2, α-2-antiplasmin, and bikunin. Regarding localization, uPA, uPAR, and PAI-1 are not generally expressed by quiescent endothelium, whereas tPA has been detected in the quiescent endothelium of normal human tissues. In contrast, uPA, uPAR, and PAI-1 are all expressed during angiogenesis in vivo. uPA and uPAR appear to be expressed by ECs, and, depending on the situation, PAI-1 is expressed either by ECs or by stromal cells *(106).* These in vivo observations were further supported by results obtained in vitro. Cultured ECs expressed uPA, uPAR, tPA, and PAI-1 and their expression profiles were regulated by angiogenic factors such as VEGF and basic fibroblast growth factor (bFGF *(106).* Hypoxia, a major stimulus of angiogenesis was also reported to increase uPAR and PAI-1 in ECs *(107).* An interesting observation was that VEGF induced uPA and tPA in ECs derived from the microvasculature but not in cells derived from the aorta *(108).* We studied the gene expression of uPA, tPA, PAI-1, and uPAR in normal brain and in an intracerebral CNS-1 glioblastoma model as well as in isolated ECs from these tissues by reverse transcription polymerase chain reaction (Fig. 2B). We also performed a plasminogen-zymography assay

A Hyperpermeability of the blood-brain barrier in brain tumor



B Increased mRNA levels of uPA system components and extracellular matrix (ECM) protein receptors

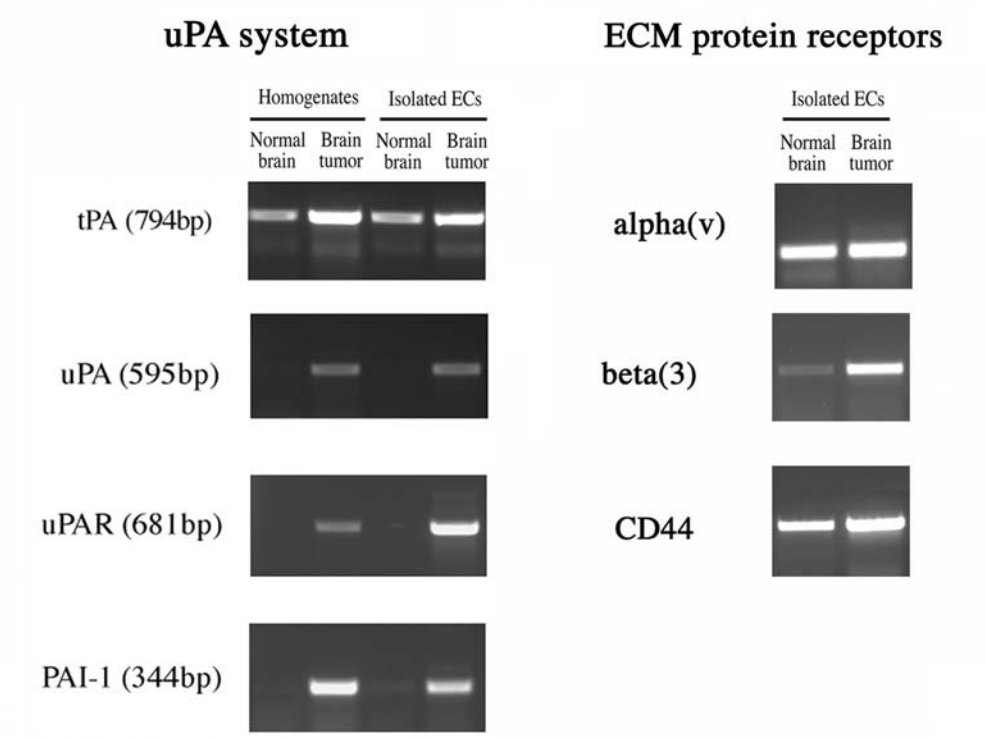


Fig. 2. Schematic representation of normal brain capillary and brain tumoral EC. **(A)** Normal brain ECs are surrounded by pericytes and their close association of ECs with the astrocyte foot processes and basement membrane of capillaries is important for the development and maintenance of BBB properties. Angiogenic factors and paracrine regulation by glioma cells modify the phenotype of brain tumoral ECs and lead to a leakage of the BBB. The basement membrane is either absent or present profound structural abnormalities. **(B)** Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of the uPA/tPA system and extracellular matrix protein receptors was performed using RNA samples isolated from homogenates and from isolated ECs of normal brain and brain tumor. Results show that components of the uPA system and ECM protein receptors are upregulated in ECs from brain tumors.

to measure the activity of tPA and uPA in the same samples. We observed upregulation of tPA, uPA, PAI-1, and uPAR in ECs isolated from CNS-1 tumors. In light of those results, antiangiogenic inhibitor therapies that target components of these systems may represent attractive strategies.

Activation of MMPs is also crucial in glioma invasion and angiogenesis *(109).* The molecular regulation of the extracellular matrix proteolysis that occurs during angiogenesis and in glioma migration/invasion is accomplished largely through the action of soluble and membrane-bound MMPs *(110,111).* We used a gelatin-zymography assay to measure the levels of pro- and activated MMP activity in intracerebral and subcutaneous CNS-1 glioblastoma models and in isolated ECs from these tumors. No gelatinase activity was detected within normal brain homogenates. However, considerable differences in gelatinase activity were seen between ECs isolated from the three tissues. In the CNS-1 glioblastoma model, brain tumor cells primarily expressed a MMP-2 activity, whereas ECs generally expressed a MMP-9 activity *(82).* Studies using *in situ* hybridization and immunohistochemistry also showed that in human gliomas, MMP-2 expression was primarily detected in glioma cells, whereas MMP-9 expression was predominantly found in vascular structures *(112,113).* Interestingly, it has been reported that the cerebrospinal fluid (CSF) of patients with malignant gliomas contains MMP-2 and MMP-9, whereas only MMP-2 is found in the CSF of healthy patients *(114).* Our study supports the idea that MMP-9, and not MMP-2, is the major matrix-degrading enzyme expressed by angiogenic ECs. Collectively, these results show that the tumor cells surrounding ECs in gliomas are able to influence the invasive phenotype of the ECs. Strong molecular differences in the phenotypes of normal and tumoral brain endothelium were observed, as shown by differences in the expression of important targets for brain cancer therapy, such as P-glycoprotein and MMPs. The establishment of specific tumor cell properties was shown to depend on tumor cell implantation at their histological origin *(115).* We demonstrated that the same is true for ECs within tumors by showing that ECs differ phenotypically based on whether the tumor cells were inoculated orthotopically or ectotopically *(82).*

Signal Transduction

ECs frequently display multiple alterations in signal transduction pathways, leading to either cell survival or apoptosis. In particular, several G protein-coupled receptor agonists have been shown to play a role in angiogenesis. Among these, endothelin (ET)-1, by acting directly on EC, affects different stages of neovascularization *(116).* Indeed, ET-1 can modulate proliferation, migration, invasion, protease production, and morphogenesis and positively (but indirectly) modulates angiogenesis through the induction of VEGF. Thus, ET-1 and its receptors

(ET_A and ET_B have been implicated in carcinogenesis through both autocrine and paracrine regulation. In the cases of highly vascularized human glioblastomas, ET-1 is a survival/anti-apoptotic factor that is produced by tumor vasculature mainly by acting via the ET_B receptor found in most cancer cells *(117).* In vessel samples from patients with cerebrovascular disease as well as cerebral neoplasms, ET_B receptor messenger RNA was detected more frequently *(117).* Because of this receptor's crucial role and involvement in angiogenesis, it has been suggested that new therapeutic strategies using specific ET-receptor antagonists could improve anticancer treatment by inhibiting both neovascularization and tumor cell growth *(118).*

One of the most striking phenotypical changes observed was a drastic decrease in caveolin-1 expression in brain tumoral ECs *(119).* Caveolin-1 expression was associated with the extent of cell differentiation *(120)* and was downregulated in rapidly dividing cells *(121)* and in many oncogenically transformed and cancerous cells *(122).* On the other hand, upregulation of caveolin-1 expression was observed in confluent cells and in terminally differentiated cells *(123).* In vitro, it was shown that caveolin-1 expression was regulated during capillary formation, with the highest expression found just before the stabilization of the vessels network *(124).* Downregulation of caveolin-1 may affect the activity of several proteins that are reported to be closely coupled with caveolin-1. We observed increased extracellular signal-regulated kinase (ERK)1/2 phosphorylation in ECs isolated from brain tumors. Activation of ERKs occurs in response to growth factors and phorbol esters and is associated with proliferation and differentiation. ERK1/2 and other components of the Ras-ERK mitogenic pathway are reported to be localized in caveolae *(125).* Demonstration of this regulation in an in vivo system was recently provided using the caveolin-1 null mice model. More specifically, hyperactivation of the p42/44 mitogen-activated protein kinase (MAPK) cascade was demonstrated in heart tissue *(126).* In gliomas, it has been shown that the ERK/MAPK activation may contribute to the neoplastic glial phenotype *(127).* Our results demonstrate that the constitutive activation of the ERK pathway also occurs in glial vascular endothelium. Furthermore, a link was observed in vitro between glioma invasion and ERK activation, with a decrease in glioma cell invasion associated with downregulation of MMP-9 after stable transfection of a mutated ERK *(128).* We previously reported an upregulation of MMP-9 activity in ECs from brain tumors *(82).* Activation of the ERK pathway, as reported here, may correlate with this MMP-9 upregulation. Those results again demonstrate that glioma invasion is associated not only with tumoral cell behavior but also with ECs modulation.

Brain tumor capillaries are also known to be hyperpermeable, causing brain tumor-associated edema. The model proposed to explain this phenomenon is based on tight junction opening associated with VEGF secretion by tumor cells. It was reported that the VEGF receptor VEGFR-2 was localized in endothelial caveolae and associated with caveolin-1. Moreover, caveolin-1 acted as a negative regulator of VEGFR-2 activity *(129).* The loss of brain tumor EC caveolin-1 expression may certainly be one of the molecular mechanisms associated with blood–tumor hyperpermeability. Such observations may have significant implications for the development of antiangiogenic therapies.

Extracellular Matrix Protein Receptors

Angiogenesis and invasion in malignant gliomas share common regulatory mechanisms in which integrins play a crucial role as extracellular matrix protein receptors. In particular, integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ were shown to be necessary for tumor-induced angiogenesis *(130).* However, $\alpha_V\beta_3$ integrins usually are not expressed in normal brain but are expressed in astrocytes and ECs of gliomas, where expression correlates with tumor grade. Moreover, in vitro and in vivo studies showed that IS201, a specific inhibitor for $\alpha_V\beta_3$, has antiangiogenic,

antimitotic, and antimigratory properties and reduces glioma growth *(131).*

Future Strategies

Combined Ionizing Radiation/ Antiangiogenesis Therapies

Despite its efficacy in certain cases, radiotherapy may give rise to secondary tumors that are more invasive and resistant to radiation than the primary tumors that generated them. The molecular basis for this problem may be explained in part, by recent studies reporting increased invasiveness of glioma and pancreatic cancer cells following irradiation *(132–134).* In these studies, the gene expression and proteolytic activity of soluble and membrane-bound MMPs were enhanced by irradiation. In addition, other studies showed that ultraviolet-irradiation *(135)* and ionizing radiation (IR) *(136)* increased the gene expression of Egr-1, a nuclear transcription factor that regulates several biological functions, including cell proliferation and programmed cell death *(137,138),* and that is known to regulate membrane type-1-MMP gene expression *(139,140).* It is well-known that proteolytic remodeling of the extracellular matrix by MMPs is necessary for cells to mobilize within it. Thus, it has been suggested that irradiation may activate the invasive and ECM-adhesive properties of cancer cells through MMP and cell-surface integrin expression *(141,142).* Several lines of evidence indicate that integrins are also a key factor in the interactions of ECs with extracellular matrix components. Studies have clearly established that IR activates cell-surface expression of vascular adhesion molecules *(143)* and that integrin β_3 is activated and accumulated in the lumen of irradiated tumor blood vessels *(142).* These observations led to the generation of β_3-binding proteins that were shown to bind to tumors following exposure to IR. This strategy may eventually allow the targetting of drug delivery to IR-induced neoantigens in tumor neovasculature *(144,145).*

Because angiogenesis is required for a tumor mass to expand and become malignant, it would be reasonable to use radiotherapy to target tumor-derived blood vessels. Until recently, ECs apoptosis in response to radiotherapy was suggested to regulate angiogenesis-dependent tumor growth *(146,147).* However, very little is known about the molecular and cellular events necessary for ECs to escape IR-induced apoptosis. Low-energy laser irradiation was recently shown to promote angiogenesis in an infarcted rat heart and in the chick chorio allantoic membrane (CAM) model *(148)* and may be attributable to upregulation of the nitric oxide pathway in ECs *(149).* Low doses of γ-radiation given to tumor-bearing mice were also found to induce fibroblast growth and angiogenesis prior to tumor recurrence *(150).* These results indicate that irradiation stimulates neovascularization. Because ECs are directly involved in angiogenesis, they certainly would play a central role in IR-enhanced tumor neovascularization.

As mentioned previously, we observed a drastic decrease in caveolin-1 expression in brain tumoral ECs *(119).* Because patients with glioma are often submitted to radiotherapy, we investigated the effects of radiation on the molecular regulation that we identified in the tumoral vasculature. After irradiation, caveolin-1 expression in tumor ECs tends to return to the level in normal brain ECs. This observation suggests that irradiation may have stimulated the maturation of the remaining tumoral capillary network. Therefore, caveolin-1 expression could be a marker for vasculature state at a defined time in the angiogenic process. Histopathological evaluation of a tumor after irradiation showed a large tissular necrotic center (*see* Fig. 3A). However, immunohistochemical study of the remaining tumor vascularization showed an increase in tumor cell density around newly formed vessels in the parenchyma adjacent to the tumor center following irradiation (*see* Fig. 3B). This observation indicates that there is an increased perivascular spreading of tumor cells and suggests increased dissemination of the tumor. Interference with tumor blood vessels through antiangiogenesis or vascular targeting

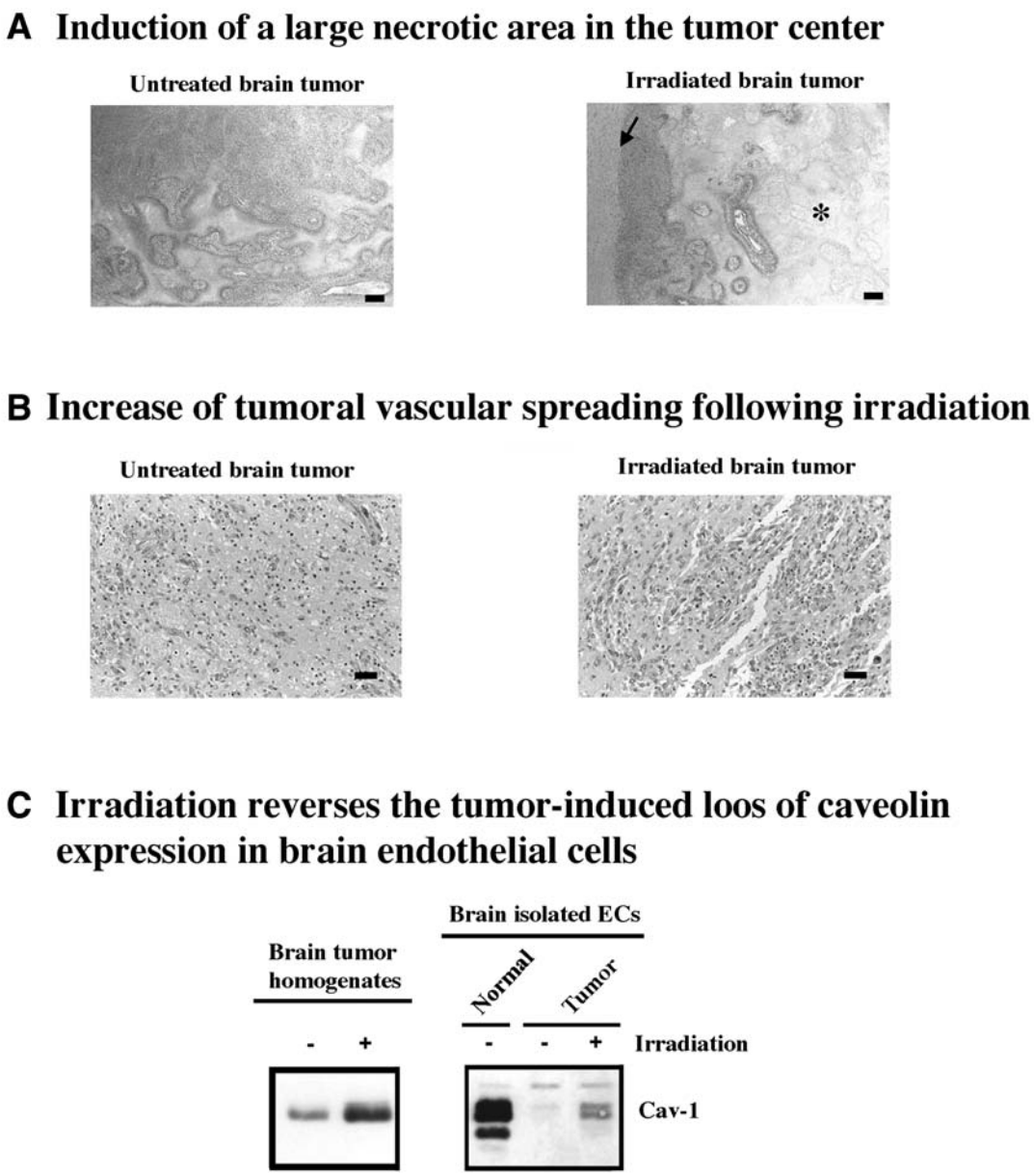


Fig. 3. Effect of radiotherapy on histopathological appearance of CNS-1 tumors. **(A)** Hematoxylin & eosin stain of untreated and irradiated tumors. Irradiation induces a large increase in necrotic areas in the tumor center (asterisk). The tumor brain interface is visible (arrow) (scale bars: 100 µm). **(B)** Effect of irradiation on tumor vascularization and Cav-1 expression. Factor VIII immunostaining of normal parenchyma and tumoral tissue. High magnification photomicrographs were taken at the edge of the tumors to be able to evaluate tumor infiltrative growth pattern and neovascularization level. High perivascular tumor cell density and increased factor VIII cytoplasmic staining is observed after radiotherapy (scale bars: 40 µm). **(C)** Immunodetection of caveolin-1 in brain tumor homogenates and in ECs isolated from control and irradiated brain tumors. Tissue homogenates and isolated ECs were lyzed and subjected to Western blot analysis using caveolin-1 specific antibody.

can indirectly suppress tumor growth. Because tumor cells are dependent on proliferating ECs for survival, it is tempting to target newly forming blood vessels as part of antiangiogenic therapeutic approaches. Accordingly, single doses of radiation were recently shown to preferentially damage the ECs *(146,147),* which could have profound implications for cancer therapy.

Therefore, targeting the vasculature of solid tumors using antiangiogenic agents in parallel with IR seems to be a promising and selective novel treatment *(151).* For instance, combining IR with angiostatin, a proteolytic fragment of plasminogen, improved tumor eradication *(152–154).* However, most of the recent data documented the use of synthetic agents in combination with radiotherapy. An alkylating agent such as temozolomide was shown to prevent irradiation-induced glioma cell invasion *(133).* More recently, the orally available VEGF receptor inhibitor PTK787 *(155),* combined with IR, also was shown to decrease EC proliferation and the number of microvessels in tumor xenografts. Other antiangiogenic agents, such as SU5416 (an inhibitor of VEGF receptor) and SU6668 (an inhibitor for VEGF, fibroblast growth factor [FGF], and PDGF receptors) also were recently shown to increase the antitumor effects of fractionated IR *(154).* We recently reported that the naturally occurring green tea catechin epigallocatechin gallate (EGCg) similarly and very effectively inhibited the VEGF receptor tyrosine kinase activity in ECs *(156).* Both the clinical potential of natural, dietary compounds to decrease the incidence of several cancers as well as the multiple anticancer activities associated with one of the dietary-derived sources (green tea) were recently reviewed *(157,158).* We also showed that the IR-induced tubulogenesis in ECs was antagonized by EGCg *(159).* Therefore, it is tempting to hypothesize that such inhibitory mechanisms may be specifically responsible for the action of EGCg and other VEGFR inhibitors in synergy with IR.

Some other promising synthetic agents include thalidomide *(160),* gemcitabine, paclitaxel, docetaxel, irinotecan, and vinorelbine *(161),* as well as rofecoxib (Vioxx), a specific COX-2 inhibitor that was found to inhibit EC function in combination with IR *(162).* These agents have shown improved toxicity profiles and appear to be effective both as single agents and in combination with other treatments to target angiogenesis-dependent malignancies. However, the radiosensitizing ability of these agents has thus far shown limited efficacy in the standard treatments for patients with a number of types of cancer.

Bone Marrow-Derived Stromal Cells

BMSCs represent a subpopulation of nonhematopoietic pluripotent cells within the bone marrow microenvironment and frequently are referred to as mesenchymal stem cells because of their ability to differentiate into many mesenchymal phenotypes *(163).* In contrast to their hematopoietic counterparts, BMSCs demonstrate a strikingly enhanced ability to adhere to tissue-culture surfaces and to differentiate in culture into osteogenic, chondrogenic, tendonogenic, adipogenic, and myogenic lineages *(164).* More recently, it was confirmed that infused BMSCs may selectively reach tumor sites, proliferate there, and participate in the formation of tumor stroma *(165).* Although it is still debatable whether BMSCs infused via the systemic circulation are capable of any engraftment *(166),* recent evidence suggests that BMSCs have the ability to cross the BBB *(167)* to migrate throughout the forebrain and cerebellum and thus be potentially useful as vectors for treating a variety of CNS disorders *(168).* Accordingly, we recently provided molecular and cellular evidence that hypoxic environment such as that encountered within tumors regulated several angiogenic properties of BMSCs *(169).* However, molecular studies of the phenotypical and functional properties of BMSCs in neovascularization and their role in microvascular network remodeling in response to tumor angiogenic factors have received little attention. The recently reported unorthodox plasticity and endothelial-like phenotype of

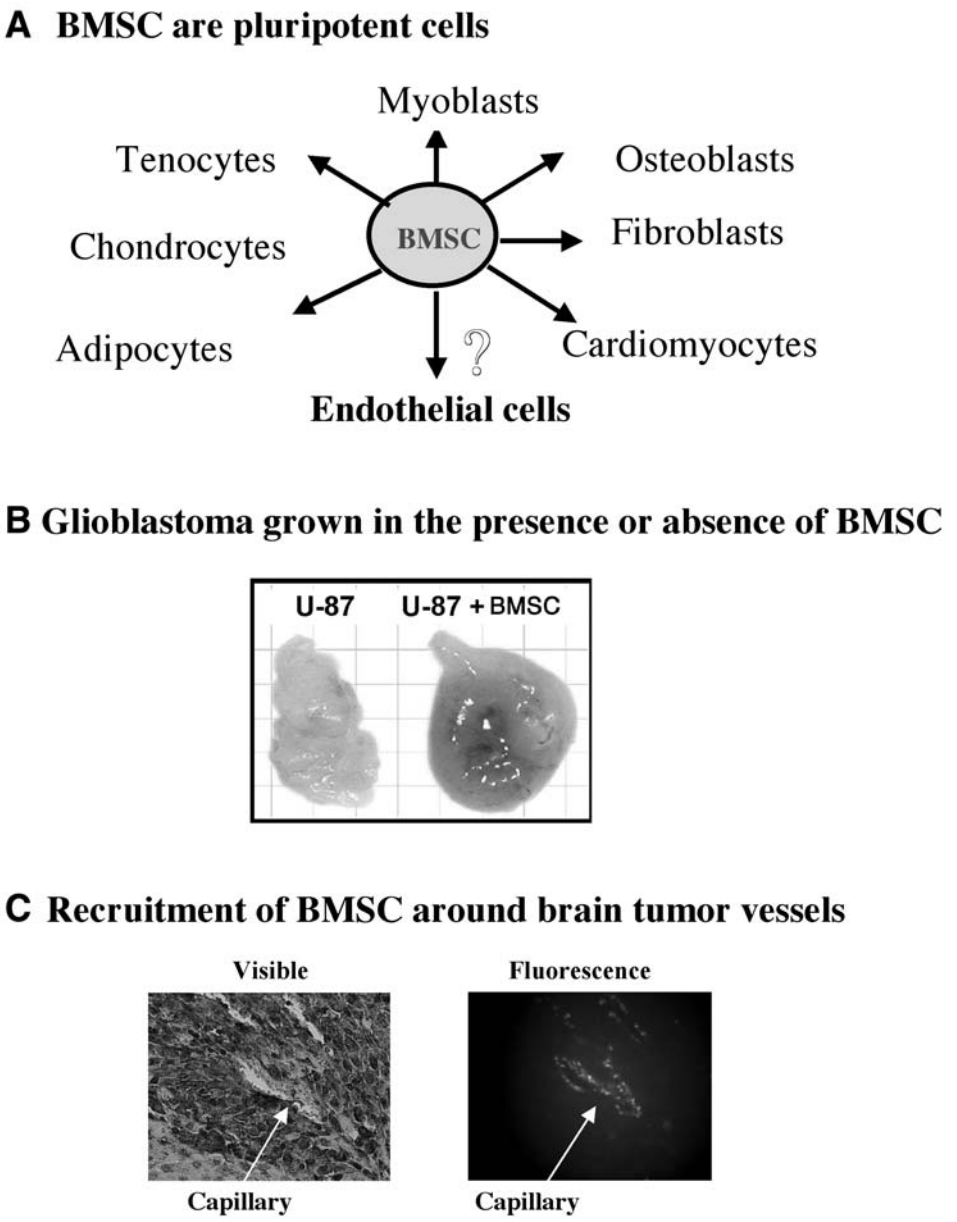


Fig. 4. Involvement of BMSC in angiogenesis and brain tumor development. **(A)** BMSCs are pluripotent cells with a strikingly enhanced ability to differentiate into various type of cells. **(B)** Coinjection of human glioblastoma (U-87) with BMSCs increases the growth and the vascularization of the tumor. Tumors were dissected and photographs taken 30 d after cells injection. **(C)** Following coinjection of green fluorescent protein (GFP)-positive BMSCs with U-87, immunofluorescence detection shows that a portion of the GFP-positive BMSCs are found around brain tumor vessels. Thus, BMSCs may participate in the vascularization of an subcutaneously implanted U-87 glioma-derived tumor.

BMSCs *(170,171)* may provide new insights into their potential role in tumor vascularization. This is further strengthened by the observation that BMSCs may have the ability to be recruited at active sites of angiogenesis, indicating that they could be involved in host-derived angiogenic response in vivo *(172,173).* These observations are consistent with a recent study suggesting that BMSCs also participate in angiogenesis and arteriogenesis *de novo (173)* as well as in the vascularization of a subcutaneously implanted U-87 glioma-derived tumor (*see* Fig. 4B).

Antiangiogenic Gene Therapy

Gene therapy is a therapeutic strategy that may be able to exploit the new discoveries in the field of angiogenesis. Formulation of new blood vessels, which is highly activated in tumors, may serve as an attractant for cellular vehicles. It is assumed that antiangiogenic cancer therapy requires prolonged administration of the drug to the patient. Gene therapy has the potential to produce the therapeutic agent in high concentrations in a local area for a sustained period, thereby avoiding problems associated with long-term administration of recombinant proteins, monoclonal antibodies, or antiangiogenic drugs. Free viral vectors (mutated adenovirus or retrovirus) expressing natural antiangiogenic factors have been employed in experimental glioma tumors *(174,175).* Other gene transfer methods have been used, such as engineered C6 glioma cells that endogenously express mouse endostatin *(176).* Genetically modified ECs can also be stably engrafted to growing gliomas, suggesting that EC implantation may provide a means of delivering therapeutic genes to brain neoplasms and other solid tumors *(177–179).* As antiangiogenic therapy against experimental glioblastoma using genetically engineered cells has already been described *(180),* one can hypothesize that the use of BMSCs transduced using retroviral vectors to secrete antiangiogenic molecules may prove to be efficient in clinical applications targeting neoplastic disorders.

Conclusions

The development of efficient therapies for brain tumors requires better knowledge about the molecular, functional, and anatomical properties of the vascular bed as the number of molecules with antiangiogenic properties increases. Various issues must be addressed when establishing the efficacy of a given antiangiogenic treatment. These include the identification of adequate surrogate markers for evaluating the therapeutic efficacy, monitoring tumor growth, and determining the angiogenic status to define therapeutic windows for antiangiogenic brain tumor therapies. New technologies and experimental approaches described in this article review may allow researchers to systematically define the unique molecular profile of brain ECs, which orchestrates and sustains the BBB properties, and to assess the influences of various environmental and developmental stimuli. These recent advances and findings provide new insights into both the extent and causes of EC diversity and into pathologies associated with BBB dysfunction. Therefore, brain ECs have now become crucial pharmacological targets of various strategies for the treatment of neuropathologies, including brain tumors.

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