Laboratory Investigation (2004) 84, 397–405

www.laboratoryinvestigation.org

Mini Review

Vaso-occlusive and prothrombotic mechanisms associated with tumor hypoxia, necrosis, and accelerated growth in glioblastoma

Daniel J Brat[1] and Erwin G Van Meir[2]

[1]*Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA and* [2]*Departments of Neurosurgery and Hematology/Oncology and Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, USA*

Glioblastoma (GBM) has explosive biologic properties with rapid clinical progression leading to death. Its distinguishing pathologic features, necrosis with surrounding pseudopalisades and microvascular hyperplasia, are believed to be instrumental to its accelerated growth. Microvascular hyperplasia arises in response to the secretion of proangiogenic factors by hypoxic pseudopalisades and allows for peripheral neoplastic expansion. Mechanisms underlying necrosis and hypoxia remain obscure, but vaso-occlusive and prothrombotic contributions could be substantial. Recent investigations on the origin of pseudopalisades suggest that this morphologic phenomenon is created by a tumor cell population actively migrating away from a central hypoxic region and that, in at least a significant subset, hypoxia-induced migration appears due to vaso-occlusion caused by intravascular thrombosis. Both vascular endothelial growth factor induced vascular permeability to plasma coagulation factors and the increased neoplastic expression of tissue factor likely contribute to a prothrombotic state favoring intravascular thrombosis. In addition to prothrombotic mechanisms, vaso-occlusion could also result from angiopoietin-2-mediated endothelial cell apoptosis and vascular regression, which follows neoplastic co-option of native vessels in animal models of gliomas. Vaso-occlusive and prothrombotic mechanisms in GBM could readily explain the presence of pseudopalisades and coagulative necrosis in tissue sections, the emergence of central contrast enhancement and its rapid peripheral expansion on neuroimaging, and the dramatic shift to an accelerated rate of clinical progression. Since the hypoxic induction of angiogenesis appears to support further neoplastic growth, therapeutic targeting of the underlying vascular pathology and thrombosis could provide a new means to prolong time to progression.
Laboratory Investigation (2004) **84,** 397–405, advance online publication, 1 March 2004; doi:10.1038/labinvest.3700070

Keywords: glioblastoma; astrocytoma; hypoxia; necrosis; thrombosis; angiogenesis; brain tumor

Glioblastoma (GBM; WHO (World Health Organization) grade IV) is the most common and highest-grade primary brain tumor and has a truly dismal prognosis.[1] Following surgical resection and radiation therapy—the current therapeutic gold standard—mean survival is only 50 weeks.[2] Perhaps more telling of its explosive natural behavior, survival of patients treated by surgical resection alone averages 14 weeks.[3] Lower-grade astrocytomas (ie WHO grade II and III astrocytomas) are also ultimately fatal, but have substantially slower growth rates and longer survivals (3–8 years). With rare exception, patients with lower-grade tumors will eventually die from progression of their tumors to GBM, with short survival periods following this transition. Thus, whether arising *de novo* or from a lower-grade precursor, transition to GBM represents an abrupt turning point, with rapid clinical progression leading to death. The biologic properties that make the infiltrating astrocytomas fatal are known: their diffuse infiltration makes complete surgical resection nearly impossible; they are generally resistant to adjuvant therapies; and they eventually invade nervous system structures critical for life. In contrast, reasons for the explosive growth following malignant progression to GBM have not been fully explained. This review focuses on an emerging

Correspondence: DJ Brat, MD, PhD, Department of Pathology and Laboratory Medicine, Emory University Hospital, H-176, 1364 Clifton Road, NE, Atlanta, GA 30322, USA.
E-mail: dbrat@emory.edu
Received 12 January 2004; accepted 12 January 2004; published online 1 March 2004

concept regarding mechanisms responsible for the accelerated growth in GBM, emphasizing a link between intravascular thrombosis, hypoxia, necrosis, and angiogenesis.

Accelerated growth in GBM

Neuroimaging and pathologic features that distinguish GBMs from lower-grade astrocytomas (grades II and III) provide clues to their escalated growth potential. By magnetic resonance imaging (MRI), lower-grade lesions are mildly expansile and show signal abnormalities—best seen on T2-weighted and FLAIR images—reflecting vasogenic edema generated in response to infiltrative tumor cells (Figure 1). These tumors show minimal or no enhancement following administration of intravenous contrast agents due to an intact blood–brain barrier and a lack of tumor necrosis. In tissue sections, grade II and III astrocytomas consist of neoplastic cells diffusely infiltrating brain parenchyma. Cell density, nuclear anaplasia, and proliferation indices increase as tumors progress. Measurements of the radial growth rates of grade II astrocytomas AAs in patients who have not received adjuvant therapy demonstrate a modest annual increase of 2 mm/year.[4,5] Slow, persistent expansion characterizes these grade II–III astrocytomas.

Growth patterns change dramatically following transition to GBM. Radial growth rates can accelerate to values nearly 10 times greater than those in grade II astrocytomas.[5] Moreover, a central, contrast-enhancing component emerges from within the infiltrative astrocytoma and rapidly expands outward (Figure 1). In tissue sections, the pathologic features that distinguish GBM from lower-grade astrocytomas are found near the contrast-enhancing rim and include (1) necrotic foci, usually with evidence of peripheral cellular pseudopalisading, and (2) microvascular hyperplasia.[2] Pseudopalisades are dense collections of tumor cells surrounding necrosis that are pathophysiologically linked with adjacent microvascular hyperplasia (Figure 2). The latter is an exuberant form of angiogenesis that arises in response to the secretion of proangiogenic factors (VEGF, interleukin-8) by pseudopalisades.[6] Pseudopalisading necrosis and microvascular hyperplasia are two of the most powerful predictors of poor prognosis among the diffuse gliomas.[7] Rather than mere morphologic markers, these structures are almost certainly mechanistically instrumental in the accelerated growth properties that characterize the grade III to IV transition.

Angiogenesis supports peripheral tumor growth

While necrosis has long been recognized as a marker of aggressive behavior in diffuse gliomas, *by itself* it does not explain rapid tumor progression. Indeed, tumor cell death is the goal of most adjuvant therapies. Rather, pseudopalisades that surround necrosis are intimately related to microvascular hyperplasia, which provides a new vasculature for rapid neoplastic expansion. Pseudopalisading cells are hypoxic and express high levels of hypoxia-inducible regulators of angiogenesis including vascular endothelial growth factor (VEGF).[8,9] VEGF concentrations have been found to be 200–300-fold higher in the cystic fluid of human GBMs than in serum.[10] Such high levels result from hypoxia-inducible factor (HIF)-mediated transcription of the *VEGF* gene by hypoxic pseudopalisades.[11,12] Inhibition of this pathway suppresses tumor growth.[13] Once expressed and secreted, extracellular VEGF binds to its high-affinity receptors, VEGFR-1 and VEGFR-2, which are upregulated on endothelial

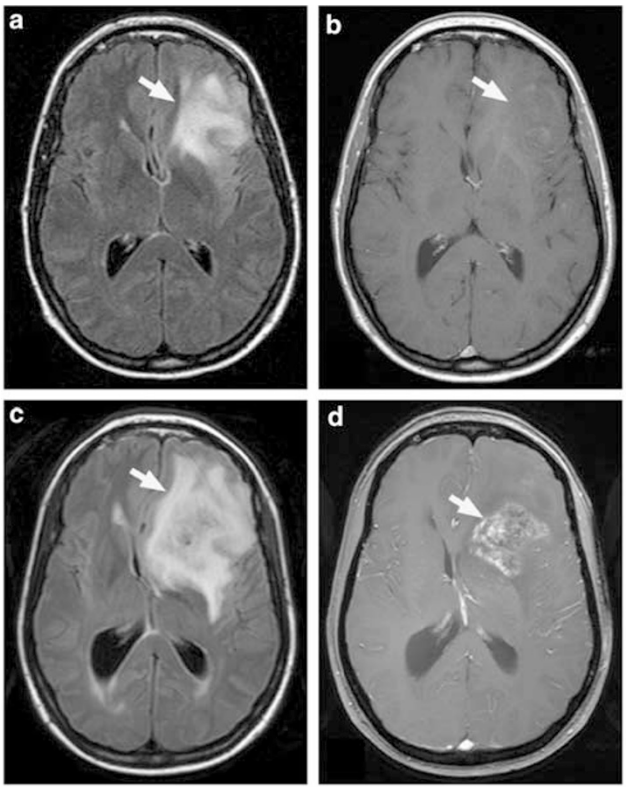

Figure 1 AA (WHO grade III) and GBM (WHO grade IV) have distinct growth patterns by MRI. (**a**) A 47-year-old woman with seizures was found on MRI to have a left frontal lobe lesion, which on biopsy proved to be an AA. Axial MR images shows increased signal intensity on FLAIR imaging, representing vasogenic edema in response to infiltrating tumor cells (arrow). (**b**) Following the administration of contrast agents, most AAs do not show enhancement on T1-weighted images due to an intact blood–brain barrier and a lack of central necrosis (arrow). (**c**) After 1 year, the same patient developed worsening symptoms and was rescanned, revealing progression to GBM. GBMs also have hyperintense regions at their periphery on FLAIR imaging, indicative of diffusely infiltrating tumor cells (arrow). (**d**) A distinguishing feature is the emergence of a contrast-enhancing component centrally, which rapidly expands radially (arrow). Note the degree of midline shift in the GBM that was not present in the AA.

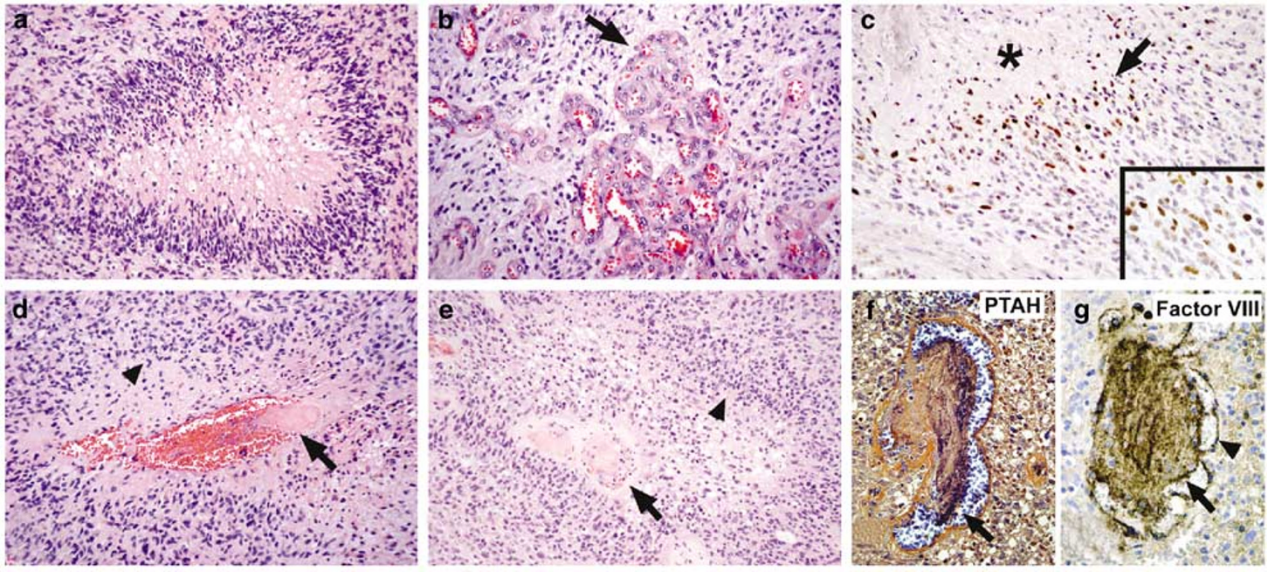

Figure 2 Pathologic features of GBM. (**a**) Pseudopalisades are characterized by an accumulation of tumor cells around central necrosis and typically have internal, peripheral zones of fibrillarity. (**b**) Microvascular hyperplasia (arrow) is a form of angiogenesis that is induced by hypoxic pseudopalisading cells and is usually present in regions adjacent to necrosis. (**c**) Low and high (inset) magnification of immunohistochemistry for HIF-1α performed on a human GBM demonstrating increased nuclear staining in pseudopalisades (arrow) around necrosis (asterisk) but not adjacent astrocytoma. (**d**), (**e**) Intravascular thrombosis (arrows) within pseudopalisades at different stages of development. (**d**) A central vessel occluded by thrombus (arrow) is dilated proximal to the occlusion and surrounded by delicate fibrillarity and a low density of tumor cells, most likely due to migration of pseudopalisading cells (arrowhead) away from perivascular hypoxia. (**e**) Necrosis is present adjacent to a thrombosed vessel (arrow) within a developing pseudopalisade (arrowhead). (**f**), (**g**) Fibrin within an unorganized intravascular thrombus in a GBM stains with PTAH (arrow), while aggregated platelets (arrow) and vascular walls (arrowhead) are immunoreactive for factor VIII.

cells of high-grade gliomas, but not in normal brain.[14] Receptor binding to VEGF, in turn, leads to angiogenesis in hypoxic regions adjacent to pseudopalisades, eventually leading to a vascular density in GBMs that is among the highest of all human neoplasms. The precise type of angiogenesis, microvascular hyperplasia, is characterized by numerous, enlarged, rapidly dividing endothelial cells, pericytes and smooth muscle cells that form tufted microaggregates at the leading edge of sprouting vessels.[15] In its most florid form, angiogenesis takes the shape of 'glomeruloid bodies'—a feature that is most characteristic of GBM, but is also an independent marker of poor prognosis in prostate cancer, breast cancer, and melanoma.[16] Since necrosis and hypoxia are located in GBM's core and near the contrast-enhancing rim, the most biologically relevant hypoxia-induced angiogenesis occurs futher peripherally, favoring neoplastic growth outward. The permissive nature of the central nervous system parenchymal matrix to diffuse infiltration by individual glioma cells allows for this burst of peripheral expansion.[17]

Necrogenesis in GBM

The relationship between pseudopalisades and angiogenesis has been fairly well established, yet mechanisms initiating cell death (necrosis and/or apoptosis) and pseudopalisade formation have not. Cell death is fundamental to GBM, occurring as apoptosis, programmmed death of individual cells, and coagulative necrosis, the sheet-like coalescence of dying tissue caused by extrinsic factors. Apoptosis can be initiated through mechanisms that include death receptor (DR) ligation by members of the tumor necrosis factor (TNF) family. Best characterized in GBM are FasL (CD95L)/Fas (CD95) and TNF-related apoptosis-inducing ligand (TRAIL)/DR4/5. TRAIL induces apoptosis most effectively by binding to DR5 and signaling through its cytoplasmic death domains, ultimately activating caspase-8.[18,19] Both Fas and FasL messenger RNA (mRNA) levels are higher in astrocytomas than normal brain and correlate with tumor grade.[20,21] Most Fas expression in GBM is within pseudopalisades corresponding to their higher levels of apoptosis.[22,23] Contact between tumor cells expressing FasL and those expressing Fas may facilitate apoptosis.[24] Overall, however, levels of apoptosis are generally low in malignant gliomas; apoptosis accounts for a slim minority of cell death; and apoptotic rates do not correlate with prognosis.[25,26]

In contrast, coagulative necrosis represents the majority of cell death in GBMs and its degree is inversely related to patient survival.[2,27,28] The mere presence of focal necrosis remains one of the strongest independent markers of poor prognosis.[7,29,30] Coagulative necrosis is seen

pathologically as either microscopic foci with surrounding pseudopalisading cells ('pseudopalisading necrosis') or as large confluent expanses of dying tumor and nontumor elements. Pseudopalisades are characteristic of GBM and likely due to tumor and stromal characteristics. When small ($<100\,\mu$m), they often surround fibrillar zones (without frank necrosis); when larger ($>500\,\mu$m), they always contain central necrosis and most show peripheral fibrillarity.[2] Their morphologic spectrum suggests they evolve over time to incorporate larger areas. Establishing mechanisms underlying pseudopalisade formation and its role in necrosis is critical since they potentially could precede all forms of coagulative necrosis in GBM.

Classic infarction could be involved, in which tumor necrosis would result from the metabolic demands of an enlarging tumor exceeding its nutrient supply.[31] Reduced oxidative phosphorylation and intracellular ATP culminate in breached membrane integrity and proteolysis. Infarction could also arise secondary to a compromised vascular supply. Increased interstitial pressure due to vasogenic edema is typical of GBM and could promote vascular collapse. Neoplastic cells themselves could alter vascular integrity by invading perivascular spaces and vascular walls.[32] Lastly, intravascular thrombosis—the formation of a fibrin-platelet clot—occurs frequently in GBM and could cause vaso-occlusion and infarction. Procoagulation factors such as tissue factor (TF) and plasminogen activator inhibitor 1 (PAI-1) are increased in GBM and correlate with the extent of necrosis.[33,34] Levels of tissue type plasminogen activator (tPA), which lyses fibrin clots, are decreased.[35] An environment favoring intravascular thrombosis could result in compromised blood flow, ischemia, and necrosis.

What is the pathogenesis of pseudopalisades?

Recent studies have shed light on the initial events of pseudopalisade formation, which may in turn explain necrogenesis.[36] These investigations entertained and then formally excluded other potential explanations that might result in an increased tumor cell density around a central area of necrosis. First, pseudopalisading cells were found to be *less* proliferative than adjacent astrocytoma cells, indicating that they are not rapidly dividing cells that have 'outgrown their blood supply' as has been widely believed. Second, they were not due to an influx of other, non-neoplastic cells such as inflammatory cells, but rather composed predominantly of astrocytic tumor cells. Third, pseudopalisades showed *increased* levels of apoptosis compared to adjacent astrocytoma, indicating that they do not accumulate due to a survival advantage. Instead, pseudopalisades are most likely formed by a wave of actively migrating tumor cells that are moving away from a central area of hypoxia. Supporting this contention, cells in pseudopalisades exhibit an adaptive response to hypoxia as evidenced by their nuclear overexpression of HIF-1α (Figure 2c);[36,37] hypoxic GBM cells migrate more rapidly than normoxic cells *in vitro*; and both hypoxic cells *in vitro* and pseudopalisades in GBM tissue sections show expression of extracellular matrix proteases associated with invasion, including MMP-2 and uPAR.[38–43] Secretion of MMP-2 activity by gliomas is markedly increased under hypoxic conditions. Together, the evidence suggests that pseudopalisades are formed by hypoxic, actively migrating neoplastic cells that have imposed themselves on a less mobile population.

At least two mechanisms could explain hypoxia-induced migration that leads to pseudopalisades around necrosis. One possibility is that cells at greatest distance from arterial supplies become hypoxic after a critical point in tumor growth due to increased metabolic demands (Figure 3, Mechanism 1). Migration outward towards nearby vessels would leave a central zone filled with fibrillar processes as well as nonmigrated cells that eventually become necrotic. An alternative explanation is that blood vessels within the neoplasm become occluded or collapse, resulting in perivascular tumor hypoxia and cellular migration away, both at the site of occlusion, and proximal and distal to it (Figure 3, Mechanism 2). In favor of the latter, many pseudopalisades have a long, narrow, and winding ('serpiginous') pattern when viewed in longitudinal tissue sections, suggesting an underlying vascular substrate associated with their emergence. Chief among arguments opposing vaso-occlusion as a mechanism of necrosis is that pseudopalisades do not always appear to contain a central blood vessel,

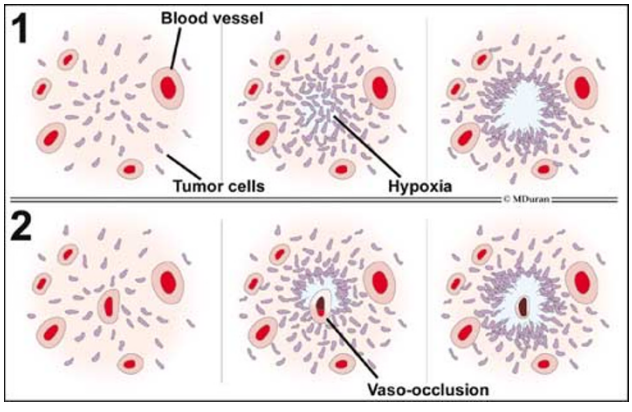

Figure 3 Potential mechanisms of pseudopalisade formation due to hypoxia-induced migration. (1) One possibility is that tumor cells at greatest distance from arterial supplies become hypoxic (shaded in blue) after a critical point in tumor growth due to increased metabolic demands and migrate toward peripheral vessels, leaving a central clear zone. (2) Alternatively, vascular occlusion or collapse within the neoplasm could lead to central, perivascular hypoxia, followed by tumor cell migration toward a viable blood supply. Illustration by Mica Duran.

occluded or not. However, the morphologic identification of such vascular structures is impossible in many instances given the extensive necrosis within pseudopalisades. In addition, tissue sampling most likely accounts for an under-representation of dysfunctional vascular structures within pseudopalisades. The idea that vascular pathology precedes pseudopalisading necrosis in GBMs deserves further attention because both experimental models and pathologic observations support such a mechanism.

Mechanisms of vascular pathology in GBM

Vascular Regression

Experimental animal models of gliomas have suggested that the initial, preangiogenic stages of neoplastic growth actually involve regression of native blood vessels. New tumor cells first gain access to a vascular supply by 'co-opting' the host's blood vessels.[44,45] In response to co-option, vascular endothelial cells upregulate angiopoietin-2 (Ang-2) by mechanisms that are poorly understood. Ang-2 acts in an autocrine fashion on tumoral blood vessels as a Tie-2 receptor antagonist. In the absence of VEGF, Tie-2 receptor blockage leads to vascular destabilization, endothelial cell apoptosis, and vascular regression. Only neoplastic foci that can induce angiogenesis in this setting remain viable and grow. Typically, this involves the HIF-dependent expression of VEGF. In human specimens, Ang-2 is expressed by endothelial cells of high-grade gliomas but not low-grade gliomas or normal brain.[46,47] Its expression precedes endothelial apoptosis and the disruption of endothelial–extracellular matrix interaction, indicating that it could cause vascular injury.[45] Other arguments hold that Ang-2 induces vascular changes, but does not induce apoptosis. For example, based on intravital epifluorescence microscopy of VEGF, VEGFR-2, and Ang-2 in growing gliomas, Ang-2 expression was found to correlate with vascular remodeling and sprouting, but not with apoptosis.[48] Indeed, it is clear that Ang-2 mediates vascular remodeling in the presence of VEGF.[49] However, the weight of evidence indicates that Ang-2 antagonism of Tie-2 in the absence of VEGF has more severe effects on endothelium, causing apoptosis and regression.[50,51] Ang-2-mediated vascular pathology could initiate the hypoxic conditions that precede tumor cell migration and pseudopalisade formation in GBM.

Intravascular Thrombosis and Vaso-occlusion

Intravascular thrombosis, either through engagement of intrinsic or extrinsic pathways of coagulation, could substantially contribute to vaso-occlusive mechanisms underlying necrosis in GBM, whether secondary to Ang-2-mediated vascular events or independently. A strong relationship between abnormal blood clotting and human malignancy is well established.[52] Patients with GBM are at high risk for developing deep vein thrombosis in the lower extremities as well as pulmonary thromboembolism, indicating a profound systemic disturbance in coagulation.[53] Perhaps less appreciated, intravascular thrombosis is a frequent finding both macroscopically and microscopically within GBM tissue, noted histologically in over 90% of well-sampled cases.[33,36,54] Importantly, the frequencies of intravascular thrombosis in neoplastic tissue and at distant sites are much higher in patients with GBM than AA. Critical prothrombotic events must occur in this transition.

A recent morphologic survey of vascular pathology and intravascular thrombosis within human GBM specimens found that over 50% of pseudopalisades had evidence of a central vascular lumen, either viable, degenerating, or thrombosed.[36] In all, 20% of pseudopalisades contained a vessel with intravascular thrombosis as evidenced by complete luminal occlusion by an unorganized platelet and fibrin clot (Figure 2d–g). Analysis of pseudopalisade sizes and shapes led to the conclusion that tissue sampling and tangential sections could account for an underestimation of the true frequency of intravascular thrombosis within pseudopalisades, resulting in a historic underappreciation of its relevance to necrosis in GBM. Thus, we believe a substantial subset of pseudopalisades around necrosis represents hypoxic tumor cells migrating from vaso-occlusion secondary to thrombosis. There is a strong possibility that this mechanism is more generally relevant to coagulative necrosis in GBM.

What might cause increased thrombosis within GBM? At the transition of AA to GBM, numerous tumoral and vascular changes likely promote blood clotting. Normal vessels of the central nervous system show minuscule levels of protein diffusion though their walls due to a highly restrictive blood–brain barrier. The latter is formed primarily by endothelial tight junctions, but also has contributions from astrocytic foot plates, extracellular matrix, and endothelial–pericytic interactions.[55,56] This barrier becomes breached in GBM, which can be visualized radiologically by their high degree of contrast enhancement. Such enhancement is due to the increased permeability of vessel walls to both intravenously injected contrast agents and to proteins that bind them, such as albumin.[57,58] As a result, contrast agents leak into tumoral but not normal brain tissue. Microscopically, there is increased fenestration of vessel walls, pericyte detachment, and alteration of the extracellular matrix.[59] All events that initate this increased permeability have not been completely defined, but VEGF expression by neoplastic cells is a known cause of increased vascular leakage.[60,61] The end result is that plasma coagulation factors enter tissue spaces where they

are activated, leading to the formation of a fibrin plug and platelet aggregation.

Tissue factor

A critical regulator of tissue hemostasis and a potent stimulant of thrombosis is TF, a 47 kDa transmembrane glycoprotein receptor.[62] In normal tissue, TF is expressed almost exclusively by stromal cells. A disruption of vascular integrity is necessary for TF to bind to its activating ligand from the plasma, factor VII/VIIa, in order to initiate thrombosis. The end result of TF/factor VIIa activation is the generation of thrombin from prothrombin, causing platelet aggregation, fibrin deposition, and local hemostasis. The tight regulation of TF expression that is present in normal tissue is lost in pathologic conditions including neoplasia. A variety of cancers show increased expression of TF by neoplastic cells, stroma, and endothelium. A direct correlation between TF levels and tumor grade has been noted for multiple tumor types,[63–65] including gliomas.[34] TF protein is expressed in over 90% of malignant astrocytomas, but only in 10% of grade I and II astrocytomas. *In situ* hybridization localized its expression to neoplastic cells. Similarly, a reverse transcriptase-polymerase chain reaction study found that, on average, TF mRNA levels were 500 × greater in astrocytomas than normal brain tissue and that its levels correlated with histologic grade.[66] Precise mechanisms responsible for increased TF expression by gliomas are not known. Tissue hypoxia leads to increased rates of thrombosis as well as increased TF expression by mononuclear phagocytes. The latter depends on Egr1-mediated transcription rather than HIF.[67–69] Whether tumor hypoxia leads to increased TF expression in gliomas is unknown. Nonetheless, its high expression in GBM could explain the strong tendency toward thrombosis.

Activation of plasma coagulation factors, thrombin in particular, has biologic significance beyond clot formation. Thrombin is a potent physiologic activator of protease-activated receptors (PARs), a family of G-protein-coupled transmembrane receptors. PAR1, the family's prototype, is activated when thrombin cleaves its amino-terminal extracellular domain and unmasks a new N-terminus, which then serves as the receptor's ligand.[70] Activated PAR1 tranduces intracellular signals by coupling to G-proteins, predominantly Gαi, Gαq, and Gα12/13.[71] Secondary signals are generated through Rho, phospholipase C (IP3 and diacylglycerol), and inhibition of adenyl cyclase. Although PAR1 is expressed at low levels in most normal epithelia, it is aberrantly overexpressed by a variety of carcinomas including those of breast, colon, lung, and stomach.[72,73] PAR1 activation can transform cells and is able to enhance tumorigenicity, in large part by signaling through Gαq and Gα13.[74,75] It is also clear that PAR1 activation promotes invasive and metastatic properties of malignant cells.[72,76] Mechanisms of increased invasion include its ability to direct cytoskeletal actin rearrangements, phosphorylation of focal adhesion kinases, and recruitment of $\alpha v\beta 5$ integrin to contact sites.[77] PAR1 protein is present in both human and mouse brain, mostly in astrocytes, where it can be activated by thrombin.[78,79] More recent investigations of human GBM cell lines and short-term cultures derived from human specimens have demonstrated PAR1 expression on tumor cells. Its activation by thrombin and PAR1 agonists causes increased phospho-inositol (PI) hydrolysis and calcium mobilization, presumably coupling through Gαq.[79] More work will be required to determine the biologic relevance of TF activation of thrombin and consequent PAR1 signaling in gliomas.

Conclusion

Pseudopalisades and the ensuing microvascular hyperplasia that are associated with accelerated growth in GBM may result from the following sequence (Figure 4): (1) vascular occlusion, possibly related to Ang-2-mediated endothelial apoptosis and often associated with intravascular thrombosis; (2) hypoxia in regions surrounding vascular pathology; (3) outward migration of glioma cells away from hypoxia creating a peripherally moving wave (pseudopalisade); (4) death of nonmigrated cells leading to central necrosis; (5) secretion of soluble

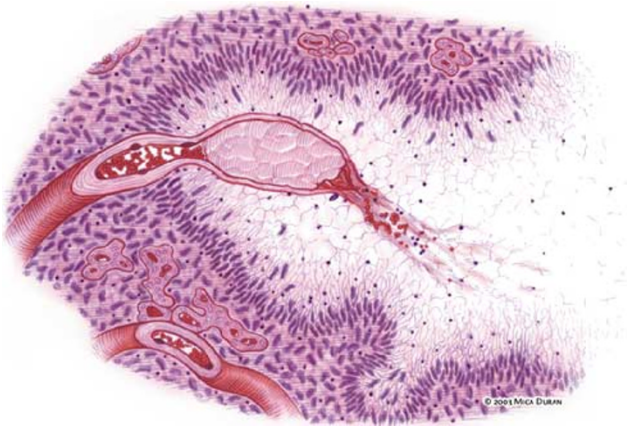

Figure 4 Schematic representation of pseudopalisade formation in GBM. Vaso-occlusion/collapse and intravascular thrombosis lead to tissue hypoxia in the perivascular region. Hypoxic tumor cells then migrate away along cellular processes, leaving a fibrillar center initially. Hypoxia-induced cellular migration also occurs at sites proximal and distal to vaso-occlusion and thrombosis due to a lack of functional blood flow. Tumor cells that do not migrate become hypoxic and undergo apoptosis or necrosis, eventually leaving an enlarging central necrotic zone. Vessels distant to the vascular occlusion become necrotic and degenerate. Hypoxic pseudopalisading cells show increased HIF-mediated transcription and VEGF secretion, leading to microvascular hyperplasia nearby. Illustration by Mica Duran.

proangiogenic factors (VEGF, IL-8) by hypoxic pseudopalisading cells; (6) an exuberant angiogenic response creating microvascular proliferation in regions peripheral to central hypoxia; (7) enhanced outward expansion of infiltrating tumor cells toward a new vasculature. Since both necrosis and glomeruloid vascular proliferation are also markers of poor prognosis in other types of cancer,[15,16] the identification of their underlying mechanisms may have more general implications for tumor angiogenesis and malignant progression. Once identified, the pathophysiologic triggers underlying vaso-occlusion will become attractive, novel targets for antitumor therapy.

Acknowledgement

This work was supported in part by US Public Health Service National Institutes of Health (NIH) Grants NS-42934 (DJB), CA-86335, CA-87830, and NS-41403 (EGVM).

References

1 CBTRUS. Statistical Report: Primary Brain Tumors in the United States, 1995–1999. Central Brain Tumor Registry of the United States: Chicago, IL, 2002.
2 Kleihues P, Burger PC, Collins VP, *et al.* Glioblastoma. In: Kleihues P, Cavenee WK (eds). Pathology and Genetics of Tumours of the Nervous System, 2nd edn. International Agency for Research on Cancer: Lyon, 2000, pp 29–39.
3 Taveras JM, Thompson HG, Pool JL. Should we treat glioblastoma multiforme? A study of survival in 425 cases. Am J Roentg 1962;87:473–479.
4 Mandonnet E, Delattre JY, Tanguy ML, *et al.* Continuous growth of mean tumor diameter in a subset of grade II gliomas. Ann Neurol 2003;53:524–528.
5 Swanson KR, Bridge C, Murray JD, *et al.* Virtual and real brain tumors: using mathematical modeling to quantify glioma growth and invasion. J Neurol Sci 2003;216:1–10.
6 Brat DJ, Castellano-Sanchez A, Kaur B, *et al.* Genetic and biologic progression in astrocytomas and their relation to angiogenic dysregulation. Adv Anat Pathol 2002;9:24–36.
7 Daumas-Duport C, Scheithauer B, O'Fallon J, *et al.* Grading of astrocytomas. A simple and reproducible method. Cancer 1988;62:2152–2165.
8 Plate KH, Breier G, Weich HA, *et al.* Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. Nature 1992;359:845–848.
9 Plate KH. Mechanisms of angiogenesis in the brain. J Neuropathol Exp Neurol 1999;58:313–320.
10 Takano S, Yoshii Y, Kondo S, *et al.* Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. Cancer Res 1996;56:2185–2190.
11 Shweiki D, Itin A, Soffer D, *et al.* Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1999;359:843–845.
12 Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001;7:345–350.
13 Kung AL, Wang S, Klco JM, *et al.* Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat Med 2000;6:1335–1340.
14 Plate KH, Breier G, Weich HA, *et al.* Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible *in vivo* regulatory mechanisms. Int J Cancer 1994;59:520–529.
15 Brat DJ, Van Meir EG. Glomeruloid microvascular proliferation orchestrated by VPF/VEGF: a new world of angiogenesis research. Am J Pathol 2001;158:789–796.
16 Straume O, Chappuis PO, Salvesen HB, *et al.* Prognostic importance of glomeruloid microvascular proliferation indicates an aggressive angiogenic phenotype in human cancers. Cancer Res 2002;62:6808–6811.
17 Bellail A, Hunter SB, Brat DJ, *et al.* Microregional extracellular molecular heterogeneity in brain modulates glioma cell invasion. Int J Biochem Cell Biol, (in press).
18 Rieger J, Naumann U, Glaser T, *et al.* APO2 ligand: a novel lethal weapon against malignant glioma? FEBS Lett 1998;427:124–128.
19 Hao C, Beguinot F, Condorelli G, *et al.* Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis in human malignant glioma cells. Cancer Res 2001;61:1162–1170.
20 Tachibana O, Nakazawa H, Lampe J, *et al.* Expression of Fas/APO-1 during the progression of astrocytomas. Cancer Res 1995;55:5528–5530.
21 Gratas C, Tohma Y, Van Meir EG, *et al.* Fas ligand expression in glioblastoma cell lines and primary astrocytic brain tumors. Brain Pathol 1997;7:863–869.
22 Tachibana O, Lampe J, Kleihues P, *et al.* Preferential expression of Fas/APO1 (CD95) and apoptotic cell death in perinecrotic cells of glioblastoma multiforme. Acta Neuropathol (Berl) 1996;92:431–434.
23 Takekawa Y, Sawada T, Sakurai I. Expression of apoptosis and its related protein in astrocytic tumors. Brain Tumor Pathol 1999;16:11–16.
24 Saas P, Walker PR, Hahne M, *et al.* Fas ligand expression by astrocytoma *in vivo*: maintaining immune privilege in the brain? J Clin Invest 1997;99:1173–1178.
25 Migheli A, Cavalla P, Marino S, *et al.* A study of apoptosis in normal and pathologic nervous tissue after in situ end-labeling of DNA strand breaks. J Neuropathol Exp Neurol 1994;53:606–616.
26 Schiffer D, Cavalla P, Migheli A, *et al.* Apoptosis and cell proliferation in human neuroepithelial tumors. Neurosci Lett 1995;195:81–84.
27 Nelson JS, Tsukada Y, Schoenfeld D, *et al.* Necrosis as a prognostic criterion in malignant supratentorial, astrocytic gliomas. Cancer 1983;52:550–554.
28 Lacroix M, Abi-Said D, Fourney DR, *et al.* A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 2001;95:190–198.

29 Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. Cancer 1987;59:1617–1625.
30 Curran Jr WJ, Scott CB, Horton J, *et al.* Recursive partitioning analysis of prognostic factors in three radiation therapy oncology group malignant glioma trials. J Natl Cancer Inst 1993;85:704–710.
31 Raza SM, Lang FF, Aggarwal BB, *et al.* Necrosis and glioblastoma: a friend or a foe? A review and a hypothesis. Neurosurgery 2002;51:2–12.
32 Dinda AK, Sarkar C, Roy S, *et al.* A transmission and scanning electron microscopic study of tumoral and peritumoral microblood vessels in human gliomas. J Neurooncol 1993;16:149–158.
33 Sawaya R, Yamamoto M, Ramo OJ, *et al.* Plasminogen activator inhibitor-1 in brain tumors: relation to malignancy and necrosis. Neurosurgery 1995;36: 375–380.
34 Hamada K, Kuratsu J, Saitoh Y, *et al.* Expression of tissue factor correlates with grade of malignancy in human glioma. Cancer 1996;77:1877–1883.
35 Sawaya R, Ramo OJ, Shi ML, *et al.* Biological significance of tissue plasminogen activator content in brain tumors. J Neurosurg 1991;74:480–486.
36 Brat DJ, Castellano-Sanchez A, Hunter SB, *et al.* Pseudopalisading cells in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by a rapidly migrating population. Cancer Res 2004;64:910–919.
37 Zagzag D, Zhong H, Scalzitti JM, *et al.* Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. Cancer 2000;88:2606–2618.
38 Krishnamachary B, Berg-Dixon S, Kelly B, *et al.* Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res 2003;63: 1138–1143.
39 Pennacchietti S, Michieli P, Galluzzo M, *et al.* Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3: 347–361.
40 Ben-Yosef Y, Lahat N, Shapiro S, *et al.* Regulation of endothelial matrix metalloproteinase-2 by hypoxia/ reoxygenation. Circ Res 2002;90:784–791.
41 Graham CH, Forsdike J, Fitzgerald CJ, *et al.* Hypoxia-mediated stimulation of carcinoma cell invasiveness via upregulation of urokinase receptor expression. Int J Cancer 1999;80:617–623.
42 Yamamoto M, Mohanam S, Sawaya R, *et al.* Differential expression of membrane-type matrix metalloproteinase and its correlation with gelatinase A activation in human malignant brain tumors *in vivo* and *in vitro*. Cancer Res 1996;56:384–392.
43 Mori T, Abe T, Wakabayashi Y, *et al.* Up-regulation of urokinase-type plasminogen activator and its receptor correlates with enhanced invasion activity of human glioma cells mediated by transforming growth factor-alpha or basic fibroblast growth factor. J Neurooncol 2000;46:115–123.
44 Holash J, Maisonpierre PC, Compton D, *et al.* Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284: 1994–1998.
45 Zagzag D, Amirnovin R, Greco MA, *et al.* Vascular apoptosis and involution in gliomas precede neovascularization: a novel concept for glioma and angiogenesis. Lab Invest 2000;80:837–849.
46 Zagzag D, Hooper A, Friedlander DR, *et al. In situ* expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. Exp Neurol 1999;159:391–400.
47 Stratmann A, Risau W, Plate KH. Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis. Am J Pathol 1998;153:1459–1466.
48 Vajkoczy P, Farhadi M, Gaumann A, *et al.* Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. Clin Invest 2002;109:777–785.
49 Zhang L, Yang N, Park JW, *et al.* Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. Cancer Res 2003;63:3403–3412.
50 Yu Q, Stamenkovic I. Angiopoietin-2 is implicated in the regulation of tumor angiogenesis. Am J Pathol 2001;158:563–570. 53.
51 Maisonpierre PC, Suri C, Jones PF, *et al.* Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science 1997;277:55–60.
52 Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. Thromb Res 2001;102:V215–V224.
53 Walsh DC, Kakkar AK. Thromboembolism in brain tumors. Curr Opin Pulm Med 2001;7:326–331.
54 Rodas RA, Fenstermaker RA, McKeever PE, *et al.* Correlation of intraluminal thrombosis in brain tumor vessels with postoperative thrombotic complications: a preliminary report. J Neurosurg 1998; 89:200–205.
55 Kniesel U, Wolburg H. Tight junctions of the blood–brain barrier. Cell Mol Neurobiol 2000;20:57–76.
56 Long DM. Capillary ultrastructure and the blood–brain barrier in human malignant brain tumors. J Neurosurg 1970;32:127–144.
57 Zhu XP, Li KL, Kamaly-Asl ID, *et al.* Quantification of endothelial permeability, leakage space, and blood volume in brain tumors using combined T1 and T2* contrast-enhanced dynamic MR imaging. J Magn Reson Imaging 2000;11:575–585.
58 Sugahara T, Korogi Y, Kochi M, *et al.* Correlation of MR imaging-determined cerebral blood volume maps with histologic and angiographic determination of vascularity of gliomas. AJR Am J Roentgenol 1998;171: 1479–1486.
59 Rascher G, Fischmann A, Kroger S, *et al.* Extracellular matrix and the blood–brain barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin. Acta Neuropathol (Berl) 2002;104:85–91.
60 Senger DR, Galli SJ, Dvorak AM, *et al.* Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219: 983–985.
61 Fischer S, Clauss M, Wiesnet M, *et al.* Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. Am J Physiol 1999;276: C812–C820.
62 Versteeg HH, Peppelenbosch MP, Spek CA. Tissue factor signal transduction in angiogenesis. Carcinogenesis 2003;24:1009–1013.
63 Contrino J, Hair G, Kreutzer DL, *et al. In situ* detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. Nat Med 1996;2:209–215.

64 Vrana JA, Stang MT, Grande JP, *et al.* Expression of tissue factor in tumor stroma correlates with progression to invasive human breast cancer: paracrine regulation by carcinoma cell-derived members of the transforming growth factor beta family. Cancer Res 1996;56:5063–5070.
65 Seto S, Onodera H, Kaido T, *et al.* Tissue factor expression in human colorectal carcinoma: correlation with hepatic metastasis and impact on prognosis. Cancer 2000;88:295–301.
66 Guan M, Jin J, Su B, *et al.* Tissue factor expression and angiogenesis in human glioma. Clin Biochem 2002;35:321–325.
67 Yan SF, Mackman N, Kisiel W, *et al.* Hypoxia/hypoxemia-induced activation of the procoagulant pathways and the pathogenesis of ischemia-associated thrombosis. Arterioscler Thromb Vasc Biol 1999;19:2029–2035.
68 Lawson CA, Yan SD, Yan SF, *et al.* Monocytes and tissue factor promote thrombosis in a murine model of oxygen deprivation. J Clin Invest 1997;99:1729–1738.
69 Yan SF, Lu J, Zou YS, *et al.* Hypoxia-associated induction of early growth response-1 gene expression. Biol Chem 1999;274:15030–15040.
70 Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258–264.
71 Macfarlane SR, Seatter MJ, Kanke T, *et al.* Pharmacol Rev 2001;53:245–282.
72 Even-Ram S, Uziely B, Cohen P, *et al.* Thrombin receptor overexpression in malignant and physiological invasion processes. Nat Med 1998;4:909–914.
73 Darmoul D, Gratio V, Devaud H, *et al.* Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells. Am J Pathol 2003;162:1503–1513.
74 Martin CB, Mahon GM, Klinger MB, *et al.* The thrombin receptor, PAR-1, causes transformation by activation of Rho-mediated signaling pathways. Oncogene 2001;20:1953–1963.
75 Marinissen MJ, Servitja JM, Offermanns S, *et al.* The thrombin receptor PAR-1 signals through Gq and G13-initiated MAPK cascades regulating c-jun expression to induce cell transformation. J Biol Chem 2003;278:46814–46825.
76 Yin YJ, Salah Z, Grisaru-Granovsky S, *et al.* Human protease-activated receptor 1 expression in malignant epithelia: a role in invasiveness. Arterioscler Thromb Vasc Biol 2003;23:940–944.
77 Even-Ram SC, Maoz M, Pokroy E, *et al.* Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the alpha vbeta 5 integrin. J Biol Chem 2001;276:10952–10962.
78 Junge CE, Sugawara T, Mannaioni G, *et al.* The contribution of protease activated receptor-1 to neuronal damage caused by transient focal ischemia. Proc Natl Acad Sci USA 2003;100:13019–13024.
79 Junge CE, Lee CJ, Hubbard KB, *et al.* Protease activated receptor-1 (PAR1) in human brain: localization and functional expression in astrocytes. Exper Neurol, (in press).