J Neuropathol Exp Neurol
Vol. 65, No. 6
June 2006
pp. 529–539
Copyright © 2006 by the American Association of Neuropathologists, Inc.

REVIEW ARTICLE

'Pseudopalisading' Necrosis in Glioblastoma: A Familiar Morphologic Feature That Links Vascular Pathology, Hypoxia, and Angiogenesis

Yuan Rong, MD, PhD, Donald L. Durden, MD, PhD, Erwin G. Van Meir, PhD, and Daniel J. Brat, MD, PhD

Abstract

Glioblastoma (GBM) is a highly malignant, rapidly progressive astrocytoma that is distinguished pathologically from lower grade tumors by necrosis and microvascular hyperplasia. Necrotic foci are typically surrounded by "pseudopalisading" cells—a configuration that is relatively unique to malignant gliomas and has long been recognized as an ominous prognostic feature. Precise mechanisms that relate morphology to biologic behavior have not been described. Recent investigations have demonstrated that pseudopalisades are severely hypoxic, overexpress hypoxia-inducible factor (HIF-1), and secrete proangiogenic factors such as VEGF and IL-8. Thus, the microvascular hyperplasia in GBM that provides a new vasculature and promotes peripheral tumor expansion is tightly linked with the emergence of pseudopalisades. Both pathologic observations and experimental evidence have indicated that the development of hypoxia and necrosis within astrocytomas could arise secondary to vaso-occlusion and intravascular thrombosis. This emerging model suggests that pseudopalisades represent a wave of tumor cells actively migrating away from central hypoxia that arises after a vascular insult. Experimental glioma models have shown that endothelial apoptosis, perhaps resulting from angiopoetin-2, initiates vascular pathology, whereas observations in human tumors have clearly demonstrated that intravascular thrombosis develops with high frequency in the transition to GBM. Tissue factor, the main cellular initiator of thrombosis, is dramatically upregulated in response to *PTEN* loss and hypoxia in human GBM and could promote a prothrombotic environment that precipitates these events. A prothrombotic environment also activates the family of protease activated receptors (PARs) on tumor cells, which are G-protein-coupled and enhance invasive and proangiogenic properties. Vaso-occlusive and prothrombotic mechanisms in GBM could readily explain the presence of pseudopalisading necrosis in tissue sections, the rapid peripheral expansion on neuroimaging, and the dramatic shift to an accelerated rate of clinical progression resulting from hypoxia-induced angiogenesis.

Key Words: Angiogenesis, Glioblastoma, Hypoxia, IL-8, Necrosis, Protease activated receptor, Thrombosis, Tissue factor, Vascular endothelial growth factor (VEGF).

INTRODUCTION

Glioblastoma (GBM; World Health Organization [WHO] grade IV) is the highest grade astrocytoma and has a dismal prognosis (1–3). Mean survival after the most advanced treatment, including neurosurgery, radiotherapy, and chemotherapy, is only 60 weeks (4). When patients receive only surgical resection, but are not treated with adjuvant therapy, mean survival is a mere 14 weeks, underscoring the tremendous natural growth properties of these tumors (5). Lower grade infiltrative astrocytomas (i.e. WHO grade II and III astrocytomas) are also ultimately fatal but have substantially slower growth rates and longer survivals (3–8 years) (6, 7). Only after lower grade tumors have progressed to GBM do they demonstrate accelerated growth and rapid progression to death. Reasons for the abrupt onset of explosive growth properties that follow malignant transformation to GBM have not been adequately explained. This review proposes a mechanism that accounts for the rapid clinical progression of GBM, emphasizing the central role of the pseudopalisade as a link among an underlying vascular pathology, the development of hypoxia and hypoxia-induced angiogenesis, and outward tumor expansion.

Accelerated Growth in Glioblastoma

The biologic properties of GBM (grade IV) are quite distinct from those of lower grade astrocytomas (grade II and III) and suggest that it represents more than an incremental step in malignancy. The unique neuroimaging and pathologic features that emerge during the transition to GBM provide the best insight into potential mechanisms responsible for the enhanced growth properties. By magnetic resonance imaging (MRI), grade II and III astrocytomas show hyperintense T2-weighted (or FLAIR) signal abnormalities,

From the Departments of Pathology and Laboratory Medicine (YR, DJB), Pediatrics (DLD), Hematology/Oncology (DLD, EGVM), and Neurosurgery (EGVM), Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia.

Send correspondence and reprint requests to: Daniel J. Brat, MD, PhD, Department of Pathology and Laboratory Medicine, Emory University Hospital, H-176, 1364 Clifton Rd. NE, Atlanta, GA 30322; E-mail: dbrat@emory.edu

Supported in part by U.S. Public Health Service National Institutes of Health (NIH) Awards NS-42934, CA-109382, and NS053727 (DJB), CA-86335, CA-87830 (EGVM), The Georgia Cancer Coalition (DLD), the Musella Foundation (EGVM), and The Pediatric Brain Tumor Foundation of the US (EGVM).

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

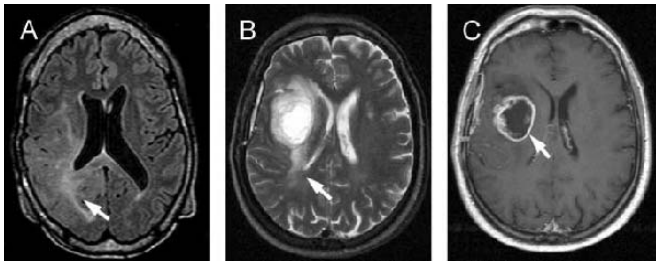

FIGURE 1. Anaplastic astrocytoma (AA; World Health Organization [WHO] grade III) and glioblastoma (GBM; WHO grade IV) have distinct growth patterns as demonstrated on magnetic resonance imaging (MRI). **(A)** Axial MRI of AA shows expansion of the involved brain and increased signal intensity on FLAIR imaging, reflecting the vasogenic edema that arises in response to infiltrating tumor cells (arrow). After the administration of contrast agents, most AAs demonstrate modest or no enhancement resulting from an intact blood–brain barrier and a lack of central necrosis. **(B)** Axial MRI of GBM also demonstrates hyperintense regions at the tumor periphery on T2-weighted imaging, indicative of diffusely infiltrating cells (arrow). **(C)** A distinguishing feature is the emergence of a central contrast-enhancing component, which contains a necrotic center and a leading edge that rapidly expands outward (arrow).

reflecting vasogenic edema generated in response to diffuse infiltration by individual tumor cells (Fig. 1). Lower grade tumors expand the involved brain but show mild or no contrast enhancement, suggesting an intact blood–brain barrier and a lack of tumor necrosis (8, 9). Radial growth rates are modest, with annual increases in diameter of 2 to 4 mm/year (10, 11). Histologic sections of grade II–III tumors reflect the imaging properties: neoplastic cells are seen diffusely infiltrating between neuronal and glial processes, leading to architectural distortion and edema (7, 12). As astrocytomas advance through the pathologic spectrum from the lower end of grade II to the upper end of grade III, the degree of nuclear anaplasia increases and the proliferative capacity creeps upward, resulting in a more densely cellular tumor with greater malignant potential (6). Thus, grade II–III astrocytomas represent a continuum of gradually increasing tumor grade and growth with clinical properties generally correlating with the malignancy of individual tumor cells.

Tumor dynamics change dramatically after transition to GBM, suggesting a fundamentally altered neoplasm. Radial growth rates can accelerate to values nearly 10 times greater than those in grade II astrocytomas (10, 11). MRI typically reveals a central, contrast-enhancing component ("ring-enhancing mass") emerging from within the infiltrative astrocytoma and rapidly expanding outward, causing a much larger T2-weighted signal abnormality in the tumor's periphery (Fig. 1) (8, 9). The histopathologic features that distinguish GBM from lower grade astrocytomas are found near this contrast-enhancing rim and include 1) foci of necrosis, usually with evidence of surrounding cellular pseudopalisades ("pseudopalisading necrosis"), and 2) microvascular hyperplasia, a form of angiogenesis morphologically recognized as endothelial proliferation within newly sprouted vessels (Fig. 2) (2, 6, 13, 14). In contrast to lower grade astrocytomas, these 2 diagnostic findings of GBM are largely independent of tumor cell morphology, yet carry an inordinate degree of prognostic power (15, 16). Rather than mere markers, these structures are more likely to be mechanistically linked to the accelerated growth properties that characterize the grade III to IV transition. Why should these 2 tumoral elements be so predictive of rapid growth?

An emerging model of tumor progression may explain the development of pseudopalisades, the relationship between pseudopalisades and angiogenesis, and the strong association between pseudopalisades and aggressive clinical behavior (Figs. 3 and 4) (6, 13, 17, 18). This model hypothesizes a sequence that begins with an infiltrating astrocytoma of moderate to high cellularity (i.e. grade III astrocytoma) and, as shown in Figure 4, continues with 1) vascular occlusion within the tumor that is often associated with intravascular thrombosis; 2) hypoxia in regions surrounding vascular pathology; 3) outward migration of tumor cells away from hypoxia, creating a peripherally moving wave (pseudopalisade) and central necrosis; 4) secretion of hypoxia-inducible, proangiogenic factors (vascular endothelial growth factor [VEGF], IL-8) by pseudopalisading cells; 5) an exuberant angiogenic response creating microvascular proliferation in regions adjacent to central hypoxia; and 6) accelerated outward expansion of tumor cells toward a new vasculature. The global growth properties of GBM within the brain reflect a coalescence of these microscopic processes and result in a peripherally expanding tumor with a large degree of central necrosis.

Pseudopalisades Are Actively Migrating Tumor Cells

Everyone agrees that pseudopalisades are hypercellular zones that surround necrotic foci in GBM (Fig. 2) (2, 14). Experienced neuropathologists can affirm that the centers of the smallest pseudopalisades sometimes contain only fibrillar processes and apoptotic cells but lack frank coagulative necrosis. Analysis of the shapes and sizes of pseudopalisades suggests that these structures evolve and enlarge over time, giving rise to wider and wider expanses of coagulative necrosis (19). Pseudopalisading of cells around central degeneration has been recognized for nearly a century as both a defining feature of GBM and a morphologic finding that predicts aggressive behavior (20). Although discussions of their etiology are scant, a commonly held belief has been that pseudopalisades represent a rim of residual tumor cells around a centrally degenerating clone of highly proliferative cells. Indeed, the term "pseudopalisade" itself implies that cells have not truly aggregated around necrosis, but only give this impression as a result of the absence of a central hypercellular zone. The idea of a highly malignant clone developing from within a lower grade tumor and undergoing central degeneration fits nicely with prevailing models of tumorigenesis that emphasized a stepwise progression of morphologic features and genetic alterations.

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

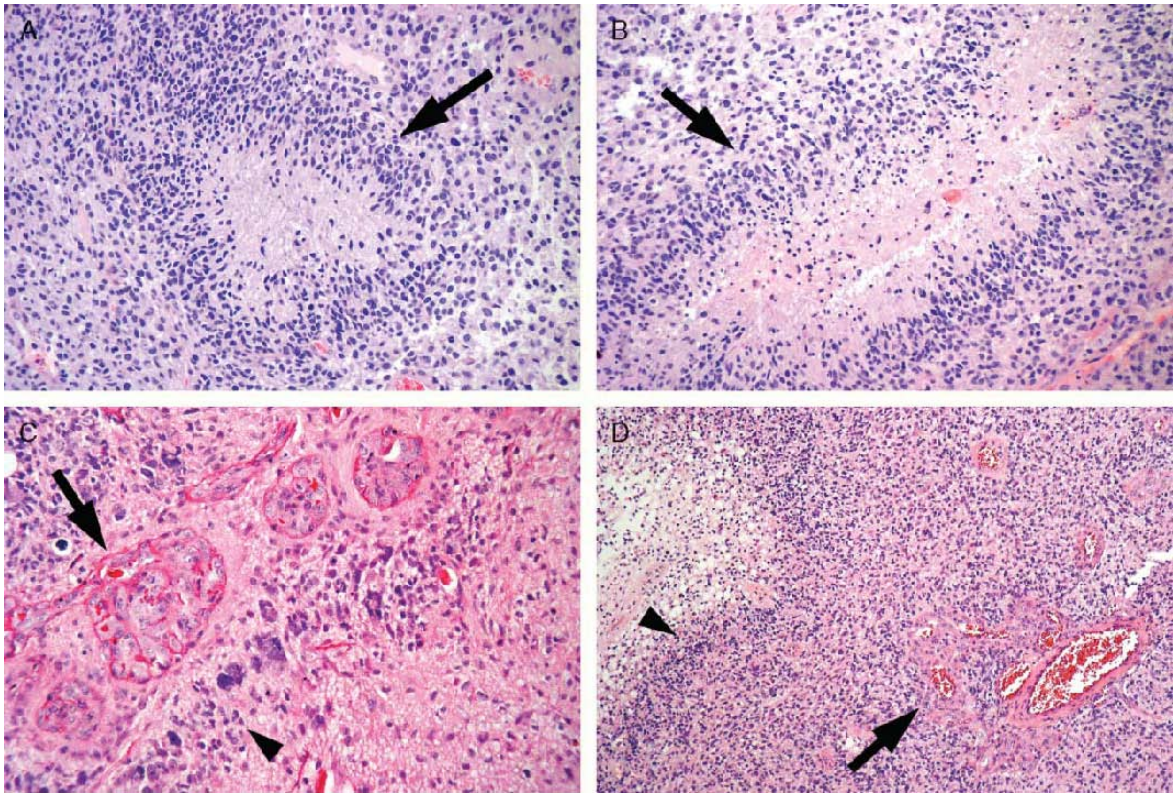

FIGURE 2. Pathologic features of glioblastoma. **(A)** Pseudopalisades (arrow) are characterized by an accumulation of tumor cells around a central clear zone. Some smaller pseudopalisades have internal fibrillarity and apoptotic cells but lack coagulative tumor necrosis. **(B)** Larger pseudopalisades (arrow) contain regions of necrosis as well as apoptotic cells and some have an elongate shape that suggests an underlying vascular substrate. **(C, D)** Microvascular hyperplasia (arrow) is a form of angiogenesis that is induced by hypoxic pseudopalisading cells in glioblastoma (arrowhead) and can be noted in regions adjacent to necrosis. **(D)** Low-magnification view of pseudopalisading necrosis (arrowhead) and microvascular hyperplasia (arrow) demonstrating a wave of tumor cells migrating toward the emerging vasculature (left to right).

Recent studies have questioned this assumption and attempted to more precisely define underlying mechanisms of necrosis and pseudopalisading in GBM (19). These began by considering that this hypercellular population around central necrosis could represent 1) a clone of rapid proliferating neoplastic cells that "outgrew its blood supply" and underwent central necrosis (clonal expansion theory); 2) a population of neoplastic cells that was resistant to apoptosis and accumulated as a result of increased cell survival; 3) a mixed population of neoplastic and inflammatory cells adjacent to necrosis; or 4) a population of rapidly moving tumor cells that superimposed themselves on a more stationary population, causing increased cell density. Perhaps surprisingly, these investigations determined that pseudopalisading cells were *less* proliferative than adjacent tumor, indicating they do not likely accumulate as a result of clonal expansion (19, 21). Second, pseudopalisades are composed almost entirely of tumor cells and do not include a significant population of nonneoplastic cells (e.g. inflammatory cells). Third, pseudopalisades show *increased* levels of apoptosis compared with adjacent tumor, indicating they do not accumulate as a result of a survival advantage (19, 22).

Instead, these investigations concluded that pseudopalisades represent a wave of actively migrating tumor cells that are moving away from an area of central hypoxia. Pseudopalisading cells are known to be hypoxic, as demonstrated by their dramatic upregulation of hypoxia inducible factor-1 (HIF-1), a nuclear transcription factor that orchestrates the cell's adaptive response to low oxygen (Fig. 3) (19, 23–25). Gene expression studies performed on microdissected pseudopalisading cells from human GBMs have demonstrated upregulated gene transcripts in this population that suggest a response to a hypoxic microenvironment, including those related to glycolysis, angiogenesis, and cell-cycle control (26). Hypoxic GBM cells in culture that have similar upregulation of HIF-1α are more highly migratory than normoxic cells and HIF-1 itself mediates many of the critical promigratory mechanisms in gliomas and other neoplastic cells (19, 27–29). Hypoxic upregulation of c-Met, a tyrosine kinase receptor, as well as the potentiation of hepatocyte growth factor (HGF) signaling through c-Met, could account for at least some of the promigratory effects of HIF-1 activation (29, 30). Moreover, hypoxic pseudopalisades express increased levels of extracellular matrix proteases associated with invasion, including MMP-2 and uPAR (12, 19, 27, 29, 31, 32). Thus, the combined evidence suggests that the pseudopalisades in GBM are formed by a population of hypoxic, actively migrating neoplastic cells that have imposed themselves on a

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

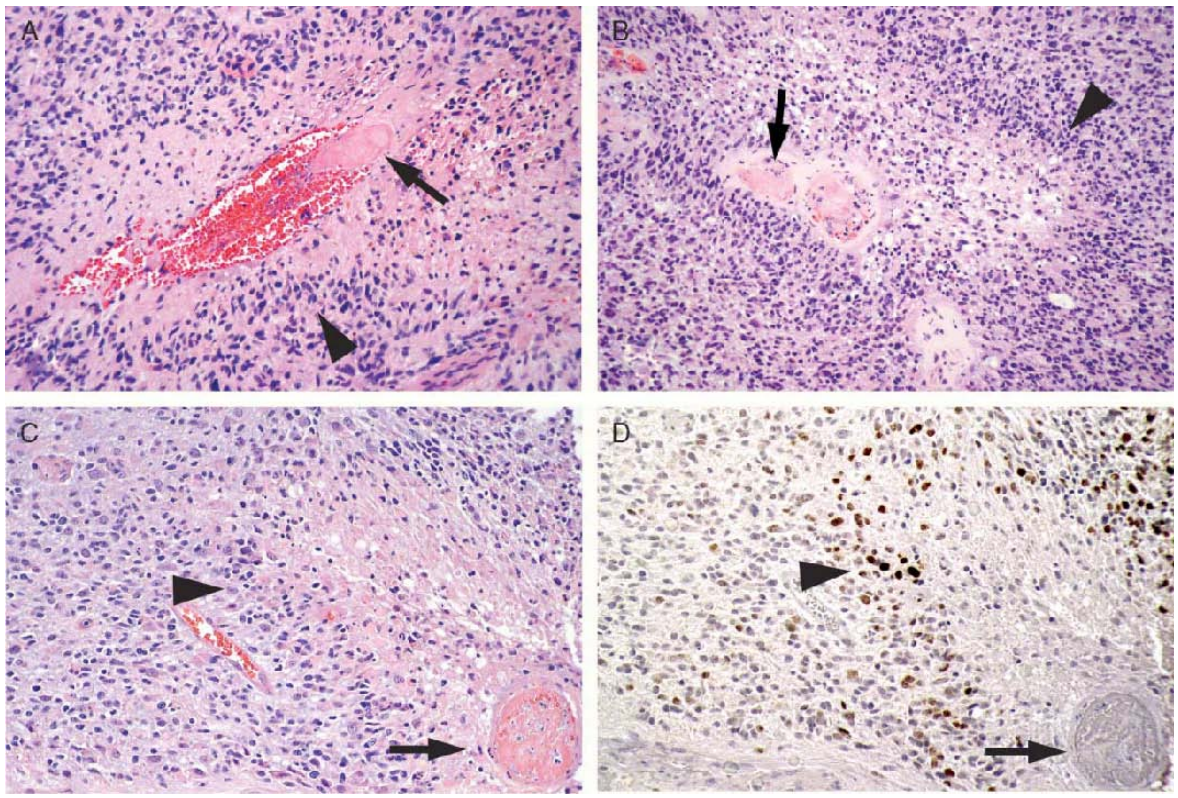

FIGURE 3. Intravascular thrombosis in glioblastoma (GBM). **(A)** A central vessel within a GBM is occluded by intravascular thrombus (arrow). The vessel is dilated proximal to the occlusion and surrounded by delicate fibrillarity and scattered apoptotic cell, most likely representing the initial stages of pseudopalisade formation (arrowhead). **(B)** As the pseudopalisading front of tumor cells (arrowhead) enlarges around a central thrombosed vessel (arrow), perivascular necrosis becomes more prominent. **(C)** Hematoxylin and eosin staining of a GBM demonstrates intravascular thrombosis occluding and distending a vessel (arrow) within the center of a pseudopalisade (arrowhead). **(D)** Immunohistochemistry for HIF-1α of a serial tissue section shows increased nuclear staining in pseudopalisades, indicating an adaptive response to hypoxia (arrowhead).

less mobile population, thereby creating a hypercellular zone around an evolving area of central necrosis.

Pseudopalisades Are Hypoxic Tumor Cells Migrating Away From Vascular Pathology

The hypoxia that leads to increased tumor cell migration to form pseudopalisades could result from limitations in vascular perfusion within the tumor (i.e. disruption of the blood supply) or from reduced oxygen diffusion within the neoplasm, in part as a result of increased metabolic demands of a growing tumor (13). Attenuated perfusion would lead to cell migration away from central blood vessels that no longer provides the necessary oxygen supply. Limitations in oxygen diffusion, on the other hand, would cause tumor cells at greatest distance from arterial supplies to become hypoxic and migrate toward viable vessels. These mechanisms are not mutually exclusive. However, a growing body of experimental and observational evidence favors the hypothesis that pseudopalisades represent tumor cells migrating away from a dysfunctional vasculature (17–19). Most pseudopalisades that are captured photographically and find their way into textbooks are ring-like or ovoid (Fig. 2). However, these structures often have a long, narrow, and winding (i.e. serpiginous) pattern when viewed in longitudinal tissue sections–a pattern highly suggestive of an underlying vascular substrate (2). Perhaps less appreciated, abnormal vessels can often be noted within the lumina of at least a subset of pseudopalisades. A comprehensive survey of human GBM specimens found that over 50% of pseudopalisades had evidence of a central vascular lumen that was either degenerating or thrombosed (19). A full 20% contained a vessel with complete luminal occlusion caused by intravascular thrombosis (Fig. 3). Analysis of pseudopalisade sizes and shapes led to the conclusion that tissue sampling and tangential sections results in an underestimation of the true frequency of vascular pathology and intravascular thrombosis within pseudopalisades, which has led to an underappreciation of their relevance to necrosis in GBM (Fig. 5). Thus, pseudopalisades around necrosis appear to represent hypoxic tumor cells migrating away from vaso-occlusion and thrombosis.

Initiators of Vascular Pathology

Experimental models support a vaso-occlusive model of glioma progression and indicate that the initial, preangiogenic stages of tumor growth involves–perhaps counterintuitively–

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

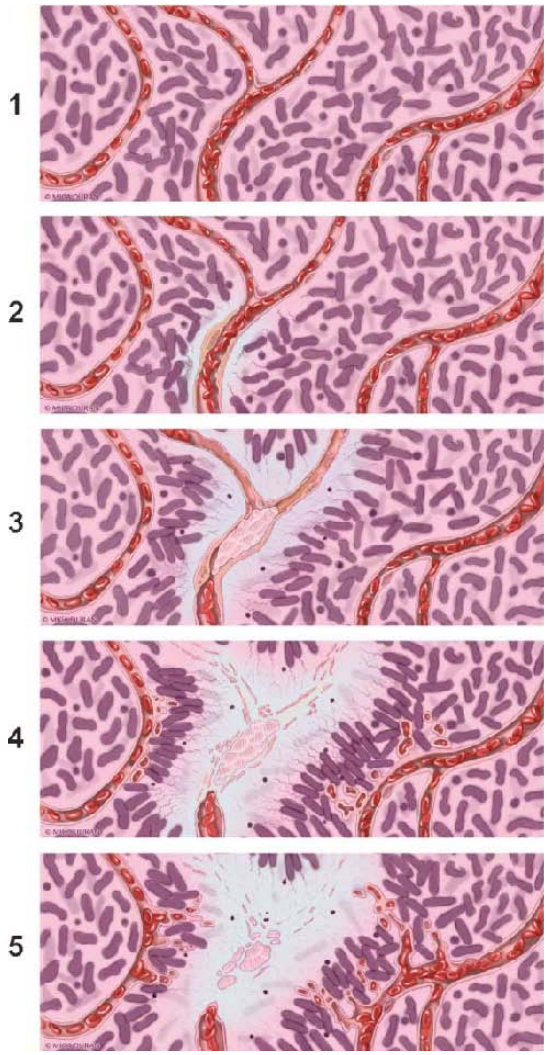

FIGURE 4. Potential mechanism of pseudopalisade formation. 1) In grade III astrocytoma, tumor cells with moderate to high density infiltrate through the central nervous system and receive oxygen and nutrient supplies through intact native blood vessels. 2) A vascular insult occurs as a result of tumor growth and causes endothelial injury and vascular leakiness. 3) Endothelial injury and the expression of procoagulant factors by the neoplasm result in intravascular thrombosis and increasing hypoxia in regions surrounding vascular pathology (light blue). Tumor cells begin to migrate away from hypoxia, creating a peripherally moving wave that is seen microscopically as pseudopalisading cells. 4) The zone of hypoxia and central necrosis expands, whereas the hypoxic tumor cells of pseudopalisades secrete proangiogenic factors (VEGF, IL-8). 5) Microvascular proliferation in regions adjacent to central hypoxia causes an accelerated outward expansion of tumor cells toward a new vasculature. Illustration by Mica Duran.

the regression of native blood vessels. New tumor cells such as those in grade II astrocytoma first gain access to oxygen and nutrients through a vascular supply by "co-opting" the host's blood vessels (17, 18). In response, vascular endothelial cells eventually undergo a number of changes that include hypertrophy, discohesion, and even apoptosis (Fig. 6). Endothelial damage may be initiated by the effects of angiopoietin-2 (Ang-2) on endothelial cell. Ang-2 is thought to act in an autocrine fashion on tumoral blood vessels as a Tie-2 receptor antagonist. In the absence of VEGF, Tie-2 blockage leads to vascular destabilization, endothelial cell apoptosis and vascular regression (17, 33). In human specimens, Ang-2 is expressed by endothelial cells of high-grade gliomas but not low-grade gliomas or normal brain, and its upregulation precedes endothelial apoptosis, suggesting that it could cause vascular injury (18, 34, 35). Other arguments hold that Ang-2 causes structural changes of vessels that are required for angiogenesis but does not induce apoptosis (36, 37). Factors released from glioma cells after genetic alteration (*EGFR* amplification or *PTEN* loss) such as VEGF and TNF-α could also precipitate vascular injury and thrombosis. VEGF induces changes in vascular permeability, whereas both VEGF and TNF-α have been demonstrated to induce endothelial tissue factor expression through activation of the transcription factor Egr-1.

Intravascular Thrombosis Accentuates and Propagates Tumor Hypoxia

Although precise initiators of vascular pathology in GBM continue to be studied, it is becoming clear that

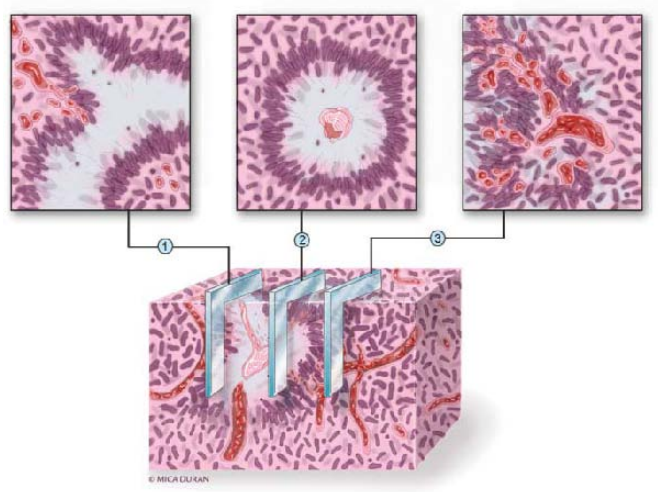

FIGURE 5. Three-dimensional schematic representation of pseudopalisade formation in glioblastoma. Vaso-occlusion/collapse and intravascular thrombosis lead to tissue hypoxia in the perivascular region and eventually results in central necrosis (light blue) surrounded by pseudopalisading tumor cells. Representative sections through the region of necrosis will often show pseudopalisades that do not contain central vascular pathology resulting from 2-dimensional sampling and central degeneration (left panel). Other sections will show a pathologic central vessel that is distorted, thrombosed, or degenerating, depending on the relation of the tissue section to the vascular pathology (central panel). The finding of microvascular pathology in tissue sections will sometimes suggest that tumor hypoxia and/or necrosis are nearby but not caught in the plane of section (right panel). Illustration by Mica Duran.

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

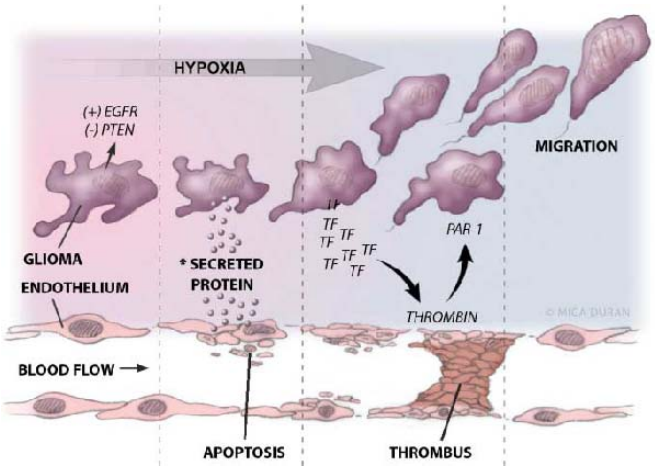

FIGURE 6. Proposed vaso-occlusive and prothrombotic mechanisms of pseudopalisade formation in glioblastoma. 1) Genetic alterations in tumor cells, including *EGFR* amplification and *PTEN* loss, are hypothesized to cause secretion of factors that promote vascular leakiness, endothelial apoptosis, or endothelial tissue factor expression. 2) Both genetic alterations and hypoxia cause increased expression of tissue factor (TF) by tumor cells, which strongly promotes thrombosis. The resulting tumor hypoxia and the activation of PAR-1 by thrombin cause increased tumor cell migration away from occluded vessels to form pseudopalisades. 3) Pseudopalisades in turn drive neoplastic progression by inducing angiogenesis through the secretion of proangiogenic factors, including VEGF and IL-8.

intravascular thrombosis within these neoplasms can accentuate and propagate tumoral hypoxia and necrosis. A strong relationship between abnormal blood clotting and human malignancy is well established and gliomas are no exception (38). Indeed, patients with GBM are at high risk for developing systemic disorders of coagulation and nearly 30% have deep vein thrombosis or pulmonary thromboembolism (39). Intravascular thrombosis within the tumoral tissue of GBM is a frequent intraoperative finding by the neurosurgeon. Even more impressively, thrombosed vessels within resected GBM specimens can almost always be identified under the microscope (noted histologically in over 90% of GBMs) (19). Both the frequencies of associated systemic coagulopathy and the finding of intravascular thrombosis within neoplastic tissue are much higher in GBMs (grade IV) than AAs (grade III), indicating that critical prothrombotic events must occur in this transition (19, 39, 40). The development of intravascular thrombosis within GBM and the emergence of pseudopalisades around hypoxic zones are too coincidental to be dismissed as unrelated epiphenomena. Intravascular thrombosis could directly initiate or propagate hypoxia and necrosis in GBM (41).

Multiple factors likely contribute to intravascular thrombosis in GBM, including abnormal blood flow within a distorted vasculature, increased interstitial edema, dysregulation of pro- and anticoagulant factors, and access of plasma clotting factors to tumoral tissue. Normal central nervous system blood vessels allow only limited diffusion through their walls resulting from a highly restrictive blood–brain barrier, which is formed primarily by endothelial tight junctions, but also has contributions from astrocytic foot plates, extracellular matrix, and endothelial–pericytic interactions (42). This barrier becomes breached in GBM and can be visualized radiologically by the presence of contrast enhancement resulting from increased vascular permeability to contrast agents (e.g. gadolinium) and to proteins that bind them such as albumin (Fig. 1) (8, 9). Damaged vessels appear fenestrated, show detachment of pericytes, and exhibit extracellular matrix alterations (42). All the factors that contribute to increased permeability have not been defined, but VEGF secretion by neoplastic cells is known to cause vascular leakage (43, 44). One result is to bring plasma coagulation factors such as factor VII into the tissue spaces where they are activated and result in thrombosis.

Plasma Coagulation Is Promoted by Tissue Factor

The number of plasma clotting factors that are dysregulated in GBM and favor thrombosis is large and growing. For example, the procoagulant plasminogen activator inhibitor 1 (PAI-1) is markedly elevated in GBMs, whereas the expression of anticoagulants such as the fibrinolytic tissue type plasminogen activator (tPA) is decreased (40, 45). One of the most highly upregulated prothrombotic factors in GBM is tissue factor (TF), a 47-kDa transmembrane glycoprotein receptor that is a critical regulator of tissue hemostasis and one of the body's most potent stimulants of thrombosis (46). In normal tissue, TF is expressed almost exclusively by stromal cells, and it usually takes a disruption of vascular integrity to cause TF to bind to its activating ligand from the plasma, factor VII/VIIa (47). In turn, TF/factor VIIa activation promotes the generation of thrombin from prothrombin, ultimately leading to platelet aggregation, fibrin deposition, and local hemostasis. The normally tight regulation of TF is lost in a variety of pathologic conditions, including neoplasia, and numerous cancers show increased expression by tumor cells, stroma, and endothelium (48). A direct correlation between TF levels and tumor grade has been noted for multiple tumor types (49, 50), including gliomas (51, 52). Indeed, TF is highly expressed by over 90% of malignant astrocytomas, but only 10% of grade I and II astrocytomas (51). The prothrombotic effects of TF at the cell surface are largely mediated through downstream activation of coagulation proteases factor VII (VIIa), factor X (Xa), and thrombin. As a transmembrane receptor, TF also transduces independent intracellular signals through its cytoplasmic tail that promote tumorigenesis, including activation of p38 MAP kinase and Rac-1 and interactions with the actin-binding protein ABP-280 (53, 54). Intracellular signaling mechanisms induced by TF strongly promote tumorigenesis through proangiogenic and prometastatic mechanisms (46, 55, 56).

PTEN Loss and Hypoxia Cause Increased Tissue Factor Expression in Glioblastoma

Recent investigations have attempted to define the genetic and physiologic triggers that might cause increased

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

TF expression and thrombosis in human malignancy (Fig. 6) (57–60). Genetic events that arise during astrocytoma progression have been well characterized and include *PTEN* and *TP53* mutations, *p16(CDKN2A)* and $p14^{ARF}$ deletions, *EGFR* and *MDM2* amplifications (61). Among these, *EGFR* amplification and *PTEN* mutations are prime candidates to explore for regulation of prothrombotic factors because they occur precisely during transition from AA to GBM, when thrombosis and pseudopalisades emerge (59, 62). *PTEN* is a tumor suppressor located at 10q23.3 (63). Inactivating mutations *PTEN* occur in 30% to 40% of GBMs and gene inactivation through promoter methylation leads to lost expression of PTEN in over 70% (64–66). The effects of PTEN on TF expression and procoagulant properties by malignant gliomas were recently studied by introducing a wild-type *PTEN* gene into a *PTEN* null glioma cell line (U87MG). The expression TF protein at the cell surface of glioma cells was dramatically suppressed by *PTEN*, which in turn led to prolonged plasma clotting times using in vitro measures of coagulation. Although many of *PTEN*'s biologic effects depend on its lipid phosphastase activity and ability to antagonize phospho-inositol (PI)-3 kinase, these studies indicated that regulation of TF depended at least in part on *PTEN*'s protein phosphatase activity. Potential downstream signaling mechanisms relevant to the control of TF by *PTEN* were investigated using a series of human astrocytes that were sequentially infected with E6/E7/hTERT, Ras, and Akt. This series of astrocytes have been used to recapitulate astrocytoma progression both in vitro and in vivo (67, 68). Cells transfected with either Akt or Ras showed upregulation of TF, whereas those transformed with combined Ras and Akt showed the highest TF expression, suggesting that both signaling pathways may participate as downstream regulators of *PTEN* (57, 58). Other investigations have emphasized that activated forms of Ras are critical for the expression of TF and its tumorigenic effects (58). Thus, *PTEN* loss during astrocytoma progression likely leads to increased TF expression and plasma coagulation, both through Akt/Ras-dependent and protein phosphatase-dependent mechanism.

In addition to genetic regulation, TF is also strongly upregulated by hypoxia in GBM (57). Hypoxic GBM cells placed directly into human plasma cause a marked acceleration of plasma clotting times compared with normoxic cells. This effect can be prevented by both the preincubation of cells with inhibitory antibodies to TF and by using plasma that lacks factor VII, strongly implicating TF-dependent mechanisms. Similar hypoxic conditions also cause a rapid increase in TF mRNA and protein expression by GBM cells in vitro, which are modestly suppressed by *PTEN* expression. Within human GBM specimens, the severely hypoxic pseudopalisading cells around necrosis show the highest level of TF expression, corroborating *in vitro* studies. Mechanisms responsible for the hypoxic upregulation of TF are challenging to investigate because the TF promoter contains binding sites for a variety of transcriptional regulators that can be induced by hypoxia, including Egr-1, Sp1, NF-κB, and AP-1 (69). The accumulated evidence in animal models and *in vitro* indicates that Egr-1 is the transcription factor that is most important to the hypoxic upregulation of TF and that these mechanisms do not depend on the increased expression of HIF-1 (70, 71). Thus, both *PTEN* loss and hypoxia upregulate TF expression and promote plasma clotting by GBM cells in vitro, which might suggest that these mechanisms promote intravascular thrombosis and pseudopalisading necrosis in the transition from AA to GBM.

Protease-Activated Receptor-1 Is Activated by Thrombin in Gliomas

Activation of plasma coagulation factors by TF has biologic significance beyond clot formation. Thrombin, factor VIIa, and factor Xa are proteases that act as potent physiological activators of protease-activated receptors (PARs), a family of G-protein coupled, transmembrane receptors. PAR1, the family's prototype, is activated most strongly by thrombin, which cleaves the aminoterminal extracellular domain of PAR1 and unmasks a new N-terminus, which then serves as the receptor's ligand (72). Both PAR1 and PAR2 can also be activated by factors VIIa and factor Xa. Activated PAR transduces intracellular signals by coupling through G-proteins, predominantly Gαi, Gαq, and Gα12/13. Secondary signals are generated through Rho, phopholipase C (IP3 and diacylglycerol), and inhibition of adenyl cyclase. Although PARs are expressed at low levels in most normal epithelia, they are aberrantly overexpressed by a variety of carcinomas, including those of breast, colon, lung, and stomach (73–75). PAR1 activation can transform cells and is able to enhance tumorigenicity, in large part by signaling through Gαq and Gα13 (76, 77). It is also clear that PAR1 and PAR2 activation by coagulation factors promotes invasive and metastatic properties of malignant cells (75, 78–80). Mechanisms of increased invasion include its ability to direct cytoskeletal actin rearrangements, phosphorylation of focal adhesion kinases, and recruitment of αvβ5 integrin to contact sites (74).

PAR1 protein is present in both the human and mouse central nervous system, mostly in astrocytes, where it can be activated by thrombin (81, 82). Investigations of human GBM cell lines and short-term cultures of resected human GBM specimens have demonstrated that PAR1 is present on the surface of tumor cells and that it can be activated by both thrombin and PAR1 agonists. Such activation leads to increased phospho-inositol (PI) hydrolysis and calcium mobilization, presumably coupling through Gαq (82). Although more evidence is required to determine the biologic relevance of TF activation of thrombin and consequent PAR1 signaling in gliomas, it is highly probable that the activation of PAR1 by procoagulant proteases direct the migration of tumor cells in a manner similar to other malignancies. In the context of human GBM, activation would be expected to direct migration away from vaso-occlusion and hypoxia to form pseudopalisades (Fig. 6).

Angiogenesis Supports Peripheral Tumor Growth

If emerging models of GBM progression are valid, then vascular pathology may underlie the development of hypoxia and necrosis in GBM. Although necrosis has long been recognized as a marker of aggressive behavior in diffuse gliomas, *by itself* it does not explain rapid tumor progression

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

(14, 16). Indeed, tumor cell death is the goal of most adjuvant therapies. Instead, pseudopalisades that surround necrosis in GBM are intimately related to microvascular hyperplasia, a defining morphologic feature of GBM that is most often noted in regions directly adjacent to pseudopalisades (Figs. 2 and 4) (6, 13, 23). This exuberant angiogenic response attempts to lay down a new vasculature for rapid neoplastic expansion, yet the proper function of these distorted vessels has not been established. The formation of new blood vessels from preexisting ones is tightly regulated process and follows a complex sequence in response to pro- and antiangiogenic factors. Initial phases require increased vascular permeability of parent vessels, extravasation of plasma, and deposition of proangiogenic matrix proteins. In response to the mitogenic effects of proangiogenic cytokines, endothelial cells proliferate and migrate along a chemotactic gradient into the extracellular matrix. Once established, endothelial cells form tubes with a central lumen, elaborate a basement membrane, and eventually recruit pericytes and smooth muscle cells to surround the mature vessels.

One of the most critical proangiogenic factors produced by pseudopalisades that are responsible for directing nearby angiogenesis in GBM is VEGF. As noted previously, pseudopalisading cells are severely hypoxic and express high levels of hypoxia-inducible transcription factors, including HIF-1. The *VEGF* gene contains a hypoxia-responsive element within its promoter that binds HIF-1, thereby activating transcription (24, 83–85). VEGF concentrations in the cystic fluid of human GBMs can reach levels that are 200- to 300-fold higher than in serum (86). Inhibition of this the HIF/VEGF pathway suppresses tumor growth experimentally (87). Once expressed and secreted, extracellular VEGF binds to its high-affinity receptors, VEGFR-1 and VEGFR-2, which are upregulated on endothelial cells of high grade gliomas but not present in normal brain (84). Receptor activation then leads to angiogenesis in regions adjacent to pseudopalisades, eventually leading to a vascular density in GBMs that is among the highest of all human neoplasms (Fig. 3).

A second proangiogenic factor that is highly upregulated in GBMs is interleukin-8 (IL-8, CXCL8) (88). Much like VEGF, hypoxia/anoxia strongly stimulates IL-8 expression and its expression is also found at highest levels within the pseudopalisades of GBM (89, 90). Unlike VEGF, IL-8 has a more punctate distribution within pseudopalisades and it remains unclear if tumor cells or scattered infiltrating macrophages are most responsible for the majority of its expression. Hypoxic upregulation of IL-8 is not directly the result of HIF activation, because there is no hypoxia responsive element within its promoter. Rather, the IL-8 promoter contains binding sites for other transcription factors, including NF-κB, AP-1, and C-EBP/NF-IL-6. AP-1 appears to mediate much of IL-8's upregulation by hypoxia/anoxia (91). IL-8 is also strongly upregulated by tumor cells in response to activation of factor VIIa by TF. Such overexpression of IL-8 by neoplastic cells may have autocrine effects on the malignant behavior of tumor cells (i.e. invasion or metastasis) in addition to inducing angiogenesis (78). The IL-8 receptors that could potentially contribute to IL-8 mediated tumorigenic and angiogenic responses in GBM include CXCR1 and CXCR2, both of which are G-protein-coupled.

Lastly, hepatocyte growth factor (HGF) is upregulated in human astrocytomas and its expression levels correlate with tumor grade (30, 92, 93). Multiple studies have shown that HGF has strong proangiogenic effects in vitro as measured by its ability to cause endothelial proliferation, migration, matrix invasion, and tubule formation (30). In addition to its own angiogenic effects, HGF causes the upregulation and secretion of VEGF by glioma cells (94). Investigation of human astrocytoma specimens by immunohistochemistry and in situ hybridization has localized HGF receptor c-Met to both endothelial cells and tumor cells with increasing expression in both compartments found in higher grade tumors (95, 96). Overexpression of HGF in xenografted gliomas leads to increased angiogenesis and tumor growth, whereas antagonism of this signaling cascade leads to inhibited angiogenesis and an antitumor effect (97–99). Although hypoxia has been shown to induce the expression of c-Met and promote the effects of HGF on c-Met signaling, the expression of HGF in pseudopalisading cells of glioblastoma has not yet been demonstrated (26, 29).

The precise type of angiogenesis that is most evident in GBM, 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 (Fig. 3) (13). 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 other forms of cancer (100). Because necrosis and hypoxia are located in the GBM's core and near the contrast-enhancing rim, hypoxia-induced angiogenesis occurs further 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 (12).

CONCLUSION

Pseudopalisades and the ensuing microvascular hyperplasia that are associated with accelerated growth in GBM may result from the following sequence (Figs. 4 and 6): 1) vascular occlusion, possibly related to 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 proangiogenic factors (VEGF, IL-8) by hypoxic pseudopalisading cells; 6) an exuberant angiogenic response creating microvascular proliferation in regions peripheral to central hypoxia; and 7) enhanced outward expansion of infiltrating tumor cells toward a new vasculature. These mechanisms could readily explain the dramatic change in behavior as tumors transition to the GBM histology. Because both necrosis and vascular proliferation are also markers of poor prognosis in other types of cancer, the identification of their underlying mechanisms may have more general implications for

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

tumor angiogenesis and malignant progression. Once identified, the pathophysiological triggers underlying vaso-occlusion will become attractive, novel targets for antitumor therapy.

REFERENCES

1. *Statistical Report: Primary Brain Tumors in the United States 1995–1999.* Chicago: Central Brain Tumor Registry of the United States (CBTRUS), 2002
2. Kleihues P, Burger PC, Collins VP, Newcomb EW, Ohgaki H, Cavenee WK. *Pathology and Genetics of Tumours of the Nervous Systems.* Lyon, France: International Agency for Research on Cancer, 2000:29–39
3. Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathol (Berl) 2005;109:93–108
4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–96
5. Shapiro WR, Young DF. Treatment of malignant glioma. A controlled study of chemotherapy and irradiation. Arch Neurol 1976;33: 494–500
6. Brat DJ, Castellano-Sanchez A, Kaur B, Van Meir EG. Genetic and biologic progression in astrocytomas and their relation to angiogenic dysregulation. Adv Anat Pathol 2002;9:24–36
7. Gupta M, Djalilvand A, Brat DJ. Clarifying the diffuse gliomas: An update on the morphologic features and markers that discriminate oligodendroglioma from astrocytoma. Am J Clin Pathol 2005;124: 755–68
8. Henson JW, Gaviani P, Gonzalez RG. MRI in treatment of adult gliomas. Lancet Oncol 2005;6:167–75
9. 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;11:575–85
10. Mandonnet E, Delattre JY, Tanguy ML, et al. Continuous growth of mean tumor diameter in a subset of grade II gliomas. Ann Neurol 2003;53:524–28
11. Swanson KR, Bridge C, Murray JD, Alvord EC Jr. Virtual and real brain tumors: Using mathematical modeling to quantify glioma growth and invasion. J Neurol Sci 2003;216:1–10
12. Bellail AC, Hunter SB, Brat DJ, Tan C, Van Meir EG. Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. Int J Biochem Cell Biol 2004;36:1046–69
13. Brat DJ, Van Meir EG. Glomeruloid microvascular proliferation orchestrated by VPF/VEGF: A new world of angiogenesis research. Am J Pathol 2001;158:789–96
14. Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. Cancer 1987;59: 1617–25
15. Nelson JS, Tsukada Y, Schoenfeld D, Fulling K, Lamarche J, Peress N. Necrosis as a prognostic criterion in malignant supratentorial, astrocytic gliomas. Cancer 1983;52:550–54
16. Raza SM, Lang FF, Aggarwal BB, Fuller GN, Wildrick DM, Sawaya R. Necrosis and glioblastoma: A friend or a foe? A review and a hypothesis. Neurosurgery 2002;51:2–12; discussion 12–13
17. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994–98
18. Zagzag D, Amirnovin R, Greco MA, et al. Vascular apoptosis and involution in gliomas precede neovascularization: A novel concept for glioma growth and angiogenesis. Lab Invest 2000;80:837–49
19. Brat DJ, Castellano-Sanchez AA, Hunter SB, et al. Pseudopalisades in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by an actively migrating cell population. Cancer Res 2004; 64:920–27
20. Bailey P, Cushing H. *Tumors of the Glioma Group.* Philadelphia: Lippincott,1926
21. Schiffer D, Cavalla P, Migheli A, et al. Apoptosis and cell proliferation in human neuroepithelial tumors. Neurosci Lett 1995;195:81–84
22. Tachibana O, Lampe J, Kleihues P, Ohgaki H. Preferential expression of Fas/APO1 (CD95) and apoptotic cell death in perinecrotic cells of glioblastoma multiforme. Acta Neuropathol (Berl) 1996;92:431–34
23. Brat DJ, Mapstone TB. Malignant glioma physiology: Cellular response to hypoxia and its role in tumor progression. Ann Intern Med 2003;138:659–68
24. Semenza GL. Hypoxia-inducible factor 1: Oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001;7:345–50
25. Zagzag D, Zhong H, Scalzitti JM, Laughner E, Simons JW, Semenza GL. Expression of hypoxia-inducible factor 1alpha in brain tumors: Association with angiogenesis, invasion, and progression. Cancer 2000;88:2606–18
26. Dong S, Nutt CL, Betensky RA, et al. Histology-based expression profiling yields novel prognostic markers in human glioblastoma. J Neuropathol Exp Neurol 2005;64:948–55
27. Graham CH, Forsdike J, Fitzgerald CJ, Macdonald-Goodfellow S. Hypoxia-mediated stimulation of carcinoma cell invasiveness via upregulation of urokinase receptor expression. Int J Cancer 1999;80: 617–23
28. Krishnamachary B, Berg-Dixon S, Kelly B, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res 2003;63:1138–43
29. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3:347–61
30. Abounader R, Laterra J. Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. Neurooncology 2005;7:436–51
31. 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;46:115–23
32. 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;56:384–92
33. 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
34. 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;153:1459–66
35. 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
36. Vajkoczy P, Farhadi M, Gaumann A, et al. Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. J Clin Invest 2002;109:777–85
37. 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;63:3403–12
38. Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. Thromb Res 2001;102:V215–24
39. Walsh DC, Kakkar AK. Thromboembolism in brain tumors. Curr Opin Pulm Med 2001;7:326–31
40. Sawaya R, Yamamoto M, Ramo OJ, Shi ML, Rayford A, Rao JS. Plasminogen activator inhibitor-1 in brain tumors: Relation to malignancy and necrosis. Neurosurgery 1995;36:375–80; discussion 380–71
41. Brat DJ, Van Meir EG. Vaso-occlusive and prothrombotic mechanisms associated with tumor hypoxia, necrosis, and accelerated growth in glioblastoma. Lab Invest 2004;84:397–405
42. 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;16:1492–58
43. Fischer S, Clauss M, Wiesnet M, Renz D, Schaper W, Karliczek GF. Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. Am J Physiol 1999;276:C812–20
44. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983–85

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

45. Sawaya R, Ramo OJ, Shi ML, Mandybur G. Biological significance of tissue plasminogen activator content in brain tumors. J Neurosurg 1991;74:480–86
46. Versteeg HH, Peppelenbosch MP, Spek CA. Tissue factor signal transduction in angiogenesis. Carcinogenesis 2003;24:1009–13
47. Morrissey JH. Tissue factor: an enzyme cofactor and a true receptor. Thromb Haemost 2001;86:66–74
48. Rickles FR, Patierno S, Fernandez PM. Tissue factor, thrombin, and cancer. Chest 2003;124:58S–68S
49. Contrino J, Hair G, Kreutzer DL, Rickles FR. In situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. Nat Med 1996;2:209–15
50. 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
51. Guan M, Jin J, Su B, Liu WW, Lu Y. Tissue factor expression and angiogenesis in human glioma. Clin Biochem 2002;35:321–25
52. Hamada K, Kuratsu J, Saitoh Y, Takeshima H, Nishi T, Ushio Y. Expression of tissue factor correlates with grade of malignancy in human glioma. Cancer 1996;77:1877–83
53. Ott I, Weigand B, Michl R, et al. Tissue factor cytoplasmic domain stimulates migration by activation of the GTPase Rac1 and the mitogen-activated protein kinase p38. Circulation 2005;111:349–55
54. Ott I, Fischer EG, Miyagi Y, Mueller BM, Ruf W. A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280. J Cell Biol 1998;140:1241–53
55. Belting M, Dorrell MI, Sandgren S, et al. Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. Nat Med 2004; 10:502–9
56. Versteeg HH, Spek CA, Peppelenbosch MP, Richel DJ. Tissue factor and cancer metastasis: The role of intracellular and extracellular signaling pathways. Mol Med 2004;10:6–11
57. Rong Y, Post DE, Pieper RO, Durden DL, Van Meir EG, Brat DJ. PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. Cancer Res 2005;65:1406–13
58. Yu JL, May L, Lhotak V, et al. Oncogenic events regulate tissue factor expression in colorectal cancer cells: Implications for tumor progression and angiogenesis. Blood 2005;105:1734–41
59. Rak J, Milsom C, May L, Klement P, Yu J. Tissue factor in cancer and angiogenesis: The molecular link between genetic tumor progression, tumor neovascularization, and cancer coagulopathy. Semin Thromb Hemost 2006;32:54–70
60. Boccaccio C, Sabatino G, Medico E, et al. The MET oncogene drives a genetic programme linking cancer to haemostasis. Nature 2005;434: 396–400
61. Hunter SB, Brat DJ, Olson JJ, Von Deimling A, Zhou W, Van Meir EG. Alterations in molecular pathways of diffusely infiltrating glial neoplasms: Application to tumor classification and anti-tumor therapy. Int J Oncol 2003;23:857–69
62. Yu JL, May L, Klement P, Weitz JI, Rak J. Oncogenes as regulators of tissue factor expression in cancer: implications for tumor angiogenesis and anti-cancer therapy. Semin Thromb Hemost2004;30:21–30
63. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943–47
64. Wang SI, Puc J, Li J, et al. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res 1997;57:4183–86
65. Rasheed BK, Stenzel TT, McLendon RE, et al. PTEN gene mutations are seen in high-grade but not in low-grade gliomas. Cancer Res 1997; 57:4187–90
66. Duerr EM, Rollbrocker B, Hayashi Y, et al. PTEN mutations in gliomas and glioneuronal tumors. Oncogene 1998;16:2259–64
67. Sonoda Y, Ozawa T, Hirose Y, et al. Formation of intracranial tumors by genetically modified human astrocytes defines four pathways critical in the development of human anaplastic astrocytoma. Cancer Res 2001;61:4956–60
68. Sonoda Y, Ozawa T, Aldape KD, Deen DF, Berger MS, Pieper RO. Akt pathway activation converts anaplastic astrocytoma to glioblastoma multiforme in a human astrocyte model of glioma. Cancer Res 2001;61:6674–78
69. Mackman N. Regulation of the tissue factor gene. Thromb Haemost 1997;78:747–54
70. Yan SF, Mackman N, Kisiel W, Stern DM, Pinsky DJ. Hypoxia/hypoxemia-induced activation of the procoagulant pathways and the pathogenesis of ischemia-associated thrombosis. Arterioscler Thromb Vasc Biol 1999;19:2029–35
71. Yan SF, Lu J, Zou YS. et al. Hypoxia-associated induction of early growth response-1 gene expression. J Biol Chem 1999;274:15030–40
72. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258–64
73. Darmoul D, Gratio V, Devaud H, Lehy T, Laburthe M. 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;162:1503–13
74. 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;276:10952–62
75. Even-Ram S, Uziely B, Cohen P, et al. Thrombin receptor overexpression in malignant and physiological invasion processes. Nat Med 1998;4:909–14
76. Marinissen MJ, Servitja JM, Offermanns S, Simon MI, Gutkind JS. Thrombin protease-activated receptor-1 signals through Gq- and G13-initiated MAPK cascades regulating c-Jun expression to induce cell transformation. J Biol Chem 2003;278:46814–25
77. Wang H, Ubl JJ, Stricker R, Reiser G. Thrombin (PAR-1)-induced proliferation in astrocytes via MAPK involves multiple signaling pathways. Am J Physiol Cell Physiol 2002;283:C1351–64
78. Hjortoe GM, Petersen LC, Albrektsen T, et al. Tissue factor–factor VIIa-specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated by PAR-2 and results in increased cell migration. Blood 2004;103:3029–37
79. Nguyen QD, De Wever O, Bruyneel E, et al. Commutators of PAR-1 signaling in cancer cell invasion reveal an essential role of the Rho-Rho kinase axis and tumor microenvironment. Oncogene 2005;24:8240–51
80. Morris DR, Ding Y, Ricks TK, Gullapalli A, Wolfe BL, Trejo J. Protease-activated receptor-2 is essential for factor VIIa and Xa-induced signaling, migration, and invasion of breast cancer cells. Cancer Res 2006;66:307–14
81. Junge CE, Sugawara T, Mannaioni G, et al. The contribution of protease-activated receptor 1 to neuronal damage caused by transient focal cerebral ischemia.Proc Natl Acad Sci U S A2003;100:13019–24
82. Junge CE, Lee CJ, Hubbard KB, et al. Protease-activated receptor-1 in human brain: Localization and functional expression in astrocytes. Exp Neurol 2004;188:94–103
83. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845–48
84. Plate KH. Mechanisms of angiogenesis in the brain. J Neuropathol Exp Neurol 1999;58:313–20
85. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843–45
86. 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 Res1996;56:2185–90
87. Kung AL, Wang S, Klco JM, Kaelin WG, Livingston DM. Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat Med 2000;6:1335–40
88. Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neurooncology 2005;7:122–33
89. Desbaillets I, Diserens AC, Tribolet N, Hamou MF, Van Meir EG. Upregulation of interleukin 8 by oxygen-deprived cells in glioblastoma suggests a role in leukocyte activation, chemotaxis, and angiogenesis. J Exp Med 1997;186:1201–12
90. Desbaillets I, Diserens AC, de Tribolet N, Hamou MF, Van Meir EG. Regulation of interleukin-8 expression by reduced oxygen pressure in human glioblastoma. Oncogene 1999;18:1447–56
91. Garkavtsev I, Kozin SV, Chernova O, et al. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature 2004;428:328–32
92. Lamszus K, Laterra J, Westphal M, Rosen EM. Scatter factor/hepatocyte growth factor (SF/HGF) content and function in human gliomas. Int J Dev Neurosci 1999;17:517–30

© 2006 American Association of Neuropathologists, Inc.

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.

93. Lamszus K, Schmidt NO, Jin L, et al. Scatter factor promotes motility of human glioma and neuromicrovascular endothelial cells. Int J Cancer 1998;75:19–28
94. Moriyama T, Kataoka H, Hamasuna R, et al. Up-regulation of vascular endothelial growth factor induced by hepatocyte growth factor/scatter factor stimulation in human glioma cells. Biochem Biophys Res Commun 1998;249:73–77
95. Koochekpour S, Jeffers M, Rulong S, et al. Met and hepatocyte growth factor/scatter factor expression in human gliomas. Cancer Res 1997; 57:5391–98
96. Kunkel P, Muller S, Schirmacher P, et al. Expression and localization of scatter factor/hepatocyte growth factor in human astrocytomas. Neurooncology 2001;3:82–88
97. Brockmann MA, Papadimitriou A, Brandt M, Fillbrandt R, Westphal M, Lamszus K. Inhibition of intracerebral glioblastoma growth by local treatment with the scatter factor/hepatocyte growth factor-antagonist NK4. Clin Cancer Res 2003;9:4578–85
98. Laterra J, Nam M, Rosen E, et al. Scatter factor/hepatocyte growth factor gene transfer enhances glioma growth and angiogenesis in vivo. Lab Invest 1997;76:565–77
99. Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett 2005;225:1–26
100. 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;62:6808–11

Downloaded from https://academic.oup.com/jnen/article/65/6/529/2645248 by guest on 20 August 2022

© 2006 American Association of Neuropathologists, Inc.

Copyright © 2006 by the American Association of Neuropathologists, Inc. Unauthorized reproduction of this article is prohibited.