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"...*those left behind.*" Biology and Oncology of Invasive Glioma Cells

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Abstract

Although significant technical advances in surgical and radiation treatment for brain tumors have emerged in recent years, their impact on clinical outcome for patients has been disappointing. A fundamental source of the management challenge presented by glioma patients is the insidious propensity of the malignant cells to invade into adjacent normal brain. Invasive tumor cells escape surgical removal and geographically dodge lethal radiation exposure. Recent improved understanding of the biochemistry and molecular determinants of glioma cell invasion provide valuable insight to the underlying biological features of the disease, as well as illuminating possible new therapeutic targets. Heightened commitment to migrate and invade is accompanied by a glioma cell's reduced proliferative activity. The microenvironmental manipulations coincident to invasion and migration may also impact the glioma cell's response to cytotoxic treatments. These collateral aspects of the glioma cell invasive phenotype should be further explored and exploited as novel antiglioma therapies.

Keywords: glioma, invasion, adhesion molecules, migration, proliferation.

Introduction

"...those left behind." The clinical course of glioma patients after surgery is determined by residual, invasive tumor cells.

Gliomas are a particularly lethal solid tumor arising from support cells in the central nervous system. The incidence of primary malignant brain tumors is approximately 5.8 cases per 100,000 person-years in the general population, and the overall survival rate 5 years after diagnosis is approximately one in four (1). Survival rates deteriorate substantially for older patients, who are also the group most frequently diagnosed with primary central nervous system (CNS) tumors. The most common primary CNS tumor is also the most malignant, glioblastoma multiforme (GBM), which claims its victims' lives most typically within 1 year of diagnosis. Survival statistics for patients with malignant CNS tumors have not shown any change for the better over the past 20 years (Figure 1).

Advances in neurosurgery and neuroradiology have established the present management practices for brain tumor patients, but these have reached their practical limits. After surgical resection of a glioma, the residual pool of invasive cells gives rise to a recurrent tumor, which in 96% of the cases arises immediately adjacent to the resection margin or within 2 cm from the resection cavity (2,3). This pattern of local treatment failure most likely is due to residual tumor cells peripheral to the removed highly cellular part of the lesion. Recurrence near the site of tumor resection may also be due to changes in the ECM coincident with scar tissue. In this sense, control of the disease by local treatment strategies (for example applied into the resection cavity) may reduce the rate of local failure and may increase the time to local progression, hopefully translating into prolonged survival time.

Recently interesting local treatment approaches such as implantation of biodegradable biopolymers containing BCNU (Gliadel) (Guilford Pharmaceuticals, Inc., Baltimore, MD), HSV-Tk gene therapy, which uses injection of vector-producing cells into the walls of a resection cavity, and convection-enhanced drug delivery have demonstrated some potential in early clinical trials (4,5). The HSV-Tk study has been discontinued on the basis of proven ineffectiveness, but considering the distant spread of the disease, not infrequently to the contralateral hemisphere, gliomas can hardly be called a local disease (6,7). It is not surprising that with local therapies investigators now observe cases of more distant satellite lesions resembling multifocal disease when tumors recur. This pattern of recurrence reveals the true potential of glioma migration and invasion, suggesting that given time, these tumors will spread throughout the entire brain.

A relationship exists between the extent of surgical debulking of GBM and increased patient survival (Figure 2). This clinical observation suggests two fundamental linked realities in the natural history of gliomas. One reality is that the greater the extent of surgical removal of GBM tissue, the more a patient's survival is extended. The second, and simultaneous, reality is that regardless of how extensive the resection, GBM recurrence is essentially inevitable, highlighting the clinical threat posed by invasive glioma cells. The ultimate total anatomical tumor resection may be considered

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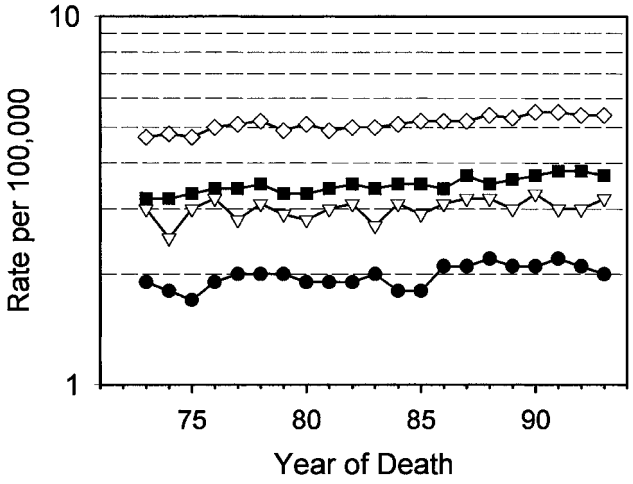

Figure 1. *Mortality rates for primary malignant brain tumors in the United States for the 20-year interval 1973 to 1993. No significant appreciable improvement in these mortality rates has been made in these two decades, highlighting the difficulty in therapeutic advances for brain tumor patients. (Symbols: Diamond, white men; square, white women; triangle, black men; and circle, black women) (Adapted from Ref. (171); with permission).*

to be hemispherectomy, for which no survival advantage could be demonstrated (8–11); consequently, it is no longer performed.

Despite this prowess for invasion, gliomas are systemically nonmetastatic (12). Development of management strategies for a nonmetastatic disease such as glioma, has a high likelihood of ushering in a profound improvement in clinical outcome for patients. Thus, sober reflection on the biology of local brain invasion and possible means to exploit this behavior are crucial in developing new treatments for this disease. In the future, therapies that target the invasive mechanisms of glioma cells may be useful adjuncts to other local strategies to limit further spread of the disease and possibly render invasive cells more susceptible to other cytoreductive treatments.

Biology of Invasive Glioma Cells

The molecular genetic aberrations described for glial tumors are elaborated in several recent reviews and texts (13–16). Mounting evidence provides strong impetus to conclude that as specific genetic mutations and/or deletions accumulate, so descends the tumor cell's behavior into more malignant grades (17–19). These molecular genetic studies derive their information from resected tumor lesions, and these specimens, even at their rim, are typical of a bulky, crowded, tumor mass.

The degree of heterogeneity between different gliomas of the same grade and even within an individual glioma sample from the same patient is remarkable (20,21). Absent from surveys on the molecular changes in gliomas, however, are the genotypes of the invasive glioma cells, which, only when regrown as a solid recurrent tumor, become the subject of such study (22–24). Morphologically, however, there is clear evidence that the invasive cells giving rise to recurrence are likely to be the small, anaplastic glioma cells beyond the peritumoral rim (2).

Glioma invasion is a very dynamic process that impacts multiple features of the glioma cells. In any one period of time, such as at the time of biopsy, invasion and proliferation behaviors of glioma cells will prove to be regionally and temporally variable. It may not be surprising that single-look biopsy analysis fails to demonstrate an association between proliferation and invasion (25). Despite very low proliferating indices of distantly invasive glioma cells (26), or even an inability to morphologically identify glioma cells distant from the rim of obvious tumor, it is, nonetheless, possible to harvest clonogenic glioma cells from such noncontiguous sites (27). The clonogenic potential of these most invasive glioma cells, referred to as "guerrilla cells" by Pilkington (28), is the ultimate cause for tumor recurrence. How invasive glioma cells survive in the setting of invasion, evading immune detection (29), thwarting cytotoxic therapies (30,31), and deferring commitment to proliferation (32), remains largely unknown.

Recently, the biology of glial cell invasion, both developmentally and in diseased states, has advanced, allowing the assembly of a paradigm of the functional constraints on migrating glial cells.

Invasion of Normal Astrocytes

Developmentally, glioblasts emerge in the subventricular zone of the brain as O-2A progenitor cells (type 2 astrocytes) or as type 1 astrocytes (33). The centripetal spread of radial glia into the cortex is accompanied by elaboration of directional matrix fibers composed of laminin, tenascin, and

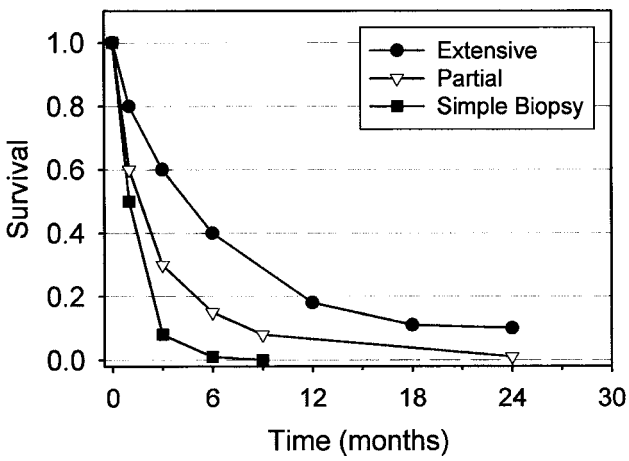

Figure 2. *Relationship between extent of GBM removed by surgery and patient survival. Survival curves illustrate outcome for 172 patients receiving extensive surgical resection (circles), 301 patients receiving partial resection (triangles), and 130 patients having biopsy procedures (squares) as the sole treatment for GBM. The greater the extent of tumor removed, the longer patients survive. GBM is a lethal disease irrespective of how aggressive the management strategy becomes. (Adapted from Ref. (172); with permission).*

vitronectin, as well as proteoglycans on which the movement of subsequent progeny glial cells is facilitated (34–38).

The motility of glial cells during development is a manifestation of contact with the matrix as well as dynamic cell-to-cell interactions mediated through specific receptors. The gap junction protein on astrocytes, connexin 43, shows a pronounced role in glial cell behavior, including communication pathways (39) and cell proliferation (40,41). Knockout mice lacking an ability to express connexin 43 develop viable offspring, but the brains show architectural aberrations consistent with abnormal astrocyte migration (42). Phosphorylation states of connexin proteins may regulate cell to cell communication (43); oncogene transfection of glial cells leads to reduced phosphorylation of connexins (44).

The neural cell adhesion molecule, NCAM, which binds to NCAM on adjacent cells, promotes astrocyte migration (45). Over the course of development, the level of NCAM on astrocytes diminishes profoundly (46) with consequences on both astrocyte motility and the ability to support neurite outgrowth (47,48).

Soluble factors, like many growth factors, are actually potent activators of astrocyte migration. Transforming growth factor (TGF) beta, basis fibroblast growth factor, epidermal growth factor, and TGF-α each promote astrocyte migration (49). The earliest glial cells, the radial glia, maintain their migratory capacity under the influence of soluble factors elaborated by the embryonic brain (50). Mature astrocytes adopt radial glia morphology when exposed to these same factors.

Although the term *glia* refers to early anatomical description of these cells as a sort of brain "glue," adult astrocytes retain their ability to migrate. After trauma, stroke, or other disease conditions characterized by necrotic brain cells, astrocytes migrate and proliferate to form scar tissue. When propagated in cell culture, nontransformed astrocytes isolated from normal brain adopt successful migratory behavior and some limited ability to self-renew (51–53). In the setting of brain tumors, gliotic scarring is not uncommon. Such cellular remodeling of the brain by normal astrocytes, when appropriately activated, indicates that invasion is a normal and regulated behavior of astrocytes (54).

Transplantation studies of brain tissue or brain cell cultures into developing or adult brain also highlight the motility propensity of astrocytes. The migration of such implanted astrocytes is invariably most pronounced in the white matter tracts (55,56). Young, developmentally immature brains sustain more extensive infiltration by transplanted astrocytes (57,58), consistent with a loss of plasticity in the mature brain. In a complementary manner, if immature glial cells are induced to differentiate *in vitro*, their invasive potential as an intracranial transplant is also diminished (59).

These findings indicate that reduced migratory potential accompanies astrocyte differentiation and that the fully mature brain is a structure somewhat resistant to cell percolation. Hormonal induction of specific integrin matrix receptors may re-engage the migratory behavior of mature astrocytes on specific substrates (60) pointing to an ability to recall developmental programs by fully differentiated cells.

Invasion of Transformed Astrocytes

Migration and invasion as key features of glioma malignancy From the natural patterns of glioma dissemination, it is evident that white matter is the preferred route for glioma cell invasion (61–63). Given the increase in understanding cellular interactions with the immediate environment, an emerging paradigm of how cell-substrate interactions influence much of cell behavior is emerging (64). Histologically, disseminating glioma cells percolate into normal brain parenchyma (Figure 3). These invasive cells comprise only a small fraction of the total tumor mass, yet they have adapted to, or been selected for, egress from the healthy, hyperdense cellular tumor edge. Because of their low abundance, compounded by the inherent cellular and genetic heterogeneity of glial tumors, these "seeds of recurrence" may be especially difficult to investigate. Alternative strategies for gaining access to the molecular genetics of glioma invasion may be to use selection criteria for generating subpopulations of cells with the desired phenotype (65) or to employ laser capture microdissection of the specified cells from fresh biopsy material (66–68). Experimental and clinical studies describe various mechanistic determinants of glioma invasion.

Cell adhesion receptors Interactions between glioma cells and their cellular and extracellular matrix environment are mediated by adhesion molecules, which are a diverse collection of cell-surface receptors. Expression of adhesion molecules is organ specific and this might account for the preferential seeding of tumors in certain organs. Adhesion molecules are classified according to their functional behavior, which is derived from the amino acid sequence and receptor ligand(s); the four major categories of such cell adhesion molecules are integrins, cadherins, selectins, and the immunoglobulin superfamily. The reported changes in these various receptors for glioma invasion are summarized in Figure 4.

Integrins Integrins are transmembrane glycoproteins that act as receptors for specific amino acid sequences found in extracellular matrix (ECM) proteins, or for membrane-bound counterreceptors on other cells (69). Because of the ubiquity of ECM throughout tissues, integrins establish both the texture of solid tissue and mediate much of the motility behavior of normal and transformed cells. Although the ECM of the brain remains incompletely catalogued, integrins on glioma cells argue for a role of cell to ECM interactions in invasion. About 20% of the total volume of the central nervous system is composed of extracellular space, which is largely filled by complex macromolecules constituting the ECM (70). Molecules in the matrix, which include fibronectin, collagens, proteoglycans, and so on. influence a number of cellular functions including adhesion, migration, proliferation and differentiation. Migration of glioma cells is dependent on ligands in the matrix (71,72). Laminin and collagen type IV

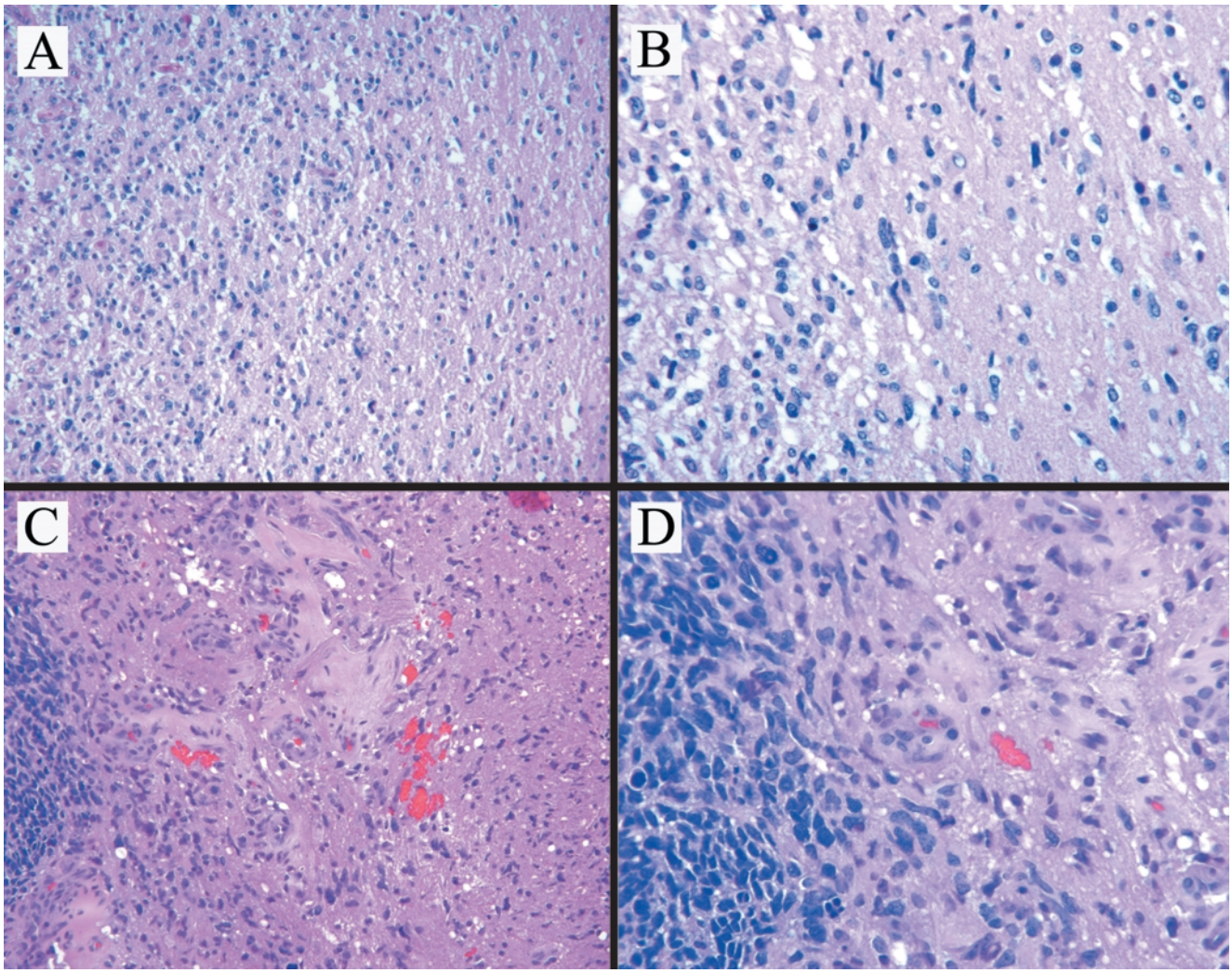

Figure 3. *Histologic appearance of infiltrating edge from high grade astrocytoma. Two different specimens are evaluated at low power (A and C) and high power (B and D) microscopy. The first case (A and B) shows diffusive infiltration of tumor cells into the brain parenchyma (B), with evidence of a very gradual gradient of declining tumor cell density moving left-to-right in the field. The second specimen (C and D) presents with a cellular tumor core and a well-delineated tumor rim (C), but at higher magnification (D) the centripetal dissemination of tumor cells can be seen. Typical glioma cell morphological heterogeneity is also evident in the higher magnification images.*

have been shown to be permissive substrates for astrocytoma migration (73), whereas fibronectin and vitronectin are less permissive (73,74). When labeled human glioma cells are seeded onto sections of normal human brain, rapid adhesion and migration occur following along anatomical structures containing matrix glycoproteins, predominantly isoforms of laminin and collagen type IV (75). Other laboratories suggest that vitronectin may be an ECM of consequence to neovascularization in GBMs (76).

There have been extensive analyses of interactions between glioma cells, their ECM and the expression of integrins (71,77), all of which have been correlated to the migratory abilities of the cells. Individual cells can vary their adhesive properties by selective expression of integrins (78). Cells are also able to modulate the binding properties of integrins. Normal astrocytes have been shown to express integrin subunits α2, α3, α6, β1, and β4, whereas subunits α4, α5, αv, β2, and β3 are consistently absent in these cells; neoplastic astrocytes show increased expression and/or neoexpression of these subunits (79). Ohnishi and colleagues (80) demonstrated that migration of glioma cells can be stimulated by fibronectin and the degree of expression of the α5 integrin subunit correlated well with the strength of glioma cell adhesion to fibronectin. Studies of primary tumor cells showed that the intensity of glioma cell adhesion to fibronectin was negatively correlated with the degree of tumor invasion (81). Laminin has been shown to be a strong promoter of glioma cell migration out of multicellular spheroids (82). Blocking the $\alpha 3\beta 1$ integrin receptor significantly reduces migration on laminin (83). Antibody-blocking studies with rat C6 glioma cells suggest that migration and invasion are mediated by β1 integrin laminin receptors (84). Deryugina and colleagues (85) have shown that only a subset of integrin receptors that are involved in cell adhesion is required to mediate migration, and these integrins are ligand specific. For example, glioma cell migration on

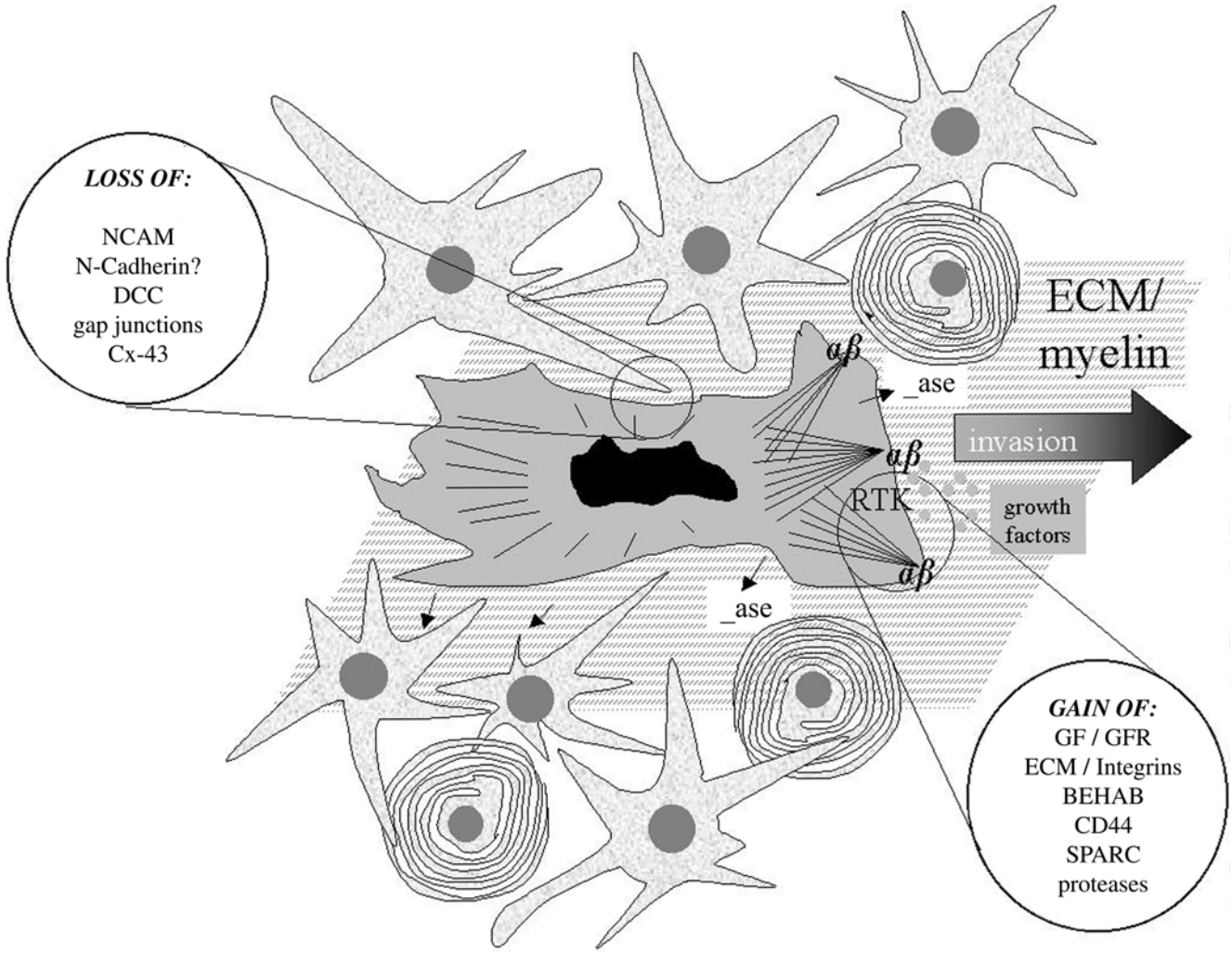

Figure 4. *Schematic diagram of modifications in gene expression (loss or gain) that promote the invasive phenotype of glioma cells. The microenvironment of astrocytes, oligodendrocytes, and ECM is the backdrop against which opportunistic or tumor-derived reactions lead to glioma cell locomotion. The overview depicts the various reported determinants of invasion gleaned from reports on the different receptors or proteins and does not intend to infer that the changes are linked, sequential or concordant. It is likely that glioma cells use common mechanisms of invasion, which include detachment consequences as well as attachment events in their successful penetration into brain parenchyma. The biochemical determinants of invasion into white matter are not necessarily the same as those needed for invasion in the perivascular space.*

fibronectin was critically dependent on αv integrins, whereas tenascin-mediated cell migration was dependent on β1 integrins.

We have shown that the matrix glycoprotein tenascin is able to provoke an antimigratory response in glioma cells, and that this phenotype is mediated by an αv-containing integrin (86). The migratory phenotype of glioma cells may be directly influenced by manipulating the expression of the αv gene as demonstrated by antibody-blocking studies as well as antisense strategies (87). Yamamoto and colleagues (88) showed that inappropriate sialylation of integrin $\alpha 3\beta 1$ can change focal adhesion as well as adhesion-mediated signal transduction and block glioma cell invasion *in vitro*.

By using purified matrix proteins of different adhesiveness such as laminin, collagen, fibronectin, and vitronectin, it has been shown that migration rates of glioma cells positively correlate with adhesiveness to the protein used for migration experiments (71). Furthermore, the degree of adhesion to a specific matrix protein (i.e., merosin) correlates to the migration rate for various cell lines. A direct linear correlation was demonstrated, suggesting that migration rates on this substrate are contingent on the degree of substrate adhesion (89). In a somewhat artificial system with CHO B2 cells which are deficient in fibronectin receptor ($\alpha_{IIb}\beta_3$), transfection with inducible expression constructs coding for variants of the fibronectin receptor having different affinities to ligand, Palacek and colleagues (90,91) demonstrated that maximum migration speed is dependent on three determinants: 1) the degree of matrix ligand density, 2) integrin receptor expression levels, and 3) integrin-ligand binding affinities. In their study a biphasic migration response curve was observed when cell detachment force to fibronectin substrate was plotted against migration speed. Optimal migration occurred at a cell-substratum detachment force of $2–4\times10^{-8}$ N. At high ligand concentration or very high levels of receptor expression (resulting in high substrate binding force),

migration speed decreased. These findings would suggest two possible mechanisms may explain loss of matrix control over tumor cell motility. Tumor cells may either loose high expression of constitutive matrix receptors, or, alternatively, gain matrix receptor function that may facilitate motility on a given substrate. However, among 13 glioma cell lines tested, no instance was observed that demonstrated very high substrate binding affinity resulting in a decreased migration rate. Integrin affinity is known to be regulated on matrix ligand binding by intracellular mechanisms (92) and may be co-regulated by the signaling cascades of other receptor pathways such as G protein receptors, including the thromboxane receptor (93). It is possible that integrin-transfected CHO cells lack the intracellular mechanisms normally required for modulation of receptor affinity coordinated by motile cells. Therefore, decreased CHO cell motility at high substrate detachment forces may be due to an artificial substrate affinity that does not occur when integrin receptors are expressed *de novo* or in the context of a normal signaling cascade initiated by native integrins.

Novel approaches to arrest local invasion of tumors by selectively activating antimigratory integrins and potentially blocking migration-enhancing integrins may have a profound impact on future therapeutic strategies (94,95) including inducing apoptosis (96,97).

Cadherins Cadherins are calcium-dependent, homotypic adhesion receptors. They play an important role in the determination of tissue organization. Decreased cadherin expression in epithelial tumors is associated with a more malignant and highly invasive phenotype (98–100). A similar biological association was described for glial tumors (101), although not corroborated in another study (102). Malignant meningiomas, like other mesenchymal tumors (i.e., sarcomas), manifest decreased cadherin expression compared with their benign counterparts (88).

Selectins Selectins are proteins that bind specifically to carbohydrates on the cell surface and mediate heterotypic cell interactions via calcium dependent recognition of sialyated glycans. While ligands for the currently known selectins are incompletely identified, it appears that signals transmitted by selectins can regulate gene expression in some types of cells (103). Although not appearing to play a role in brain development or glial cell biology, these receptors have demonstrated significance in lymphocyte homing and immune regulation and may be involved in glioma escape from effective immune reactions (104).

Immunoglobulin superfamily The *immunoglobulin superfamily* includes a diverse array of cell adhesion receptors including NCAM (the neural cell adhesion molecule), ICAM-1 (the intercellular adhesion molecule-1), and DCC. NCAM may modulate subtle changes in the invasion pathways of glioma cells (105). The expression of ICAM-1 is enhanced in GBM cells *in vitro* by cytokines (106). Such receptors modulate visibility to immune detection and may not subserve tumor cell locomotion. The tumor suppresser gene DCC encodes a protein with significant homology to members of the immunoglobulin superfamily and is likely to function as an adhesion receptor (107,108), including binding to the CNS matrix protein netrin (109). DCC has been shown to induce differentiation and control cell proliferation (110). A correlation has been noted between loss of DCC expression and glioma progression: Malignant gliomas have reduced expression of DCC, whereas low-grade astrocytomas are predominantly DCC-positive (111–113), implying that DCC may play a role in glioma progression (114,115).

CD44 The transmembrane glycoprotein CD44 is involved in development of the nervous system (116–119). Because its ligand, hyaluronic acid, is a glycosaminoglycan abundant in the CNS, much speculation exists regarding CD44 as a glioma marker (120). CD44 has been reported to mediate glioma migration and invasion (121–124). Elimination of the expression of the intermediate filament specific for astrocytes, glial fibrillary acidic protein (GFAP), which may be construed as a dedifferentiation event (125), is accompanied by an increase in CD44 expression (126). A firm link between CD44 expression and human glioma invasion has not been demonstrated, but confrontation cultures between glioma spheroids and normal brain aggregates provoke enhanced CD44 expression at the interface (127), and suppressed CD44 expression compromises the invasion of transplanted human glioma cells in the brains of nude mice (128). Hyaluronate is also specifically bound by receptor for hyaluronan-mediated motility (RHAMM) (129), which modulates many behaviors of glioma cells including locomotion (130,131). RHAMM has recently demonstrated links to kinase signal transduction (132) and to modification of the cellular response to growth factors (133).

BEHAB After the discovery of a brain enriched hyaluronan binding protein, BEHAB (134), and the demonstration of its expression during development of the CNS (135), its role as ECM substrate for cell adhesion (136), and its heightened expression in glioma biopsy specimens (137), consideration was focused on its role in glioma biology. Proteolytic cleavage of BEHAB activates glioma cell migration and invasion *in vitro* (138,139), suggesting a role for this receptor in glioma pathobiology.

SPARC Very recently, by using subtractive hybridization techniques to identify genes inordinately expressed in varying grades of glioma specimens, Rempel identified a cell-matrix protein, SPARC, as a candidate mediator of glioma invasion (140). Interestingly, this protein shows heightened localization in the invasive regions of glioma specimens. Because the protein is also expressed by reactive astrocytes, this may be an indication for a fundamental role in cell locomotion in the brain.

Gap junction communication An early event reported in transformation is the loss of gap junctional communication, mediated by reduced expression levels or decreased

assembly of connexin proteins (141). These receptors are not responsible for stable cell-cell contacts, but rather mediate cell-to-cell communication through diffusible, small molecular weight, cytosolic molecules. As such, their role in cell locomotion is likely to be indirect. For glioma cells, the levels of connexin expression correlate with proliferation (142). Connexin transfection into glioma cells retards proliferation (143) and can even revert the transformed phenotype (144). The motility rate of glioma cells is inversely correlated with levels of connexin expression (16).

Proteolytic Remodeling of the Extracellular Matrix

Locomotion of cells necessitates the availability of a space through which to travel. Enzymatic remodeling of the ECM is crucial for sustaining a cell's trajectory. A growing inventory of secreted proteolytic enzymes is being identified as produced by glioma cells (145–148), which may serve to solubilize the ECM, creating a conduit for invasion. A specific metalloprotease produced by glioma cells has been isolated that modifies CNS myelin, transforming an otherwise nonpermissive substrate into one that facilitates migration (149). This protease promotes the adhesion and migration of neurons (150), astrocyte precursor cells (151), and glioma cells (152). The establishment of specific secreted proteolytic enzymes from glioma cells and the presence of appropriate substrates as ECM barriers or motility anchors, however, has not been achieved.

Receptor Biology and the Glioma Cell Phenotype

In addition to their influence in various aspects of cellular structure and function, cell adhesion receptors regulate cell growth and differentiation by initiating intracellular biochemical signaling cascades via signal transduction pathways. Integrins are capable of transmitting biochemical signals from the ECM to the cell interior (153–155). Integrin-mediated signals overlap considerably with those induced by cytokine and growth factor receptors, most notably the pathway for receptor tyrosine kinase signal transduction (156). Cadherin-initiated signaling events have also been shown to impinge on the receptor tyrosine kinase pathway, as well as the G-protein pathway (157).

There is a relative paucity of information on the role of selectins and the immunoglobulin superfamily as signal transducers, when compared with the numerous studies with integrins and cadherins. However, it is clear that novel aspects of signal transduction involving cell adhesion molecules will most likely impinge on known signaling pathways. The morbidity and mortality associated with cancer as a disease are primarily consequences of the spread of cancer cells. Modulation of adhesion molecule expression in gliomas may afford the possibility of altering cell-cell interactions as well as cell-ECM interactions, which may be avenues of manipulating local invasion in these neoplasms.

Collectively, invasive glioma cells demonstrate loss of adhesion factors that anchor cells in the tissue while also gaining biochemical machinery conducive to the migratory behavior (Figure 4). It is likely that these biochemical changes are coordinated and responsive to microenvironmental factors. To the extent glioma cells modify and respond to conditions in their immediate environment, the invasion process can be considered to be opportunistic.

Going and Growing

The dichotomy between differentiation and proliferation of both normal and tumor cells is well accepted. Astrocytoma cells follow this mutually exclusive behavior in that induced differentiation leads to suppressed growth (158). Treatment of glioma cells with phorbol ester (an agent typically associated with differentiation induction) leads to suppressed growth but increased migration and enhanced invasion (159). Antifolate chemotherapeutic agents also suppress growth, but migration suppression became evident only after an order of magnitude higher drug concentration (160), consistent with a disparate response by the cells to growth and migration effects from the same treatment. Remarkably, specific receptor–mediated responses to TGF-β_1 by human glioma cells demonstrate growth inhibition and migration stimulation (131). Extracellular matrix proteins that activate glioma cell motility behavior suppress proliferation (23,32,161,162). Results of studies using tumor cyst fluids demonstrate that autocrine factors generated in the tumor bed lead to proliferative and migratory responses with markedly dissimilar response profiles (163); subsequent work identified scatter factor as a potent component of such fluids (164). Interleukin-10, however, had identical dose-optima for both growth and migration stimulation (165).

When human glioma cells are evaluated for their proliferative activity while under differing migration conditions, it becomes evident that environmental determinants of migration influence growth. Figure 5 demonstrates the proliferative labeling index with anti-myb-1 antibodies to reveal the interplay between migration and proliferation. When human glioma cells deposit as a confluent monolayer on a nonspecific substrate for adhesion but nonpermissive for motility, the cells adopt a uniformly active proliferative commitment (Figure 5A). In contrast, when these same cells are seeded onto laminin, which profoundly accelerates cell migration, the cells most engaged to migrate are only very infrequently found to be in cell cycle (Figure 5B). Tumor cells harvested from the vital core of a GBM will rapidly grow to large colonies in soft agar, whereas cells plated from regions of invaded brain will develop colonies of smaller size and reduced number of cells. When tested for migration *in vitro*, cells from invaded brain show higher motility rates compared with cells from the solid tumor, suggesting that invasive glioma cells are more highly migratory but show inherently decreased proliferative capacity (unpublished observations). Such an observation parallels the clinical presentation of gliomas in that the invasive, solitary cells do not manifest any proliferative activity until they generate a recurrent, satellite lesion.

A growing body of evidence from studies using treatments ranging from growth factors, cytotoxic chemotherapeutic agents, and ECM proteins, begin to drive a recognition of the

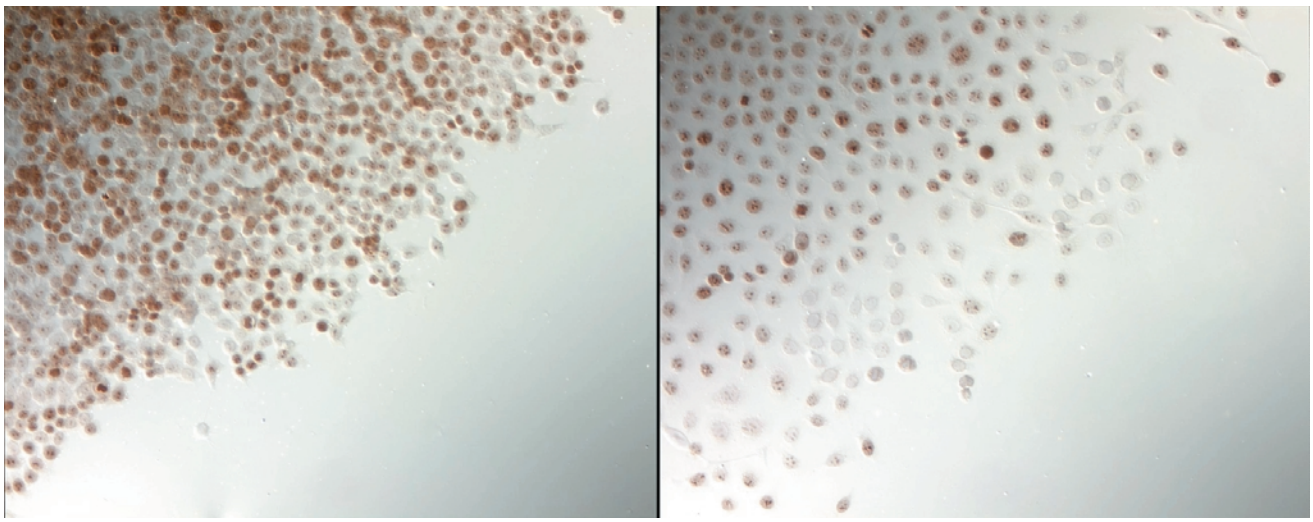

Figure 5. *Proliferation index of stationary (A) and migratory (B) human glioma cells assessed by immunocytochemistry for MIB-1. Cells were deposited as confluent discs of cells (74) onto substrates of tissue culture–treated glass or laminin that were blocked with bovine serum albumin, then the population of cells was monitored for lateral dissemination from the initial site. Cells were fixed after 48 hours of culture, probed with anti–MIB-1 antibodies (DAKO, Carpinteria, CA), treated with biotinylated anti-mouse secondary antibodies (Pierce, Rockford, IL), then incubated with strepavidin-HRP (Amersham Pharmacia Biotech, Piscataway, NJ). Reaction with DAB produces a brown insoluble product where the primary antibody bound MIB-1. Cells on glass (A) manifest minimal migration evidenced by the small intercellular space and the tightness of the perimeter cells to one another. The population of nonmotile cells is uniformly labeled by anti–MIB-1, indicating that the population of cells is largely in cell cycle. Cells on laminin (B) were in a sustained migration mode evidenced by the increased intercellular spaces and the dispersion of the perimeter cells away from the initial site of seeding. These motile cells, especially those at the periphery, show greatly diminished proliferation commitment.*

remarkable ability of glioma cells to shift between cell division or cell locomotion and the mutually exclusive basis of these options in any one time frame (166).

Oncology of Invasive Glioma Cells

Invasive glioma cells escape surgical removal and focused radiation treatment. Because of their commitment to locomotion instead of reproduction, these cells are not readily identified with radiological imaging techniques. As the repertoire of migration and invasion biochemistry becomes understood, various means by which to control this pathologic entity may present themselves. However, it must be recognized that at the time of clinical presentation, invasive glioma cells have already disseminated into the brain parenchyma, and development of anti-invasive treatments will likely prove to be too little, too late in making an impact on patient survival. Heightened commitment to migration by glioma cells is accompanied by a diminution of proliferation. One significant implication of the dichotomy between "go and grow" is that therapies designed to arrest or retard glioma cell invasion are likely to accelerate proliferation (Figure 6). We propose that this would be an unwelcomed and unfavorable outcome to anti-invasive therapies.

Two alternative approaches present themselves for a new oncology of invasive glioma cells. The first would be to attempt to exploit collateral changes in cell behavior coincident to manipulation of cell migration, and the second would be to specifically focus on the invasive glioma cells as a therapeutic target.

For the first strategy, it is recognized that biochemical, pharmacological, and biophysical alterations in the cells' microenvironment alter the executed genetic program of the cells. For interventions that directly interfere with migration, or that exploit down-stream signaling mediators of the invasive phenotype, it may be feasible to show an accompanying change in the cells' responsiveness to toxic therapies. Herein, anti-invasive treatments may not be an

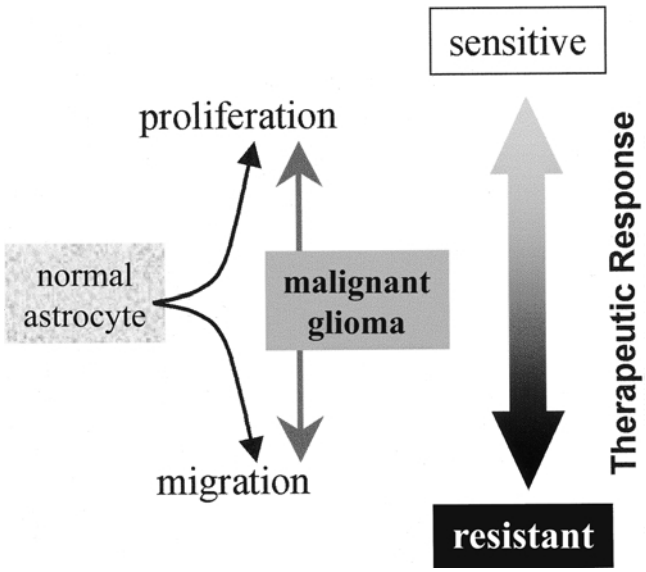

Figure 6. *Oncology of invasive glioma cells. A dichotomy exists between proliferation commitment and the migratory phenotype of normal and malignant glial cells. Arrested migration leads to more proliferation, whereas suppressed proliferation shifts glioma cells to a more migratory phenotype. Highly migratory glioma cells also show a relative resistance to cytotoxic insult (chemotherapy or radiation therapy), whereas nonmigratory glioma cells in the proliferative pool are more responsive to these treatments. Recognition of the role migration has on both proliferation and response to therapy, and identification of means by which to manipulate motility behavior to exploit therapeutic gain, should provide new opportunities for brain tumor treatments.*

effective direct intervention, but rather may shift the invading cells into a more responsive mode to other therapies. Coordinated invasion-arrest in the setting of radiation, chemotherapy, or certain gene therapies would serve to gain therapeutic advantage.

Secondly, in the context of the inverse link between proliferative and motile behaviors of glioma cells, we posit consideration of therapies that promote glioma cell migration and invasion (so called taxis therapies). Various ECM proteins have been demonstrated to activate glioma cell motility (71,167). It is recognized that ECM proteins engage specific receptors on the cell surface and that such receptor occupancy triggers specific signal transduction reactions within the cell. It is not inconceivable that such a signaling cascade would include in its repertoire the controlled prolongation of various phases of cell cycle or even proliferation arrest. In addition to such insoluble matrix molecules that engage the motility machinery of the cell, soluble factors function as motility activation agents (23,168–170). The roster of such compounds includes epidermal growth factor, platelet derived growth factor, scatter factor or hepatic growth factor, and insulin like growth factor. Although many of these are typically considered to be agents that stimulate cell proliferation, at appropriate concentrations or in conjunction with appropriate ECM ligands, these compounds provoke motility responses in cells.

Certain combinations of both soluble and insoluble agents that engage the migratory response of glioma cells could be harnessed for use as taxis therapy. In this approach, following initial surgical resection of a primary glioma, the resection cavity would be installed with a bioengineered cannula whose luminal surface was modified to present to wandering glioma cells an anchored substrate that activates glioma cell motility. The farther cells moved up the cannula, the higher the ligand density would become to activate migration. Concurrently, a solution containing chemotactic concentrations of a specific factor or a cocktail of factors that attract the glioma cells would be pumped into the resection cavity through the cannula. As glioma cells chemotactically reinvade the primary tumor bed, they would encounter the cannula, inducing their egress. These strategies which activate glioma cell motility also suppress proliferation, providing a therapeutic benefit.

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