

Cost of Migration: Invasion of Malignant Gliomas and Implications for Treatment

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Abstract: Tumors of glial origin consist of a core mass and a penumbra of invasive, single cells, decreasing in numbers towards the periphery and still detectable several centimeters away from the core lesion. Several decades ago, the diffuse nature of malignant gliomas was recognized by neurosurgeons when super-radical resections using hemispherectomies failed to eradicate these tumors. Local invasiveness eventually leads to regrowth of a recurrent tumor predominantly adjacent to the resection cavity, which is not significantly altered by radiation or chemotherapy. This raises the question of whether invasive glioma cells activate cellular programs that render these cells resistant to conventional treatments. Clinical and experimental data demonstrate that glioma invasion is determined by several independent mechanisms that facilitate the spread of these tumors along different anatomic and molecular structures. A common denominator of this cellular behavior

may be cell motility. Gene-expression profiling showed upregulation of genes related to motility, and functional studies demonstrated that cell motility contributes to the invasive phenotype of malignant gliomas. There is accumulating evidence that invasive glioma cells show a decreased proliferation rate and a relative resistance to apoptosis, which may contribute to chemotherapy and radiation resistance. Interestingly, interference with cell motility by different strategies results in increased susceptibility to apoptosis, indicating that this dynamic relationship can potentially be exploited as an anti-invasive treatment paradigm. In this review, we discuss mechanisms of glioma invasion, characteristics of the invasive cell, and consequences of this cellular phenotype for surgical resection, oncologic treatments, and future perspectives for anti-invasive strategies.

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INVASIVE PHENOTYPE OF MALIGNANT GLIOMAS: A CLINICAL CHALLENGE

With the development of new technical adjuncts for surgical management of gliomas, such as neuronavigation, functional imaging, and cortical mapping, a renewed interest in a more radical surgical removal of these tumors has emerged, and it has been hypothesized that more complete resection of tumors may translate into prolonged survival.¹ However, the inherent tendency of glial tumors to widely disseminate within normal brain has to be regarded as a natural limit to this concept. With the exception of benign ependymomas, the rare subependymomas, gangliocytomas, dysembryoplastic neuroectodermal tumors, subependymal giant-cell astrocytomas, and some nodular pilocytic astrocytomas, all other glial tumors are invasive. The degree of invasiveness does not necessarily correlate with the grade of malignancy.² Low-grade astrocytomas often show extensive infiltration of normal brain, which limits surgical resection and eventually leads to recurrence and progression of the disease. The invasion of high-grade gliomas follows similar anatomic structures, but the dynamics of this process

seem to be more rapid. After surgical removal of a malignant glioma, invariably a recurrent tumor will manifest; in more than 95% of the cases, the tumor will manifest within 2 to 3 centimeters of the resection cavity,^{3,4} and frequently immediately adjacent to the resection cavity (Fig 1). Distant satellite lesions several centimeters away from the site of the initial presentation may also be found. These lesions may also occur in the contralateral hemisphere and may increasingly become apparent in the presence of a controlled primary site as documented for recent experimental local treatments.⁵ These types of multifocal lesions have been reported to represent 1% to 10% of cases at the initial presentation,⁶⁻⁹ with a significantly higher incidence in later stages of the disease.¹⁰ Many of these tumors may not be truly multifocal but rather may reflect manifestations of more rapidly proliferating foci within a larger area of microscopically invaded brain (Fig 2).

The dissemination of malignant gliomas has been investigated extensively in the past. Analyzing a series of 120 untreated gliomas, Scherer⁹ demonstrated 60 years ago that most glial tumors show an infiltrative growth pattern. The invasion of normal brain by malignant brain tumors was found to be associated with distinct anatomic structures following myelinated axons, the basement membranes of blood vessels or other basement membrane-like structures, and the subependyma.^{9,11} It was noted that, because of a high affinity to myelinated fiber tracts, invasive tumor cells could be found several centimeters away from the bulk lesion, sometimes with extensions into the contralateral hemisphere.

INVASIVE PHENOTYPE: EARLY RECOGNITION AND SURGICAL COST

This infiltrative biologic behavior was recognized early by neurosurgeons. In the 1920s, Walter E. Dandy^{12,13} performed a hemispherectomy for a "tumor, which was clearly of the infil-

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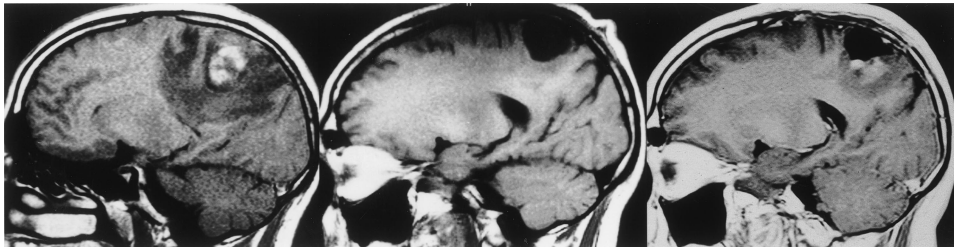


Fig 1. Local recurrence of malignant gliomas. A right parietal anaplastic astrocytoma (left) was operated, and a postoperative magnetic resonance imaging (MRI) scan confirmed complete removal (middle). Sixty-Gy radiation therapy was administered. Twelve months later, a routine MRI showed a recurrent tumor immediately adjacent to the resection cavity (right).

trating type” and stated that, “one could perhaps have resected the territory immediately surrounding the tumor, but this would have produced precisely the same disturbances as resection of the hemisphere.” Even though this and four other cases were flawed, with perioperative complications, one patient survived more than 2 years. In 1949, Bell and Karnosh¹⁴ reported a 10-year follow-up of a patient who had undergone a hemispherectomy for a cystic right frontal astrocytoma and revisited survival data of cases previously treated for gliomas with this type of super-radical procedure. Documented tumor recurrence after hemispherectomy was reported after 3 and 15 months and after 3 years for two more cases.¹⁴⁻¹⁶ In desperate recognition of the infiltrative nature of the glioma disease, French performed hemispherectomies in seven glioma patients at the cost of hemiplegia or hemiparesis and found histologic extension of the tumor beyond the limits of the resection in all cases (cited in MacCarty¹⁷). To identify gliomas that potentially could be treated with wide resections, Matsukado et al¹⁸ examined 100 consecutive cases of malignant gliomas. The authors reported that histologic examination at autopsy demonstrated bilateral extension in 47 tumors, and only 36 tumors were limited to one hemisphere and did not involve diencephalic structures. The authors concluded that, theoretically, in these selected cases, a radical surgical removal accepting a high morbidity should have been possible. Experiments exploring the feasibility of extensive surgical removal of hemispheres in primates and canines had demonstrated encouraging results regarding survival and preservation of cranial nerve function.^{19,20} These findings renewed interest in performing wide excisions including parts of basal ganglia and thalamus for malignant gliomas. However, several authors have shared the experience that super-radical removal using hemispherectomy does not ensure eradication of the disease.^{21,22} In fact, the benefits of radical surgical resection for

patients with malignant gliomas remain somewhat controversial even today.

For a long time, radiation oncologists treated malignant glioma as a whole-brain disease and, subsequently, irradiated the whole brain, which demonstrated prolonged survival over surgical treatment alone.^{23,24} A shift to increasingly localized fields of radiation was prompted by concerns over the cost of delayed toxic effects of radiation on normal brain, which led to brain necrosis and cognitive impairment.²⁵ Whole-brain radiation combined with a boost to a reduced volume proved as effective as whole-brain irradiation.²⁶ Subsequently, it was shown that tumor recurrence in the majority of cases occurred within the field of the tumor boost rather than at distant sites.⁴ This led to the conclusion that radiation, like surgery, cannot eradicate the invasive fraction of tumor cells and, therefore, that whole-brain radiation and its neurologic costs were not justified.

Finally, chemotherapy as well was used to treat the whole brain to eliminate disseminated tumor cells remaining after surgery. However, with the exception of oligodendrogliomas, systemic chemotherapy has not been a major breakthrough in the treatment of malignant gliomas, and survival benefit has generally been months. Several attempts have been made to improve the delivery of compounds and to increase their local concentration. Intra-arterial delivery of carmustine or cisplatin showed little additional efficacy but produced local toxicity and a high rate of complications that were possibly a result of high toxicity to vascular endothelial cells and limited control over drug delivery to specific vascular territories.²⁷⁻²⁹ Further attempts to increase drug delivery to brain tumors by opening the blood-brain barrier again increased the tissue concentration of the drugs.³⁰ Administration of carboplatin after modification of the blood-brain barrier by analogs of bradykinin (RMP-7) has demonstrated significant activity in recurrent glioblastomas.³¹

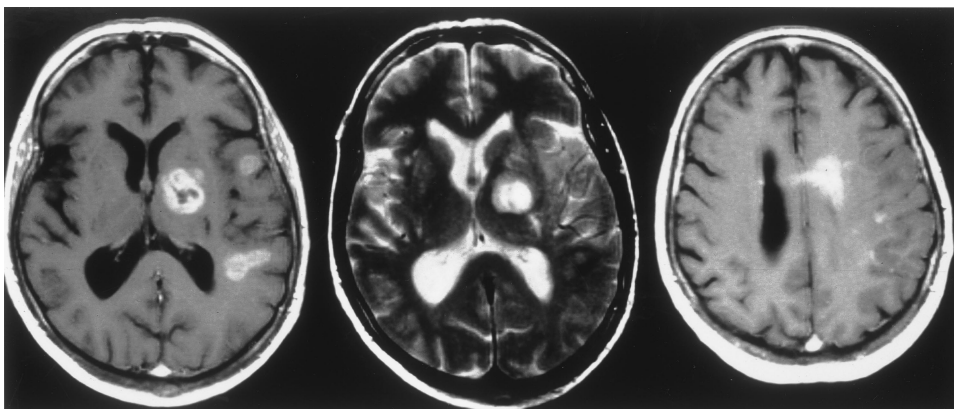


Fig 2. Multifocal glioblastoma. The T1-weighted contrast-enhanced magnetic resonance imaging scan demonstrates distinct areas of contrast enhancement (left). The corresponding T2 image shows that these foci locate within a larger area of abnormal signal intensity (middle). This hyperintense area represents both edema and tumor infiltration. Extension of the tumor following the myelinated fiber tracts of the corpus callosum has occurred (right).

However, increased tissue concentrations of these highly toxic compounds may be associated with a high risk for leukoencephalopathy, as demonstrated for the intra-arterial delivery of alkylating substances.³² A dilemma for current chemotherapy protocols seems to be that high concentrations of compounds will have to be delivered to a large volume of brain to effectively treat invasive glioma cells that apparently are not very susceptible to these agents. Maximizing the treatment effect of surgery, radiation, and chemotherapy on a widely disseminated disease comes at the cost of impairment of neurologic function and quality of life because of the large volume of normal brain within the target volume. To reduce this cost of quality of life for the patient, the infiltrative portion of the glioma has to be understood in its biology to be targeted separately and specifically. First, the question has to be asked whether invasive glioma cells have a different biology, different phenotype, and different cellular programs than the proliferating tumor core.

HISTOPATHOLOGY OF INVASIVE GLIOMA CELLS

Typically, malignant gliomas show an area of central necrosis surrounded by a highly cellular rim of viable tumor. Macroscopically, the highly cellular tumor may appear to be well delineated from adjacent brain, but histologic examination invariably demonstrates a zone of infiltrated brain. Preceding the massive invasion and destruction of adjacent structures, single invasive cells can be identified following myelinated fiber tracts where an intrafascicular, perifascicular, and interfibrillary pattern may occur with minimal damage to pre-existing neuronal structures, making the early stages of invasion a rather gentle process. Furthermore, invasive cells will migrate along basement membranes of blood vessels and within the perivascular spaces.⁹ The glial part of the vascular basement membrane may be disrupted and penetrated, but the endothelial basement membrane stays largely intact with no intravasation of tumor cells.³³⁻³⁶ Vascular basement membranes, the glial limitans externa, and the ependyma share a similar composition of matrix glycoproteins and can also be found as structures that will support dissemination of invasive glioma cells. In contrast, white matter shows an entirely different composition of matrix components (reviewed in Giese and Westphal³⁷). The ability of invasive glioma cells to use these diverse anatomic structures and molecular substrates raises the question of whether independent mechanisms and phenotypes of invasive cells are involved in dissemination following specific pathways (Fig 3A-3C).

Burger and Kleihues³⁸ analyzed the cellular topography of untreated glioblastomas. They reported that, based on the distribution of well-differentiated and anaplastic cells, the following three principal categories could be defined: glioblastomas emerging from a background of better-differentiated astrocytic neoplasms, tumors with intimate mixtures of poorly and well-differentiated cells, and neoplasms with no components of better-differentiated cells. Later, Kleihues and Ohgaki³⁹ demonstrated that, in fact, some glioblastomas represent the end stage of malignant progression of differentiated astrocytomas, whereas a second type arises *de novo*. These two tumor entities are defined by different cascades of mutational events and show different epidemiology and prognosis. The infiltrated area adjacent to the highly cellular and contrast-enhancing mass may vary

from 1 mm to several centimeters depending on neighboring anatomic structures clearly promoting or restricting invasion. Compact myelinated fiber tracts tangential to a highly cellular region may show little infiltration, whereas, on invasion of such fiber tracts, single invasive cells have been demonstrated up to 6 cm from the hypercellular area of the tumor.⁴⁰ This observation would indicate that, in addition to cellular and molecular aspects, a component of mechanical resistance influences the pattern of tumor spread in brain.

Small anaplastic cells and small fibrillated (small bipolar cells with fibrillated eosinophilic cytoplasm) cells seem to be the most freely infiltrating cell types, and small anaplastic cells are most often the sole cell type in subarachnoid and ventricular implants.⁴¹ These cells also most often contribute to mass effect, show high proliferation rates by 3H-thymidine *in vivo* uptake, and most successfully establish tumors in transplantation models.⁴²⁻⁴⁵ In a postmortem series of 50 glioblastomas, small anaplastic cells were the predominant invasive cell type, but in some cases, pleomorphic astrocytes and gemistocytic astrocytes were the major cell type in the invaded brain.⁴¹ Glioma cells with different morphologic features may invade white matter and contribute to tumor dissemination. Clearly, however, these studies have demonstrated that anaplastic phenotypes dominate other routes of invasion (eg, spread along the CSF pathways or into the subarachnoid space) rather than white matter invasion. Again, this would demonstrate that, in addition to distinct anatomic routes, invasion is defined by distinct morphologic phenotypes that, to a certain extent, determine the pattern of dissemination. However, these observations do not answer the question of whether invasive cells acquire specific cellular programs, making them functionally distinct from cells of the tumor core.

MOLECULAR AND CELLULAR CHARACTERISTICS OF THE MIGRATORY GLIOMA CELL

The advent of gene-expression profiling has opened up the opportunity to gain insight into the underlying genetic basis of tumors, including gliomas.^{46,47} These studies afford evaluation of the collateral changes in the expression and function of apparently normal genes against the backdrop of mutations and dysregulated genes (oncogenes and tumor suppressor genes) that are currently understood to underlie malignant transformation. Initial studies characterizing brain tumors indicate that genes functioning in proliferation and cell/matrix regulatory pathways are frequently dysregulated in gliomas; quantitative profiling reveals certain patterns of expression that allow distinction of patient subgroups with short or long intervals to tumor recurrence.⁴⁸ In addition, these studies offer an unanticipated glimpse into putative fundamental distinctions between tumor types, with provocative implications toward underlying molecular pathologies such as that of the spliceosome, which differentiates astrocytic from oligodendrocytic lineage neoplasms.⁴⁹ Curiously, gene-expression profiling focused on the apoptosis pathway is disappointing when used to probe for possible explanations of short- versus long-term surviving patients with glioblastoma.⁵⁰ Nonetheless, diffuse gliomas studied by these techniques sort themselves into distinct molecular subgroups that match conventional histologic criteria and may lead to the

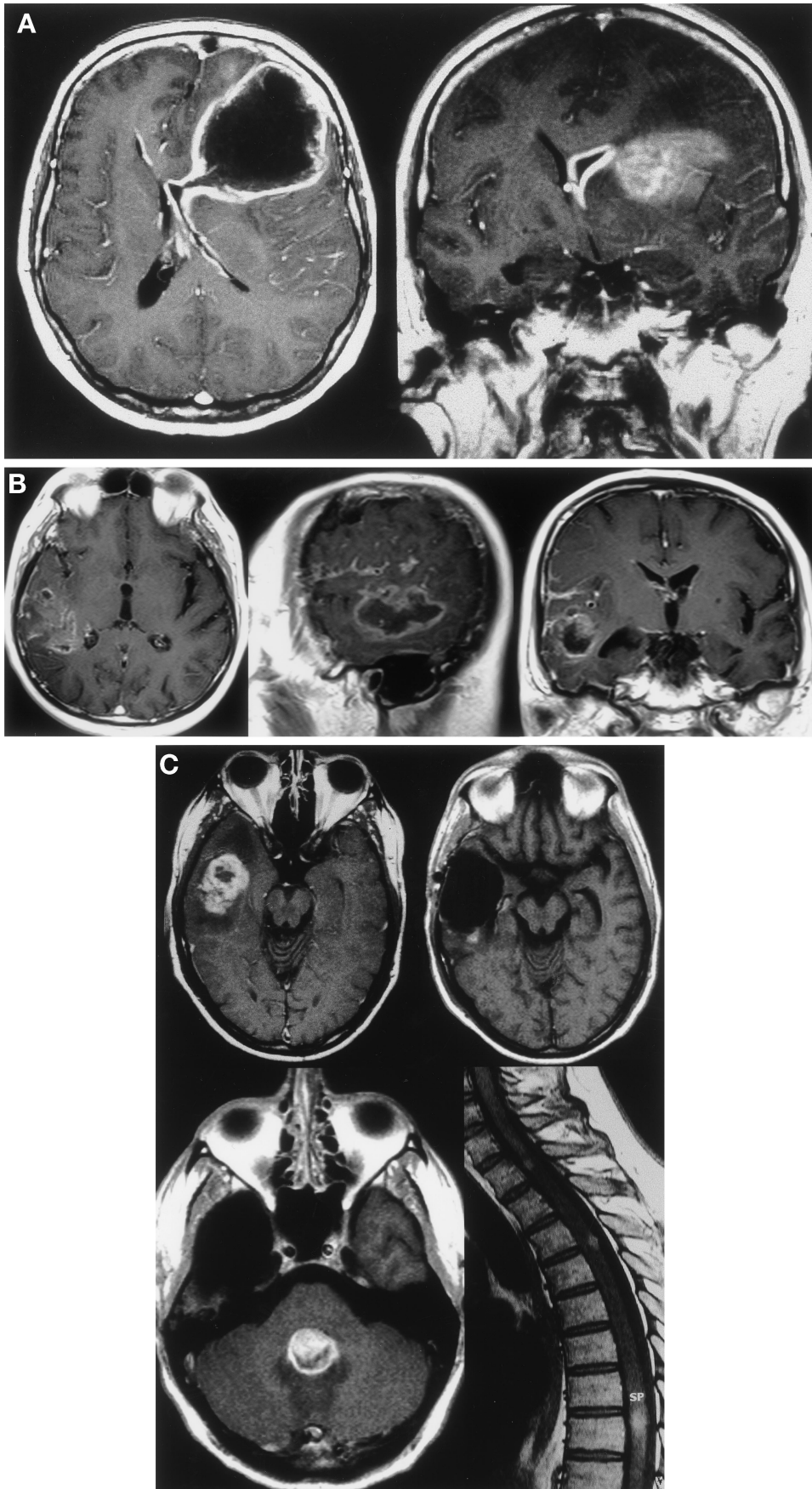


Fig 3. Pattern of spread in malignant gliomas and dissemination of gliomas along the cerebrospinal fluid pathway. (A) Gliomas located near the ventricular system may follow the subependymal layers. A certain preference for specific routes must exist for individual tumors. This tumor shows extensive infiltration of the ventricular system but no infiltration of the corpus callosum (right). (B) This temporal glioblastoma shows extensive subpial and subarachnoid infiltration of the cortical surface. Because of the large surface area invaded, any attempt of resection has to remain incomplete (from left: axial, sagittal, and coronal gadolinium-enhanced magnetic resonance imaging scan). (C) A complete removal of this glioblastoma was achieved (upper left). At 6 months, a local recurrence (upper right) and a metastatic lesion in the fourth ventricle occurred (lower left). At 8 months, spinal dissemination led to paraparesis.

identification of novel vulnerabilities of some tumors.⁵¹ Because the malignant cell characterizing the diffuse glioma phenotype remains an elusive therapeutic target, dedicated understanding of migratory and invasive molecular pathologies may be especially supportive for a new treatment paradigm of gliomas.

Motility

Invasion is a complex process, generally perceived as a multistep process.⁵² The initial step requires receptor-mediated adhesion of tumor cells to matrix proteins, followed by a second phase of degradation of matrix by tumor-secreted proteases, which can be categorized into three classes. The proteolytic activity of matrix-metalloproteinases has been correlated with invasiveness in tumors of various tissue types and may be an important mediator of glioma invasion. However, serine- and cysteine-proteases have also been implicated (reviewed in Binder and Berger⁵³ and Uhm et al⁵⁴).

Protease degradation of extracellular matrix creates an intercellular space into which invading cells can migrate by an active mechanism that requires membrane synthesis, receptor turnover, and rearrangement of cytoskeletal elements. This process also requires dissociation from adjacent cells and detachment from previous matrix adhesion sites. It has been suggested that dissemination of glioma cells in brain may not represent true invasion, but rather may be a result of growth pressure in the perivascular spaces or may represent a passive distribution of cells by the flux of CSF.⁵⁵ However, glioma cells in vitro are highly migratory cells, and the migration rate of these cells can be profoundly regulated by extracellular matrix components.⁵⁶⁻⁵⁸ Motility rates positively correlate with substrate adhesion, demonstrating that matrix receptors, which link the cellular membrane to the extracellular space, facilitate migration by providing adhesion and traction on a given matrix substrate.⁵⁹⁻⁶¹ These observations do not allow a definite conclusion that motility phenomena are involved in glioma invasion, even though motility rates reported in vitro roughly correlate with the calculated expansion rates of tumors modeled from imaging data of glioblastomas.⁶²

Silbergeld and Chicoine⁶³ established cell lines from the tumor core and invaded brain and quantified motility in vitro using a radial dish assay, but they found no increased motility in cell lines established from invaded brain. Recently, a study was presented by Mariani et al⁶⁴ who harvested 20,000 glioma cells by a laser-capture microdissection technique from invaded brain as well as the highly cellular tumor mass of a human glioblastoma specimen. Differential gene-expression profiles of invading and noninvading tumor cells were analyzed, and some 60 candidate genes differentially expressed in invasive cells were identified. One gene overexpressed in invasive cells was identified as *P311*, which localizes to focal adhesion areas and serves a structural function by linking the F-actinin bundles through a chain of linking proteins to the cell membrane. This process plays a critical role in cell motility.⁶⁵ Overexpression of this gene was confirmed in a number of glioblastoma specimens, and antisense oligonucleotide treatment decreased motility of glioma cells in a monolayer-migration assay. This demonstrates that mechanisms regulating cell motility contribute to the invasive phenotype of malignant gliomas. In fact, it is likely that

mechanisms regulating motility may represent key elements of the invasive cascade. Considering the dissimilarity of anatomic routes taken by invasive cells and the requirement for specific receptor systems to recognize and use entirely different matrix environments, migration may turn out to be a common denominator of invasion independent of the route taken.⁶⁶

Migration and Proliferation

A question that may be of great importance for the treatment of invasive cells remaining after surgical resection is whether these cells differ in their proliferation rate from cells of the tumor core because this would directly influence their susceptibility to cytotoxic therapy. Within a glioma cell population migrating on a permissive substrate in vitro, the front of migrating cells shows a decreased proliferation rate compared with the highly cellular center of the colony. Consistent with this observation, extracellular matrix that supports a migratory phenotype retards the growth rate of the cell population.⁶⁷ Soluble motogens may have similar effects on the balance between cell motility and glioma proliferation.⁶⁸ For example, transforming growth factor beta 1 stimulates glioma invasion while suppressing proliferation.⁶⁹ Apparently, motility is not exclusively controlled by exogenous stimuli such as matrix or soluble motility factors. A subpopulation of glioma cells selected for migration from an established cell line demonstrated a permanently increased migration rate compared with the parental cell line. Not surprisingly, the growth rate in this selected highly migratory subpopulation was decreased compared with the less migratory nonselected cell line.⁷⁰ This experimental evidence indicates that there may be an inherent and inverse correlation of cell motility and proliferation of a cell population. What triggers the switch of phenotype from an infiltrative, restive wanderer to a proliferative, less motile cell?

Regulation of motility is tightly controlled by matrix adhesion.⁶⁰ Cell-matrix adhesion is mediated by transmembrane receptors such as integrins.⁷¹ Integrin activation includes tyrosine phosphorylation of focal adhesion kinase, which is involved in the assembly of a large signaling complex associated with the cytoplasmic tail of the receptor.⁷² This signaling complex includes Src family kinases, cytoskeletal proteins, such as alpha-actinin, talin, paxillin, and p130^{Cas}, and other elements of downstream signal transduction molecules, such as MAP-kinase, Ras, NF- κ B, PI 3-K, PKC, JNK, and other kinases associated with the regulation of the cell cycle.⁷³⁻⁷⁶ On the basis of this convergence of shared signal transduction cascades used by both integrins and mitogenic factors, proliferation and migration are likely to be coordinated cellular behaviors. In this sense, invasive glioma cells may represent a less proliferative fraction of the tumor. These data are paralleled by some in vivo observations.

The analysis of proliferation markers in cells obtained along the trajectory of stereotactic glioma biopsies has indicated that invasive cells show a lower proliferation rate.⁷⁷ Schiffer et al⁷⁸ determined the proliferation index of human malignant gliomas focusing on the invasive cells in distinct anatomic regions. This study demonstrated that a relationship between invasion and proliferation may not be as straightforward as indicated by results obtained from in vitro experiments. Invasive cells in white matter and infiltrated cortex showed a decreased proliferation index compared with solid tumor, but a decreasing gradient

along invaded white matter could not be demonstrated for all specimens. However, invading tumor cells far from the solid tumor mass tended to have a low proliferation rate. In contrast, subarachnoidal and subpial tumor collections and tumor cells clustering within the perivascular space of leptomeningeal vessels showed high proliferation. These studies are challenged by the fact that it may be difficult to determine whether a labeled cell is actively invading, and they do not argue against a dichotomy between migration and proliferation. Subpial and perivascular growth, in contrast to white matter invasion, may represent a mechanism not related to the migratory capacity of an invasive cell.

Relationship of Cell Migration, Proliferation, and Apoptotic Predisposition

If invasive glioma cells activate a predominantly migratory cellular phenotype with a temporarily lowered proliferation rate, then these cells may also be relatively resistant to conventional cytotoxic treatments, which frequently are directed against proliferating cells. In fact, there is increasing evidence that invasive glioma cells with a low propensity to proliferate also may be resistant to apoptosis. In general, a positive correlation has been found between the proliferation rate of a cell population and a preapoptotic disposition. In neoplastic cells, a shift to an activation of the cell-proliferation machinery may prime a proapoptotic program that, unless counterbalanced by appropriate survival signals, removes the affected cell.⁷⁹ Conversely, one would hypothesize that the invasive phenotype with a lowered proliferation rate may protect invasive cells from apoptosis. In fact, Cho and Klemke⁸⁰ have demonstrated suppressed apoptosis in tumor cells during the invasive process through collagen gels. Mariani et al⁸¹ showed that, in glioma cells stimulated to migrate, cassettes of genes associated with cellular proliferation and apoptosis are downregulated, whereas a number of antiapoptotic genes are upregulated. Furthermore, laser-capture microdissected glioma cells from the invasive front of a glioblastoma overexpress the death-associated protein 3.⁸² The functional evaluation of this protein in glioma cells showed that overexpressing cells are resistant to camptothecin-induced apoptosis. Antisense treatment decreased cell migration and rendered the cells susceptible to apoptosis. A relationship between these dichotomous cellular behaviors, migration and proliferation/apoptosis, must represent dynamic events. Identification of the conversion points of these signaling pathways may allow for the influencing of the balance between these phenotypes (Fig 4). For invasive glioma cells, few such conversion points of signaling cascades have been identified.

Differential gene expression analysis showed overexpression of human thromboxane synthase in highly migratory glioma cells.⁷⁰ Subsequently, expression of this enzyme was demonstrated in gliomas *in vitro* and *in vivo*. Furthermore, specific thromboxane synthase inhibitors block glioma migration.⁸³ Thromboxane (THX) synthase, an enzyme of the arachidonic acid metabolism, mediates conversion of PGH₂ (prostaglandine H₂) to THX A₂, which is then converted to a stable but biologically inactive metabolite THX B₂. THX A₂ binds to an $\alpha_q\beta\gamma$ heterotrimeric G protein receptor that has

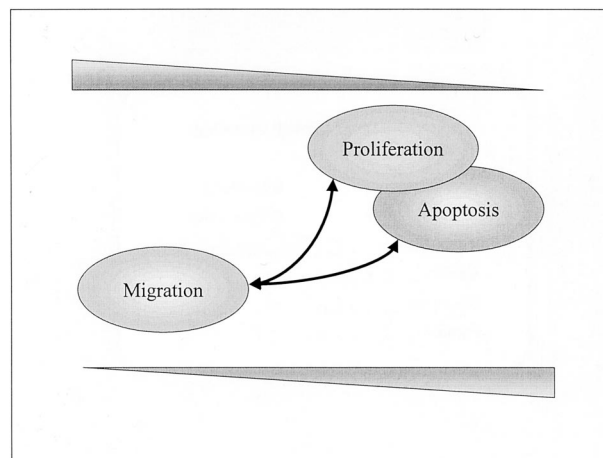


Fig 4. Dichotomy of migration and proliferation. In a cell population, migration and proliferation are antagonistic cellular behaviors. The permissiveness of a cellular environment may promote migration while decreasing proliferation and the pro-apoptotic disposition. Influencing this dynamic balance offers an intriguing paradigm for anti-invasive strategies directed against invading glioma cells.

been demonstrated in glia and glioma cells.⁸⁴⁻⁸⁶ Ligand binding leads to activation of phospholipase C β and the subsequent rise of cytosolic Ca²⁺, which activates the protein kinases PKC and PYK2.⁸⁷ These kinases lead to phosphorylation of the focal adhesion kinase pp125 FAK, which is an important element of integrin activation and integrin signaling.⁸⁸ This pathway is linked to activation of Ras, Rho, and Rac, which theoretically influences both cytoskeletal rearrangements as a prerequisite for adhesion and migration as well as transcription and proliferation. Therefore, this signaling pathway may modulate matrix-dependent adhesion and influence cell migration and proliferation. Interestingly, after THX synthase-inhibitor treatment, the decrease of motility rates is paralleled by increased caspase activity followed by intracellular DNA fragmentation and subsequent apoptotic cell death.⁸⁹ These data demonstrate that pharmacologic inhibitors may block the migratory phenotype of glioma cells and prime an apoptotic program, which was also documented by an increased susceptibility of treated cells to apoptosis induced by other agents such as camptothecin or carmustine.

The relevance of the above mechanism for the treatment of malignant gliomas remains open, but this relationship of migration/proliferation and apoptosis may present an example of a novel entry point for modification of the cellular behavior of invasive cells. Anti-invasive therapies based on this concept may not only inhibit further spread of an invading glial tumor but may also provide therapeutic targets specifically sensitizing invasive cells to conventional treatments, inducing apoptosis. Obviously, gliomas at the time of diagnosis are widely disseminated as a consequence of a highly invasive phenotype. The invasive cells retain the ability to revert to a proliferative cellular program and, in principle, seem to be able to establish a recurrent tumor most likely from a single cell. Whereas single invading glioma cells do not seem to depend on tumor-associated angiogenesis, regrowth of a recurrent tumor mass critically depends on neovascularization.

VASCULAR INDUCTION AND ANGIOGENESIS IN GLIOBLASTOMAS

Tumor-Associated Angiogenesis

The highly vascular nature of glioblastomas has led to the speculation that progression to secondary glioblastomas requires tumor-associated angiogenesis.⁹⁰ Considerable research has been directed toward developing agents that inhibit angiogenesis.⁹¹ This is based on the hypothesis that neovascularization is a prerequisite for local expansion of tumor colonies beyond the size (0.125 mm²) restricted by oxygen and nutrient diffusion.⁹¹ Angiogenesis is the process by which new capillaries sprout from pre-existing blood vessels. It is distinct from vasculogenesis in that it entails endothelial cell proliferation and migration, rather than the differentiation of endothelial cells from stem cells.⁹² The process of angiogenesis begins with the degradation of the basement membrane by proteases secreted by activated endothelial cells, which will migrate and proliferate, leading to the formation of solid endothelial cell sprouts into the stroma. Then vascular loops and capillary tubes are formed, tight junctions develop, and a new basement membrane is laid. A balance between proangiogenic and antiangiogenic factors tightly regulates angiogenesis. The angiogenic pathways leading to tumor vascularization can be divided into two general phases: the prevascular phase (often referred to as the angiogenic switch) and the vascular phase.⁹³

Angiogenesis in Gliomas

Although the mechanism governing the angiogenic surge observed in glioblastomas has yet to be identified, evidence indicates a pivotal role of vascular endothelial growth factor (VEGF).^{94,95} It has been shown that the rapid growth of glioblastomas results in focal ischemia and hypoxia, which in turn induces angiogenesis mediated by VEGF activity.⁹⁶ This assumption is based predominantly on observations of increased VEGF expression in perinecrotic regions and at the invasion zone between tumor and normal brain tissue.⁹⁶ Furthermore, it has also been shown that both VEGF and platelet-derived growth factor (PDGF) are greatly overexpressed by tumor cells in hypoxic rat and human glioblastoma multiforme tumors and that the VEGF receptors are overexpressed in tumor endothelium.^{97,98} Macrovasculature in glioblastoma are typically composed of a single layer of endothelial cells and pericytes, the latter of which are enclosed within the basement membrane. Pericytes commonly surround vessels that are less than 30 μ m in lumen diameter.⁹⁹ Furthermore, pericytes contain smooth muscle actin and are structurally and functionally similar to smooth muscle cells.¹⁰⁰ In fact, they may also differentiate into smooth muscle cells.¹⁰¹ These mesenchymal cells are essential in maintaining the function and structure of the vessels. It has been shown that endothelial cells produce many growth factors, such as PDGF, and that PDGF stimulates proliferation of smooth muscle cells and pericytes.¹⁰² Therefore, it has been suggested that endothelial cell proliferation in glioblastomas is driven by tumor-derived VEGF, whereas smooth muscle-cell and pericyte proliferation is driven by endothelial-derived and perhaps also tumor-derived PDGF.^{103,104} In contrast, it has also been shown that tumor cells are able to target and use pre-existing host vessels, a mechanism

known as co-optation.¹⁰⁵ This co-optation of host vessels ultimately leads to an induction of angiopoietin-2 expression by host endothelial cells, which in turn leads to disruption of the normal pericyte capping by the retraction of the astrocytic end-feet away from the endothelial cells. The net result is the regression of pre-existing vessels, tumor necrosis, and the subsequent onset of hypoxia-induced VEGF-mediated angiogenesis.¹⁰⁵ Interestingly, experiments blocking angiogenesis by antibodies directed against the VEGF receptor-2 in experimental gliomas demonstrated that a decrease in microvessel density was paralleled by an increase in satellite tumor formations and pronounced migration of tumor cells over long distances following the host vasculature.¹⁰⁶ These observations indicate that, under certain conditions, hypoxia and angiogenesis may influence the invasive behavior of glial tumor cells.

CONSEQUENCES OF THE INVASIVE CELLULAR PROGRAMS FOR SURGICAL TREATMENTS

Quigley and Maroon¹⁰⁷ reviewed 20 reports involving more than 5,600 patients treated over a period of 30 years. Only four studies found a positive correlation between surgical resection and survival, whereas most major series including thousands of patients did not. Even those studies that did identify a positive effect of resection recognized other variables that ranked higher in significance. In one such study, survival was increased by four times in patients younger than 45 years of age versus those who were 65 years of age or older.¹⁰⁸ Age and neurologic performance have been confirmed as high-ranking predictors of outcome by several authors, both in multivariate analyses of large patient cohorts and in studies of long-term survivors.¹⁰⁹⁻¹¹⁵ Further analysis of many of the older studies revealed several limitations, such as failure to control for patient age, functional status, tumor location, histologic criteria, selection bias, selective use of radiation, and retrospective analysis. Furthermore, the extent of resection in all cases was based on the surgeon's estimate. However, the surgeon's estimate regarding complete removal of visible tumor may only correlate with clearance of enhancement on MRI (magnetic resonance imaging) in approximately 30% of cases.¹¹⁶ Therefore, several authors have pointed out the necessity for an objective determination of residual tumor by postoperative neuroimaging.^{1,116-122} Subsequently, a significant correlation between survival and no residual contrast enhancement on postoperative MRI was established.¹¹⁶ Lacroix et al¹ showed that, on the basis of a large number of patients treated for malignant gliomas, resection of more than 98% of the contrast-enhancing lesion results in a significantly prolonged survival. This indicates that aggressive removal of macroscopic tumor could influence early regrowth of tumors, and an increasing number of authors now conclude that the extent of tumor resection may be a decisive prognostic factor (Fig 5).^{1,108,117,119,123-127} However, it is important to emphasize that, until now, no data from randomized controlled trials are available to support the assertion that aggressive surgical resection prolongs survival.^{128,129}

Salford et al¹³⁰ analyzed common denominators of long-term survivors with documented malignant gliomas. The authors reported that cases of long-term survival occurred in patients that had received wide macroscopically radical resections, but long-

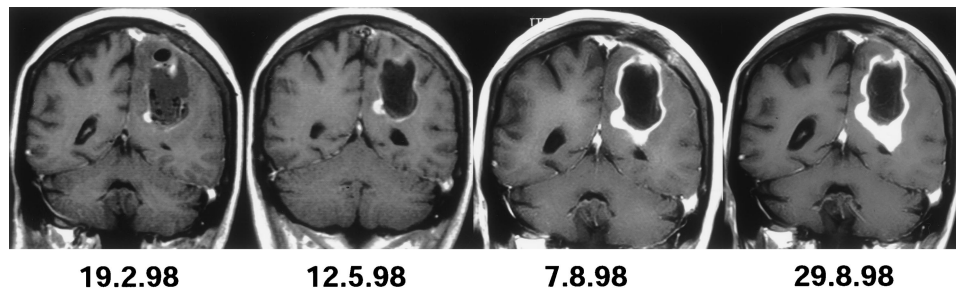


Fig 5. Progression of a glioblastoma after incomplete resection. After resection of a left parietal glioblastoma, postoperative magnetic resonance imaging demonstrated a small tumor remnant that, despite radiation therapy, showed continuous progression. This has prompted an interest in technical adjuncts for intraoperative tumor detection and is the rationale for intracavitary therapies.

term survival also occurred in patients with documented subtotal removal and even in a patient who had biopsy only. These data obtained from a small number of rare cases indicate that, in addition to the remaining tumor load, biologic factors are important in determining long-term outcome. A more recent study comparing stereotactic biopsy followed by external-beam radiation in 58 patients versus surgical resection and radiation in 57 patients failed to show prolongation of mean survival for patients that had surgical removal of malignant gliomas.¹³¹ However, cases with mass-effect radiation in the absence of surgical decompression resulted in a transient decrease of neurologic performance. These findings highlight the intuitive feeling of many neurosurgeons that surgical removal of malignant gliomas is a well-made decision when severe space-occupying lesions cause deterioration of neurologic performance and in patients for whom the total removal of the contrast-enhancing areas of a tumor can be achieved.

This controversy and the fact that, historically, a positive association of surgical resection and survival has been so difficult to demonstrate indicates that the overall effect of resection on growth kinetics of malignant gliomas must be moderate. Mathematical modeling has attempted to clarify the role of surgical cytoreduction in an infiltrative disease. One such model assumes that glial tumors are diffuse with a high growth rate and that, after a "gross total removal" or even after a maximal excision, a shell of infiltrating tumor cells remains that will rapidly regenerate a space-occupying tumor mass.⁶² Simulating the extent of resection by calculating concentric resection boundaries (no resection, total resection of equivalent to macroscopically detectable tumor, or super-radical resection beyond the boundaries of the core lesion), a hypothetical survival time was calculated until growth of a potentially lethal tumor volume. This simulation indicated that, on the basis of regrowth kinetics, there is an advantage of surgical cytoreduction, but prolongation of time to regrowth of a critical tumor mass is significantly prolonged only for super-radical resections. For example, in this model of growth dynamics, no resection will lead to a hypothetical median survival of 33.4 weeks. A simulation of gross total resection increases the median survival to 35.0 weeks only, whereas resections 0.5 and 1.0 cm beyond the macroscopic tumor result in 41.1 and 52.6 weeks, respectively, until calculated regrowth of a lethal tumor mass. These findings argue that, considering the effect of cytoreduction, removal of macroscopic tumor has a rather modest effect on mass and volume development in malignant gliomas. This also implies that the invasive (noncontrast-enhancing on MRI) portion of the lesion signifi-

cantly determines development of mass effect in the course of the disease.

The immediate goals of surgical treatment of malignant gliomas, therefore, remain the establishment of histologic diagnosis, reduction of space occupation, improvement of neurologic status, and creation of a time window for other adjuvant treatments.¹³² Considering that the effect of surgery on survival may be modest, the costs imposed on the patients must be minimal. The postoperative morbidity obviously depends on size and location of a lesion. For larger patient populations, an overall morbidity of 8% and a mortality less than 3% are estimated.¹³³ Ciric et al¹³⁴ demonstrated that total or near-total removal of a lesion was likely to improve the neurologic status at a morbidity of 3%. In contrast, in this series, subtotal removal had a 40% risk of morbidity, with brain edema and hemorrhage accounting for most of the postoperative complications.

In addition to perioperative complications, there may be a certain risk for oncologic complication precipitated by surgical intervention. Dissemination of malignant gliomas via the CSF pathways and subarachnoid metastasis are reported in 15% to 25% of malignant gliomas.¹³⁵⁻¹³⁷ Some reports indicated that tumor location adjacent to the CSF pathways or surgical entry of the ventricular system may be a predisposing factor.¹³⁸⁻¹⁴² However, a study evaluating pre- and postoperative computed tomography and MRI findings demonstrated no association of symptomatic CSF seeding with tumor location or surgical ventricular entry.¹⁴³ Interestingly, the extent of spinal and leptomeningeal dissemination of glioblastomas seems to be independent of the degree of parenchymal tumor invasion. Onda et al¹⁴⁴ reported that extensive ventricular dissemination and leptomeningeal spread occurred within tumors demonstrating minimal infiltration of adjacent white matter, whereas tumors with massive parenchymal invasion and spread into the contralateral hemisphere and brainstem showed minimal or no dissemination along the CSF pathways. These observations indicate that this phenomenon may reflect biologic mechanisms, such as loss of intercellular adhesion and cell shedding, related to the classical mechanisms of metastasis rather than to mechanisms of local invasion. Interestingly, a loss of glial fibrillary acid protein expression in tumor cells from leptomeningeal deposits has been observed, indicating a loss of astrocytic differentiation that may be related to this phenomenon.^{135,144}

Metastasis outside the neuraxis is exceedingly rare. In a series of 8,000 neuroectodermal tumors at autopsy, 23 cases with extra axial metastasis were glioblastomas.¹⁴⁵ The organs involved in order of frequency were lung, liver, lymph nodes, bone, pleura,

kidney, and others.¹⁴⁶ Most of the few cases reported followed surgical intervention¹⁴⁷; however, there are scattered reports that metastasis may occur spontaneously.^{148,149} Most likely, surgical intervention does not significantly contribute to progression of the disease in terms of dissemination or metastatic events. However, there may be other risks caused by surgical alterations related to changed local conditions at the resection site.

Despite a far dissemination of infiltrating glioma cells in the brain, the vast majority of tumors recur immediately adjacent to the resection cavity or within 2 cm around the resection cavity.^{3,4} An unsolved question is whether surgical trauma induces formation of a glial scar that may contribute to this predominantly local pattern of recurrence by alteration of the local environment of invasive tumor cells. In general, epidemiologic studies have failed to show a correlation between head injury causing traumatic glial scars and malignant brain tumors.^{150,151} Only six brain tumors were reported among 14,445 cases of missile injuries (incidence = 0.04%),¹⁵² and a prospective study observing 2,953 patients with head injuries over a period of 29,859 patient-years did not show a difference in the incidence of brain tumors.¹⁵³ But scar formation may influence the cellular behavior and the growth characteristics of invasive cells that pre-exist in an area undergoing such transformation.

Traumatic injury to the CNS initiates reactive gliosis and angiogenesis with increased local expression of several potent mitogens as well as altered expression of insoluble extracellular matrix components.¹⁵⁴ For instance, both fibroblast growth factor and insulin-like growth factor have been shown to induce glioma cell proliferation (reviewed in Hamel and Westphal¹⁵⁵). The glial scar is rapidly formed after injury by fibroblasts, hematogenous cells, and microglia, and is formed predominantly by reactive astrocytes. The vigorous response of these cells leads to profound changes in the composition of the extracellular matrix, which is radically different from the matrix of normal brain. Within the lesion site, extensive deposits of basement membrane components are laid down¹⁵⁶ with increased density around blood vessels and also adjacent to astrocytic processes.¹⁵⁷ The *de novo* formation of matrix proteins includes the glycoprotein laminin, fibronectin, tenascin C, collagen type IV, vitronectin, and a large family of proteoglycans, such as chondroitin sulfate, heparan sulfate, keratan sulfate, and others.¹⁵⁸

When purified components of such a matrix are tested *in vitro*, various and sometime conflicting effects on glioma growth and

motility have been observed.^{37,56} Glycosaminoglycans in scar tissue have demonstrated predominantly inhibitory effects on motility in several cellular systems, including gliomas. Clearly, small amounts of nonpermissive components can render an otherwise permissive matrix inhibitory for cell migration.³⁷ For example, glioma cells seeded onto laminin-coated surfaces show up to 70-fold induction of motility rates over baseline values. The addition of small amounts of vitronectin or secreted protein acidic and rich in cysteine were able to render a laminin matrix nonpermissive.⁶¹ Inhibition of glioma migration *in vitro* by a nonpermissive matrix significantly increased proliferation rates.⁶⁷ These *in vitro* data indicate that the glial scar may decrease the permissiveness of the extracellular matrix environment. This may inhibit the invasive phenotype of the cells, which in turn may promote cell growth within the surgically created glial scar. However, this speculation is based solely on *in vitro* evidence, and further investigations are needed to clarify this important issue.

IMPLICATIONS FOR LOCAL THERAPIES

Despite advances in surgical and oncologic brain-tumor therapy, there has been little progress in improving the survival of patients with malignant gliomas, and a median survival time of 12 to 14 months can still be anticipated. Invasive cells remaining after surgical resection significantly contribute to the demise of the patients. It is apparent that progressive neurologic dysfunction may occur despite the absence of a mass effect or a recurrent bulk disease. A number of glioma patients die without evidence of a significant tumor load because of neurologic deterioration, indicating that the infiltrative disease significantly contributes to the poor course of glioma patients.¹⁰ Any successful treatment strategy will have to treat the invasive portion of the tumor and the core lesion.

Because of the large area invaded by glioma cells, a relatively large brain volume (if not the whole brain) will have to be covered by a treatment directed against invasive cells. Local treatment strategies, for example, applied to the resection cavity may reduce the rate of early local failure and may, thus, increase the time to local progression. For most of these strategies, the volume of treated brain is relatively small. Local chemotherapy protocols using degradable polymers depend on the diffusion of the delivered drugs to the brain parenchyma, which presumably is within millimeters around the site of implantation.

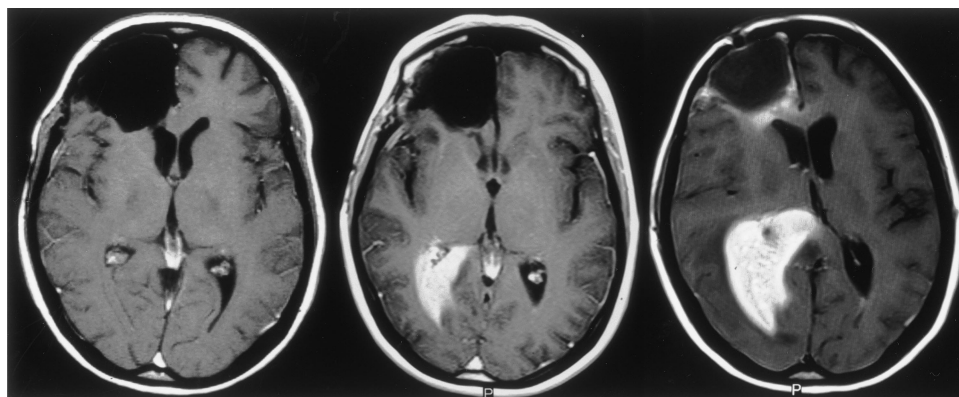


Fig 6. Local control of malignant gliomas. Assuming efficacy of compounds administered locally, the question remains whether local control of malignant gliomas is going to significantly impact the course of these widely disseminated tumors. A right frontal glioblastoma was removed by a wide resection (left). At 3 months, the tumor was controlled locally, but a large diffuse contrast-enhancing area in the temporal and occipital white matter appeared (middle), which rapidly progressed within 1 month (right). This capacity to invade illustrates that gliomas affect the brain as a whole and, therefore, require a whole-brain treatment.

Increased permeability of tumor vessels is the basis of edema, which follows a directional flow from the bulk mass of the tumor to the periphery, where it is subsequently absorbed and cleared.¹⁵⁹ This bulk flow can be enhanced and exploited for delivery of large molecules by implantation of catheters into the tumor followed by a positive-pressure infusion. This concept of a convection-enhanced delivery has demonstrated that experimental compounds can reach a large volume of brain by a mechanism that is not limited to passive diffusion.^{160,161} Because removal of a brain tumor usually results in fast resolution of edema and a reversal of fluid flow directed toward the resection cavity, this treatment will only be successful in tumors not scheduled for resection. With the exception of convection-enhanced delivery, most local therapies will not reach a large enough volume of brain to account for the invasive front of a glial tumor. As attractive as local treatment strategies are today, their perspective in the long run will be limited in the absence of collateral anti-invasive concepts (Fig 6).

FUTURE PERSPECTIVES IN ANTI-INVASIVE THERAPIES

Experimental anti-invasive therapies for malignant glioma conceptually are aimed at containment of the disease to improve the efficacy of local treatments. However, this faces the limitation of a widely disseminated disease at the time of diagnosis. Attempts to limit further spread of the disease may be of little value in a situation in which even a hemispherectomy cannot

eradicate the tumor. The biology of invasive glioma cells seems to render this fraction of the tumor relatively resistant to conventional approaches such as radiation and chemotherapy. Therefore, specifically targeting invasive glioma cells remains an interesting concept because invasiveness must be a common denominator of a major fraction of tumor cells remaining after surgical resection. Experimental evidence now indicates that glioma invasion may be regulated by distinct and possibly independent molecular trigger mechanisms. Therefore, downstream effector molecules of the invasion process may represent the most attractive treatment targets. Matrix-metalloproteinases may be such a point of convergence for regulatory pathways downstream from growth factors, cytokines, and oncogenes.⁵⁴ Cellular locomotion may be another common molecular denominator of this complex cellular behavior. The signaling events in motility and invasion of glioma cells are currently under intense investigation. It already has become clear that motility shares common signaling pathways with proliferative and apoptotic events. A better understanding of these interdependent cellular programs will provide entry points that may allow modification of a cell's preference to migrate and invade versus a proapoptotic state. These approaches could specifically use mechanisms of invasion to sensitize cells to induction of cell death.

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