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From Expert Review of Neurotherapeutics Glioblastoma Therapy: Going Beyond Hercules Columns



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Abstract and Introduction

Abstract

Glioblastoma multiforme is the most common primary brain tumor in adults. Median survival from the time of diagnosis is 14 months, with less than 5% of patients surviving 5 years. Despite advances in deciphering the complex biology of these tumors, the overall prognosis has only slightly improved in the past three decades. The clinical failure of many therapeutic approaches can be explained by the following considerations: the location of tumors within the brain presents a special set of challenges, including ability of drugs to cross the BBB; cancer cells have unstable genetic structures, very susceptible to mutations; cancer cells have an amalgam of different genetic defects that respond in different ways to any given treatment agent; and, infiltrating and apparently normal but 'activated' cells are evident in the brain surrounding the main tumor. In this way, the biologic phenomena of the 'normal brain' adjacent to the enhanced tumor could allow us to understand the first steps of cancerogenesis and, consequently, to interfere with the pathways responsible for tumor growth and recurrence.

Introduction

Glioblastoma (GBM) is the most common primary brain tumor in adults. Primary GBM, which comprises more than 90% of cases, arises *de novo*, whereas secondary GBM progresses from previously diagnosed low-grade gliomas.^[1] Despite implementation of intensive therapeutic strategies and supportive care, patients with newly diagnosed GBM have a median survival of approximately 14 months from intervention, with a 5-year survival rate of less than 5%.

Molecular studies have identified important genetic events in human GBMs, including the following: inactivation of the p53

and retinoblastoma tumor suppressor pathways; dysregulation of growth factor signaling via amplification and mutational activation of receptor tyrosine kinase genes; and, activation of the phosphatidylinositol-3-OH kinase and the MAPK pathway.^[1] Recently, two key papers from Vogelstein's lab^[2,3] identified a highly interconnected network of aberrations (Figure 1), including three major pathways: receptor tyrosine kinase signaling and the p53/retinoblastoma tumor suppressor pathways. Moreover, these authors identified *IDH1* mutations and their potential clinical importance. First, mutations in *IDH1* preferentially occurred in younger GBM patients. Second, mutations in *IDH1* were found in nearly all of the patients with secondary GBMs (mutations in five of six secondary GBM patients, compared with seven of 99 patients with primary GBMs). Third, patients with *IDH1* mutations had a significantly improved prognosis, with a median overall survival of 3.8 years compared with 1.1 years for patients with wild-type *IDH1*.

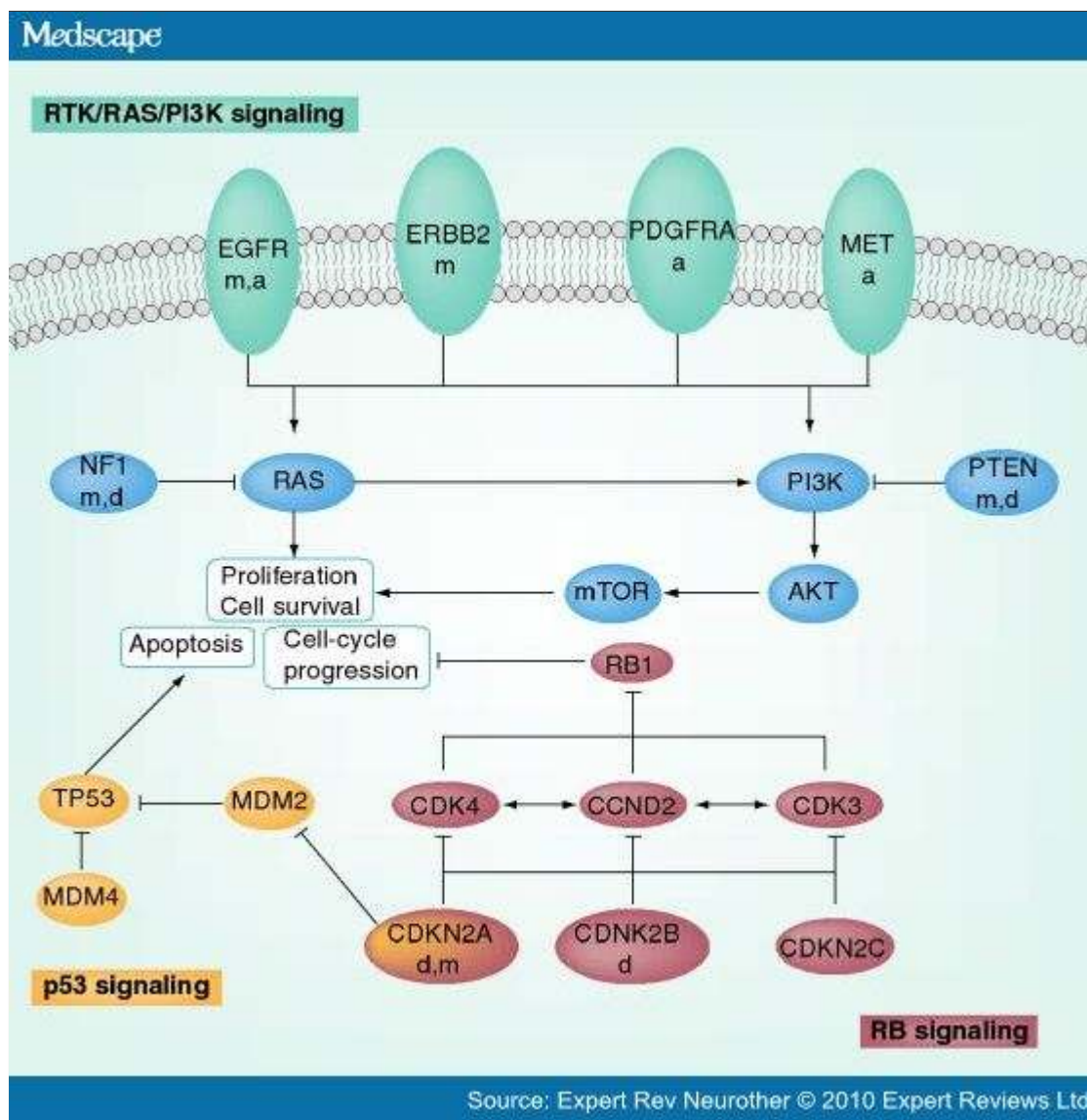


Figure 1. Genetic alterations and pathways involved in tumorigenesis.

a: Amplification; d: Deletion; EGFR: EGF receptor; m: Mutation; PDGFRA: Platelet derived growth factor receptor α ; RB: Retinoblastoma tumor suppressor protein. Modified with permission from Cancer Genome Atlas Research Network.^[3]

Surgery should be the first therapeutic modality for GBM and the optimal goal is complete resection. Gross tumor resection immediately decompresses the brain and, due to the consequent reduction in neoplastic cells in the surgical cavity, probably increases the likelihood of response to radiotherapy and/or chemotherapy; it may, moreover, delay progression.^[4] Therefore, all patients should undergo tumor resection that is as extensive as possible.^[4] However, as GBM is infiltrative, complete resection is virtually impossible and relapse is almost inevitable. The extent of surgery is limited by the extensiveness of the tumor and/or the presence of eloquent areas, and so these patients can only undergo partial resection, which makes a worse prognosis more likely.^[5] Postoperative external beam radiation delivered conformally to the tumor volume (now commonly determined by both MRI contrast-enhancement and surrounding T2 signal hyperintensity) is now standard adjuvant treatment for GBM; 60 Gy in 30 fractions are delivered for a total of 6

weeks, to a target volume defined as a 2–3 cm ring of tissue surrounding the perimeter of the contrast-enhancing lesion.^[6] A randomized study conducted on 77 GBM patients older than the age of 70 years has demonstrated a survival advantage of radiotherapy over the best supportive care, without reducing the quality of life or cognition.^[7] The use of radioenhancers is still being investigated. Motexafin gadolinium (MGd) is a putative radiation enhancer initially evaluated in patients with brain metastases. In a preliminary Phase I trial study, MGd was administered in a 2–6-week course (10–22 doses) concomitant with radiotherapy in patients with GBM, demonstrating a median survival of 17.6 months. In a case-matched analysis, the MGd patients had a median survival of 16.1 months (n = 31), compared with the matched Radiation Therapy Oncology Group database patients, with a median survival of 11.8 months.^[8]

In the last 40 years, several randomized clinical trials have examined the role of adjuvant chemotherapy in improving the survival of brain tumor patients. Chemotherapeutic agents have been administered before ('neo-adjuvant'), during ('concomitant') or after ('adjuvant') radiotherapy. The addition of temozolomide to radiotherapy, resulting in a survival benefit with minimal additional toxicity, has become the standard treatment for newly diagnosed GBM. Methylguanine methyltransferase (MGMT) excision repair enzyme has been associated with tumor resistance, because it may reverse, in part, the impact of alkylating drugs by removing alkyl groups from the O6 position of guanine. Inactivation of the *MGMT* gene in the tumor tissue by methylation of the promoter region has been associated with good outcomes in malignant glioma.^[9] The final results of a trial with a median follow-up of more than 5 years, demonstrated that the methylation of the *MGMT* promoter is the strongest predictor for the outcome and benefit of temozolomide chemotherapy.^[10] Temozolomide has proved to be a proautophagic cytotoxic drug.^[11] Several potential common targets in apoptosis and autophagy resistance pathways, specifically, mTOR, class I PI3K and Akt, have been identified in GBM. The same authors found that temozolomide decreased the expression levels of hypoxia inducible factor-1 α , ID-1, ID-2 and c-Myc in the glioma models that they investigated, all of which played major roles in angiogenesis and the switch to hypoxic metabolism. These changes could be at least partly responsible for the impairment of angiogenesis.^[12] The current standard of care, that is, the Stupp protocol, has led to a significant improvement in patient survival. This protocol consists of radiotherapy plus continuous daily temozolomide (75 mg per square meter of body-surface area per day, 7 days per week from the first to the last day of radiotherapy), followed by six cycles of adjuvant temozolomide (150–200 mg per square meter for 5 days during each 28-day cycle).^[10] However, following the introduction of the new standard of care for newly diagnosed GBM patients, with radiotherapy and concomitant/adjuvant temozolomide, new first- and second-line treatments are under evaluation. For this reason, even in the absence of clear data, a nitrosourea-based chemotherapy should be considered as a reasonable option,^[13] as well as a temozolomide rechallenge for patients that did not progress during temozolomide treatment.^[14]

Targeted Therapy: EGF Receptor

Several targeted therapies have been evaluated during the last 5 years (Table 1), in particular against the EGF receptor (EGFR), which is mutated in 45% of GBM. Recent studies propose that coexpression of EGFRvIII and phosphatase and tensin homolog (mutated in multiple advanced cancers and in 36% of GBM) in the tumor is crucial for response to EGFR inhibitors.^[15] However, in a study by Haas-Kogan and coworkers, none of the responders expressed EGFRvIII, while response was associated with EGFR amplification and expression, and low levels of protein kinase B/Akt phosphorylation.^[16] These molecular studies were performed on tissue from the initial resection, which may not reflect the molecular pattern at recurrence after variable treatment regimens. Molecular analysis of tumor tissue samples available from resection during treatment with the EGFR inhibitors erlotinib or gefitinib seems to suggest inefficient inhibition of EGFR phosphorylation.^[17]

Table 1. Glioblastoma therapy: brief literature review.

Drug (commercial name)	Target	Patient population	Survival	Ref.
Gefitinib (Iressa) or Erlotinib (Tarceva)	EGFR	49 recurrent GBM	Median time to progression: responders: 9.7 months; nonresponders: 1.7 months. Responsivity strongly associated with coexpression by the tumor of EGFRvIII and PTEN	[16]
Gefitinib	EGFR	53 recurrent GBM	Median OS time from treatment initiation: 39.4 weeks	[18]

Erlotinib	EGFR	110 recurrent GBM	Control arm (temozolomide): 6-month PFS: 24%; treatment arm (erlotinib): 6-month PFS: 11.4%	[19]
Bevacizumab (Avastin)	VEGF	35 recurrent GBM	Bevacizumab and irinotecan (a topoisomerase 1 inhibitor-Campto): 6-month PFS: 46%; 6-month OS: 77%	[20]
AZD2171 (Cediranib)	VEGF	16 recurrent GBM	6-month PFS: 25.6%	[21]
CCI-779 (Temosolimus)	mTOR	43 recurrent GBM	Median time to progression: 9 weeks	[22]
CCI-779	mTOR	65 recurrent GBM	6-month PFS: 7.8%	[23]
Imatinib	PDGFR	51 recurrent GBM	6-month PFS: 9.8%	[26]
Imatinib and hydroxyurea	PDGFR	30 progressive GBM	6-month and 2-year PFS: 32 and 16%	[29]
BCNU wafers (Gliadel)	Alkylating agent	207 newly diagnosed GBM	Median survival: 13.5 months for the BCNU group and 11.4 months for placebo group	[30]
NK cells		Nine recurrent malignant gliomas (16 cycles)	Three partial responses, two minor responses, four no changes and seven progressive disease	[35]
Dendritic cells		24 recurrent malignant gliomas	OS of patients with grade 4 glioma: 480 days, significantly better than that in the control group	[40]
Vaccine (patient's tumor cells with Newcastle disease virus)		23 newly diagnosed GBM	OS of vaccinated patients: 100 weeks	[46]

EGFR: EGF receptor; GBM: Glioblastoma; NK: Natural killer; OS: Overall survival; PDGFR: PDGF receptor; PFS: Progression-free survival; PTEN: Phosphatase and tensin homolog.

In a Phase II gefitinib trial on a series of 53 patients with recurrent GBM, the 6-month progression-free survival (6-PFS) was 13% (seven of 53 patients).^[18] More recently, a large, well-conducted, randomized Phase II study by the European Organisation for Research and Treatment of Cancer (EORTC 26034 trial) compared first-line erlotinib with either temozolomide or BCNU as standard treatments, and the study confirmed that the results are disappointing when the EGFR inhibitor is given as a single agent for recurrent disease: 6-PFS was 12% in the erlotinib arm and 24% in the control arm.^[19]

Targeted Therapy: VEGF, mTOR, PDGF & BCNU

Antiangiogenic treatments appear promising. The treatment with a VEGF-neutralizing antibody, bevacizumab (Avastin), administered in combination with irinotecan demonstrated a 6-PFS of 46%.^[20] Because VEGF (also known as the vascular permeability factor) regulates vascular permeability, targeting VEGF with bevacizumab may decrease contrast leakage into the tumor, thus maximizing a radiographic response. Other antiangiogenic drugs, such as AZD2171 (Cediranib), an oral tyrosine kinase inhibitor of VEGF receptors, have been evaluated in a Phase II trial in patients with recurrent GBM, providing significant clinical benefit in alleviating edema, and a 6-PFS of 25.6%.^[21] Another target for new compounds has been mTOR, an intracellular mediator of cell-surface receptors and Akt-mediated signaling. Two trials on temsirolimus in patients with recurrent GBM have now been completed and demonstrated a 6-PFS of 2.5 and 7.8%, respectively.^[22,23]

PDGF receptor overexpression has been described in low-grade and transformed glioma and in up to two-thirds of GBM cases.^[24] Imatinib, another small-molecule tyrosine kinase inhibitor, has been shown to block c-Kit and the PDGF receptor.^[25] This was the rationale for conducting trials in patients with recurrent glioma. The EORTC has conducted separate trials for patients with anaplastic astrocytoma and GBM.^[26,27] At 6 months, 15% of patients were stable or had responded, comparing favorably with EORTC contemporary controls from similar trials with other agents.^[28] Superior efficacy was suggested for the combination of imatinib and hydroxyurea in a single, uncontrolled trial that requires

confirmation.^[29]

The implantation of carmustine-impregnated polymers (Gliadel®) represents another approach for GBM treatment. In a recent trial in patients with newly diagnosed high-grade glioma, the carmustine-containing wafers were placed in patients undergoing surgical resection or tumor debulking. The median survival time was 13.9 months (vs 11.6 months in the group treated with placebo; $p = 0.03$).^[30] Considering that these were all patients selected for tumor resection, the survival rates have to be considered poor. Moreover, the apparent survival benefit disappeared when only GBM patients were considered (exclusion of 21 patients with anaplastic histologies and 12 patients with ineligible tumors), and there appeared to be no significant difference in progression-free survival, which makes these data difficult to interpret. Recent and ongoing studies are evaluating carmustine-containing wafers with a higher drug concentration.^[31]

Immunotherapy

Until now, most current brain tumor research has focused on the molecular and cellular analysis of the bulk tumoral mass, which typically comprises of morphologically different cells. It has recently been suggested that tumorigenicity and resistance to therapy might not be a feature of all the cells of the bulk tumoral mass, but rather of a selected population of cells with stem-like phenotype.^[32,33] The chemoresistance potential is highly preserved by immature cells, whereas the angiogenic potential is, to a larger extent, a property of mature cells.^[34]

Several reports, however, show that patients with GBM can spontaneously develop an antitumoral immunity and that activated lymphocytes do cross the BBB. Natural killer cells are highly efficient in the cellular immune response against malignant tumors, without restriction of major histocompatibility complex (MHC). In nine patients with recurrent malignant glioma, it was demonstrated that natural killer cell-rich effector cell therapy was safe and partially effective.^[35] Various sources of antigens can be used to induce T-cell response by dendritic cells, such as synthetic peptides, stripped peptides derived from tumor MHC class 1 molecules, tumor lysates, fused dendritic cells and tumor cells.

Loading dendritic cells with tumor-derived material, such as eluted peptides or tumor lysate, bypasses the limiting step of identifying a relevant tumor antigen and in theory reduces the risk of selecting an antigen-negative clone escape, as multiple antigens are selected. The safety and feasibility of such dendritic cell therapy have been supported by many Phase I clinical trials, with several of these reporting antitumor cytotoxicity and radiologic responses in some patients.^[36–39] Moreover, loading dendritic cells with eluted peptides requires a large culture of autologous tumor cells, a time-consuming and hazardous process. To overcome this limitation, some trials do not load dendritic cells with eluted peptides, but with tumor lysate. In a Phase I/II trial, 24 patients with recurrent malignant gliomas were treated with intradermal injections of dendritic cells loaded with tumor lysate. Some patients also received intratumor injections. For intradermal injections in some patients, dendritic cells were also matured with OK-432, since mature dendritic cells are believed to induce stronger immune responses. One partial response and three minor responses were observed. When compared with a control population, survival increased, especially in patients who were treated with mature dendritic cells and who received dendritic cells both intradermally and intratumorally.^[40]

Gliomas secrete immunosuppressive cytokines and therefore impair cytotoxic T-lymphocyte-based therapies. Gliomas also induce production of Fas ligand by microglia; this in turn induces apoptosis in T cells.^[41] Microarray comparison between native and chemoresistant U87 cells reveals significant upregulation of IL-1 β , IL-8 and VEGF in chemoresistant cells. Transfection of native cells with the *IL-1 β* gene results in resistance to apoptosis induction.^[42] Targeting tumor-associated antigens with dendritic cells may be an effective way to combat GBM.^[43] Tyrosinase-related peptide (TRP) is expressed on the surface of glioma cells and is targeted by endogenous cytotoxic T cells. After immunotherapy, TRP levels of the remaining cells go down and the tumors become more sensitive to temozolomide and carboplatin. Transfection of glioma cells with TRP makes them more resistant to temozolomide. TRP appears to regulate chemosensitivity without altering known conventional mediators of chemoresistance.^[44]

Some immunization protocols use autologous cells that have been genetically modified to render them more immunogenic, for example, IL-4-transfected fibroblasts, B7-2 and granulocyte-macrophage colony-stimulating factor-transfected tumor cells or glioma cells infected with the Newcastle disease virus.^[45] In a well-designed Phase II study, 23 patients with GBMs were immunized intradermally with tumor cells infected with the Newcastle disease virus after the patients had completed radiotherapy.^[46] One complete radiologic response was seen in a patient who had residual tumor after surgery, and the median survival was approximately 23 months, much higher than is usually seen in GBM (12–15 months). These very promising results need to be confirmed in a randomized study.

Altogether, active immunotherapy has shown some undeniable successes with the induction of anti-tumor cytotoxicity and

some radiologic responses. The main limitation of such an approach is the requirement for tumor material or tumor cultures. In addition, several issues, such as the source of antigens (e.g., tumor lysates, peptides, genetically modified cells), dendritic cell maturation status and the frequency and route of injections, must be optimized before this strategy can be an acceptable therapy.

Expert Commentary & Five-year View

The therapeutic perspectives for GBM are mostly based on the biology of the tumor mass, where hundreds of cell, protein and gene alterations exist. The hope for improving the knowledge of these malignancies is centered on the successful clinical application of current high-throughput technologies. For example, genomics and proteomics represent the forefront of cancer research and a powerful approach to systematically identify large volumes of data that can be used to study the basis of oncology. Moreover, the amount of biologic information increases daily. This exponential and fast cultural growth allows us to define new therapeutic targets, but also determines a knowledge fragmentation that could take the researchers very far from the early alterations that characterize tumor development. The current standard of care and the new experimental therapeutic approaches for newly diagnosed and recurrent GBM are based on studies conducted on the enhanced lesion. All the efforts made by several scientists look like a puzzle representing a labyrinth. (Figure 2). In this game all the players are concentrated at the centre of the puzzle, and numerous benchmarks (linked among them) are located along a widespread network. Each player follows some benchmarks in order to identify the best pathway to reach the entry site, but several routes cross each other making it very difficult to find the solution of the maze. The peripheral portions of the puzzle have rather fewer paths and few benchmarks (compared with the center of the puzzle). Therefore, the players should start constructing the puzzle from those points, which are easier to recognize.

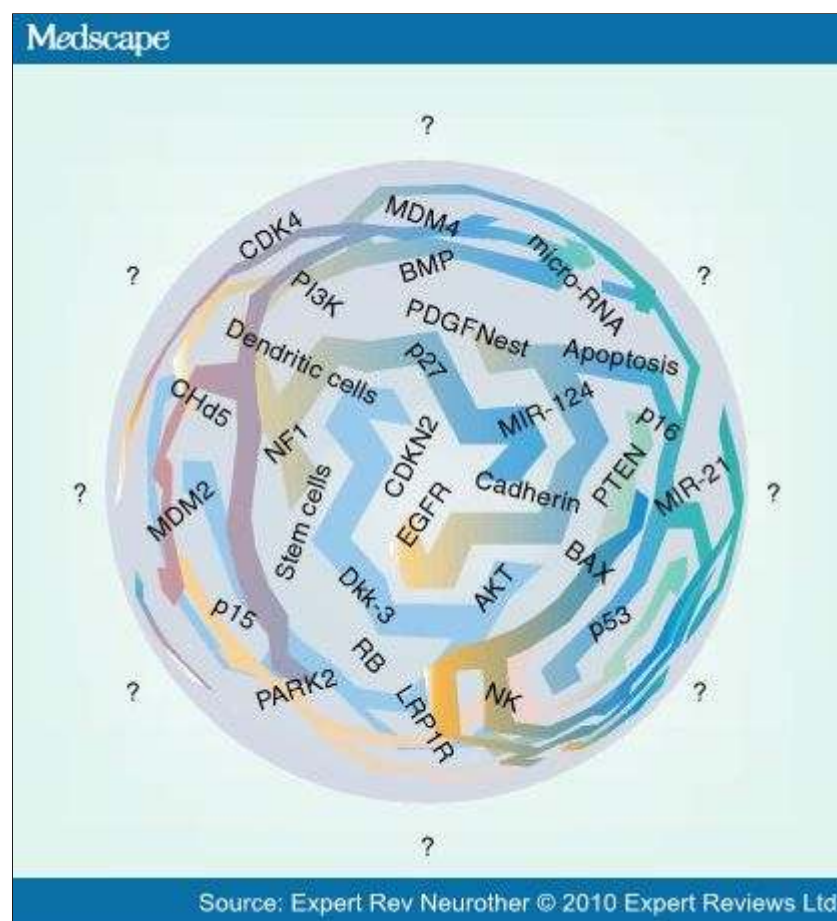


Figure 2. The 'labyrinth' of glioblastoma.

Paraphrasing the puzzle metaphor, the center is the enhanced tumor lesion and the periphery the white matter that surrounds it. After surgical resection, GBM recurs adjacent to the resection margin or within 2–3 cm from the resection cavity in 96% of cases.^[47]

The most vexing problem that precludes surgical cure of GBM is the infiltrative behavior of these lesions. Even tumors that appear to be circumscribed have isolated tumor cells around the border, and these tumor cells may extend as far as 4–5 cm from the epicenter of the lesion. Silbergeld and Chicoine demonstrated that malignant tumor cells could grow in tissue

culture from surgical specimens obtained at a minimum of 4 cm from the macroscopic and radiologic margin of the tumor.^[48] Little is known about the distinct biology of invasive GBM cells *in situ*, but their diffuse infiltration suggests that the activation of genetic and cellular processes distinguishes them from cells in the tumor core.^[49–51] Cell migration is a complex combination of multiple molecular processes, including the alteration of tumor cell adhesion to a modified extracellular matrix, the secretion of proteases by the cells and modifications to the actin cytoskeleton. A number of signaling pathways can be constitutively activated in migrating glioma cells (PI3K, Akt, mTOR, NF- κ B and autophagy), thus making these cells resistant to apoptosis and cytotoxic insults.^[52]

Glioblastoma invasion is a very dynamic process that has an impact on multiple features of the glioma cells. Invasion and proliferation behaviors of GBM cells will prove to be regionally and temporally variable.

Despite very low proliferating indices of distantly invasive GBM cells or even an inability to morphologically identify glioma cells that are distant from the rim of obvious tumor, nonetheless, it is possible to harvest clonogenic glioma cells from such noncontiguous sites.^[53] Tumor cell infiltration is not identifiable with a sequence of MRIs. As demonstrated in a recent study, infiltrating cells are not always detected in the T2 component, where signal alteration is similar in tissue with and without infiltration.^[53] Moreover MAPKs (ERK and JNK) overexpression has been detected in endothelial cells and in apparently normal glial cells at 2–3 cm from the macroscopic border of the enhanced lesions.^[54,55] Activated ERK1/2 expression in GBM and in 'apparently normal' peritumor tissue has also been detected.^[54]

The expression of these markers independently of neoplastic cells, strongly suggests that these areas are changing to tumor tissue. According to the emerging hypothesis that brain neoplasias may derive from neural stem cells, nestin (a marker for neural stem cells in the adult mammalian brain) was detected inside tissue samples obtained from white matter surrounding GBM.^[55] Nestin was detected on tumor cells (which can be either migrated from the GBM or represent the initiation of independent foci of transformed cells), reactive glial cells and normal cells. Nestin immunoreactivity of apparently normal cells may support the idea of an induced premalignant state, which may lead to a full transformation. Nestin immunoreactivity has also been found in the endothelium of tumor vessels and microvessels. This supports the hypothesis that these areas are undergoing an increase in blood supply, which is critical to GBM progression. The study of the brain adjacent to tumor allowed the role of neural cancer stem cells to be highlighted.^[56] Neural cancer stem cell lines were obtained from the tumor (T-GBM) and from the peritumor tissue (P-GBM). Both T and P cells were multipotent and, following cloning and differentiation, gave rise to a progeny expressing neuronal, astroglial and oligodendroglial antigens. Notwithstanding, their growth properties were extremely different. Under identical conditions, T-GBM cells established typical neurosphere cultures, whereas P-GBM cells grew exclusively as a monolayer. Clearly, T-GBM cells displayed faster growth kinetics and a much higher clonogenic index, demonstrating that their cancer stem cell content was larger than that in P-GBMs. On the contrary, expression of the CD133 antigen, whose role as a GBM stem cell marker remains controversial, was similar between T- and P-GBM. As expected, upon orthotopic transplantation of as many as 300,000 T-GBM cells/animal, GBM-like lesions arose in 100% of the immunodeficient SCID recipients. Conversely, even transplantation of such a high number of P-GBM cells never established any tumor. All animals transplanted with P-GBM survived for 15 months, with no sign of neurological alterations, whereas 100% of the mice receiving T-GBM cells died 45–60 days after transplantation.

These findings should prompt an active search for such candidate ancestor cells within primary GBM surgery specimens, as this could shed light on the specific and critical aberration/mutation steps that must occur in neural cells for them to acquire cancer stem cell features and, particularly, tumor-initiating ability. These data strongly sustain the importance of tumor microenvironment as a contributing factor to gene expression changes. Cells at the tumor core (i.e., highly cellulated, enhanced tumor) are densely packed, proliferative and may determine considerable hypoxia leading to extensive areas of necrosis. Individually infiltrating cells interact with the extracellular matrix and surrounding cells, exchanging signals as they invade. Interactions with such diverse microenvironments is likely to contribute significantly to the initiation and maintenance of transcription profiles. Brain tissue surrounding the tumor must be considered as a site of a precarious biologic balance where the apparent absence of visible pathology is the result of a transitional conflict between oncogene and oncosuppressor factors. This view has been recently confirmed by studies on the expression of microRNA (potent post-transcriptional regulators of protein coding genes). A clear and opposite regulation of these nucleotides was detected. In this way, oncogene microRNAs were underexpressed in the periphery compared with tumor areas. Converse behavior was observed for oncosuppressor microRNAs.^[57,58]

In conclusion, we propose that the biologic expression profile of tissue surrounding tumor reveals a unique pattern of 'quiescent' cancer cells. A tumor resection including, when possible, the apparent normal tissue, should be performed in order to improve our biologic knowledge. This could be done in a minority of cases, considering that tumors often develop

in eloquent areas where large resection is difficult. Molecular and cellular differences between the tumor and the periphery may explain why these cells are unlikely to respond to conventional therapies against proliferative tumor, that is, the tumor core that presently represents the reference tissue for the biologic analysis of this disease. Understanding the pathophysiology of peripheral cells may lead to novel therapies that not only address the tumor core, but also this distinct subpopulation of cells.

Sidebar

Key Issues

- Glioblastoma multiforme is the most common primary malignant neoplasm of the CNS in adults, with a median survival of approximately 1 year.
- Standard treatment is surgery (preferably gross total removal) followed by radiation therapy (60 Gy administered in fractions) and concomitant/adjuvant temozolomide (an alkylating agent).
- Methylguanine methyltransferase (an excision repair enzyme) methylation status identifies patients most likely to benefit from the addition of temozolomide.
- Several second-tier therapies are under evaluation, such as EGF receptor inhibitors (erlotinib), VEGF antibodies (bevacizumab), PDGF receptor antibodies (imatinib) and locally implanted carmustine-impregnated wafers. Immunotherapy (e.g., with dendritic cells loaded with tumor lysates) represents another intriguing approach.
- The failure of present therapies can be explained by the complex biology of these tumors, with cancer cells very susceptible to mutations and with a great variety of genetic alterations. Moreover, infiltrating cancer cells and 'activated normal' cells are evident in the brain surrounding the main tumor.
- Recent studies addressing the white matter adjacent to tumor found that cells, even if morphologically normal, expressed several biological alterations, thus demonstrating a precancerous state of that area.
- The knowledge of the biologic phenomena of 'normal brain' adjacent to tumor could allow us to understand the first steps of carcinogenesis and, consequently, to interfere with the pathways responsible for tumor growth and recurrence.

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