Molecular Profiling in Glioblastoma: Prelude to Personalized Treatment

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The purpose of this review is to provide an up-to-date summary of the current knowledge and understanding of the molecular alterations and pathways relevant to the clinical outcome of glioblastoma patients and their potential use in designing personalized treatment for these patients. This article also discusses the potential of molecular profiling as a diagnostic modality, possible therapeutic implications of MGMT promoter methylation, the targeted inhibition of angiogenesis, and assessment of the tumor's molecular background with respect to PI3K/AKT pathway activation and associated molecules (EGFR, EGFRvIII, PTEN).

Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults [1], and despite important advances in the knowledge of molecular abnormalities related to its biologic behavior and new treatment options, GBM remains among the most aggressive [2] and most devastating types of cancer. The current treatment includes maximal surgical removal of the tumor mass (complete resection is often impossible because of the widely invasive nature of this tumor [3]), followed by external-beam radiotherapy with concurrent and adjuvant chemotherapy [4]. These therapies represent aggressive treatment modalities with a considerable impact on the patient's quality of life [5]. The prognosis for glioblastoma patients remains very poor, with median survival times in the range of 12 to 15 months [6]. However, clinical outcomes are highly variable with respect to patient-related factors [7], pattern of disease development (primary vs secondary GBM) [8], and the resistance mechanisms to treatment. Consideration of the genetic heterogeneity of GBM suggests the existence of multiple genetic subsets of this tumor, with specific molecular features determining tumor behavior. Identifying the determinant molecular pathways and mechanisms directly related may affect clinical outcome. Individual use of targeted therapies based on the actual molecular profile of the patient's tumor may eventually result in better treatment options for patients with GBM [9].

Molecular Tools for Diagnosing GBM

An accurate diagnosis is required for adequately treating disease and assessing prognosis for any patient. Tumor diagnosis should predict the biologic behavior and corresponding outcome of the tumor [10]. Currently, GBM is confirmed by histopathologic assessment [11], which affects therapeutic decisions and prognostic estimation more than any other known variable [12]. The most commonly used histopathologic diagnosis scheme is based on World Health Organization criteria. However, despite several studies proving the clinical value of these criteria, there is considerable interobserver disagreement with respect to tumor typing and grading [13]. Therefore, despite the decades of effort leading to our current standard histologic classification, many malignant gliomas remain diagnostically challenging [12]. This implies that molecular characterization of tumors is essential not only to guide therapy and predict outcome but also to diagnose glioblastomas in the first place [14].

In the late 1990s, genetic analyses led to the discovery that allelic loss of chromosomal arms 1p and 19q in anaplastic oligodendroglial tumors is significantly associated with an improved clinical course in affected patients [15]. The deletion of 1p predicts chemosensitivity to procarbazine-lomustine-vincristine (PCV) chemotherapy, and combined 1p/10q loss is associated with enhanced chemosensitivity and longer progression-free survival (PFS) [16•]. The actual biologic and molecular basis for why this deletion is so influential on patient survival is unclear. It is possible that the favorable phenotype may result from a combination of factors (eg, inverse relation to the *TP53* mutation, cell of origin) for which the allelic loss is merely a convenient marker [16•].

Certain genetic subsets have been defined in glioblastomas, including the mutually exclusive alterations of the *TP53* gene and epidermal growth factor receptor (EGFR): EGFR gene amplification occurs less frequently in glioblastomas with *TP53* mutation or allelic loss of chromosome 17p [17]. The evidence suggests that EGFR overexpression and mutations of the p53 tumor suppressor gene appear to be mutually exclusive events defining two different genetic pathways in the evolution of glioblastoma, the common phenotypic end point [18]. The pathway including *TP53* inactivation is characteristic of secondary glioblastomas arising in younger adults, whereas tumors with EGFR amplification most commonly occur in older patients with primary glioblastoma [17]. Because age is one of the crucial patient-related factors determining clinical outcome in GBM patients, the correlation of specific molecular alterations with patient age is of significant clinical interest.

Several studies have revealed distinct molecular signatures that reliably group gliomas according to survival prediction [19]. In total, 44 genes were described to determine the tumor's prognostic cluster, including genes that regulate cell adhesion, proliferation, motility, and extracellular matrix proteins, indicating a multifactorial determination of patient prognosis. Several markers were confirmed to correlate independently with adverse clinical outcome in GBM patients, including survivin, an apoptosis inhibitor abundantly expressed in various tumors. Chakravarti et al. [20] showed that patients with GBM had a higher rate of survivin positivity than those with non-GBM tumors ($P < 0.001$) and that survivin also has significant prognostic value. A later study showed that survivin plays a critical role in mediating radiation resistance in primary GBM cells [21].

Current Chemotherapeutic Standard of Care and MGMT Promoter Methylation

For a long time, the standard of care for patients with GBM was surgical resection followed by radiation therapy (RT); nitrosourea-based chemotherapy was found to have minimal benefit on patient outcome. Recently, however, there has been a significant shift toward therapy with the oral imidazotetrazine derivative temozolomide (TMZ) [16•]. TMZ is an alkylating agent shown in randomized clinical trials to improve patient survival through concurrent and adjuvant administration [22]. Its primary mechanism of action is thought to be methylation of the O6 position of guanine in DNA, leading to production of lethal methylguanine adducts. This process is reversed by the DNA repair enzyme MGMT, which repairs methylguanine adducts by transferring the methyl group at the O6 position to a cysteine residue of the enzyme, leading to restoration of the originally methylated nucleotide. It has therefore been hypothesized that the actual cytotoxic effect of TMZ is inversely related to MGMT activity and that a decrease in MGMT expression consequently leads to an increase in tumor sensitivity to TMZ.

The major mechanisms for MGMT inactivation in GBM and other tumors include the epigenetic silencing of the *MGMT* gene as the result of promoter hypermethylation. Considering TMZ's primary toxic effects, including myelosuppression, nausea, and vomiting [23], basing treatment decisions on the individual patient's genetic background can minimize these adverse effects in patients unlikely to benefit from TMZ administration.

At the protein level, MGMT expression is heterogeneous within tumors and even within tumor regions. A multicenter study revealed that MGMT promoter methylation status has predictive value if determined by genetic tools but that assessment of protein expression is uninformative [24]. A translational research study was performed on tumor tissue from the randomized trial described earlier. MGMT gene promoter methylation was assessed in a representative subgroup of 206 patients, 45% of whom had tumors with a methylated MGMT promoter [25]. In patients with a methylated MGMT promoter treated with TMZ/RT followed by TMZ, the median survival was 22 months, with a 2-year survival rate of 46%. In comparison, patients with a methylated MGMT promoter treated with RT alone had a median survival of 15 months, with a 2-year survival rate of 23%. The worst outcome was observed in patients with an unmethylated MGMT promoter treated with RT only: their median survival was 12 months, with a 2-year survival rate less than 2%. In the setting of TMZ/RT followed by TMZ, the median survival for patients with an unmethylated MGMT promoter was 13 months, with a 2-year survival rate of 14% (difference not statistically significant). The relative improvement in survival of patients with a methylated versus unmethylated MGMT promoter treated in the RT-alone arm can be explained by the administration of alkylating agent chemotherapy after tumor progression. Indeed, 72% of the patients received salvage chemotherapy at tumor progression. In analyzing the time to tumor progression, no difference can be detected between the treatment arms, except for the patients with methylated tumors treated with TMZ/RT followed by TMZ.

Targeted Inhibition of the Tumor's Angiogenic Potential

Tumor growth and survival depend on an appropriate supply of oxygen and metabolic nutrients, provided by blood vessels. Because GBM generally is a highly vascularized tumor, depriving it of a blood supply by preventing angiogenesis seems to be a promising direction for further therapeutic investigation. The process of angiogenesis is highly complex, requiring interaction of various angiogenic factors and pathways (Fig. 1), many of which are

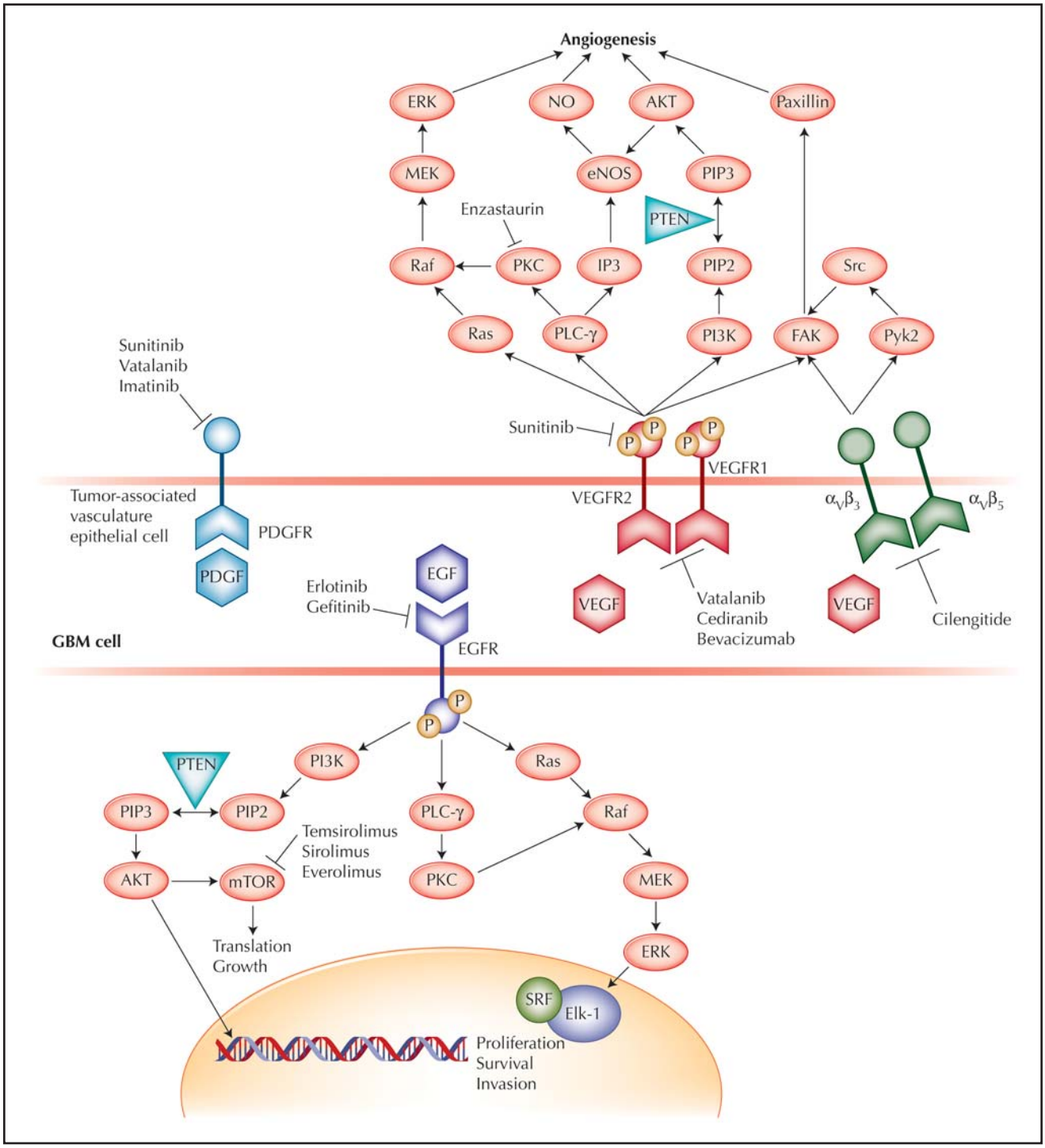

Figure 1. Current molecular targets in glioblastoma multiforme (GBM) signaling pathways. Growth factor (eg, epidermal growth factor [EGF]) binds to receptor tyrosine kinase, stimulating PI3K activation, which changes PIP2 to PIP3 (process reversed by phosphatase tensin homologue on chromosome 10 [PTEN]), leading to activation of the downstream AKT and mammalian target of rapamycin (mTOR). Simultaneously, phospholipase C-γ (PLC-γ) is activated, leading to Raf activation (downstream substrate of Ras) via protein kinase C (PKC) stimulation; parallel to this, tyrosine kinase activates the Ras directly. Ras (key effector of the MAPK pathway) activates extracellular signal-regulated kinase (ERK) by the upstream MEK, and ERK activation leads to formation of ternary complex of serum response factor (SRF) and Elk-1, promoting transcription in the nucleus. Activation of platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and vascular endothelial growth factor receptor (VEGFR) by their cognate growth factors in epithelial cells leads to activation of the same pathways. PLC-γ also activates inositol triphosphate (IP3), which in turn activates endothelial nitric oxide synthase (eNOS), resulting in nitric oxide (NO) genesis. Focal adhesion kinase (FAK) is activated directly by VEGFR2, by integrins, or by downstream substrate of integrins—Pyk2 and subsequently FAK activate paxillin. All processes lead to angiogenesis promotion.

currently targetable by specific inhibitors, as described in the following sections. The clinical effects and observations of discussed inhibitors are displayed in Table 1.

Targeting integrins with specific inhibitors

Integrins are cell-surface adhesion molecules and receptors involved in signal transmission for various processes, such as cell migration, invasion, and proliferation. During angiogenesis, integrins are essential for endothelial cell migration, proliferation, and survival [26•], and their inhibition may produce potent antitumor effects. Many integrin inhibitors, including cilengitide, a synthetic pentapeptide binding to the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors identified as specific for tumor angiogenesis, have been tested in clinical trials for cancer patients [26•].

The preliminary results of a phase 2 clinical trial of cilengitide added to standard chemotherapy and RT in patients with newly diagnosed GBM suggested potential efficacy, with little or no additional toxicity, in a subgroup of patients [26•]. Interestingly, in a subset of patients with recurrent GBM receiving cilengitide after previous TMZ and RT, cilengitide demonstrated long-term disease stabilization [27].

Inhibition of vascular endothelial growth factor

Of the many proangiogenic factors involved in GBM progression, vascular endothelial growth factor (VEGF) may play a crucial role in the hypoxia-induced angiogenesis cascade, and the potential clinical efficacy of targeting VEGF and its receptors in treating GBM patients is currently undergoing broad clinical assessment.

VEGF has been shown to be upregulated in GBM, with higher levels directly linked to worse patient prognosis. On the molecular level, upregulation occurs in response to several biologic factors and processes, including hypoxia (through hypoxia-inducible factor-1), platelet-derived growth factor (PDGF), epidermal growth factor, transforming growth factor-β, interleukin-1β, and tumor necrosis factor-α [28••]. The biologic effect of VEGF is elicited through two receptors with tyrosine kinase activity, VEGFR-1 and VEGFR-2. The main receptor, VEGFR-2, is expressed in the vascular endothelia of GBM and mediates endothelial cell signaling through the activation of various pathways, including Ras/Raf/MEK/MAPK, PI3K/AKT/PKB, and protein kinase C (PKC)-β [28••]. Hence, several strategies to target VEGF have been designed, including tyrosine kinase inhibitors (TKIs) and anti-VEGF and anti–VEGFR-2 monoclonal antibodies, some of which have been translated into actual clinical studies.

Bevacizumab

Bevacizumab, a recombinant humanized monoclonal antibody targeting VEGF, recently was approved for use in colorectal cancer based on a significant survival benefit following its addition to fluorouracil chemotherapy [29•]. This observation may have been the result of the agent's antiangiogenic effect on the cancer stem cell niche. Recently, an unusually high radiographic response rate (60%) was reported with the combination of bevacizumab and irinotecan in GBM patients with recurrent disease [30]. Furthermore, a noncomparative clinical study of the effect of bevacizumab alone or in combination with irinotecan in recurrent glioblastoma showed a 6-month PFS of more than 9 months for patients receiving bevacizumab alone or with irinotecan, providing encouraging evidence of bevacizumab's significant activity in GBM patients [31].

Vatalanib

Vatalanib targets all known VEGF receptors (VEGFRs) and also inhibits PDGF receptor (PDGFR), which is frequently overexpressed in gliomas and implicated in GBM angiogenesis [32], providing a promising molecular background for its potential role in inhibiting the growth of the tumor's vasculature. In two recent studies in recurrent glioblastoma, vatalanib monotherapy was associated with disease stability in a subset of patients (with disease stabilized for a median of 3 months) [28••]; in patients receiving vatalanib/TMZ combination therapy, the median time to progression was 16 weeks [28••]. A randomized phase 2 trial by the European Organisation for Research and Treatment of Cancer (EORTC 26041-22041) evaluated the therapeutic efficacy of vatalanib given concurrently with or after TMZ and RT; the results are pending.

Cediranib

In a clinical study with correlative imaging and biologic end points, cediranib (AZD2171), an oral TKI targeting all VEGFRs, led to tumor vasculature normalization and edema alleviation in patients with recurrent glioblastoma [33•]. Although these effects were shown to be reversible over time, this study demonstrated the importance of exploring related biologic and radiographic markers. A phase 3 multicenter study is under way to evaluate cediranib in combination with lomustine (a nitrosourea agent that crosses the blood–brain barrier) in recurrent malignant brain tumors.

Protein kinase C inhibition

On the molecular level, membrane growth factor receptors activate the complex intracellular PKC signaling cascade. PKC is a family of 14 serine-threonine protein tyrosine kinases, and its overactivity has been associated with angiogenesis, growth, and proliferation [34•] in various tumors, including glioblastomas. PKC has been directly linked to various pathways, including the Ras/MAPK and PI3K/AKT signaling pathways, and is considered a major molecule in the VEGF signaling cascade [28••].

In preclinical studies, the PKC inhibitor enzastaurin was associated with glioma cell apoptosis, a decrease in proliferation, and angiogenesis suppression, promising

Table 1. Clinical trials of targeted therapies in glioblastoma multiforme

Study	Target	Function	Agent	Phase	Outcome/clinical observation	Comment
Reardon et al. [27]	Integrins	Angiogenesis	Cilengitide	1/2	Limited long-term disease stabilization in selected patients with recurrent GBM	Effect after previous RT and TMZ
Cloughesy et al. [31]	VEGF	Angiogenesis	Bevacizumab	1/2/3	Prolonged PFS in recurrent GBM	Effect alone or with irinotecan
Omuro et al. [28••]			Vatalanib	1/2	Limited disease stability in subset of patients with recurrent GBM	Results of combination with TMZ and RT pending
Batchelor et al. [33•]			Cediranib	1/2/3	Edema alleviation and vasculature normalization in recurrent GBM	Effects reversed over time
de Groot and Gilbert [34•]	PKC	Angiogenesis	Enzastaurin	1/2/3	Prolonged PFS in recurrent GBM in phase 2	Phase 1/2 in combination with TMZ and RT currently ongoing
Stupp et al. [26•]					Phase 3 terminated due to lack of efficacy	
Marosi et al. [37]; Viola et al. [38]	PDGF	Angiogenesis	Imatinib	2/3	Prolonged PFS, disease stabilization in recurrent disease	In patients expressing PDGFR
Chaskis et al. [41]			Sunitinib	2	Reduction of tumor blood flow in recurrent high-grade glioma	Studies of combination with other targeted agents ongoing
Mellinghoff et al. [43]; Van den Bent et al. [44]; Chakravarti et al. [45]	EGFR	Proliferation, survival	Gefitinib, erlotinib	1/2	No overall effect; clear benefit in selected patients with recurrent GBM	Neither PTEN nor EGFRvIII was proven to be a predictor of response
Cloughesy et al. [46]	mTOR	Cell cycle progression, tumor growth	Sirolimus	1/2	Limited anticancer activity in PTEN-deficient GBM	PTEN loss is not a predictor of response
Omuro et al. [28••]			Temsirolimus	1/2	No effect in recurrent GBM	EGFR, PTEN loss/presence, AKT, and AKT phosphorylation are of no value for response prediction; study of combination with erlotinib ongoing
Omuro et al. [28••]			Everolimus	2	No effect in recurrent GBM; limited radiographic response in selected patients in combination with gefitinib	PTEN loss is not a predictor of response

EGFR—epidermal growth factor receptor; GBM—glioblastoma multiforme; mTOR—mammalian target of rapamycin; PDGF—platelet-derived growth factor; PDGFR—PDGF receptor; PFS—progression-free survival; PKC—protein kinase C; PTEN—phosphatase tensin homologue on chromosome 10; RT—radiation therapy; TMZ—temozolomide; VEGF—vascular endothelial growth factor.

results that have led to clinical studies. The results from a phase 2 study in patients with recurrent glioma were encouraging, showing a 30% response rate and an overall median PFS of 5 months [34•]. However, a phase 3 trial in patients with recurrent disease was terminated before completion because of lack of efficacy of PKC inhibitor monotherapy and concerns regarding a possible inferior outcome [26•]. Nevertheless, in a phase 1/2 study in patients with GBM or gliosarcoma, PKC inhibition combined with standard TMZ chemotherapy and RT was effective and well tolerated; however, the final results are not yet available [35].

Platelet-derived growth factor inhibition

A frequent observation in malignant gliomas is increased expression of PDGF and its receptor, which leads to cell proliferation, invasion, and resistance to apoptosis because of PDGFR activation. This process initiates the signaling cascades that comprise the Ras/Raf/MAPK and PI3K/AKT pathways. Because PDGFR is also involved in angiogenesis, it is considered an attractive target for molecular inhibition.

Imatinib mesylate

As the result of promising preclinical results from PDGFR inhibition in glioblastoma xenograft models, several clinical studies in GBM patients have been conducted using imatinib mesylate. This agent, which targets bcr-abl and c-kit as well as PDGFR, has already been approved for certain indications, such as chronic myeloid leukemia [28••].

Initial studies exploring the efficacy of imatinib as a single agent in malignant gliomas were somewhat disappointing [28••], with a phase 2 trial showing imatinib to be virtually ineffective in GBM patients (6-month PFS of 3%) [36]. However, results from clinical trials that also assessed PDGF expression revealed much more encouraging data. One study demonstrated 32.4% 6-month PFS in patients with malignant gliomas expressing PDGFR [37]. A phase 2 study of high-dose imatinib in patients with recurrent GBM with immunohistochemistry-confirmed PDGFR positivity demonstrated disease stabilization in a significant proportion [38], further emphasizing the point that selection of patients based on the molecular profile of their tumor will maximize therapeutic benefit.

Other studies assessed the efficacy of imatinib combined with other cytotoxic agents. In one study, the combination of imatinib, hydroxyurea, and vatalanib was safe and well tolerated, with an encouraging rate of radiographic response [39]. A phase 1 trial of combination TMZ/imatinib therapy in GBM patients showed a median PFS of 41.7 weeks among those with stable disease at enrollment [40].

Sunitinib

Sunitinib, an oral multitargeted TKI that selectively inhibits VEGFR-2, PDGFR, and several other kinases, showed promising preclinical results in GBM in vivo models [32]. A phase 2 study in patients with recurrent high-grade glioma demonstrated a reduction in the ratio of lesion to normal white matter cerebral blood flow in most patients [41]; however, actual tumor regression was observed less frequently. The results from several ongoing clinical studies on sunitinib and other antiangiogenic TKIs (eg, sorafenib) are highly anticipated.

Targeted Inhibition of Intracellular Signals

Increased understanding of GBM oncogenesis has led to an increase in potential therapeutic targets and the emergence of inhibitors of suspected key mediators in the recognized pathways. The malignant behavior of GBM is driven by several mechanisms involved in antiapoptotic and mitogenic signal transmission, cell migration, and invasion promotion [26•]; the various elements thought to play a crucial role in these pathways (eg, receptor tyrosine kinases) are frequently amplified or overexpressed in glioblastoma, leading to pathologic activation of these processes (Fig. 1). Clinical effects and correlations of discussed molecular targets of inhibition are summarized in Table 1.

Inhibition of the epidermal growth factor receptor

EGFR, a transmembrane protein with tyrosine kinase activity, is involved most prominently in the signal mediation of several pathways, including Ras/MAPK, PI3K/AKT, and PKC. It represents an attractive target for inhibition because of its frequent, almost exclusive overexpression in glioblastomas compared with other human gliomas [42]. Hence, EGFR analysis at the protein or genetic level and EGFR's molecular inhibition have gained much attention, resulting in several clinical studies assessing this protein's efficacy and molecular profile. The correlative data acquired so far suggest that besides EGFR's expression, its other molecular features—many of which remain enigmatic—also may represent predictors of response to treatment with EGFR inhibitors [26•,34•].

Gefitinib and erlotinib

The therapeutic efficacy of the EGFR TKIs gefitinib and erlotinib has been investigated in several clinical studies in recurrent glioblastoma, with some effect [26•]. However, the molecular correlations that may predict response remain controversial. A clear correlation between phosphatase tensin homologue on chromosome 10 (PTEN) retention and response rate was noted in patients with recurrent disease. In addition, expression of the EGFR mutant form EGFRvIII (the constitutively active form of EGFR that lacks exons 2 to 7 and is nearly unique to glioma cells [16•]) was also demonstrated to be necessary for GBM sensitivity to EGFR inhibitors [43]. Nonetheless, the investigators noted PTEN loss in several responsive patients, one of whom also lacked

EGFRvIII. The role of EGFRvIII in predicting response was not confirmed in other studies, including a phase 2 trial of erlotinib versus standard chemotherapy in recurrent GBM, which showed insufficient activity of erlotinib regardless of EGFRvIII mutation status [44]. Investigators comparing the combination of gefitinib and RT with RT alone showed no significant survival benefit in the gefitinib-treated patients [45].

Although data from studies assessing the efficacy of EGFR TKIs in the general population of glioblastoma patients may be discouraging, these studies did not adequately address the molecular profile that would reliably indicate the tumor's responsivity to this therapeutic strategy, which was proven effective in a relatively small number of selected patients. If the appropriate molecular profile is eventually identified, new studies could be designed for selected patients having predefined molecular correlations with legitimate anticipation of results showing higher efficacy of EGFR inhibition. Nonetheless, a synergistic effect of combination therapy involving inhibition of EGFR and other molecular targets was demonstrated in vitro and may be clinically significant, as discussed in the following sections.

Mammalian target of rapamycin and the synergistic effect of its inhibition

The transition between EGFR activation at the cell surface and its eventual oncogenic effect in the nucleus takes place over a complex and potentially excessive signal transduction network, with the PI3K/AKT pathway perhaps being the most prominent cascade. Greater PI3K activity has been associated with increased RT resistance [9]. Activation of the pathway is triggered by stimulation not only of EGFR, but of several other growth factor receptors, including PDGFR, fibroblast growth factor receptor, and insulin-like growth factor-1 receptor [28••], illustrating the inevitably complex background that determines pathway regulation in actual tumors.

PI3K activation leads to PIP2 in PIP3 transformation—a process regulated by PTEN (lost in 70% of GBM)—and PIP3 promotes phosphorylation of AKT. Because mammalian target of rapamycin (mTOR), a downstream mediator of AKT, regulates cell cycle progression and can control the levels of hypoxia-inducible factor-1α involved in angiogenesis, it has become an attractive therapeutic target.

Sirolimus

Sirolimus (also known as rapamycin), a macrolide antibiotic routinely used as an immunosuppressant, binds to FK-binding protein 12 in the cytosol, creating a complex that directly inhibits mTOR. Preclinical research indicated that PTEN loss sensitizes tumors to mTOR inhibition, providing the rationale for a phase 1 trial in patients with recurrent PTEN-deficient glioblastoma. Although this study revealed some anticancer activity in this patient population, the eventual clinical resistance to mTOR inhibition was not cell intrinsic [46]. Several studies are assessing a therapeutic approach combining sirolimus with EGFR inhibitors in patients with recurrent GBM; however, the results so far have revealed only modest toxicity and antitumor activity [47,48].

Temsirolimus

Temsirolimus is a soluble propyl ester analogue of sirolimus with a similar mechanism of action: inhibition of the mTORC1/raptor complex. It is worth noting that this inhibitory mechanism unexpectedly increases PI3K/AKT activity, likely diminishing the actual antitumor effect. This process may be the result of certain tumor "escape mechanisms" due to activation of alternative pathways that may undermine the therapeutic effectivity of mTOR if it is not modulated further [34•].

Two phase 2 studies assessed the effect of temsirolimus monotherapy on recurrent GBM; both demonstrated disappointing results, with no improvement in patient response rates or PFS [28••]. Moreover, EGFR amplification, PTEN deletion assessed by fluorescence in situ hybridization, PTEN expression confirmed by immunohistochemistry, and AKT and AKT phosphorylation assessment were of no value in predicting response. Therefore, these studies did not identify any markers that might be helpful in developing a treatment plan for a specific patient population. Results are awaited from a phase 1/2 trial exploring the combination of temsirolimus and erlotinib.

Everolimus

Everolimus, another sirolimus derivative used as an immunosuppressant, is under investigation to assess its activity in GBM as a single agent or in combination with TMZ. Simultaneous VEGF/EGFR and mTOR inhibition showed an increased survival benefit in xenograft models in vitro [49], but this observation was not confirmed by clinical studies. Preliminary results from a study using gefitinib and everolimus in patients with recurrent glioblastoma revealed no improvement in median overall survival or PFS, although 31% of the patients had some radiographic response [28••].

The role of PTEN loss as a predictor of response to everolimus therapy was tested in xenograft models. Not surprisingly, the results suggest that PTEN loss is insufficient to reliably predict response to mTOR inhibition in patients with GBM, which again may be the result of inconsistent activation of PI3K/AKT/mTOR or interplaying pathways [50].

Conclusions

In recent years, there has been increasing interest in identifying and understanding the tumor-specific pathways

in GBM and their translation into targeted therapies, leading to important and conclusive findings [23]. These strategies, however, have not made a major impact on the overall outcome for patients with GBM. Rather, the recent discoveries have underscored the tumor complexities and challenges in refining diagnosis and treatment.

Future investigation likely will focus on describing the specific molecular pathways and oncogenic mechanisms underpinning tumor resistance to treatment and tumor recurrence. Further elucidation of the underlying molecular profile of the tumor will help determine individual treatment outcomes. The knowledge already gained regarding aberrant pathways involved in GBM has led to the development of new therapeutic agents, many of which are being tested in clinical trials [9]. Nevertheless, larger studies are needed before highly specific and individualized patient treatment becomes routine in the oncologic practice.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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