Molecular Predictors in Glioblastoma

Toward Personalized Therapy

Howard Colman, MD, PhD; Ken Aldape, MD

Recent therapeutic advances have improved standard treatment for patients with newly diagnosed glioblastoma. Unfortunately, even with these improvements, only a fraction of patients derive significant benefit and experience prolonged survival. These findings are consistent with long-standing clinical and recent molecular evidence that subtypes of glioblastoma exist with differing survival rates and response to treatment. However, patients with newly diagnosed glioblastoma are currently treated in a uniform fashion, without regard for potential underlying differences in molecular alterations or prognosis. In this review, we will discuss recent progress toward the identification of robust and clinically relevant molecular subgroups of glioblastoma and initial steps in using this information to individualize therapy and overcome treatment resistance.

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Glioblastoma is the most common primary brain tumor in adults. Glioblastoma is also highly lethal, with only a small percentage of patients experiencing prolonged survival. Historically, patients with glioblastoma have been treated with maximal surgical resection followed by external beam radiation therapy. Many patients have also received chemotherapy (most commonly an alkylating agent) either in the adjuvant setting after radiation therapy or at recurrence. However, until recently there was little evidence showing a survival benefit of chemotherapy in glioblastoma. This situation changed in 2005 with the publication of results from a phase 3 clinical trial, which demonstrated the efficacy of chemotherapy for significantly improving overall survival. In this study, the oral alkylating agent temozolomide given concurrently with radiation and for 6 months following significantly improved median survival over radiation therapy alone (14.6 vs 12.1 months).1 The findings of this trial have changed the standard of care for patients with glioblastoma to include concurrent adjuvant temozolomide in the treatment of newly diagnosed glioblastoma. However, despite the significant overall benefit of temozolomide chemoradiation, it is clear that only a subset of patients experience a durable response to this regimen. Approximately one-quarter of patients treated with temozolomide chemoradiation will survive 2 years from diagnosis.1 Molecular subtypes of glioblastoma exist, and it is likely that differences in key molecular alterations underlie the variability in response to standard therapy. However, there are currently no validated molecular markers that will distinguish patients likely to respond from those likely to be refractory to current standard therapy.

These considerations point to 2 related but difficult tasks facing the neuro-oncology field. First, how can treatment be optimized for the individual patient? Since many patients have tumors refractory to standard therapy, identifying these patients prospectively could allow them to be treated with alternative and/or additional regimens with which they may have a better chance of a durable response. Second, what are the common characteristics of refractory tumors? Molecular signatures that present in tumors resistant to standard treatment may point to new tar-

Author Affiliations: Departments of Neuro-oncology (Dr Colman) and Pathology (Dr Aldape), and Brain Tumor Center (Drs Colman and Aldape), University of Texas M. D. Anderson Cancer Center, Houston.
gets for therapy tailored to the patients whose tumors harbor these changes. To make headway in these areas, it will be necessary to (1) develop sensitive and specific markers that will prospectively distinguish those patients who are likely to experience a durable response to standard therapy from those who will not and (2) to identify molecular markers and mechanisms that underlie treatment resistance to define new therapeutic targets in those patients for whom current standard therapy will have limited or no benefit. In this review, we will summarize some of the recent clinical and molecular advances in these areas as they apply to the molecular classification and treatment of glioblastoma.

MOLECULAR ALTERATIONS AND TARGETED THERAPY IN ONCOLOGY

During the past decade or more, there has been a dramatic shift in drug development and clinical investigation in oncology away from traditional cytotoxic chemotherapies and toward biologic drugs targeted at particular molecular alterations. This shift is based in part on significant successes of molecularly targeted therapy, including imatinib mesylate for chronic myelogenous leukemia, which targets the BCR-ABL fusion protein (Food and Drug Administration [FDA] approval in 2001), and trastuzumab for metastatic breast cancers that exhibit HER2 gene amplification (FDA approval in 1998). The emphasis on identifying specific molecular targets in particular tumors or subtypes of tumors has been greatly aided by the development of high throughput methods for the identification of alterations in the DNA, RNA, and (more recently) protein levels. In addition, these high throughput methods have allowed the development of marker panels to prospectively identify distinct groups of patients with differing prognoses or responses to particular therapies. For example, initial screening using gene expression microarrays and subsequent validation in independent samples were used to develop multigene assays to predict risk of recurrence or survival in patients with breast cancer, and one of these assays recently became the first such complex molecular assay approved by the FDA. Based on these successes, attempts are ongoing to identify specific molecular markers of treatment response or patient outcome in glioblastoma. In addition, better definition of specific molecular subclasses of glioblastoma could set the stage for both selective treatment of particular subgroups with particular signal transduction drugs currently in development (eg, receptor tyrosine kinase or angiogenesis inhibitors) or identifying novel therapeutic targets specific to particular tumor subtypes.

MOLECULAR MARKERS AND SUBTYPES IN GlioblASTOMA

The current classification of gliomas is based on histopathology alone. However, it is becoming increasingly clear that tumors that share identical histopathologic features can actually represent multiple distinct molecular phenotypes. A more detailed molecular understanding of these tumors is thus crucial for improved classification, outcome prediction, and the potential for tailoring specific treatments to individual tumor types or patients. The clinical importance of molecular subtype in high-grade gliomas is exemplified by the significant association of chromosome 1p/19q loss with improved prognosis and treatment response in anaplastic oligodendroglioma, which was recently validated in two phase 3 trials. While rigorous validation is currently lacking for particular molecular markers of prognosis or treatment response in glioblastoma, a number of candidate markers have been identified that have the potential for significant clinical impact.

MGMT-Promoter Methylation

Temozolomide is an alkylating agent that adds methyl groups to DNA and is currently the standard of care for first-line chemotherapy for glioblastoma. The MGMT gene (OMIM *156569) encodes a protein that reverses the addition of a methyl group to DNA and thus represents 1 potential mechanism of resistance to temozolomide treatment. In a recent phase 3 study, an analysis of MGMT-promoter methylation status was performed on a subset of tumor samples and a significant association between MGMT-promoter methylation (which decreases protein expression) and improved patient outcome from treatment was found. Among the patients in the combined temozolomide and radiation therapy arm, those with MGMT-promoter–methylated tumors experienced a 2-year survival rate of 46% compared with 14% among patients with unmethylated tumors. It was noted that in the radiation therapy–only arm, patients with MGMT-promoter–methylated tumors also experienced improved 2-year survival rates compared with patients with unmethylated tumors (23% vs 2%, respectively). It is currently unclear whether the relationship between MGMT status and outcomes in the radiation therapy–only treatment arm is due to the fact that many of these patients received salvage temozolomide therapy at recurrence. While MGMT is promising as a marker, it is important to note several limitations of MGMT-promoter methylation status that preclude its current use as a definitive predictive marker. First, the MGMT methylation studies were performed retrospectively on a subset of patients limited to those for whom adequate paraffin tissue was available and thus requires prospective validation in an independent clinical trial. Second, although the difference in 2-year survival in the temozolomide chemoradiation group based on MGMT status was highly statistically significant, approximately half (54%) of the patients in the MGMT-promoter–methylated group did not survive for 2 years, and 14% of the patients with the unfavorable marker did experience a favorable (>2-year survival) outcome after temozolomide chemoradiation therapy. Finally, the presence of the MGMT tumor methylation was associated with a survival benefit in both the temozolomide chemoradiation and radiation therapy–only treatment arms, suggesting that it may partially be a prognostic (associated with tumor natural history) rather than a truly predictive marker (associated with response to a specific therapy).
**PTEN/PI3-K/AKT, MAPK, and EGFR/EGFRvIII Pathway Alterations and Outcome in Glioblastoma**

Loss of chromosome 10 and amplification of regions of chromosome 7 are common cytogenetic alterations in glioblastoma. Detailed investigations of these alterations have identified specific genes and pathways affected by these alterations, with recent data highlighting their potential clinical importance. Loss of chromosome 10 is associated with mutation or deletion of the PTEN gene in glioblastoma and other cancers. The loss of PTEN leads to increased activation of the phosphatidylinositol-3-kinase (PI3-K)/protein kinase B (AKT) pathway, which has been associated with many biologic functions in cancers, including increased invasion and proliferation and decreased apoptosis. Data from our group and others indicate that loss of chromosome 10 or activation of the PI3-K/AKT pathway is associated with worse outcome and treatment resistance in glioblastoma. 

Our analysis of activation of individual members of the PI3-K/AKT and mitogen-activated protein kinase (MAPK) pathways in a retrospective set of glioblastomas demonstrated that activation of MAPK and members of the AKT pathway (mammalian target of rapamycin, p70s6k1) were associated with poor outcomes in glioblastoma. Furthermore, multivariate analysis demonstrated that an increased level of phosphorylated MAPK was the strongest predictor of both poor survival and radiation response (Table). The development of specific inhibitors targeting various alterations in the PI3-K/AKT pathway make glioblastoma a promising therapeutic target for these agents and a number of them are currently in clinical trials alone or in combination with cytotoxic drugs.

Amplification of the EGFR gene has been identified as an important alteration associated with chromosome 7 gain in glioblastoma. In addition, a subset of glioblastoma tumors with EGFR amplification harbors a specific mutation of the epidermal growth factor receptor (EGFR) known as EGFR variant III (EGFRvIII). The prognostic associations of EGFR or EGFRvIII are complex, with outcome association varying with patient age. Two recent studies highlight the potential interaction between the EGFR/EGFRvIII pathway and the PTEN/PI3-K/AKT pathways in response to specific inhibitors of the EGFR in glioblastoma. Although the overall clinical benefit of EGFR inhibitors in glioblastoma has been disappointing, these results suggest the possibility that prospective identification of subsets of patients with higher likelihood of benefit may be possible in future studies.

**GENE EXPRESSION PROFILING STUDIES IDENTIFY MOLECULAR SUBTYPES OF GLIOBLASTOMA**

While investigations of individual gene or protein alterations can provide potentially important clinical markers of outcome or as therapeutic targets, new techniques, including gene expression profiling using microarray-based platforms, provide a powerful approach to identify novel molecular alterations associated with molecular subtypes of tumors or clinical outcome. In addition, the biologic information obtained through these large-scale screens has the potential to identify novel therapeutic targets or pathways. Several microarray-based studies comparing gene expression profiles with patient outcome in glioblastoma have been published. Our group and others have identified several molecular subtypes of glioblastoma based on this gene expression data. Using unsupervised analysis methods to cluster glioblastoma tumors, we identified 3 distinct molecular groups of glioblastoma based on the pattern of genes with the highest expression in each cluster: proneural, proliferative, and mesenchymal (Figure 1). Analysis across these groups demonstrated that the survival of patients in the proneural group was significantly longer than patients in either the proliferative or mesenchymal groups (Figure 1B). The robustness of these molecular subtypes and their survival associations were confirmed using data from an independent sample set (Figure 1C). The reproducibility of these molecular groups across separate data sets indicates that these likely reflect true molecular subtypes of glioblastoma rather than spurious clusters related to biological or technical bias specific to an individual data set. High expression of mesenchymal genes was also correlated with increased expression of angiogenic genes, such as...
as VEGF, VEGFR1, VEGFR2, and PECAM1, as well as increased proliferation of vascular cells. We also found that tumors initially classified as proneural or proliferative tended to shift toward the mesenchymal/angiogenic phenotype at recurrence.11 These data demonstrate that robust molecular subtypes of glioblastoma can be identified across independent data sets using gene expression profiles and that the mesenchymal/angiogenic phenotype may represent the majority of aggressive newly diagnosed and recurrent glioblastoma tumors.

MESENCHYMAL TRANSITION IN TREATMENT-REFRACTORY GLIOBLASTOMA

The epithelial-to-mesenchymal transition has been described in a number of epithelial tumors as a process in which tumor cells lose epithelial characteristics and normal cell-cell interactions and acquire a mesenchymal and more invasive phenotype.23 The molecular mechanisms that regulate epithelial-to-mesenchymal transition in cancer are complex and often show tissue specificity. Pathways that have been described to promote epithelial-to-mesenchymal transition include transforming growth factor β, Wnt, PI3-K/akt, EGFR, and Ras.24-29 While a mesenchymal transition has not been formally described in the literature as a general property of gliomas, the unusual but well-documented entity of gliosarcoma suggests that it indeed occurs. While the original description of gliosarcoma postulated that the glial and mesenchymal components arise from different cell types, more recent evidence indicates a single cell of origin,30 suggesting that the mesenchymal component results from transdifferentiation of the glial component. Examination of microarray data obtained by us9,11 as well as others22 indicates that high expression of mesenchymal/angiogenic genes is common in glioblastoma and associated with worse outcomes.11,22 Furthermore, these findings suggest that the histopathologic entity of gliosarcoma may simply represent the extreme example of this phenomenon. Given these observations, the availability of agents that target the PI3-K/akt, Ras/Raf, and transforming growth factor β pathways or other pathways associated with epithelial-to-mesenchymal transition, alone or combined with agents that target angiogenesis, may represent a rational ap-
proach for tumor-specific therapy of glioblastomas, demonstrating this molecular signature.

ROLE OF STEM-LIKE CELLS IN INITIATION AND TREATMENT RESISTANCE IN GliOBLASTOMA

Growing evidence indicates that a number of human tumors are generated and maintained by a small subset of tumor cells, referred to as cancer stem cells, that share several properties with normal stem cells, including unlimited self-renewal and multilineage differentiation.\(^{31-34}\) These cancer stem cells may also underlie the relative resistance to treatment in cancer.\(^{35-37}\) While controversy remains regarding the optimal definition and cellular markers of cancer stem cells, recent data demonstrate that cells satisfying the consensus definition of cancer stem cells\(^{38}\) can be isolated from human glioblastoma tumors and expanded in vitro as suspension neurosphere cultures.\(^{33,39-42}\) These glioblastoma stem-like cells are characterized by the ability to proliferate in serum-free media containing the mitogens epidermal growth factor and fibroblast growth factor and have been shown to recapitulate many histologic features of the parent tumor when injected into the brains of nude mice.\(^{33,39-42}\) Comparison of primary cultures of glioblastoma demonstrate that stem-like cells maintained as neurospheres more closely mirror the histologic phenotype and genotype of the parent tumor compared with matched primary cultures grown under adherent conditions in serum.\(^{39}\) Expression of the cell surface marker CD133 has been reported as a marker for tumor-initiating cells from brain tumor neurosphere cultures from glioblastoma as well as other tumor types.\(^{43}\) Furthermore, recent studies demonstrate that glioblastoma stem-like cells expressing high levels of CD133 are more resistant to both radiation and temozolomide.\(^{45,46}\) Our studies of glioblastoma stem cells isolated from individual patients demonstrate that many neurosphere cultures express high levels of mesenchymal/angiogenic genes. An example of the striking similarities between expression in the human tumors and in the glioblastoma stem cell/neurosphere cultures of one of these mesenchymal genes, CHI3L1 (also known as YKL-40),\(^{9,10}\) is shown in Figure 2. These observations, along with those of other groups,\(^{47}\) suggest that expression of mesenchymal markers in glioblastoma may be due at least in part to the lineage plasticity inherent in tumor stem cells. Thus, better understanding of the mechanisms regulating glioblastoma stem-cell self-renewal, tumorigenesis, and differentiation may reveal new insights into glioblastoma biology and could identify novel approaches to target the key cells potentially responsible for the treatment resistance of these tumors.

IDENTIFICATION OF A CONSENSUS GENE EXPRESSION PREDICTOR IN GliOBLASTOMA

While the finding of distinct gene expression profiles associated with prognosis represents an advance in the molecular classification of these tumors, the clinical application of gene expression signatures in glioblastoma requires additional development and validation. It should be noted that while functional similarities exist between several published gene expression studies in glioblastoma, these publications are noteworthy more for their differences than similarities in the lists of specific top survival genes. This is likely the consequence of the multiple comparisons problem inherent to the large number of genes examined compared with the relatively low number of tumors examined in most expression microarray experiments, which leads to a high false-discovery rate.\(^{48,49}\) In addition, technical variability (eg, sample quality) and differences in statistical methods used to identify the top genes may contribute to a lack of reproducibility in gene lists.\(^{50}\) One method to overcome these limitations is to examine multiple data sets systematically to identify genes that repeatedly correlate with patient outcome; studies are ongoing to identify such a consensus gene expression predictor in glioblastoma.

TOWARD INDIVIDUALIZATION OF TREATMENT FOR GliOBLASTOMA

While prognostic markers may provide an overall indication of the natural history of the tumor in a specific patient, they do not necessarily impact patient care unless they alter treatment choice. Despite recognition of intertumor molecular heterogeneity in glioblastoma,
First, identifying the refractory patients early would allow them to make an informed decision to participate in more aggressive clinical trials with the knowledge that accepting the possibility of higher toxicity has a favorable risk-benefit ratio owing to the low likelihood of significant benefit from standard therapy alone. Second, a more detailed knowledge of the molecular alterations associated with treatment resistance would set the stage for the next generation of trials for newly diagnosed glioblastoma incorporating both molecular inclusion criteria (identifying sensitive and refractory tumors) and hypothesis-based targeted therapies for resistant tumors based on molecular phenotype. One such study design is currently in development (Figure 3) using a multi-marker predictor to prospectively stratify patients and specific signal transduction agents in addition to standard therapy to target the mesenchymal/angiogenic phenotype in glioblastoma.

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Correspondence: Howard Colman, MD, PhD, Department of Neuro-oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 431, Houston, TX 77030 (hcolmam@mdanderson.org).

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Figure 3. Proposed phase 2/3 study scheme to prospectively identify and therapeutically target distinct molecular subtypes of glioblastoma. Following initial surgery, patients begin standard temozolomide chemoradiation. During this time, the tumor tissue is analyzed using a multigene predictor optimized and validated for prediction of response to this therapy. Based on the multigene predictor score, the patient is determined to have a tumor with either a high (left) or low (right) likelihood of response. Patients in the latter category are randomized to receive standard therapy plus investigational agents (separately and combined) that target the alterations, which are related to treatment refractoriness (eg, mesenchymal/angiogenic phenotype).

REFERENCES


