Published in final edited form as:

Prog Mol Biol Transl Sci. 2011 ; 98: 187–234. doi:10.1016/B978-0-12-385506-0.00005-3.

MOLECULAR ALTERATIONS IN GLIOBLASTOMA: POTENTIAL TARGETS FOR IMMUNOTHERAPY

Azizul Haque^{1,2,3}, Naren L. Banik⁴, and Swapan K. Ray⁵

¹Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425, USA

²Hollings Cancer Center, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425, USA

³Children's Research Institute, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425, USA

⁴Department of Neurosciences, Medical University of South Carolina, 96 Jonathan Lucas Street, Charleston, SC 29425, USA

⁵Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC 29209, USA

Abstract

Glioblastoma is the most common and deadly brain tumor, possibly arising from genetic and epigenetic alterations in normal astroglial cells. Multiple cytogenetic, chromosomal, and genetic alterations have been identified in glioblastoma, with distinct expression of antigens (Ags) and biomarkers that may alter therapeutic potential of this aggressive cancer. Current therapy consists of surgical resection, followed by radiation therapy and chemotherapy. In spite of these treatments, the prognosis for glioblastoma patients is poor. Although recent studies have focused on the development of novel immunotherapeutics against glioblastoma, little is known about glioblastoma specific immune responses. A better understanding of the molecular interactions among glioblastoma tumors, host immune cells, and the tumor microenvironment may give rise to novel integrated approaches for the simultaneous control of tumor escape pathways and the activation of antitumor immune responses. This review provides a detailed overview concerning genetic alterations in glioblastoma, their effects on Ag and biomarker expression and the future design of chemoimmunotherapeutics against glioblastoma.

Keywords

glioblastoma; antigens; biomarkers; cytokines; chemotherapy; immunotherapy

*Correspondence: Dr. Azizul Haque, Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, BSB-201, Charleston, SC 29425; Tel: 843-792-9466, Fax: 843-792-2464, haque@musc.edu.

I. Introduction

Glioblastoma is the most common and aggressive primary tumor of the central nervous system (CNS) in adults (1,2). Median survival of glioblastoma patients is 10–12 months. Radiotherapy has been of key importance to the treatment of glioblastoma for decades, and the ability to focus the beam and tailor it to the irregular contours of brain tumors, while minimizing the dose to nearby critical structures through intensity-modulated or image-guided techniques has improved greatly (3). Temozolomide, an alkylating agent with simple oral administration and a favorable toxicity profile, is used in conjunction with and after radiotherapy (4). Newer surgical techniques, such as fluorescence-guided resection and neuroendoscopic approaches, have become important in the management of malignant glioblastoma. New discoveries are also being made in basic and translational research including agents that block 1 or more of the disordered tumor proliferation signaling pathways and that overcome resistance to current treatments. Targeted therapies such as antiangiogenic therapy with antivascular endothelial growth factor (anti-VEGF) antibodies are also finding their way into clinical practice (5). According to the classical genetic pathway theory, glioblastoma can be subdivided into primary and secondary tumors, reflecting the progression of low grade astrocytomas during the multistep tumorigenesis. The main molecular hallmarks of primary glioblastoma are epidermal growth factor receptor (EGFR) amplification with loss of heterozygosity (LOH) on chromosome 10q, murine double minute 2 (MDM2) oncogene amplification, p16 deletion, phosphatase and tensin homolog on chromosome ten (PTEN) mutation, and further overexpression of insulin-like growth factor binding protein 2 (IGFBP2) (6–10). In secondary glioblastoma, the main molecular hallmark is tumor protein 53 (TP53) mutations with LOH on 17p, 10q, and 19q. TP53 mutation and/or LOH on 17p and EGFR gene amplification occur in a mutually exclusive fashion in glioblastoma. Large-scale research efforts are ongoing to provide a comprehensive understanding of all the genetic alterations and gene expression changes underlying glioblastoma formation. Despite advances in molecular biology and genetics of cancer there is no effective treatment available for glioblastoma. This review will summarize the role of various Ags and biomarkers in the pathobiology of glioblastoma and their potential role in brain malignancies.

Glioblastoma molecular profiles have been analyzed that reflect a change from an invasive to an angiogenic brain tumor phenotype. Aberrant patterns of multiple gene expression and compartmentalization in glioblastoma have been associated with glioblastoma tumorigenesis and progression. Angiogenesis is a critical physiologic process that is appropriated during tumorigenesis. Little is known about how this process is specifically regulated in the brain. The cellular and molecular events that initiate and promote malignant glioblastoma development are also not completely understood, and the treatment modalities designed to promote its demise are all ultimately ineffective, leading to disease progression. Vasculogenesis and angiogenesis have been shown to play distinct roles in the pathogenesis of primary and recurrent malignant glioblastoma (11), suggesting that patient therapy should perhaps be tailored specifically against the predominant vasculature pathway at a given specific stage of glioblastoma progression. The identified proteins resulting from vasculogenesis and angiogenesis could be further exploited as biomarkers or therapeutic

targets for malignant brain tumors. Likewise, microRNAs (miRNAs) are effective post-transcriptional regulators of gene expression and are important in many biological processes, including oncogenic and tumor suppressive functions in glioblastomas (12). The manipulation of which could also yield new treatment modalities and biomarkers in glioblastoma. Recent study suggests that many cancer cell populations contain a subpopulation of self-renewing stem cells known as cancer stem cells (CSCs) (13). Unlike normal adult stem cells, CSCs can increase in number as tumor cells grow, and give rise to progeny that can be both locally invasive and colonize at distant sites. Stem cells maintain homeostasis in adult tissues via self-renewal and generation of terminally differentiated cells. Alterations in this balance can result in malignant cell growth and disease. Understanding the mechanisms by which stem cells become CSCs may lead to new therapeutic approaches.

Glioblastoma, like other malignancies, is able to overcome host immune defenses through a variety of mechanisms. The brain is believed to be an immunologically privileged region, where immune responses to tumors and cellular interactions are influenced by the presence of the selective blood brain barrier (BBB). Only activated T cells may gain entry to the brain (14). Brain infiltrating T cells may also lose activated status or may be largely immunosuppressive CD4⁺ CD25⁺ regulatory T (Treg) cells (15). One of the important features of tumor vaccines is to increase the number of tumor Ag-specific T cells *in vivo*. As the number of immune T cells increases, the effects of the vaccine usually increase, but the efficacy is dependent not only on the quantity of T cells but also on the survival of T cells. Tumor vaccines may also present Ags to the small number of T cells that are capable of recognizing and responding to the Ags, which are thereby stimulated to proliferate and increase in number (16,17). These components and factors that allow T cell activation and expansion in significant number have been identified and constructed as immunotherapeutic agents. Similarly, many of the components and factors that prohibit activation, expansion, or persistence of immune effector cells have been identified, and agents that interfere with the prohibition have also been constructed.

Several possible vaccination approaches can be envisioned for treating glioblastoma patients. One of these approaches is the isolation of patients' own tumor cells treated with cytokines and used as a whole cell vaccine. These cells are loaded on the patient's own dendritic cells (DC) cultured with cytokines, and then inoculated back to the patient (18). The advantage of this kind of cellular immunotherapy is that tumor-specific T cells may seek and kill invasive glioblastoma tumors that remain as a residue after surgical and chemotherapeutic interventions. Expansion of tumor-specific cytotoxic T cells (CTL) has also been observed in glioblastoma patients treated with an autologous DC vaccine in combination with cytokines (19). However, many patients receiving the vaccine eventually die due to glioblastoma progression, despite the presence of tumor-specific CTL. Aberrations of normal cytokine-mediated cell proliferation have also been observed in glioblastoma and there is evidence that alterations in the expression of cytokines and their receptors are most apparent in more malignant glioblastoma (16,19). Thus, in order for immunotherapy to successfully treat glioblastoma, the cells responsible for tumor progression must be targeted and eliminated by a combination of chemotherapeutics,

immune cells, and cytokines. Although recent study has provided evidence of cell-mediated anti-glioblastoma responses in a number of clinical trials, special considerations need to be accounted for devising immunotherapeutics against CNS tumors. The purpose of this review is to provide an overview of genetic alterations and appearance of Ags and biomarkers in glioblastoma, and discuss how these molecules could be targeted for devising novel chemoimmunotherapy.

II. Genetic Alterations in Glioblastoma

Glioblastoma is the most common and aggressive type of brain tumor in humans. Intensive molecular analyses have revealed a variety of deregulated genetic pathways involved in DNA damage and repair, apoptosis, cell migration, angiogenesis, and the cell cycle. This section will focus on potential new targets in glioblastoma resulting from genetic alterations. The majority of glioblastoma cases are primary brain tumors that grow rapidly without major clinical or histological evidence of a less malignant precursor lesion. These tumors mainly affect the elderly and are genetically characterized by LOH on 10q, EGFR amplification, p16^{INK4a} deletion, and PTEN mutations (Table 1) (6–10). Secondary glioblastoma tumors develop through progression from low-grade diffuse astrocytoma or anaplastic astrocytoma and are pronounced in younger patients (2, 6–8). While disruption of tumor suppressor gene TP53 is implicated in the progression of many types of human malignancies, adult glioblastoma patients with TP53 mutation may have a more severe consequence (e.g., shorter survival) than those without TP53 mutations (2, 6–8). It has also been shown that TP53 mutations, but not p53 expression, correlate with a more aggressive form of the disease. Studies have also reported that glioblastoma with TP53 mutations are more frequent in women when compared with those mutations in men, and may occur in younger patients (20). In addition, some study suggests that TP53 mutations may occur in patients of any age group, whereas EGFR amplification preferentially occurs in older patients. Thus, multiple genes are involved in the initiation of the disease, and variability occurs in different age and sex groups in the progression of glioblastoma. The median survival of secondary glioblastoma patients has been reported to be 7.8 months, significantly longer than that of primary glioblastoma patients (4.7 months). However, after careful analysis of age and disease progression, no significant difference in survival was observed in patients with primary and secondary glioblastoma. During glioblastoma progression, additional mutations and genetic alterations accumulate, which may alter disease severity and patient survival.

Primary and secondary glioblastomas can also differ significantly depending on their pattern of promoter methylation and in expression profiles at the RNA and protein levels. LOH on 10q is shown most frequent in both primary and secondary glioblastomas (2, 6–7). Studies suggest that TP53 mutations are detected early in the pathway, and frequent genetic alterations can lead to secondary glioblastoma (8–10, 21). A recent study analyzed 77 Japanese patients with primary glioblastoma and found 22% TP53 mutations, 21% PTEN mutations, 32% EGFR amplification, 42% p16^{INK4a} homozygous deletion, and 69% LOH on chromosome 10q in those patients (22). The frequencies of these genetic alterations at the population level were similar to those reported in Europe. This study noted a positive association between EGFR amplification and p16^{INK4a} deletion, and an inverse association

between TP53 mutations and p16^{INK4a} deletion in glioblastoma patients in Japan vs. European countries. The allele frequencies of polymorphisms at codon 787 CAG/CAA (Gln/Gln) in glioblastoma in Japan were significantly different from those in Europe. Collectively, this study observed a similar molecular basis in genetic alterations in Caucasians and Asians, despite different genetic backgrounds and polymorphisms in the EGFR gene. These genetic alterations in glioblastoma may also offer new potential targets for the development of a variety of antitumor therapeutics.

Tumorigenesis of human glioblastoma could be driven by several other genetic abnormalities with disruption of important molecular pathways (Table 1), such as p53/MDM2/p14^{ARF} and EGFR/PTEN/Akt/mTOR (8–10, 21). In the TP53/MDM2/p14^{ARF} pathway, TP53 mutation plays a crucial role in the development of secondary glioblastoma. TP53 mutations are also believed to be the first detectable genetic alteration in two-thirds of precursor low-grade diffuse astrocytoma and anaplastic astrocytoma. TP53 mutations also occur in primary glioblastoma, but at a lower frequency. Studies suggest that G/C to A/T mutations at CpG sites, particularly in the hotspot codons 248 and 273, seem to be an early event directly associated with malignant transformation in the pathway to secondary glioblastoma. The less specific pattern of TP53 mutations in primary glioblastoma may constitute, at least in part, secondary events due to increased genomic instability during tumor development. The P53/MDM2 pathway also plays a role in glioblastoma, where MDM2 amplification is implicated in poor prognosis of the disease (20). The association of alterations in p53 and MDM2 with the survival of patients with anaplastic astrocytoma or glioblastoma also remains controversial. Homozygous deletion of the p16^{INK4a}/p14^{ARF}/p15^{INK4b} locus is thought to be the most common genetic alterations in glioblastoma (2,6,23). Two recent studies have shown that deletion and mutation of another INK4 family member, p18^{INK4c}, may drive the pathogenesis of glioblastoma. Hypermethylation of p14^{ARF} may constitute distinct molecular pathways of astrocytoma progression, which could differ in biological behavior and clinical outcome. Loss of p14^{ARF} expression has frequently been observed in glioblastoma (76%), and this typically correlates with homozygous deletion or promoter methylation of the p14^{ARF} gene. Promoter methylation of p14^{ARF} was also shown to be more frequent in secondary glioblastoma as compared with primary glioblastoma, but no significant differences were observed in the overall frequency of p14^{ARF} alterations between glioblastoma subtypes. The p14/p19^{ARF} product of the CDKN2A gene also displays tumor suppressor activity, both in the presence and absence of p53/TP53. Genetic alterations are also frequently observed in high grade adult astrocytomas, occurring in either the p53/MDM2/p14^{ARF} or Rb/CDK4/p16^{INK4a} tumor suppressor pathways (2,6–8, 20,23). The clinical significance of other glioblastoma-associated molecular aberrations and their relationship to O⁶-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation is still unclear. Correlation of molecular findings with clinical data revealed significantly longer time to progression after onset of chemotherapy and longer overall survival of patients with MGMT-hypermethylated tumors. In contrast, MGMT protein expression, MGMT polymorphisms, and aberrations in any of the other genes and chromosomes were not significantly linked to patient outcome.

Studies suggest that the EGFR/PTEN/Akt/mTOR signaling pathway is activated in many cancers including glioblastoma, yet mTOR inhibitors have largely failed to show efficacy in

the clinic (21, 24–25). In this pathway, amplification of the gene encoding the EGFR occurs commonly in glioblastoma, leading to activation of downstream kinases including PI3K, Akt, and mTOR. While EGFR amplification may occur in about 40% of primary glioblastoma, it is rarely detected in secondary glioblastoma. Overexpression of EGFR is also thought to be common in about 60% of primary glioblastoma as compared with secondary glioblastoma (about 10%). Some study also reported little to no amplification of EGFR in younger patients with glioblastoma, suggesting that EGFR amplification is absent or very rare in pediatric glioblastoma. The amplification of EGFR is also associated with amplifications in MDM2 and CDK4 and a higher percentage of cases with promoter methylation of INK4a (21,24,26). EGFR amplification may occur independently or simultaneously with TP53 mutation but may not correlate with patient survival. Understanding the genetic profiles and molecular subtypes within primary glioblastoma may contribute toward the development of targeted drugs against glioblastoma. Study also suggests that EGFR amplicons are often mutated; EGFR variant 3 (EGFRvIII) with deletion of exons 2 to 7 is the most frequent type in glioblastoma (8–10, 21). The constitutively active EGFRvIII can enhance cell proliferation in part by downregulation of p27 through activation of the PI3K/Akt pathway. EGFR also becomes activated through the binding of EGF/transforming growth factor beta (TGF- β) to its extracellular domain, resulting in recruitment of PI3K to the cell membrane (8–10). PI3K phosphorylates PIP3, which activates downstream effector molecules such as Akt and mTOR. These events may result in cell proliferation and increased tumor cell survival by blocking apoptosis. Glioblastoma tumors often show activation of EGFR and loss of PTEN tumor suppressor (7–10), but it is not clear if these two genetic factors act together to transform brain astroglial cells. One of the obstacles in designing specific inhibitors is that glioblastoma cells frequently carry mutations in the PTEN tumor suppressor gene on 10q23.3. It has been shown that the PTEN gene is mutated in 20–40% of glioblastoma tumors. The tumor suppressor properties of PTEN are also closely related to its inhibitory effect on the PI3K-dependent activation of Akt signaling. Dissecting the molecular events associated with activation of this pathway in glioblastoma patients may help develop inhibitors to block the PI3K pathway.

Promoter methylation of p16^{INK4a}, p14^{ARF}, RB1, TIMP -3, and MGMT genes has also been analyzed in both primary and secondary glioblastomas, where secondary glioblastoma showed a higher frequency of promoter methylation than primary glioblastoma (2,6 -9). MGMT is a repair protein that specifically removes promutagenic alkyl groups from the O⁶-position of guanine in DNA. MGMT protects cells against malignancies induced by alkylating agents, and an inverse correlation has been reported between MGMT activity and tissue-specific tumorigenesis induced by alkylating agents in rodents. Repair of O⁶-alkylguanine adducts by tumor cells has been implicated in drug resistance because it reduces the cytotoxicity of alkylating chemotherapeutic agents. Loss of MGMT expression caused by methylation of promoter CpG islands was detected in 75% of secondary glioblastoma, significantly more frequently than in primary glioblastoma (36%). The difference in frequency of MGMT methylation between primary and secondary glioblastoma is clinically relevant because patients with glioblastoma containing a methylated MGMT promoter were shown to have a substantially greater benefit from adjuvant, temozolomide treatment.

Studies suggest that multiple genes expressed at a significantly higher level in primary vs. secondary glioblastomas include VEGF, fms-related tyrosine kinase 1, and IGFBP2 (10,27). Because VEGF is induced by hypoxia-inducible factor-1 (HIF-1), this difference explains the higher frequency and greater extent of necrosis in primary glioblastoma (28). Active forms of EGFR, MDM2, and MMP-9 are more frequently expressed in primary glioblastoma than secondary glioblastoma (9–10). Active MMP-9 expression is strongly correlated with EGFRvIII expression, which is also typical for primary glioblastoma. Survivin, an inhibitor of apoptosis, is also frequently expressed in 83% of primary glioblastoma while it is expressed in 46% of secondary glioblastomas (29). Achaete-scute homolog 1 (ASCL1) is overexpressed in 86% of grade II diffuse astrocytomas and 88% of secondary glioblastoma, whereas the 67% of primary glioblastoma expressed similar to or less than normal brain levels. ASCL1 upregulation was accompanied by inhibition of Notch signaling as seen by uninduced levels of Hes1, a transcriptional target of Notch1, and increased levels of Hes6, a dominant-negative inhibitor of Hes1-mediated repression of ASCL1 (30). Aberrant patterns of γ -tubulin expression and compartmentalization in glioblastoma have also been reported, and these changes might underlie perturbations in microtubule nucleation and mitosis associated with glioblastoma tumorigenesis and tumor progression (31).

A recent proteomics approach has identified additional proteins that are differentially expressed between glioblastoma subtypes. The prognosis of patients with glioblastoma may be at least partially determined by a complex interaction between age and different genetic alterations. Overexpression of IGFBP2 was recently found as the most frequent alteration in advanced stage of glioblastoma (10,25). IGFBP2 is crucial in determining the phenotypes of advanced stage of tumors, such as increased cell proliferation and invasion. IDH1 mutations are also present more often in younger glioblastoma patients (32). The identification of IDH1 mutations may have clinical value as a marker of secondary glioblastoma. Recent studies have also demonstrated that inhibition of mitochondrial IDH2 increases tumor sensitivity to certain chemotherapeutic agents. This finding provides a strong basis for the emerging development of novel gene-based diagnostic, prognostic, and therapeutic strategies for glioblastoma tumors.

III. Ags and Biomarkers in Glioblastoma

The assessment of Ags and biomarkers is needed to gain better understanding of glioblastoma pathogenesis, to help determine prognosis, and to design therapies. The majority of glioblastoma patients exhibit rapid disease progression despite aggressive surgery, radiation, and chemotherapy (1,2, 6–7). The lack of known biomarkers allowing for the assessment of the evolution and prognosis of glioblastoma is a major impediment to the clinical management of glioblastoma patients. The observed variability in Ags and biomarkers expression (Table 2) and in patient survival outcomes implies biological heterogeneity and the existence of unidentified patient categories. Study suggests that the prospero homeobox protein 1 (PROX1) is a transcription factor that plays a critical role in the development of various organs including the mammalian CNS and lymphatic system (33). PROX1 controls cell proliferation and differentiation through different transcription pathways and has both oncogenic and tumor-suppressive functions. It has been

shown that an average of 79% of glioblastoma cells express PROX1. Human epidermal growth factor receptor 2 (HER-2) is a validated immunotherapy target, and HER-2-specific T cells generated from glioblastoma patients recognizes autologous HER-2-positive tumor cells that also express CD133 biomarker (34). In this study, stimulation of HER-2-specific T cells with HER-2-positive autologous glioblastoma cells resulted in T cell proliferation and secretion of interferon-gamma (IFN- γ) and interleukin-2 (IL-2) in a HER-2-dependent manner. HER-2-specific T cells also killed both CD133 $\pm$ glioblastoma cells derived from primary HER-2-positive glioblastoma, whereas HER-2-negative tumor cells were not killed. Injection of HER-2-specific T cells induced sustained regression of autologous glioblastoma xenografts, suggesting adoptive transfer of HER-2-specific T cells may be a promising immunotherapeutic approach for patients with glioblastoma.

Studies suggest that CD44 is a major cell surface hyaluronan receptor and is implicated in the progression of a variety of cancer types (35,36). CD44 is upregulated in glioblastoma cells and its depletion blocks glioblastoma tumor growth and sensitizes tumor cells to cytotoxic drugs in vivo. Consistent with this observation, CD44 antagonists have been tested in preclinical mouse models and shown to inhibit glioblastoma growth. A recent study has provided evidence that CD44 functions upstream of the mammalian Hippo signaling pathway, promoting tumor cell growth (35). Investigating CD44 antagonists may uncover a novel CD44 signaling pathway in brain tissue malignancies, and provide a first mechanistic explanation as to how upregulation of CD44 may constitute a key event in leading to cancer cell resistance. A recent study has shown that stage-specific embryonic antigen-1 (SSEA-1) expressing glioblastoma cells are highly tumorigenic in vivo, and that this Ag can be a general biomarker in human brain tumors (37). The peripheral and local expression of IL-8 may associate with IL-6, cyclooxygenase-2 (COX-2), and VEGF expression in brain tumor patients. IL-6 and IL-8 expression levels were shown higher in the peripheral blood mononuclear cells (PBMCs) of glioblastoma patients compared with non-tumor controls (38). The coordinated expression and topographical relationship of IL-6, IL-8, COX-2, and VEGF were observed in the same tumor areas, suggesting that the angiogenic process takes place in the brain tumor microenvironment. The expression of selected neural and non-neural differentiation markers including A2B5, CD34, CD45, CD56, CD117, CD133, EGFR, GFAP, HER-2/neu, LIFR, nestin, NGFR, Pgp, and vimentin has also been analyzed in glioblastoma patients (39). All glioblastoma tumors were positive for A2B5, CD56, nestin, and vimentin. CD133, EGFR, LIFR, NGFR, and Pgp were variably expressed by brain tumor cell subpopulations. Surprisingly, CD34, CD45, CD117, GFAP, and HER-2/neu biomarkers were negative in those tumors. The expression of A2B5, CD56, NGFR, and Pgp appeared to be associated with chemoresistance whereas CD133, EGFR, and LIFR expression was characteristic of chemosensitive tumors. The activating receptor NKG2D, expressed by NK and CD8 $^{+}$ T cells, also has a role in the specific killing of transformed cells (40). Studies have shown that NKG2D expression is downregulated in NK and CD8 $^{+}$ T cells in glioblastoma patients. Expression of NKG2D on lymphocytes was significantly increased following tumor resection and correlated with an increased ability to kill NKG2D ligand-positive tumor targets. Despite the presence of soluble NKG2D ligands in the sera of glioblastoma patients, NKG2D downregulation was primarily caused by tumor cell production of TGF- β , suggesting that blocking this cytokine may have therapeutic benefit.

Recent molecular and genetic profiling studies have provided a better understanding of prognostic and predictive biomarkers of glioblastoma along with underlying mechanisms of resistance to standard therapies (Table 2). The expression of specific molecular biomarkers (e.g., MGMT status) may determine the response of the tumor to treatment and help identify the magnitude of benefit from this regimen. While MGMT promoter hypermethylation is associated with a response to alkylating chemotherapy and longer survival (41), the clinical significance of other glioblastoma-associated molecular aberrations and their relationship to MGMT promoter hypermethylation is poorly understood. A recent study revealed that the AJAP1, EMP3, and PDPN gene expression are also associated with overall survival of glioblastoma patients. By identifying further biological subtypes of glioblastoma at the molecular level, specific targeted therapies could be developed and used in the future for more individualized therapeutic regimens. The insulin-like growth factor (IGF) signaling pathway has been implicated in the progression of glioblastoma (10,42). The IGF signaling axis involves the complex coordinated actions of two ligands (IGF-I and IGF-II), three cell surface receptors (IGF-IR, IGF-IIR, and IR), IGF-binding proteins IGFBPs, and the proteases that affect the binding proteins. These interactions are thought to influence IGF bioavailability. The mitogenic effects of IGFs are mainly mediated through interactions with IGF-IR followed by the activation of its downstream effector molecules. Earlier studies have shown that human glioblastoma tumors overexpress IGFs and IGFs when compared with normal brain tissue. IGFBP isoforms can also play a role in the pathogenesis of a number of human malignancies. IGFBP isoforms (IGFBP-2, IGFBP-3, and IGFBP-5) are expressed during malignant progression of astrocytoma and the mean transcript levels of IGFBP-2 and IGFBP-3 are found significantly higher in glioblastoma tumors relative to other brain tumors. IGFBP-2 overexpression is also thought to be an invasive potential of glioblastoma cells, and is associated with poor patient prognosis. This study also found that IGFBP-3 biomarker expression and patient age were associated with shorter survival in glioblastoma. Insights into the significance of molecular, biochemical, immunochemical, and clinical markers in predicting the prognosis of glioblastoma patients may improve patient outcome.

CSCs are thought to be critical for the engraftment and long-term growth of many tumors, including glioblastoma (13). The cells are at least partially spared by traditional chemotherapies and radiation therapies, and finding new treatments that can target CSCs may be critical for improving patient survival. Studies have found an association between the expression of stem cell marker CD133 and the risk of dissemination of glioblastoma tumors and that CD133+CSCs may be implicated in the initiation of disseminated lesions (43,44). Microarray analyses of clinical specimens from glioblastoma patients also detected five potential biomarkers such as CD44, growth arrest and DNA-damage-inducible alpha (GADD45A), fibronectin 1 (FN1), CD63, and secreted phosphoprotein 1 (SPP1) (7,36). It has been shown that the Notch signaling pathway regulates normal stem cells in the brain, and that glioblastoma contains stem-like cells with higher Notch activity (30). Notch blockade by gamma-secretase inhibitors (GSIs) may reduce neurosphere growth and clonogenicity in vitro, whereas expression of an active form of Notch2 could enhance tumor growth. The putative CSC markers CD133, Nestin, BMI1, and OLIG2 were shown to be reduced when Notch signaling is blocked (30). When equal numbers of viable cells pretreated with either vehicle (DMSO) or GSI were injected subcutaneously into nude mice,

the former always formed tumors, whereas the latter did not. In vivo delivery of GSI by implantation of drug-impregnated polymer beads also effectively blocked tumor growth, and significantly prolonged survival, albeit in a relatively small cohort of animals. This study also found that Notch pathway inhibition appears to deplete stem-like cancer cells through reduced proliferation and increased apoptosis associated with decreased Akt and STAT3 phosphorylation. Blocking the Notch pathway may deplete stem-like cells in glioblastoma, suggesting that GSIs may be useful as chemotherapeutic reagents to target CSCs in malignant glioblastoma. The hairy/enhancer of split (E(spl))-related family of transcription factors (Hes and Hey) are established targets of the Notch signaling pathway, which has been implicated in different developmental processes (45). The overexpression of Hey1 in glioblastoma is thought to be linked with shorter patient survival. Thus, Hey1 expression might be used as a biomarker to distinguish glioblastoma patients with a relatively good prognosis from those at high-risk, and it could be used as a therapeutic target.

Recent study has also shown that the neurodevelopmental polysialic acid neural cell adhesion molecule (PSA-NCAM) protein is overexpressed in approximately two thirds of glioblastoma tumors at variable levels (46). In glioblastoma cell lines, PSA-NCAM expression levels are associated with one of the OLIG2 transcription factors, which are required for transformation of glioblastoma cells and represent a valuable biomarker for the prognosis of brain tumor patients. Lipid peroxidation (LPO) is an autocatalytic process caused by oxidative stress. It results in the production of 4-hydroxynonenal (HNE), which plays a crucial role in hypoxic brain injury, neuronal degeneration, and apoptosis. HNE-protein adducts have been detected in many types of tumors including glioblastoma (47). An increased expression of HNE was found in glioblastoma tumors when compared to different types of astrocytomas, suggesting that HNE might be involved in the damage of brain cells and the induction of malignancy.

It has been shown that a cell surface IgSF protein, EWI-2, is highly expressed in normal human brain cells but is considerably diminished in glioblastoma tumors and cell lines. Loss of EWI-2 expression correlates with a shorter survival time in human glioblastoma patients, suggesting that EWI-2 might be a natural inhibitor of brain tumors (48). Expression of EWI-2 in human malignant glioblastoma T98G and U87MG cell lines failed to alter two-dimensional cell proliferation but inhibited glioblastoma colony formation in soft agar, and caused diminished cell motility and invasion. At the biochemical level, EWI-2 markedly affects the organization of four molecules which play key roles in the biology of astrocytes and glioblastomas (48). EWI-2 expression causes CD9 and CD81 to become more associated with each other, whereas CD81 and other tetraspanins become less associated with MMP-2 and MT1-MMP. The expression of c-Met protein is associated with biologic features representing tumor invasiveness in patients with brain tumors (49). c-Met overexpression is also associated with shorter survival time and poor treatment response in glioblastomas, suggesting that therapeutic strategies targeting c-Met receptors may have important clinical implication. Cell division cycle 20 (CDC20) homologues are also overexpressed in glioblastoma and under expressed in low-grade tumors (50). The expression of CDC20 biomarker and its immune-reactivity can be useful for the identification of glioblastoma in small biopsies. Glioblastoma cells also express several other genes typical of normal neural stem cells. One of them is the SOX-2 gene, which is

involved in sustaining self-renewal of several stem cells, in particular neural stem cells when they acquire cancer properties (51). Thus, SOX-2, or its immediate downstream effectors, could be an ideal biomarker for glioblastoma therapy.

A recent immunochemical analysis revealed EMMPRIN and MMP-2 expression in cryo-sections of pediatric glioblastoma and normal brain tissue (52,53). The intensely positive expression rates of EMMPRIN and MMP-2 in anaplastic astrocytoma and glioblastoma tissues were significantly higher than those in normal brain and low-grade astrocytoma tissues. Patients with EMMPRIN/MMP-2 expression have been shown the lowest survival rate. Thus, EMMPRIN and MMP-2 are important biomarkers expressed differentially in normal brain and glioblastoma, and their co-expression may facilitate the prediction of glioblastoma outcomes. The astrocyte elevated gene-1 (AEG-1) expression is significantly elevated in more than 90% of diverse human brain tumor samples including glioblastoma and other astrocytic tumors (54). Knockdown of AEG-1 by siRNA has been shown to inhibit cell viability, cloning efficiency, and invasive ability of human glioblastoma U87 MG cells and rat gliosarcoma 9L cells. MMP-2 and MMP-9 are involved in AEG-1-mediated invasion of glioblastoma cells. In an orthotopic nude mouse brain tumor model, AEG-1 siRNA significantly suppressed glioblastoma cell growth (54). This study indicates that AEG-1 may play an important role in the pathogenesis of glioblastoma and that AEG-1 could represent a viable potential target for glioblastoma therapy. The non-essential amino acid neurotransmitter glycine (Gly) may serve as a biomarker for brain tumors (55). Studies using 36 biopsies from patients with brain tumors found that Gly levels were significantly elevated in glioblastoma biopsies. Tumor endothelial marker 1/endothelialin (TEM1/endothelialin) is an emerging microvascular marker implicated in tumor angiogenesis (56). The expression of TEM1/endothelialin is upregulated in primary and metastatic human brain tumors, where it is primarily localized to the tumor vasculature and a subset of tumor stromal cells. TEM1/endothelialin is expressed in 79% of brain tumors, and in 31% of glioblastomas. The cellular localization of TEM1/endothelialin and its expression profile in primary and metastatic brain tumors support efforts to therapeutically target this biomarker.

Proteases also play an important role in the invasion process and correlations among glioblastoma grading and survival. Transcripts of uPA, PAI-1, cathepsin B, and MMP-9 are significantly upregulated in glioblastoma tumors (57). The expression of cathepsin B and uPA is detected at the invasive edge of glioblastoma, whereas PAI-1 is more abundant in microvascular proliferation and pseudo-palisading cells than at the infiltrative edges. Cathepsin B and PAI-1 are also important biomarkers for the stratification of glioblastoma patients with respect to survival. Isocitrate dehydrogenase 1 (IDH1) mutations are a strong predictor of a more favorable prognosis and a highly selective molecular marker of secondary glioblastoma that complements clinical criteria for distinguishing them from primary glioblastoma (58). Caveolin-1 (cav-1) has been proposed as an immunohistochemical marker able to distinguish astroglial from oligodendroglial tumors (59). The reduction of cav-1 expression in glioblastoma cells increases their proliferative and invasive potential. Cav-1 is also expressed in high-grade glioblastoma. The glioblastoma-associated oncogene homologue 1 (GLI1) isoform has attracted much attention because of its frequent activation in many human cancers and its interactions with other signaling pathways, such as those mediated by K-Ras, TGF- β , EGFR, and protein kinase A

(60). N-Myc downstream-regulated gene-4 (NDRG4) is a largely unstudied biomarker of the tumor suppressive family, and is expressed exclusively in the heart and brain (61). NDRG4 expression is elevated in glioblastoma and is required for the viability of primary astrocytes, established glioblastoma cell lines, and both CD133 $\pm$ primary glioblastoma xenografts. While NDRG4 overexpression has no effect on cell viability, NDRG4 knockdown causes G1 cell cycle arrest followed by apoptosis. Elevated levels of the double-stranded DNA repair protein Rad51 have been predicted as an important biomarker in increased survival duration in patients with glioblastoma, at both initial tumor presentation and disease recurrence. It is also believed that the Rad51 levels correlate directly with patient survival (62).

The miRNAs are effective post-transcriptional regulators of gene expression and important in many biological processes (63). Although the oncogenic and tumor suppressive functions of several miRNAs have been characterized, the role of miRNAs in mediating glioblastoma tumor invasion and migration remains largely unexplored. The miR-10b has been identified as a miRNA highly expressed in malignant brain tissues, and may play a role in the invasion of glioblastoma cells. The miRNA profiles of glioblastoma stem and non-stem cell populations have shown an up-regulation of several miRs in the non-stem cells, including miR-451, miR-486, and miR-425, some of which may be involved in regulation of brain differentiation. Transfection of glioblastoma cells with the mature miR-451 showed dispersed neurospheres, and inhibited glioblastoma cell growth. Furthermore, transfection of miR-451 combined with Imatinib mesylate treatment had a cooperative effect in dispersal of glioblastoma neurospheres. Identification of additional miRs and target genes that regulate glioblastoma stem cells may provide new potential drugs for therapy. Tenascin-C is an extracellular matrix glycoprotein implicated in embryogenesis, wound healing, and tumor progression. Its upregulation facilitates glioblastoma invasion and reactive changes of the surrounding brain tissue (64). Although siRNA-mediated knockdown of endogenous tenascin-C does not affect proliferation of glioblastoma cells, it decreases cell migration and tumor invasion with brain tissue changes in a xenograft model. Glioblastoma cells with high tenascin-C expression may infiltrate brain tissue in an autocrine manner, which makes tenascin-C a prime target for devising anti-invasion therapy for glioblastoma.

IV. Ag-specific Immunotherapy for Glioblastoma

Immunotherapeutic strategies exploit the immune system's ability to recognize and mount a specific response against tumor cells, but not normal cells. Ag-specific immunotherapy can be applied to glioblastoma to sensitize the patient's immune cells to tumor Ags using various vaccination protocols. This section will discuss current and future strategies for Ag-specific immunotherapy for glioblastoma. Modern advances in cancer immunotherapy have led to the development of active immunotherapy that utilizes tumor-associated Ags (TAA) to induce a specific immune response against the tumor. Current methods of immunotherapy implementation are based on the principle that TAA are capable of being processed by Ag-presenting cells (APC) and inducing an activated CTL-specific immune response that targets the tumor cells. Ag processing by professional/nonprofessional APC and tumor cells results in the formation of human leukocyte antigen (HLA)-peptide complexes on their cell surfaces (65). These HLA-peptide complexes can be recognized by an Ag-specific CD8 $^{+}$ and/or

CD4⁺ T cells, inducing adaptive immune responses. Much effort has been directed towards enhancing APC activation and optimizing the processing of TAA and their association with HLA molecules (66). Preclinical studies in animal models have shown the feasibility of Ag-specific immunotherapy approaches through the utilization of TAA and induction of adaptive immune responses against many different types of tumors including brain tumors. Recently, several clinical studies using DC loaded Ags have also been initiated and have shown promising results in controlling glioblastoma (18,67). Although tumor cells express potentially immunogenic TAA, tumor vaccines often fail to clear malignant tumors because of inadequate Ag delivery and/or insufficient activation of innate immunity.

Glioblastomas are highly heterogeneous tumors and possess a considerable capacity for immune escape, with subsequent high risk of recurrence of new variants or Ag-negative tumors (68). Studies have defined a large number of Ags and protein biomarkers in malignant tissues (Table 2). Many of these TAA are shared by tumors in the skin, breast, prostate, kidney, colon, lung, and brain, but only few of them are tumor specific Ags. Cell therapies using in vitro expanded tumor-infiltrating T cells, which are sensitized to TAA in situ, have been adoptively transferred into the resection cavity of glioblastoma with promising results in some patients. TAA-specific responses have been detected in patients given repeated intratumoral inoculation of cytotoxic CD8⁺ T cells (19). Overall, the effectiveness of T cell-mediated immunotherapy for cancer depends on both an optimal immuno-stimulatory context of the therapy and the proper selection with respect to quality and quantity of the targeted tumor Ags, and, more precisely, the T cell epitopes contained in these tumor proteins.

Recent evidence suggests that combining a peptide-based therapeutic vaccination with conventional chemotherapy can uncover the full potential of the antitumor immune response. In addition, therapeutic vaccination in the preventive setting has been extremely effective in eliciting antitumor responses in preclinical tumor models and has demonstrated promise clinically in patients with minimal residual disease. While selective glioblastoma-specific Ags such as IL-13 receptor alpha 2 (IL-13R α 2) and adenosine diphosphate-ribosylation factor 4-like (ARF4L) protein have been identified (69,70), many TAAs found within glioblastoma are also expressed on other cancers such melanoma including: Mage-1, Aim-2, tyrosine's, related-related protein (Trp-1; also known as Trp-75, Trp-2), Gage, and human melanoma-associated Ag p97/GP100. Glioblastoma Ags are also found in breast, ovarian, and other malignancies. These include: HER-2/neu, B-cyclin, EphA2/Eck, Sart-1, and GnT-V, which may induce CTL as well as helper CD4⁺ T cell responses, reducing tumor burden (19). Many of these antigenic peptides are also restricted to the HLA-A2 allele: B-cyclin, EphA2, Gage-1, GP100, HER-2/neu, IL13R, Mage-1, Trp-1, and tyrosinase (19). Aim-2 is restricted to the HLA-A1 locus, whereas Sart-1 is restricted to HLA-A24 and A26 (19). An Ag profiling study using 20 human glioblastoma cell lines showed that 77%, 85%, and 78% of the U.S. Caucasian population expressed HLA-A, HLA-B, and HLA-C alleles, respectively. It appeared that all glioblastoma cell lines expressed multiple TAAs. This study found that most glioblastoma cells expressed Aim-2, B-cyclin, EphA2, GP100, β 1, 6-N-acetylglucosaminyl-transferase V (GnT-V), IL13R α 2, HER-2/neu, human telomerase reverse transcriptase (hTERT), Mage, Mart-1, Sart-1, and survivin (19). Interestingly, tumor-infiltrating CTL killed glioblastoma cells that were HLA-A2 positive

and expressed specific TAAs (Mart -1, GP100, HER-2/neu, and tyrosinase) but not HLA-A2-negative tumors and/or cells lacking specific TAA epitopes.

Effective immunotherapy will require Ag processing and tumor cell presentation of these self-Ags via HLA class I and HLA class II molecules (71). The formation of functional HLA-peptide complexes may facilitate Ag-specific T cell clonal expansion and immune recognition of glioblastoma. One therapeutic scenario would involve obtaining the tumor Ag expression profile of the patient with glioblastoma. Another possibility would involve HLA protein expression profile of the individual, potentially predicting the TAA on the patient's glioblastoma. With that knowledge, an allogeneic vaccine can be customized so that the relevant Ags are contained within the vaccine to stimulate the immune system in glioblastoma patients. Activated T cells have the added potential to mount secondary memory responses with increasing rapidity and intensity long after vaccine administration, which is crucial to eliminate microscopically disseminated glioblastoma tumors that evade surgery and cause recurrence. While most TAAs are important targets for devising Ag-specific immunotherapy, some promising TAAs are involved in cell cycle regulation, favoring malignant growth of the tumor. Two such important TAAs are telomerase and survivin (29,72). Telomerase contains telomerase reverse transcriptase (TERT) and an RNA template. Human TERT (hTERT), the catalytic subunit of telomerase, is a large protein and expressed in more than 85% of all malignancies with little or no expression in normal somatic cells. Expression of hTERT leads to telomere lengthening and cell immortalization and degradation of hTERT by proteasomes prompts telomere shortening, cancer cell senescence, and apoptosis. Recent studies have also shown promising results using hTERT as a tumor Ag. Vaccination with hTERT peptides or adoptive transfer of hTERT-specific cytotoxic T lymphocytes has been shown to induce tumor regression. In that study, helper CD4⁺ T cells were also activated by hTERT peptides. Telomerase and hTERT are remarkably upregulated in glioblastoma (72), suggesting that telomerase-based tumor vaccines could be developed for treating this cancer. By contrast, survivin is expressed in proliferating tissues and many types of tumors including brain tumors. It is a member of the inhibitor-of-apoptosis protein (IAP) family and known to regulate mitosis and to inhibit apoptosis. While expression of survivin, a prominent IAP, is increased in glioblastoma cells, the prognostic value of survivin in patients with glioblastoma is still controversial. Recent studies have shown that survivin epitopes can induce both CTL and CD4⁺ T cell responses in a number of tumor models in vitro and in vivo (73). Administration of survivin-based vaccines to experimental animals has been found to induce tumor regression in many other malignancies. Based on these factors, survivin is an attractive target for both chemotherapy and immunotherapy of glioblastoma.

Accumulating evidence suggests that a dynamic cross-talk between tumor cells and the immune system may regulate tumor progression and metastasis. Both the protective effects of the immune system against tumor cells and the escape of tumors from immune attack have led to the concept of cancer immunoediting, which implies that a bidirectional interaction between tumors and regulatory cells is ultimately responsible for orchestrating the immunosuppressive network at the tumor site. Immune escape is also regarded as a hallmark of cancer progression, with some of the more recently known strategies affecting differentiation and maturation of DC. It has been shown that the steady-state tumor

microenvironment does not contain strong stimuli for DC maturation, and it becomes dependent on CD4+ T cell help, ablation of which may fail to induce strong anti-tumor CTL responses (18). Therefore, it is not surprising that the metastatic tumor would evolve mechanisms to inhibit this safeguard against an induction of a strong CD8+ T cell response. The tumor microenvironment also contains tumor infiltrating lymphocytes (TIL) that may favor an immune response against the tumor. Recent studies have shown that a subset of the TIL consists of Treg cells as part of the tumor immune escape mechanism (74). Additional human studies have also reported that the Treg population increases in peripheral blood and tumor tissues from patients. However, the relationship between the Treg population and the prognosis of glioblastoma patients remains controversial. Multiple sources of Ags can be used to induce T cell response by DC, such as synthetic peptides, stripped peptides derived from tumor HLA class I molecules, tumor lysates, fused DC, and tumor cells (75). The ideal Ag should be widely and specifically expressed by the tumor cells and should have one or several epitopes that can bind to HLA molecules for presentation to T cells. With the advances in genomics and proteomics and the new algorithms that predict the HLA binding of peptides/epitopes, it is likely that investigators will identify more potential Ags/peptides for designing better immunotherapeutics against glioblastoma.

Given the highly infiltrative growth pattern of glioblastoma and the lack of specificity associated with currently available treatment regimens, alternative strategies designed to eradicate cancer cells while limiting collateral toxicity in normal tissues remain a priority. To this end, the development of Ag specific immunotherapy against glioblastoma cells represents a promising approach. The EGFRvIII molecules are expressed in a substantial proportion of malignant glioblastoma and other human cancers, yet are completely absent from normal tissues. Preclinical studies with a peptide vaccine directed against EGFRvIII antigenic domain showed potent cellular and humoral immunity against tumor cells, suggesting the potential role of peptide-based vaccines in devising immunotherapy against glioblastoma (76). Study using DC-based vaccine therapy in a GSC-bearing mouse model showed an efficient anti-tumor immune response against GSCs derived from glioblastoma cells (77). Vaccination with a plasmid DNA encoding murine SOX-6 induced cytotoxic CTL response specific for SOX-6-expressing glioblastoma cells, and showed protective and therapeutic antitumor responses in rodents. This study also showed that the HLA-A2-and HLA -A24-restricted SOX-6-derived CTL epitope peptides could be useful for Ag-specific immunotherapy in many glioblastoma patients.

Ag-specific cancer immunotherapy using DC-based vaccination has emerged as an attractive approach for the control of malignant diseases due to its simplicity and easy preparation. Typically, DCs reside as immature cells in almost every organ and tissue at the interface of potential pathogen entry sites. In this state, they continuously sample Ags. However, this sampling results in an effective Ag presentation only when DCs are also triggered by danger signals derived from pathogens, tissue damage, or signs of inflammation (78). As danger-triggered DCs start to mature and get activated, they upregulate chemokine receptors, which guide them to draining lymph nodes. There, the mature DCs are capable of inducing primary T cell responses due to their high levels of HLA, adhesion, and costimulatory molecule expression. Unlike other APCs, DCs are able to present and/or cross-present glioblastoma Ags via HLA class I and class II molecules. In this way, they can prime both CTL and

CD4⁺ T cells. In contrast, direct recognition of tumor cells by unprimed T cells induces T cell anergy rather than immunity as the costimulatory molecules are missing on the tumor cells (79). DCs have also been shown to be strong activators of NK cells and NKT cells, thus linking the innate and adaptive immune responses. In this way, both tumor cells with and without expression of HLA class I molecules can be killed. Studies have also developed DC vaccine approaches for treatment of glioblastoma using either crude tumor cell lysates or acid eluted peptides derived from glioblastoma cell cultures or from surgical specimens (80). In several patients, a CTL response was elicited, while in others, TILs were observed in the resection specimen obtained at relapse. Although some objective responses were observed with increased tumor-free survival, the current adjunctive approaches are not curative. Thus, movement toward testing several modalities involving combinations of biomarker specific chemotherapy coupled with specific TAA peptide-pulsed DC vaccines could be explored.

V. Biomarker-specific Chemotherapy for Glioblastoma

The identification and characterization of molecular markers in brain tumors are important for the design of biomarker specific chemotherapies for patients with glioblastoma. This section will discuss and summarize chemotherapeutic approaches against glioblastoma focusing on biomarkers and molecular targets that have produced encouraging results. Studies showed that MGMT methylation is a predictive biomarker for better response to radiotherapy, independent of temozolomide treatment, suggesting that it may be a general surrogate marker of better therapeutic response in glioblastoma (81). Investigational approaches to suppress MGMT activity include dose-intensive temozolomide regimens, which may deplete the enzyme, and combination therapy with O⁶-benzylguanine or other MGMT inhibitors (82,83). Thus far, MGMT inhibitors have had limited efficacy, at least in part because of dose-limiting myelosuppression when combined with cytotoxic chemotherapy. In addition to MGMT, the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) may promote chemotherapy resistance in patients with glioblastoma (84). PARP inhibitors such as BSI-201, ABT-888, and several others may be effective when combined with radiotherapy and cytotoxic chemotherapy. EGFR is one of the most attractive therapeutic targets in glioblastoma. The EGFR gene is amplified and overexpressed in approximately 40% of primary glioblastoma. Nearly half of tumors with EGFR amplification also have a constitutively active EGFR mutant, EGFRvIII, which has a large deletion in the extracellular domain that renders the receptor independent for signaling. This deletion also engenders a unique codon, normally absent in the wild-type receptor, thereby creating a tumor-specific epitope that can be targeted as a potential biomarker for devising novel therapeutics. Increased EGFR signaling drives tumor cell proliferation, invasiveness, motility, angiogenesis, and inhibition of apoptosis. Small-molecule EGFR inhibitors such as gefitinib and erlotinib are reported to be well tolerated in patients with glioblastoma (85). However, patient responses are infrequent and progression-free survival is not prolonged, and more investigation is needed.

Radiotherapy has been of key importance to the treatment of glioblastoma for decades and the use of temozolomide concurrently and after radiotherapy has clearly improved overall survival. However, residual tumors express new biomarkers and eventually become resistant to these therapies. The tumor's ability to repair radiation-induced injury accomplished by

aberrant or amplified growth and survival signaling pathways are being appreciated. As a standard alkylating agent, temozolomide induces DNA damage that is repaired by the DNA repair enzymes, including MGMT. It is believed that silencing of the MGMT gene promoter by methylation is associated with better tumor response to combination therapy with radiation and temozolomide. Recent clinical trials in patients with glioblastoma have evaluated irreversible EGFR inhibitors such as BIBW 2992 and PF-00299804, the dual EGFR and VEGFR inhibitor vandetanib (ZD6474), and the humanized monoclonal antibody against EGFR, nimotuzumab (86). A peptide vaccine (CDX-110) against the unique epitope of EGFRvIII has shown low toxicity profile and is being studied in combination with temozolomide in patients with newly diagnosed glioblastoma. Combinations of EGFR inhibitors with other targeted therapies, including inhibitors of mTOR and VEGFR, are also being evaluated. The PDGFR subtypes PDGF ligands are also overexpressed in glioblastoma tumors, especially in the 'proneural' subtype. This creates autocrine or paracrine loops that promote tumor cell proliferation. The PDGFR inhibitor Imatinib mesylate has been reported to have significant antitumor activity both in vitro and in orthotopic glioblastoma models (87). Unfortunately, the drug proved inactive in clinical trials, with low responses and no prolongation of progression free survival. Studies with more potent PDGFR inhibitors and agents with improved BBB penetration such as tandutinib (MLN518) are currently under investigation. In glioblastoma, PI3K/Akt/mTOR signaling is frequently increased because of receptor tyrosine kinase overactivity, mutated oncogenic PI3K subunits, and/or loss of PTEN tumor suppressor activity (88). Several mTOR inhibitors such as sirolimus (rapamycin), temsirolimus (CCI-779), everolimus (RAD001), and ridaforolimus (AP23573) have been tested with minimal activity against malignant glioblastoma. However, the use of Akt inhibitors, a dual PI3K/mTOR inhibitor, or other combination approaches could produce better results in the near future.

Hepatocyte growth factor (HGF) binds to the c-Met receptor, activating intracellular signaling cascades similar to those triggered by EGFR and PDGFR; c-Met signaling is thought to be associated with invasion. In addition to stimulating c-Met, HGF activates the EGF and VEGF pathways. Multiple c-Met inhibitors are currently under investigation. Activation of Ras requires localization to the intracellular surface of the cell membrane, a critical step that depends on farnesylation. Farnesyl transferase inhibitors (FTI) interfere with this process and have demonstrated promising activity in glioblastoma models (86). Unfortunately, the FTI tipifarnib (R115777) did not demonstrate clear evidence of efficacy in a phase 2 trial in patients with recurrent glioblastoma. Histone deacetylase (HDAC) inhibitors interfere with transcriptional regulation and can induce growth arrest, terminal differentiation, and apoptosis of tumor cells. The HDAC inhibitor vorinostat proved effective in preclinical models but only modestly prolonged the 6-month progression free survival in a phase 2 trial in patients with recurrent glioblastoma (86). Some other HDAC inhibitors (e.g., valproic acid and LBH589) targeting specific biomarkers are currently in clinical trials for treating glioblastoma.

Inhibition of angiogenesis using treatments directed toward new vessel growth in malignant glioblastoma has proven to be one of the most promising areas of targeted molecular therapy. Angiogenesis is driven primarily by tumor-secreted VEGF-A, but there are a large number of alternative secreted proangiogenic factors, including basic fibroblast growth

factor (bFGF), angiopoietins, PDGF, IL-8, and hepatocyte growth factor/scatter factor (HGF/SF) (89). Endothelial cells in the vicinity of the tumor express VEGFR2, which helps orchestrate a paracrine signaling loop for stimulating endothelial cell growth and proliferation. It has been shown that the level of VEGF production in a tumor increases with the degree of malignancy. The majority of the antiangiogenic drugs tested in clinical trials have been shown to interfere with the VEGF pathway by blocking their receptors. However, there is an increasing interest in targeting proangiogenic molecules that function by alternative mechanisms. In addition to VEGF inhibitors, small molecule inhibitors of VEGFR have been tested in recurrent glioblastoma. Cediranib (AZD2171) has been shown to inhibit all known subtypes of VEGFR and was evaluated in a phase 2 clinical trial in patients with recurrent glioblastoma (90). This study showed that the drug was largely well tolerated, with hypertension, diarrhea, and fatigue as the most common adverse effects. Treatment with cediranib reduced blood vessel size and permeability. These are the first clinical data to support the hypothesis that antiangiogenic therapy may transiently “normalize” the dilated, abnormally permeable tumor vasculature. The presumption that vascular normalization may improve chemotherapy delivery and reduce hypoxia provides a solid rationale for combining antiangiogenic therapies with chemotherapy and radiotherapy. Neuropilin-1 also facilitates HGF/SF signaling. The angiopoietins (Ang-1 and Ang-2) are involved in the stability and maintenance of the tumor vasculature (91). Binding of Ang-2 to its cognate receptor, Tie-2, may serve to destabilize vessels required for promoting angiogenesis. Ang-2 inhibitors are therefore of interest as therapeutic agents for treating glioblastoma. Notch receptors on tumor endothelial cells are also activated by transmembrane jagged and delta-like ligands on the surfaces of neighboring cells (92), suggesting that Notch inhibitors could prove effective. Inhibition of delta-like ligand 4 (Dll4) on endothelial cells in preclinical models promotes the growth of an abnormal neovasculature with reduced perfusion and tumor growth.

Brain angiogenesis inhibitor-1 (BAI1) is a brain-predominant seven-transmembrane protein that contains five antiangiogenic thrombospondin type-1 repeats (93). BAI1 is cleaved at a conserved proteolytic cleavage site releasing a soluble, 120 kDa antiangiogenic factor called vasculostatin (Vstat120). Vstat120 has been shown to inhibit in vitro angiogenesis and suppress subcutaneous tumor growth. This study found that the expression of Vstat120 markedly suppresses the intracranial growth of malignant glioblastomas, even in the presence of proangiogenic oncoprotein EGFRvIII (93). This tumor-suppressive effect was accompanied by a decrease in tumor vascular density, suggesting a potent antiangiogenic effect in the brain. A prominent feature of glioblastoma is its resistance to death receptor-mediated apoptosis. Study has shown that activation of JNK by anisomycin plus anti-Fas antibody (CH-II) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), synergistically induce cell death in human glioblastoma cell lines and tumor xenografts (94). Synergistic cell death was predominantly apoptotic involving both extrinsic and intrinsic pathways. Expression of Fas, FasL, FLIP, and Fas-associated death domain (FADD) was not changed following treatment with anisomycin+ CH -11. JNK was activated 10-to 22 -fold by anisomycin+CH-11 in U87 cells. Inhibiting JNK activation with pharmacologic inhibitors of JNKK and JNK or with dominant negative MAPK kinase 2 (MEKK2) significantly prevented cell death induced by the anisomycin+ CH -11 (95). Higher levels of

galectin-1 expression in a human orthotopic murine tumor model has been detected and revealed a consistent pattern of preferential expression in peripheral or leading tumor edges (96). Further examination of galectin-1 expression in tissues from brain tumor patients confirmed galectin-1 as a valuable biomarker and possible therapeutic target.

Tumors that progress during antiangiogenic therapy often cannot be treated successfully thereafter, and most patients die of the disease within a few months. In the cediranib study, serum levels of the proangiogenic factors bFGF, stromal-derived factor 1 alpha (SDF1 α), and soluble VEGFR2 increased at the time of failure (90). These alternative proangiogenic pathways may drive angiogenesis in the setting of VEGFR inhibition. Other preclinical and MRI data suggest that anti-VEGF therapy may promote an infiltrative tumor growth pattern with co-option of existing cerebral blood vessels. Combining antiangiogenic therapy with anti-invasion therapy may therefore delay disease progression. Studies combining cediranib (pan-VEGFR inhibitor) with cilengitide (integrin inhibitor), and bevacizumab (neutralizing VEGF antibody) with dasatinib (PDGFR β inhibitor) may produce a better outcome. Another potential mechanism of resistance to antiangiogenic therapy involves increased PDGF signaling. PDGF promotes stabilization of the neovasculature by recruiting pericytes and facilitating pericyte-endothelial cell interactions. Preclinical data suggest that dual VEGFR/PDGFR inhibition may potentiate anti-angiogenic efficacy and reduces resistance to therapy.

Drugs that inhibit single targets can also be combined to achieve multiple target inhibition. Particular interest has focused on the combination of EGFR inhibitors and mTOR inhibitors. One important obstacle to combination therapy with targeted molecular drugs is additive toxicity, which may limit the doses that patients can tolerate. For example, combinations of EGFR inhibitors with mTOR inhibitors have been associated with a high incidence of dermatologic toxicity and mucositis (86). Recent studies using molecular profiling, network analysis, and correlative studies in clinical trials have identified new targets that may drive glioblastoma growth and prevent tumor cell death. Among the promising therapeutic targets in early clinical development are c-Met, FGFR, HSP-90, HIF1 α , cyclin-dependent kinases, and many others (86). In addition to the uncertainty about which targets require inhibition, there is an ongoing controversy regarding which cell types are most important. Glioblastoma stem-like cells appear to initiate glioblastoma formation and maintain the tumor mass (97). Inhibition of unique stem cell targets such as Notch and Sonic hedgehog may be required to overcome resistance to therapy. Because multiple receptor tyrosine kinases are coactivated in glioblastoma cell lines and primary cultures, multiple kinase inhibition may be required to reduce signaling through the PI3K/Akt/mTOR pathway and decrease glioblastoma cell growth (98). A major factor that interferes with the efficacy of targeted molecular drugs in glioblastoma is insufficient penetration into the tumor tissue due to a partially intact BBB or an active drug efflux transporter. Due to the difficulty in obtaining tumor tissue in the brain tumor patient population, few clinical trials have successfully measured drug levels in tumor tissue. Our emerging knowledge of the molecular pathophysiology of malignant glioblastoma will improve therapeutic target selection in the future. Rigorous preclinical testing is needed to identify combinations of drugs and targets that are most likely to be effective and tolerable. Targets such as HSP-90 and HDAC are of particular interest as therapeutic targets because their function influences many other signaling molecules that may promote tumor cell growth and proliferation (99). Studies of resistance to

antiangiogenic therapy are needed to optimize the use of bevacizumab and other VEGF or VEGFR inhibitors. Clinical trials that incorporate tumor tissue and molecular endpoints will help us to understand why certain drugs succeed or fail in individual tumors. Although the initial results have been disappointing, targeted molecular agents hold tremendous promise.

Most of the targeted agents are small-molecule tyrosine kinase inhibitors or monoclonal antibodies. Overall survival in multiple recent single-arm studies combining targeted agents with EBRT and temozolomide for first-line treatment has been modestly superior relative to historical controls (100). Thus, combination approaches can be applied towards multiple targets, shutting down proximal and distal targets within the same pathway or shutting down targets in separate, parallel pathways. Epigenetic alterations also affect the expression of cancer genes alone, or in combination with genetic mechanisms. A subset of glioblastoma is also characterized by locus-specific and genome-wide decrease in DNA methylation, or DNA hypomethylation (101). Other epigenetic alterations, such as changes in the position of histone variants and changes in histone modifications are also likely important in the pathogenesis of glioblastoma. Surprisingly, there are very limited data about these epigenetic changes in glioblastoma. Alterations in histone modifications are especially important to understand, given that histone deacetylases are targets for drugs that are in clinical trial for glioblastoma. The technological wave of next-generation sequencing may accelerate glioblastoma epigenome profiling, allowing the direct integration of DNA methylation, histone modification, and gene expression profiles. Recent genetic profiling has identified the aminopeptidase N/CD13 inhibitor, actinonin as a secreted MMP inhibitor (102). Given that actinonin's effects against membrane-bound MMPs remain unknown and that MT1-MMP has been linked to chemotherapy and radiotherapy resistance in brain tumor development, study assessed MT1-MMP functional inhibition by actinonin in U87 glioblastoma cells. This study showed that actinonin inhibits concanavalin-A (ConA)-induced proMMP-2 activation, while it did not inhibit ConA-induced MT1-MMP gene expression, suggesting that post-transcriptional effects of the drug possibly mediated through the membrane-anchored protease regulator RECK. Specific gene silencing of MT1-MMP with siRNA also abrogated the ability of ConA to activate proMMP-2 (102). Functional recombinant MT1-MMP whose constitutive expression led to proMMP-2 activation was also efficiently antagonized by actinonin, indicating actinonin's therapeutic role in reducing brain tumor-associated angiogenesis.

Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors (29,73). In several animal model systems, downregulation of survivin or inactivation of its function has been shown to inhibit tumor growth. Strategies under investigation to target survivin include antisense oligonucleotides, siRNA, ribozymes, immunotherapy, and small molecular weight molecules. The translation of these findings to the clinic is currently ongoing with a number of Phase I/II clinical trials targeting survivin in progress. Overexpression of Gli1 correlates with glioblastoma recurrence after chemotherapy (60). Studies have shown that the HH pathway activity can promote clonogenic survival of glioblastoma cell lines in chemotherapy. Blocking the HH pathway has shown enhanced cytotoxicity of chemotherapeutic agents in glioblastoma cells, through down-regulating the expressions of MDR1, MRP1, MVP, MGMT, Bcl-2, and survivin genes (103). Thus, suppression of Gli1 expression might be an important therapeutic option

for overcoming MDR and for increasing the success of chemotherapy. Studies also suggest a significant protein nitration in human glioblastoma where nitric oxide upregulation may relate to neoplastic transformation, tumor neovascularization, induction of apoptosis, or free radical damage. Paclitaxel resistance in glioblastoma has been linked to the expression of members of the anti-apoptotic Bcl-2 family through hypoxia induced phosphorylation of Bad, thus protecting hypoxic cells from paclitaxel-induced apoptosis (104).

Microtubules are highly dynamic cytoskeletal components that are essential for many cellular functions in eukaryotes such as intracellular organization, ordered vesicle transport, and cell division (105). Glioblastoma tumors exhibit significant changes in their microtubule cytoskeleton, including aberrant expression of the class III β tubulin isotype and γ -tubulin, which are associated with the emergence of highly malignant and chemorefractory forms of brain tumors (106). It is believed that β III-tubulin expression in neuronal tumors is constitutive and differentiation-dependent, while in non-neuronal tumors it is either aberrant and/or represents dedifferentiation associated with anaplastic transformation and acquisition of progenitor-or stem cell-like phenotypic properties. The cellular distribution of β III-tubulin is significantly increased in high-grade glioblastoma (31). The epothilone compound, patupilone, has been shown promising in targeting β III-tubulin as this drug can cross the BBB. Microtubule-targeting anticancer drugs are widely used in the clinic, which often induce peripheral neuropathy, a main dose-limiting side effect. Thus, microtubules and tubulin appear as emerging biomarkers in potential therapy of glioblastoma using a new class of β III-tubulin-targeted drugs.

VI. Cytokine Based Immunotherapy for Glioblastoma

Current evidence suggests that the alteration of the immune system can contribute to the etiology of many types of cancers including glioblastoma. Although previous studies have focused on variations in candidate genes in the adaptive immune system, cytokine therapy has emerged as a critical avenue for targeting glioblastoma cells. Cytokines play a role in cell-cell communication, cellular activation, angiogenesis, immune activation, immune escape mechanisms, and a variety of other cell functions (107). The various cytokines affecting the CNS may have two major sources such as peripheral immune organs that cross the BBB, and neural cells within the CNS. Preclinical studies have shown that IL-2, IL-7, IL-15, and IL-21 can promote anti-tumor immune responses in specific settings (108). T cell-associated cytokines are usually known as pro-inflammatory (e.g., Th1, Th17) and anti-inflammatory (e.g., Th2) depending on their functions on the immune system. Th1-type pro-inflammatory cytokines include IFN- γ , IL-2, IL-12, IL-15, IL-17, IL-23, lymphotoxin (LT), and TNF- α (109). Since these cytokines influence cell-mediated immune responses, they may show anti-tumor effects in malignancies where immunosuppression is pronounced. By contrast, Th2-type cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 are shown to be elevated in many different malignancies, down regulating tumor specific immunity (110). Th2 and Th3 cytokines and their receptors are highly expressed in glioblastoma cell lines and patients with glioblastoma (111). Tumor cells may downregulate exogenous cytokines as a result of upregulation of endogenous cytokine production, and this may alter the expression of membrane receptors as well as other secondary messenger systems. Aberrations of normal cytokine-mediated cell proliferation have been observed in malignant

cells and the alterations of the expression of cytokines and their receptors are most apparent in glioblastoma tumors. Cytokines may also expand the T cell repertoire. A diverse T cell-mediated immune response directed against a broad range of tumor Ags would be preferable in minimizing the likelihood of immune escape through alterations in tumor Ags and their expression.

Many studies have contributed to our basic understanding of the physiological role and functional mechanisms of cytokines in various cancer situations. It has been reported that patients harboring glioblastoma appear to express Th2 as well as Th3 -type cytokines, which may down regulate anti-tumor immune responses (111). However, the precise origin of secretion of Th2-type cytokines in tumor microenvironment in glioblastoma patients remains controversial. There is also disagreement in the literature as to whether glioblastoma cells produce Th1-type cytokines and their receptors. In the brain, neuroinflammatory cytokines affect the growth and differentiation of both normal and malignant glial cells, with IL-1 shown to be secreted by the majority of glioblastoma cells (112). Recently, elevated levels of sphingosine kinase 1 (SphK1), but not SphK2, were correlated with a shorter survival prognosis for patients with glioblastoma multiforme. SphK1 is a lipid kinase that produces the pro-growth, anti-apoptotic sphingosine 1-phosphate, which can induce invasion of glioblastoma cells (113). This study has shown that the expression of IL-1 correlates with the expression of sphingosine kinase 1 (SphK1) in glioblastoma cells and that neutralizing anti-IL-1 antibodies inhibit both the growth and invasion of this brain tumor (113). IL-1 also upregulates SphK1 mRNA and protein levels, and activity in various glioblastoma cell lines. One of the first cytokines studied in the setting of glioblastoma was IL-2, an important T cell growth factor involved in the proliferation of CD8+ T cells and modulation of their cytotoxic activity. Various early in vitro studies supported a rationale for the use of IL-2 in patients with malignant brain tumors. This rationale was based largely on the ability of IL-2 to block the T cell depressing capacities of TGF- β , thought to be a key glioblastoma-derived immunosuppressive cytokine and growth factor. However, clinical trials utilizing IL-2 or a combination with non-specific LAK therapy did not produce satisfactory results (114). Clinical trials have also focused on the use of IFN alone or a combination with radiation therapy or chemotherapy. One study reported that up to 50% of treated patients had stable disease or tumor regression, but subsequent trials did not yield encouraging results. It is believed that many of the initial IFN trials were not well designed, and lack of uniformity regarding patient selection, imaging analysis, and tumor grading made it difficult to objectively assess whether the responses reported could truly be attributed to cytokine therapy. These trials also revealed the limitations of recombinant cytokine therapy related to issues of toxicity and short half-life. To overcome this issue, attempts were taken to manipulate cells by genetic engineering, which can secrete relevant cytokines. These cells were then transplanted into intracranial tumors, achieving a steady level and long-lasting localized production of cytokines, thereby circumventing the need to administer recombinant protein. Studies using such a therapy involving engineered glioblastoma cells secreting IL-2, IL-12, or IL-4 showed some success in rodent glioblastoma model. Retrovirally transduced glioblastoma cells producing macrophage colony-stimulating factor (M-CSF) have also been reported (115). These therapies have shown encouraging preclinical results with clear evidence confirming the development of potent antitumor

immunity. In addition, cell-based cytokine delivery paradigms using neural stem cell (NSC) have been reported (116). NSCs are capable of exhibiting potent intracranial migratory activity and are tropic for migrating glioblastoma microsatellites as they disseminate through the brain. Cytokine-secreting NSCs are shown to be capable of tracking and delivering antitumor payloads directly to these neoplastic reservoirs, suggesting that this targeted delivery may address the highly invasive and disseminated nature of intracranial glioblastomas.

Exogenous cytokines have potential adjuvant effects and, in some cases, cytokines are integral to the immunotherapy. The pro-inflammatory cytokine, TNF- α , elevates reactive oxygen species (ROS) in glioblastoma cells. TNF- α as well as ROS can play a role in regulating cytoskeletal organization and cell survival pathways (117). Treatment with TNF- α elevated Akt phosphorylation in glioblastoma cells, and Akt inhibition reversed TNF- α -mediated changes in actin cytoskeletal organization and also abrogated anchorage independent growth. Human IL-13 is a cytokine secreted by activated T cells that elicits both proinflammatory and anti-inflammatory immune responses (118). IL-13 has two types of receptors: IL-13/4R, which is present in normal cells and whose binding is shared with IL-4, and IL-13R α 2, which does not bind IL-4. IL-13R α 2 is associated with glioblastoma and is not significantly expressed in normal tissue. IL-4 and TNF- α treatments have shown variable ability to upregulate IL-13R α 2 in glioblastoma cells. Cytokine-induced IL-13R α 2 immunoreactivity localizes primarily to cell membranes, i.e. the receptor is produced and placed into the plasma membrane compartment of glioblastoma cells. An increase in IL-13R α 2 in response to EGF is mediated through the activation of the EGFR signaling pathways. EGF was found to be the most potent among the studied cytokines in up-regulating IL-13R α 2 in glioblastoma cells. Glioblastoma cells pretreated with EGF became more susceptible to the killing by an IL-13 mutant-based recombinant anti-cancer cytotoxin (119).

Cytokine based therapy and vaccination have been tested in glioblastoma patients with modest, albeit encouraging results. In the absence of a selective pressure imposed by the immune system, CNS tumors might express distinct Ag profiles that elicit immune responses by interacting with immune cells. These antitumor immune responses can be obtained in two ways, either presenting their self-Ags to the immune cells or recruiting immune cells to the tumor microenvironment. Cytokines could play a role in this process of differential expression of HLA molecules on brain APC as well as on tumors (107,120), thus influencing self-Ag presentation. Studies have proposed a number of immunotherapeutic strategies to enhance immune-augmenting cytokines (121). The generation of molecularly defined cancer vaccines requires the identification of TAA capable of eliciting an immune response with the secretion of immune stimulatory cytokines, some of which have been identified in human glioblastoma. A series of responses may also occur after cytokine activation, although their optimal function depends on several other complementary cytokines, growth factors or immune components. For example, IL-12 can promote IFN- γ release by IL-12R-expressing T and NK cells, and this may induce Th1 polarization, enhancing CTL responses in patients. IL-21 also stimulates the cytolytic functions of NK cells and induces their differentiation (122). In a murine model, it has been shown that IL-21 may promote CTL activity, and IFN- γ production, suggesting that IL-21 may represent an

optimal adjuvant for the induction of protective CTL responses and could be used in combination with other treatments. However, CTL may not be strictly required for this tumor rejection, since rejection of IL-21-transduced glioblastoma cells has also been reported in CTL depleted animals. Surprisingly, IL-21 has little activity in vitro on immune cells, yet is important in enhancing the function of T cells and NK cells in vivo. These effects are due to the ability of IL-21 to synergize with other cytokines and promote their activity (123). Recent study suggests that IL-21 may favor the differentiation of T helper cells into cells that secrete IL-17 (T_H17), and antagonize the differentiation of Treg cells. These T_H17 cells have been shown to be important in the killing of tumor cells. IL-7 has an essential role in T cell homeostasis and also plays a role in the development of NK cells, DC, and T cells. IL-2 is required for the development and secondary expansion of memory T cells. It is also critical in the development and homeostasis of Treg cells, potentially expanding activated T cells. IL-2 can enhance the activity of NK cells and T cells, and this effect can be promoted while abrogating the ability of IL-2 to expand Treg cells. IL-15 regulates the homeostasis of NK cells, NKT cells, CD8 memory T cells, and a sub-population of T cells (124). Although IL-15 is not required for the formation of memory T cells, it is dispensable for primary immune responses and maintenance of CTL by providing survival signals. IL-15 administration may cause an expansion in CTL and NK cells. In this environment, exogenous cytokines may promote effective antitumor responses.

Several possible immunotherapeutic approaches can be envisioned for treating glioblastoma patients, prolonging survival without an induction of significant autoimmunity. Expansion of tumor-specific CTL has been observed in glioblastoma patients treated with an autologous DC vaccine in combination with cytokines (125). However, many patients receiving the vaccine eventually die due to tumor progression. Exogenous cytokines can be administered transiently to antagonize immune inhibitory networks and enhance antitumor immunity. In this scenario, IL-7 and IL-15 could cause effector T cells to become refractory to the inhibitory effects of Treg cells. In order for immunotherapy to successfully treat glioblastoma, the cell types responsible for tumor progression must be targeted and eliminated by a combination of immune cells, cytokines, and other conventional treatment.

VII. Chemoimmunotherapy for Glioblastoma

The prognosis for patients with glioblastoma remains poor despite advances in surgical technique, radiation therapy, chemotherapy, and immunotherapy. This section will discuss a combination strategy using chemotherapeutics and immunotherapeutics for the treatment of glioblastoma patients. Studies suggest that the majority of glioblastomas are infiltrated by immune-effector cells, which consist primarily of CTL and macrophages. Glioblastoma-specific CTL responses can also be induced in patients using cytokines and cell-based immunotherapy (80). However, such CTL responses are often inadequate and fail to eradicate tumor growth in vivo. Systemic immunotherapy using peptide pulsed dendritic cells are capable of inducing significant anti-glioblastoma responses with negligible side effects(76). Although peptide-based immunotherapeutics appear promising, a combination of chemotherapy and immunotherapy may be introduced to successfully destroy brain tumors and prolong progression free survival of glioblastoma patients.

One of the most efficient methods developed to increase the immunogenicity of tumor cells is to force them to present their own Ags to the immune system. Unfortunately, most human tumors including glioblastoma do not express measurable HLA class II, thus, the induction of anti-tumor immune responses are mainly focused on the stimulation of CTL. Studies suggest that IFN- γ can regulate the activation, growth, and differentiation of T cells, B cells, macrophages, NK cells, endothelial cells, and fibroblasts (126). IFN- γ can also induce HLA class II molecules in a variety of tumors including glioblastoma. Depending on the dose, IFN- γ can also induce apoptotic cell death in glioblastoma as well as other tumors (127). We have recently reported that a combination of the differentiating agent all-*trans* retinoic acid (ATRA) and IFN- γ can act synergistically to induce differentiation and apoptosis, blocking the proliferation of human glioblastoma cells (128). Treatment of tumors with ATRA has been shown to exhibit increased sensitivity to HLA class I-restricted killing by CTL and NK cell-mediated lysis. ATRA treatment has been beneficial in many cancer types when administered alone or in combination with other therapies (129). ATRA may induce the expression of proteolytic and regulatory subunits of the immunoproteasome, increase the half-life of HLA class I complexes, and enhance the sensitivity of tumor cells to both HLA class I-restricted peptide-specific and HLA non-restricted lysis by CTL, NK, and NKT cells (71). ATRA may also induce systemic modulation of Ag presentation by tumor cells. In addition, ATRA treatment has been shown to modify the immunogenicity of tumor cells both in vitro and in vivo through differential regulation of HLA class I and intercellular adhesion molecule-1 (ICAM-1) molecules (130). The upregulation of ICAM-1 molecules may increase the sensitivity of glioblastoma to killing by NK cells.

Malignant tumors produce a number of cytokines (e.g., IL-4, IL-10, and TGF- β) that inhibit antitumor immune responses so as to maintain and promote tumor growth (131). Treatment of tumors with IFN- γ could reverse this tumor escape mechanism by inducing activation and differentiation of professional and by stander APC (132). The generation of anti-tumor effector cells may be accomplished by inducing apoptosis in tumor cells and subsequent immune stimulation, through the use of an agent like IFN- γ . Most of the chemotherapeutic agents are used to induce cell death and apoptosis in tumor cells. Apoptotic tumor cells could be processed by professional APC to utilize TAA for activating T cells specific for Ags expressed by neoplastic cells. This event occurs after the Ag transfer from apoptotic cells to professional APCs and is referred to as cross-presentation. Killing of glioblastoma cells may also facilitate the transfer of self-Ags from dying tumors to professional APCs or bystander cells in vivo, exploiting the cross-presentation pathway. At the moment of glioblastoma diagnosis, it is likely that there is already significant tumor burden in patients. The immune system might have failed to recognize the malignant tumor or become tolerant to the tumor. A combination of glioblastoma biomarker specific chemotherapeutics and Ag-specific immunotherapeutics together with cytokine therapy may eliminate the malignant growth of brain tumors.

VIII. Tumor-associated Molecules Influencing Glioblastoma Immunotherapy

Immune escape in cancer is increasingly recognized as a contributing factor in the failure of a natural host antitumor immune response as well as in the failure of cancer immunotherapy. Immune escape may be the result of a number of factors, including expansion of regulatory

T cells, production of immunosuppressive cytokines, downregulation of HLA and tumor-associated Ags, and upregulation of immunosuppressive molecules in tumor cells (Table 3). The genetic instability of tumors and their repeated exposure to immune selective pressures increase the potential for selection of tumor cell variants with an enhanced capacity to escape immune attack. Tumors also employ several defensive strategies that suppress and inhibit anti-tumor immune responses (Table 3). Glioblastomas are also a rich source of immunosuppressive molecules that interfere with immune recognition, rejection, and with clinical strategies of active immunotherapy. A better understanding of the mechanisms involved in eliciting anti-tumor immune responses may offer suitable targets for designing immunotherapy against glioblastoma.

1. Tumor-derived molecules contributing to immune escape of glioblastoma

1.1. Transforming growth factor-beta (TGF- β)—TGF- β has emerged as an attractive target for the therapeutic intervention of glioblastoma (40). Aberrant TGF- β overproduction in glioblastoma has been reported, which may cause potential differences in responses to anti-TGF- β therapies in distinct subgroups of glioblastoma patients. TGF- β can enhance tumor growth, invasion, angiogenesis, and immunosuppression. Downregulation of TGF- β may inhibit cytokine-induced signaling pathways and transcriptional responses in transiently transfected human glioblastoma cells. Tumorigenicity of glioblastoma cells could also be reduced by RNAi-mediated TGF- β gene silencing. TGF- β can also inhibit anti-tumor immune responses by blocking maturation and function of professional APC and the synthesis of cytotoxic molecules such as perforin, granzymes, IFN- γ , and FasL in activated CTL (133). TGF- β also plays a role in tumor tolerance by recruiting Treg cells towards the primary tumor site as a means of immune evasion (134). High biological activity of the TGF- β -Smad pathway may contribute to the malignant phenotype of glioblastoma and confers poor prognosis to the patients. Glioblastoma-initiating cells (GICs) are shown responsible for the initiation and recurrence of tumors (135). TGF- β induces the self-renewal capacity of GICs, but not of normal human neuroprogenitors, through the Smad and subsequent activation of the JAK-STAT pathways. Study also suggests that TGF- β downregulates NKG2D in the sera of glioblastoma patients, an important receptor involved in specific killing of transformed cells by CTLs, suggesting that blocking TGF- β may have further therapeutic benefit (136).

1.2. Prostaglandin E2 (PGE2)—PGE2 plays multiple roles both in the physiology and the pathophysiology of the human brain. An increased expression of PGE-synthase has been shown in a subset of human glioblastoma tumors, which catalyses the isomerization of PGH2 into PGE2 downstream of COX-2 (137). COX-2-derived PGE2 may promote tumor cell invasion, motility, and angiogenesis. PGE2 also induces immunosuppression by down regulating the production of Th1 cytokines and upregulating Th2 cytokines in the host (137). PGE2 may also inhibit the anti-tumor activity of NK cells. The sensitivity of primary cultures of glioblastoma to apoptosis was shown to be augmented by the overexpression of PGES, whereas the knockdown of its expression by shRNA decreased the apoptotic threshold in vitro and stimulated tumor growth in vivo (138). In that study, the intracellular injection of PGE2 induced a dose-dependent apoptosis in glioblastoma cultures, which was dependent on the presence of Bax, a pro-apoptotic protein. Study found a dose-and time -

dependent increase in COX-2 gene expression with elevated prostaglandin PGE₂ production in glioblastoma cells (138). The migration and invasion of glioblastoma cells was also significantly blocked by COX-2-specific inhibitor via the reduction of PGE₂.

1.3. Indoleamine 2,3-dioxygenase (IDO)—While immunotherapy has shown some promise in glioblastoma, tumor cells use different mechanisms to escape the immune responses induced by the treatment. Glioblastomas express or secrete several immunosuppressive molecules that regulate immune cell functions, including IDO (139). IDO is required for degradation of the essential amino acid tryptophan and IDO expression may create a local tryptophan shortage that starves T cells, inducing tumor tolerance (140). In immunocompetent syngeneic animals, local tryptophan degradation by IDO-expressing tumors may provide a mechanism of immune resistance. Study has shown that the pharmacological inhibition of IDO expression in malignant tumors results in tumor regression (141). It has also been shown that IDO-positive tumor-bearing mice display a reduced number of TAA specific CTL, which are required for killing of tumors (140). Because of the immunosuppressive role of IDO molecules expressed by GBM cells, blocking these molecules through immunotherapy strategies could help the efficiency of these treatments in glioblastoma patients.

1.4. Programmed death-1 (PD-1)—PD-1 is a member of the B7/CD28 family of co-stimulatory receptors. Blocking PD-1 can increase anti-tumor T cell immunity. In humans, abrogation of PD-1 can increase the number of functional cytokine-secreting CTL, presumably through increased proliferation (142). While Ag-specific T cell responses can be raised against many tumors, tumor-specific immune responses are not sufficient to eradicate tumor cells in the host. The activation and proliferation of T cells are greatly influenced by both positive and negative costimulatory receptors. PD-L1 has been described to exert costimulatory and immune regulatory functions (143). PD-1 is a receptor for PD-L1, and contains an immune receptor tyrosine-based inhibitory motif. Coligation of PD-1 and the T cell receptor leads to rapid phosphorylation of protein tyrosine phosphatase-2, attenuating T cell receptor signaling (143). Glioblastoma cells are reported to express high levels of PD-L1 in vitro and in vivo, and PD-L1 expressed on glioblastoma cells reduces their immunogenicity in vitro (143). Thus, PD-L1 is a novel mediator that may contribute to the immune inhibitory characteristics of human glioblastoma. Glioblastoma-associated PD-L1 expression was also reported as a strong inhibitor of antitumor immune responses as determined under alloreactive coculture conditions in vitro. In the presence of a neutralizing antibody to PD-L1, the levels of IFN- γ , and IL-2 produced by T cells were markedly enhanced.

1.5. Interleukin-10 (IL-10)—IL-10 expression in glioblastoma cell cultures is thought to be an ‘immunosuppressive status’ that is related to their origin and the escape of glioblastoma cells from immune surveillance and could account for the failure of immunotherapy in such tumors. IL-10 inhibits IL-2-induced T cell proliferation and the DC and macrophage activation of T cells (144). It also down regulates HLA class II protein expression on APC in the tumor microenvironment. IL-10 may also act to promote cell growth, resistance to chemotherapy, and angiogenesis (145). Study suggests that activation

of Ag-specific T cells in the presence of IL-10 drives the differentiation of these cells toward a different subpopulation that are capable of blocking T cell proliferation, altering the generation of a productive cellular immune response. In another study, IL-10 secretion from PBMCs and tumor cells of glioblastoma patients was shown to be higher as compared to healthy controls. IL-10 also contributes to the progression of brain tumors by suppressing the patient's immune response.

1.6. Galectin-1 (Gal-1)—Gal-1 is a family of mammalian beta-galactoside-binding proteins characterized by a shared characteristic amino acid sequence (146). They are expressed differentially in normal vs. neoplastic tissues and are known to play important roles in several biological processes such as cell proliferation, death, and migration (147). The involvement of Gal-1 in different steps of glioblastoma progression such as migration, angiogenesis, or chemoresistance makes them potentially good targets for the development of new drugs to combat these malignant tumors. Gal-1 is highly expressed in motile cells, and endogenous expression of Gal-1 in human glioblastoma cell lines was shown to correlate with their migratory abilities and invasiveness (147). Studies with diffuse glioblastomas demonstrated higher Gal-1 expression levels than pilocytic astrocytoma. Reducing Gal-1 expression in tumor cells by siRNA increases the anti-tumor effects of various chemotherapeutic agents, particularly temozolomide treatment. The decrease in Gal-1 expression also impairs the expression levels of other genes implicated in chemoresistance, some of which are located in the endoplasmic reticulum (ER) and whose expression is also known to be modified by hypoxia. This novel facet of Gal-1 involvement in glioblastoma biology may be amenable to therapeutic manipulation.

1.7. Growth and differentiation factor (GDF)-15—GDF-15 is a secreted protein of the TGF- β superfamily and has emerged as a candidate marker exhibiting increasing mRNA expression during malignant progression of glioblastoma (148). While GDF-15 is involved in the maintenance of pregnancy, it has been linked to other physiological and pathological conditions. Study has shown that GDF-15 is expressed by glioblastoma tumors and the expression levels in tumor tissue translate into elevated GDF-15 serum levels in glioblastoma patients. GDF-15 mRNA and protein have also been detected in human and mouse glioblastoma cells in vitro (148). Silencing of GDF-15 by RNA interference reduces the proliferation of glioblastoma cells, and enhances the susceptibility of killing by NK cells. This results in a reduced in vivo tumorigenicity and increased T cell infiltration of GDF-15-deficient glioblastoma cells in syngeneic mice. The elevation of endogenous GDF-15 may contribute to proliferation and immune escape of glioblastomas in an immunocompetent host.

1.8. Extracellular matrix (ECM) proteins—Recent studies have demonstrated that a molecular subtype of glioblastoma is characterized by overexpression of extracellular matrix (ECM)/mesenchymal components and shorter survival (149). Specifically, gene expression profiling studies revealed that matrix gla protein (MGP), whose function has traditionally been linked to inhibition of calcification of arteries and cartilages, is overexpressed in glioblastoma and associated with worse outcome. Study suggests that upregulation of MGP as well as other ECM-related components contribute to unfavorable prognosis via increased

migration (149). The rapid progression of glioblastoma is due in part to diffuse infiltration of single tumor cells into the brain parenchyma, which is thought to involve aberrant interactions between tumor cells and the ECM. Cell proliferation is also strongly regulated by ECM rigidity with cells dividing much more rapidly on rigid than compliant ECM (149). This suggests that ECM rigidity provides a transformative, microenvironmental cue that acts through actomyosin contractility to regulate the invasive properties of glioblastoma tumor cells. In primary brain tumors, glioblastoma cells invade the ECM and proliferate rapidly in the cerebral tissue, which is mainly composed of hyaluronan along with the elastin present in the basement membrane of blood vessels. The poor prognosis of glioblastoma patients is also related to diffuse brain invasion and interaction of tumor cells with ECM.

1.9. Intercellular adhesion molecule-1 (ICAM-1)—Cell-cell adhesion mediated by ICAM-1 and VCAM-1 is critical for T cell activation and leukocyte recruitment to the inflammation site (150). Interactions between ICAM-1 and LFA-1 facilitate T cell recognition of TAA presented by HLA class I molecules. Disruption of interactions between ICAM-1 and LFA-1 inhibits target cell lysis, favoring immune escape of tumors (150). ICAM-1 expression influences TAA presentation by HLA class II molecules and is required for tumor rejection in vivo (150). Soluble forms of ICAM (sICAM) are also increased in serum of many inflammatory diseases and tumors. Expression of sICAM-1 was elevated in patients with glioblastoma when compared with other tumors.

2. Accessory cells in the tumor microenvironment contributing to immune escape of glioblastoma

2.1. Myeloid-derived suppressor cells (MDSC)—MDSC have emerged as key immune modulators in various tumor models and human malignancies including glioblastoma (151). Study has shown that glioblastoma patients are immunosuppressed, and MDSC have been implicated in tumor burden. Exposure of glioblastoma tumors to MDSC-like cells such as different lineage of monocytes and macrophages may contribute to immunosuppression in glioblastoma patients. An increased immunosuppressive IL-10, TGF- β , and B7-H1 expression, decreased phagocytic ability, and increased ability to induce apoptosis in activated lymphocytes have been observed in these patients (97). Direct contact between monocytes and glioblastoma cells may also induce immunosuppressive effects. In glioblastoma patients, it has been shown that an increased circulating MDSC are detected as compared with normal donors. These MDSC are also increased in glioblastoma-conditioned monocytes in vitro. A number of studies have investigated this phenomenon without mechanistic insights into their origin and function in glioblastoma patients.

2.2. Regulatory T (Treg) cells—Studies have revealed that CD4⁺Foxp3⁺ CD25⁺ Tregs are physiologically engaged in the maintenance of immunological self-tolerance and play critical roles for the control of antitumor immune responses (152). A large number of Tregs have been found to infiltrate into brain tumors, and systemic removal of these Tregs enhances antitumor T cell responses. Tregs are recruited to tumor tissues via chemokines, such as CCL22 binding to CCR4 (152). They appear to expand and become activated in tumor tissues and in the draining lymph nodes by recognizing TAA as well as normal self-Ag expressed by tumor cells. These results indicate that cancer vaccines targeting tumor-

associated self-Ags may potentially expand/activate Tregs and hamper effective anti-tumor immune responses, and that tumor immunity can therefore be enhanced by depleting Tregs. Thus, Ag-specific Treg levels could be a potential therapeutic biomarker in many tumors including glioblastoma. Recent attempts have demonstrated that combinations of monoclonal antibodies capable of modulating Treg functions synergistically enhance anti-tumor activity and are more effective than single monoclonal antibody therapy (153). Combination therapy targeting a variety of molecules expressed in APC, effector T cells, and Tregs is envisaged to be a promising anti-cancer immunotherapy.

3. Non-classical and classical HLA molecules influencing immune recognition

Glioblastoma cells express or secrete several immunosuppressive molecules that may regulate immune cell functions. The non-classical HLA molecules such as HLA-G and HLA-E have been detected in brain tumor cells and they have a role in the induction of antitumoral immune responses (154). Recent evidence also suggests that the ectopic expression of HLA-G proteins may help tumor evasion of T and NK-mediated cell lysis (155). HLA-E is also a ligand for the immune-inhibitory NKG2A receptor expressed on NK and T cells. The expression of HLA-E was significantly higher in astrocytic tumors and glioblastoma than in normal brain (154). HLA-G and HLA-E molecules are also shown to be expressed by tumor infiltrating activated microglia and macrophages in a majority of glioblastoma patients. Many cancer cells down regulate classical HLA class I, HLA class I-related (CD1), and HLA class II molecules, which may help tumors evade immune surveillance (156). Tumor cells displaying aberrant expression of HLA molecules may also evade T cell recognition, facilitating disease progression.

IX. Conclusions

Glioblastoma is an aggressive and lethal cancer, accounting for the majority of primary brain tumors in adults. Glioblastoma tumors are characterized by alterations in genes that control cell growth, apoptosis, angiogenesis, and invasion. Multiple cytogenetic, chromosomal, and genetic alterations have been identified in glioblastomas to date, with distinct genetic patterns being associated with individual brain tumor subtypes. Some of these molecular alterations may serve as a diagnostic adjunct for tumor classification in cases with ambiguous histological features. Epigenetic alterations also affect the expression of cancer genes alone, or in combination with genetic mechanisms. A subset of brain tumors is also characterized by locus-specific and genome-wide decrease in DNA methylation and hypomethylation. Other epigenetic alterations, such as changes in the position of histone variants and changes in histone modifications are also likely important in the molecular pathology of glioblastoma, as HDACs are targets for drugs currently in clinical trials. The technological wave of next-generation sequencing will accelerate glioblastoma epigenome profiling, allowing the direct integration of DNA methylation, histone modification, and gene expression profiles. Ultimately, genomic and epigenomic data should provide new predictive markers of response and lead to more effective therapies for glioblastoma, like the effective management of cancer stem cells.

CSCs are thought to be critical for the engraftment and long-term growth of glioblastoma. The cells are at least partially spared by traditional chemotherapies and radiation therapies,

and finding new treatments that can target CSCs may be critical for improving patient survival. A deeper understanding of the molecular relationship of these tumor types is necessary to derive insights into the diagnosis, prognosis, and treatment of glioblastoma. Although genome wide profiling of expression levels with microarrays can be used to identify differentially expressed genes between these tumor types, comparative studies so far have resulted in gene lists that show little overlap. The search for new cancer genes as well as the generation of tumor-specific genomic, epigenetic, and transcriptional profiles has been advanced by the application of genome-wide array-based profiling and large-scale sequencing efforts. Recent studies employing these techniques added in complementing the picture of the alterations and pathways most frequently involved in glioblastoma formation and thus qualifying as promising targets for glioblastoma diagnostics and therapy, including improvements in chemotherapy and immunotherapy.

While the immune system tries to eradicate the tumor in an earlier phase, it often fails to do so by unknown mechanisms. Thus, the primary goal of immunotherapy should be to overcome tolerance and to re-educate the immune system when the tumor burden is reduced following surgery, radiotherapy, and chemotherapy. To overcome these profound immunologic impairments in responsiveness, compounds that are capable of modulating the local immune responses could be examined. While several vaccine reagents have been developed and tested to raise anti-tumor activity, their broad application for tumor clearance is still far away. There is substantial evidence that immune components including HLA molecules, co-stimulatory molecules, growth factors, cytokines, adjuvants, and inhibitory or suppressor molecules play vital roles in the alteration of Ag presentation, TIL, immune protection, and the clinical outcome of glioblastoma patients. Based on this evidence and previous clinical trials, special considerations need to be accounted for in devising immunotherapeutics against CNS tumors. In conclusion, this review updated recent findings on molecular alterations that significantly advanced our knowledge of the biology of human glioblastoma as well as tumor-associated biomarkers of clinical interest and therapeutic design.

Acknowledgments

This work was supported by grants from the National Institutes of Health (CA129560 and CA129560-S1) to A. Haque. We thank Bently Doonan for technical assistance.

References

1. Robins HI, Chang S, Butowski N, Mehta M. Therapeutic advances for glioblastoma multiforme: current status and future prospects. *Curr Oncol Rep.* 2007; 9:66–70. [PubMed: 17164050]
2. Khasraw M, Lassman AB. Advances in the treatment of malignant gliomas. *Curr Oncol Rep.* 2010; 12:26–33. [PubMed: 20425605]
3. Chautard E, Loubeau G, Tchirkov A, Chassagne J, Vermot-Desroches C, Morel L, Verrelle P. Akt signaling pathway: a target for radiosensitizing human malignant glioma. *Neuro Oncol.* 2010; 12:434–43. [PubMed: 20406894]
4. Aoki T, Hashimoto N, Matsutani M. Management of glioblastoma. *Expert Opin Pharmacother.* 2007; 8:3133–46. [PubMed: 18035958]
5. Norden AD, Drappatz J, Wen PY. Antiangiogenic therapies for high-grade glioma. *Nat Rev Neurol.* 2009; 5:610–20. [PubMed: 19826401]

6. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am J Pathol.* 2007; 170:1445–53. [PubMed: 17456751]
7. Nagarajan RP, Costello JF. Epigenetic mechanisms in glioblastoma multiforme. *Semin Cancer Biol.* 2009; 19:188–97. [PubMed: 19429483]
8. Huang PH, Mukasa A, Bonavia R, Flynn RA, Brewer ZE, Cavenee WK, Furnari FB, White FM. Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proc Natl Acad Sci U S A.* 2007; 104:12867–72. [PubMed: 17646646]
9. Comincini S, Paolillo M, Barbieri G, Palumbo S, Sbalchiero E, Azzalin A, Russo MA, Schinelli S. Gene expression analysis of an EGFR indirectly related pathway identified PTEN and MMP9 as reliable diagnostic markers for human glial tumor specimens. *J Biomed Biotechnol.* 2009; 2009:924565. [PubMed: 19657395]
10. Santosh V, Arivazhagan A, Sreekanthreddy P, Srinivasan H, Thota B, Srividya MR, Vrinda M, Sridevi S, Shailaja BC, Samuel C, Prasanna KV, Thennarasu K, Balasubramaniam A, Chandramouli BA, Hegde AS, Somasundaram K, Kondaiah P, Rao MR. Grade-specific expression of insulin-like growth factor-binding proteins-2, -3, and -5 in astrocytomas: IGFBP-3 emerges as a strong predictor of survival in patients with newly diagnosed glioblastoma. *Cancer Epidemiol Biomarkers Prev.* 2010; 19:1399–408. [PubMed: 20501753]
11. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest.* 2010; 120:694–705. [PubMed: 20179352]
12. Ren Y, Zhou X, Mei M, Yuan XB, Han L, Wang GX, Jia ZF, Xu P, Pu PY, Kang CS. MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer.* 2010; 10:27. [PubMed: 20113523]
13. Cheng L, Bao S, Rich JN. Potential therapeutic implications of cancer stem cells in glioblastoma. *Biochem Pharmacol.* 2010
14. Barcia C Jr, Gomez A, Gallego-Sanchez JM, Perez-Valles A, Castro MG, Lowenstein PR, Barcia C Sr, Herrero MT. Infiltrating CTLs in human glioblastoma establish immunological synapses with tumorigenic cells. *Am J Pathol.* 2009; 175:786–98. [PubMed: 19628762]
15. Waziri A. Glioblastoma-derived mechanisms of systemic immunosuppression. *Neurosurg Clin N Am.* 2010; 21:31–42. [PubMed: 19944964]
16. Rolle CE, Sengupta S, Lesniak MS. Challenges in clinical design of immunotherapy trials for malignant glioma. *Neurosurg Clin N Am.* 2010; 21:201–14. [PubMed: 19944979]
17. Yamanaka R. Cell- and peptide-based immunotherapeutic approaches for glioma. *Trends Mol Med.* 2008; 14:228–35. [PubMed: 18403264]
18. Pellegatta S, Poliani PL, Stucchi E, Corno D, Colombo CA, Orzan F, Ravanini M, Finocchiaro G. Intra-tumoral dendritic cells increase efficacy of peripheral vaccination by modulation of glioma microenvironment. *Neuro Oncol.* 2010; 12:377–88. [PubMed: 20308315]
19. Zhang JG, Eguchi J, Kruse CA, Gomez GG, Fakhrai H, Schroter S, Ma W, Hoa N, Minev B, Delgado C, Wepsic HT, Okada H, Jadus MR. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. *Clin Cancer Res.* 2007; 13:566–75. [PubMed: 17255279]
20. Zawlik I, Kita D, Vaccarella S, Mittelbronn M, Franceschi S, Ohgaki H. Common polymorphisms in the MDM2 and TP53 genes and the relationship between TP53 mutations and patient outcomes in glioblastomas. *Brain Pathol.* 2009; 19:188–94. [PubMed: 18462472]
21. Umetani N, Hoon DS. Frequent LOH at chromosome 12q22-23 and Apaf-1 inactivation in glioblastoma. *Brain Pathol.* 2004; 14:224. author reply 224. [PubMed: 15193036]
22. Fukushima T, Favreux A, Huang H, Shimizu T, Yonekawa Y, Nakazato Y, Ohagki H. Genetic alterations in primary glioblastomas in Japan. *J Neuropathol Exp Neurol.* 2006; 65:12–8. [PubMed: 16410744]
23. Newcomb EW, Alonso M, Sung T, Miller DC. Incidence of p14ARF gene deletion in high-grade adult and pediatric astrocytomas. *Hum Pathol.* 2000; 31:115–9. [PubMed: 10665922]
24. Palanichamy K, Erkinen M, Saia G, Chakravarti A. Molecular and genetic profiling in human gliomas. *Discov Med.* 2007; 7:75–81. [PubMed: 18093469]

25. Stark AM, Witzel P, Strege RJ, Hugo HH, Mehdorn HM. p53, mdm2, EGFR, and msh2 expression in paired initial and recurrent glioblastoma multiforme. *J Neurol Neurosurg Psychiatry*. 2003; 74:779–83. [PubMed: 12754350]
26. Wiedemeyer WR, Dunn IF, Quayle SN, Zhang J, Chheda MG, Dunn GP, Zhuang L, Rosenbluh J, Chen S, Xiao Y, Shapiro GI, Hahn WC, Chin L. Pattern of retinoblastoma pathway inactivation dictates response to CDK4/6 inhibition in GBM. *Proc Natl Acad Sci U S A*. 2010; 107:11501–6. [PubMed: 20534551]
27. Wiencke JK, Zheng S, Jelluma N, Tihan T, Vandenberg S, Tamguney T, Baumber R, Parsons R, Lamborn KR, Berger MS, Wrensch MR, Haas-Kogan DA, Stokoe D. Methylation of the PTEN promoter defines low-grade gliomas and secondary glioblastoma. *Neuro Oncol*. 2007; 9:271–9. [PubMed: 17504928]
28. Mendez O, Zavadil J, Esencay M, Lukyanov Y, Santovasi D, Wang SC, Newcomb EW, Zagzag D. Knock down of HIF-1 α in glioma cells reduces migration in vitro and invasion in vivo and impairs their ability to form tumor spheres. *Mol Cancer*. 2010; 9:133. [PubMed: 20515450]
29. Mellai M, Caldera V, Patrucco A, Annovazzi L, Schiffer D. Survivin expression in glioblastomas correlates with proliferation, but not with apoptosis. *Anticancer Res*. 2008; 28:109–18. [PubMed: 18383832]
30. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, Zhang J, Li YM, Maciacyk J, Nikkhah G, Dimeco F, Piccirillo S, Vescovi AL, Eberhart CG. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*. 2010; 28:5–16. [PubMed: 19904829]
31. Katsetos CD, Draberova E, Legido A, Draber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. II. gamma-Tubulin. *J Cell Physiol*. 2009; 221:514–20. [PubMed: 19650077]
32. Bleeker FE, Atai NA, Lamba S, Jonker A, Rijkeboer D, Bosch KS, Tigchelaar W, Troost D, Vandertop WP, Bardelli A, Van Noorden CJ. The prognostic IDH1(R132) mutation is associated with reduced NADP⁺-dependent IDH activity in glioblastoma. *Acta Neuropathol*. 2010; 119:487–94. [PubMed: 20127344]
33. Elsir T, Eriksson A, Orrego A, Lindstrom MS, Nister M. Expression of PROX1 is a common feature of high-grade malignant astrocytic gliomas. *J Neuropathol Exp Neurol*. 2010; 69:129–38. [PubMed: 20084020]
34. Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, Grossman RG, Heslop HE, Gottschalk S. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. *Clin Cancer Res*. 2010; 16:474–85. [PubMed: 20068073]
35. Xu Y, Stamenkovic I, Yu Q. CD44 attenuates activation of the hippo signaling pathway and is a prime therapeutic target for glioblastoma. *Cancer Res*. 2010; 70:2455–64. [PubMed: 20197461]
36. Wei KC, Huang CY, Chen PY, Feng LY, Wu TW, Chen SM, Tsai HC, Lu YJ, Tsang NM, Tseng CK, Pai PC, Shin JW. Evaluation of the prognostic value of CD44 in glioblastoma multiforme. *Anticancer Res*. 2010; 30:253–9. [PubMed: 20150644]
37. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell*. 2009; 4:440–52. [PubMed: 19427293]
38. Samaras V, Piperi C, Levidou G, Zisakis A, Kavantzias N, Themistocleous MS, Boviatsis EI, Barbatis C, Lea RW, Kalofoutis A, Korkolopoulou P. Analysis of interleukin (IL)-8 expression in human astrocytomas: associations with IL-6, cyclooxygenase-2, vascular endothelial growth factor, and microvessel morphometry. *Hum Immunol*. 2009; 70:391–7. [PubMed: 19332096]
39. Balik V, Mirossay P, Bohus P, Sulla I, Mirossay L, Sarisky M. Flow cytometry analysis of neural differentiation markers expression in human glioblastomas may predict their response to chemotherapy. *Cell Mol Neurobiol*. 2009; 29:845–58. [PubMed: 19288188]
40. Crane CA, Han SJ, Barry JJ, Ahn BJ, Lanier LL, Parsa AT. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8⁺ T cells in glioma patients. *Neuro Oncol*. 2010; 12:7–13. [PubMed: 20150362]
41. Felsberg J, Rapp M, Loeser S, Fimmers R, Stummer W, Goepfert M, Steiger HJ, Friedensdorf B, Reifenberger G, Sabel MC. Prognostic significance of molecular markers and extent of resection in primary glioblastoma patients. *Clin Cancer Res*. 2009; 15:6683–93. [PubMed: 19861461]

42. Dupont J, Pierre A, Froment P, Moreau C. The insulin-like growth factor axis in cell cycle progression. *Horm Metab Res.* 2003; 35:740–50. [PubMed: 14710353]
43. Annabi B, Laflamme C, Sina A, Lachambre MP, Beliveau R. A MT1-MMP/NF- κ B signaling axis as a checkpoint controller of COX-2 expression in CD133+ U87 glioblastoma cells. *J Neuroinflammation.* 2009; 6:8. [PubMed: 19272160]
44. Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, Modrusan Z, Nacu S, Guerrero S, Edgar KA, Wallin JJ, Lamszus K, Westphal M, Heim S, James CD, VandenBerg SR, Costello JF, Moorefield S, Cowdrey CJ, Prados M, Phillips HS. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. *Cancer Cell.* 2010; 17:362–75. [PubMed: 20385361]
45. Gaetani P, Hulleman E, Levi D, Quarto M, Scorsetti M, Helins K, Simonelli M, Colombo P, Baenay Rodriguez R. Expression of the transcription factor HEY1 in glioblastoma: a preliminary clinical study. *Tumori.* 2010; 96:97–102. [PubMed: 20437865]
46. Amoureux MC, Coulibaly B, Chinot O, Loundou A, Metellus P, Rougon G, Figarella-Branger D. Polysialic acid neural cell adhesion molecule (PSA-NCAM) is an adverse prognosis factor in glioblastoma, and regulates olig2 expression in glioma cell lines. *BMC Cancer.* 2010; 10:91. [PubMed: 20219118]
47. Juric-Sekhar G, Zarkovic K, Waeg G, Cipak A, Zarkovic N. Distribution of 4-hydroxynonenal-protein conjugates as a marker of lipid peroxidation and parameter of malignancy in astrocytic and ependymal tumors of the brain. *Tumori.* 2009; 95:762–8. [PubMed: 20210242]
48. Kolesnikova TV, Kazarov AR, Lemieux ME, Lafleur MA, Kesari S, Kung AL, Hemler ME. Glioblastoma inhibition by cell surface immunoglobulin protein EWI-2, in vitro and in vivo. *Neoplasia.* 2009; 11:77–86. 4p following 86. [PubMed: 19107234]
49. Martens T, Schmidt NO, Eckerich C, Fillbrandt R, Merchant M, Schwall R, Westphal M, Lamszus K. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. *Clin Cancer Res.* 2006; 12:6144–52. [PubMed: 17062691]
50. Marucci G, Morandi L, Magrini E, Farnedi A, Franceschi E, Miglio R, Calo D, Pession A, Foschini MP, Eusebi V. Gene expression profiling in glioblastoma and immunohistochemical evaluation of IGFBP-2 and CDC20. *Virchows Arch.* 2008; 453:599–609. [PubMed: 18953566]
51. Episkopou V. SOX2 functions in adult neural stem cells. *Trends Neurosci.* 2005; 28:219–21. [PubMed: 15866195]
52. Sameshima T, Nabeshima K, Toole BP, Yokogami K, Okada Y, Goya T, Koono M, Wakisaka S. Glioma cell extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in co-cultures with brain-derived fibroblasts. *Cancer Lett.* 2000; 157:177–84. [PubMed: 10936678]
53. Gu J, Zhang C, Chen R, Pan J, Wang Y, Ming M, Gui W, Wang D. Clinical implications and prognostic value of EMMPRIN/CD147 and MMP2 expression in pediatric gliomas. *Eur J Pediatr.* 2009; 168:705–10. [PubMed: 18795327]
54. Emdad L, Sarkar D, Lee SG, Su ZZ, Yoo BK, Dash R, Yacoub A, Fuller CE, Shah K, Dent P, Bruce JN, Fisher PB. Astrocyte elevated gene-1: a novel target for human glioma therapy. *Mol Cancer Ther.* 2010; 9:79–88. [PubMed: 20053777]
55. Righi V, Andronesi OC, Mintzopoulos D, Black PM, Tzika AA. High-resolution magic angle spinning magnetic resonance spectroscopy detects glycine as a biomarker in brain tumors. *Int J Oncol.* 2010; 36:301–6. [PubMed: 20043062]
56. Carson-Walter EB, Winans BN, Whiteman MC, Liu Y, Jarvela S, Haapasalo H, Tyler BM, Huso DL, Johnson MD, Walter KA. Characterization of TEM1/endosialin in human and murine brain tumors. *BMC Cancer.* 2009; 9:417. [PubMed: 19948061]
57. Colin C, Voutsinos-Porche B, Nanni I, Fina F, Metellus P, Intagliata D, Baeza N, Bouvier C, Delfino C, Loundou A, Chinot O, Lah T, Kos J, Martin PM, Ouafik L, Figarella-Branger D. High expression of cathepsin B and plasminogen activator inhibitor type-1 are strong predictors of survival in glioblastomas. *Acta Neuropathol.* 2009; 118:745–54. [PubMed: 19774387]
58. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. *Clin Cancer Res.* 2009; 15:6002–7. [PubMed: 19755387]

59. Barresi V, Buttarelli FR, Vitarelli EE, Arcella A, Antonelli M, Giangaspero F. Caveolin-1 expression in diffuse gliomas: correlation with the proliferation index, epidermal growth factor receptor, p53, and 1p/19q status. *Hum Pathol.* 2009; 40:1738–46. [PubMed: 19716156]
60. Lo HW, Zhu H, Cao X, Aldrich A, Ali-Osman F. A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion. *Cancer Res.* 2009; 69:6790–8. [PubMed: 19706761]
61. Schilling SH, Hjelmeland AB, Radloff DR, Liu IM, Wakeman TP, Fielhauer JR, Foster EH, Lathia JD, Rich JN, Wang XF, Datto MB. NDRG4 is required for cell cycle progression and survival in glioblastoma cells. *J Biol Chem.* 2009; 284:25160–9. [PubMed: 19592488]
62. McCord AM, Jamal M, Williams ES, Camphausen K, Tofilon PJ. CD133+ glioblastoma stem-like cells are radiosensitive with a defective DNA damage response compared with established cell lines. *Clin Cancer Res.* 2009; 15:5145–53. [PubMed: 19671863]
63. Roa W, Brunet B, Guo L, Amanie J, Fairchild A, Gabos Z, Nijjar T, Scrimger R, Yee D, Xing J. Identification of a new microRNA expression profile as a potential cancer screening tool. *Clin Invest Med.* 2010; 33:E124. [PubMed: 20370992]
64. Hirata E, Arakawa Y, Shirahata M, Yamaguchi M, Kishi Y, Okada T, Takahashi JA, Matsuda M, Hashimoto N. Endogenous tenascin-C enhances glioblastoma invasion with reactive change of surrounding brain tissue. *Cancer Sci.* 2009; 100:1451–9. [PubMed: 19459858]
65. Amria S, Cameron C, Stuart R, Haque A. Defects in HLA class II antigen presentation in B-cell lymphomas. *Leuk Lymphoma.* 2008; 49:353–5. [PubMed: 18231926]
66. Doonan BP, Haque A. HLA class II antigen presentation in prostate cancer cells: a novel approach to prostate tumor immunotherapy. *The Open Cancer Immunology Journal.* 2010; 3:1–7. [PubMed: 24163711]
67. Luptawan A, Liu G, Yu JS. Dendritic cell immunotherapy for malignant gliomas. *Rev Recent Clin Trials.* 2008; 3:10–21. [PubMed: 18474011]
68. Zhang JG, Kruse CA, Driggers L, Hoa N, Wisoff J, Allen JC, Zagzag D, Newcomb EW, Jadus MR. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. *J Neurooncol.* 2008; 88:65–76. [PubMed: 18259692]
69. Yan X, Su Z, Zhang J, Wu Z, Ye S, Lu X, Wu J, Zeng Y, Zheng W. Killing effect of interleukin-13 receptor alpha 2 (IL-13Rα2) sensitized DC-CTL cells on human glioblastoma U251 cells. *Cell Immunol.* 2010; 263:172–5. [PubMed: 20400066]
70. Chi JH, Panner A, Cachola K, Crane CA, Murray J, Pieper RO, James CD, Parsa AT. Increased expression of the glioma-associated antigen ARF4L after loss of the tumor suppressor PTEN. Laboratory investigation. *J Neurosurg.* 2008; 108:299–303. [PubMed: 18240926]
71. Haque A, Banik NL, Ray SK. Emerging role of combination of all-trans retinoic acid and interferon-gamma as chemoimmunotherapy in the management of human glioblastoma. *Neurochem Res.* 2007; 32:2203–9. [PubMed: 17676389]
72. George J, Banik NL, Ray SK. Knockdown of hTERT and concurrent treatment with interferon-gamma inhibited proliferation and invasion of human glioblastoma cell lines. *Int J Biochem Cell Biol.* 2010; 42:1164–73. [PubMed: 20394835]
73. Ciesielski MJ, Kozbor D, Castanaro CA, Barone TA, Fenstermaker RA. Therapeutic effect of a T helper cell supported CTL response induced by a survivin peptide vaccine against murine cerebral glioma. *Cancer Immunol Immunother.* 2008; 57:1827–35. [PubMed: 18438666]
74. Kosmaczewska A, Ciszak L, Potoczek S, Frydecka I. The significance of Treg cells in defective tumor immunity. *Arch Immunol Ther Exp (Warsz).* 2008; 56:181–91. [PubMed: 18512029]
75. Hatfield P, Merrick AE, West E, O'Donnell D, Selby P, Vile R, Melcher AA. Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. *J Immunother.* 2008; 31:620–32. [PubMed: 18600182]
76. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE 2nd, Lally-Goss D, McGehee-Norman S, Paolino A, Reardon DA, Friedman AH, Friedman HS, Bigner DD. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. *Mol Cancer Ther.* 2009; 8:2773–9. [PubMed: 19825799]
77. Ueda R, Ohkusu-Tsukada K, Fusaki N, Soeda A, Kawase T, Kawakami Y, Toda M. Identification of HLA-A2- and A24-restricted T-cell epitopes derived from SOX6 expressed in glioma stem cells for immunotherapy. *Int J Cancer.* 2010; 126:919–29. [PubMed: 19728337]

78. Jakubzick C, Helft J, Kaplan TJ, Randolph GJ. Optimization of methods to study pulmonary dendritic cell migration reveals distinct capacities of DC subsets to acquire soluble versus particulate antigen. *J Immunol Methods*. 2008; 337:121–31. [PubMed: 18662693]
79. Staveley-O'Carroll K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, Pardoll D, Levitsky H. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc Natl Acad Sci U S A*. 1998; 95:1178–83. [PubMed: 9448305]
80. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, Yong WH, Incardona F, Thompson RC, Riedinger MS, Zhang W, Prins RM, Black KL. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res*. 2001; 61:842–7. [PubMed: 11221866]
81. Spiegel-Kreinecker S, Pirker C, Filipits M, Lotsch D, Buchroithner J, Pichler J, Silye R, Weis S, Micksche M, Fischer J, Berger W. O6-Methylguanine DNA methyltransferase protein expression in tumor cells predicts outcome of temozolomide therapy in glioblastoma patients. *Neuro Oncol*. 2010; 12:28–36. [PubMed: 20150365]
82. Morandi L, Franceschi E, de Biase D, Marucci G, Tosoni A, Ermani M, Pession A, Tallini G, Brandes A. Promoter methylation analysis of O6-methylguanine-DNA methyltransferase in glioblastoma: detection by locked nucleic acid based quantitative PCR using an imprinted gene (SNURF) as a reference. *BMC Cancer*. 2010; 10:48. [PubMed: 20167086]
83. Jung TY, Jung S, Moon KS, Kim IY, Kang SS, Kim YH, Park CS, Lee KH. Changes of the O6-methylguanine-DNA methyltransferase promoter methylation and MGMT protein expression after adjuvant treatment in glioblastoma. *Oncol Rep*. 2010; 23:1269–76. [PubMed: 20372840]
84. Johannessen TC, Bjerkvig R, Tysnes BB. DNA repair and cancer stem-like cells--potential partners in glioma drug resistance? *Cancer Treat Rev*. 2008; 34:558–67. [PubMed: 18501520]
85. Hsu JY, Wakelee HA. Monoclonal antibodies targeting vascular endothelial growth factor: current status and future challenges in cancer therapy. *BioDrugs*. 2009; 23:289–304. [PubMed: 19754219]
86. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA Cancer J Clin*. 2010; 60:166–93. [PubMed: 20445000]
87. Razis E, Selviaridis P, Labropoulos S, Norris JL, Zhu MJ, Song DD, Kalebic T, Torrens M, Kalogera-Fountzila A, Karkavelas G, Karanastasi S, Fletcher JA, Fountzilas G. Phase II study of neoadjuvant imatinib in glioblastoma: evaluation of clinical and molecular effects of the treatment. *Clin Cancer Res*. 2009; 15:6258–66. [PubMed: 19789313]
88. Dasari VR, Kaur K, Velpula KK, Gujrati M, Fassett D, Klopfenstein JD, Dinh DH, Rao JS. Upregulation of PTEN in glioma cells by cord blood mesenchymal stem cells inhibits migration via downregulation of the PI3K/Akt pathway. *PLoS One*. 2010; 5:e10350. [PubMed: 20436671]
89. Anderson JC, McFarland BC, Gladson CL. New molecular targets in angiogenic vessels of glioblastoma tumours. *Expert Rev Mol Med*. 2008; 10:e23. [PubMed: 18684337]
90. Batchelor TT, Duda DG, di Tomaso E, Ancukiewicz M, Plotkin SR, Gerstner E, Eichler AF, Drappatz J, Hochberg FH, Benner T, Louis DN, Cohen KS, Chea H, Exarhopoulos A, Loeffler JS, Moses MA, Ivy P, Sorensen AG, Wen PY, Jain RK. Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. *J Clin Oncol*. 2010; 28:2817–23. [PubMed: 20458050]
91. Zadeh G, Koushan K, Baoping Q, Shannon P, Guha A. Role of angiopoietin-2 in regulating growth and vascularity of astrocytomas. *J Oncol*. 2010; 2010:659231. [PubMed: 20467564]
92. Kume T. Novel insights into the differential functions of Notch ligands in vascular formation. *J Angiogenes Res*. 2009; 1:8. [PubMed: 20016694]
93. Kaur B, Cork SM, Sandberg EM, Devi NS, Zhang Z, Klenotic PA, Febbraio M, Shim H, Mao H, Tucker-Burden C, Silverstein RL, Brat DJ, Olson JJ, Van Meir EG. Vasculostatin inhibits intracranial glioma growth and negatively regulates in vivo angiogenesis through a CD36-dependent mechanism. *Cancer Res*. 2009; 69:1212–20. [PubMed: 19176395]
94. Xia S, Rosen EM, Laterra J. Sensitization of glioma cells to Fas-dependent apoptosis by chemotherapy-induced oxidative stress. *Cancer Res*. 2005; 65:5248–55. [PubMed: 15958570]

95. Xia S, Li Y, Rosen EM, Lateral J. Ribotoxic stress sensitizes glioblastoma cells to death receptor induced apoptosis: requirements for c-Jun NH₂-terminal kinase and Bim. *Mol Cancer Res.* 2007; 5:783–92. [PubMed: 17699104]
96. Puchades M, Nilsson CL, Emmett MR, Aldape KD, Ji Y, Lang FF, Liu TJ, Conrad CA. Proteomic investigation of glioblastoma cell lines treated with wild-type p53 and cytotoxic chemotherapy demonstrates an association between galectin-1 and p53 expression. *J Proteome Res.* 2007; 6:869–75. [PubMed: 17269744]
97. Pan PY, Wang GX, Yin B, Ozao J, Ku T, Divino CM, Chen SH. Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. *Blood.* 2008; 111:219–28. [PubMed: 17885078]
98. Wang K, Pan L, Che X, Cui D, Li C. Sonic Hedgehog/GLI1 signaling pathway inhibition restricts cell migration and invasion in human gliomas. *Neurol Res.* 2010
99. Chinnaiyan P, Allen GW, Harari PM. Radiation and new molecular agents, part II: targeting HDAC, HSP90, IGF-1R, PI3K, and Ras. *Semin Radiat Oncol.* 2006; 16:59–64. [PubMed: 16378908]
100. Clarke J, Butowski N, Chang S. Recent advances in therapy for glioblastoma. *Arch Neurol.* 2010; 67:279–83. [PubMed: 20212224]
101. Uhlmann K, Rohde K, Zeller C, Szymas J, Vogel S, Marczynek K, Thiel G, Nurnberg P, Laird PW. Distinct methylation profiles of glioma subtypes. *Int J Cancer.* 2003; 106:52–9. [PubMed: 12794756]
102. Sina A, Lord-Dufour S, Annabi B. Cell-based evidence for aminopeptidase N/CD13 inhibitor actinonin targeting of MT1-MMP-mediated proMMP-2 activation. *Cancer Lett.* 2009; 279:171–6. [PubMed: 19264392]
103. Cui D, Xu Q, Wang K, Che X. Gli1 is a potential target for alleviating multidrug resistance of gliomas. *J Neurol Sci.* 2010; 288:156–66. [PubMed: 19818966]
104. Merighi S, Benini A, Mirandola P, Gesso S, Varian K, Leung E, McLennan S, Brandi PG, Borel PA. Hypoxia inhibits paclitaxel-induced apoptosis through adenosine-mediated phosphorylation of bad in glioblastoma cells. *Mol Pharmacol.* 2007; 72:162–72. [PubMed: 17400763]
105. Liang P, MacRae TH. Molecular chaperones and the cytoskeleton. *J Cell Sci.* 1997; 110 (Pt 13): 1431–40. [PubMed: 9224761]
106. Katsetos CD, Draberova E, Smejkalova B, Reddy G, Bertrand L, de Chadarevian JP, Legido A, Nissanov J, Baas PW, Draber P. Class III beta-tubulin and gamma-tubulin are co-expressed and form complexes in human glioblastoma cells. *Neurochem Res.* 2007; 32:1387–98. [PubMed: 17406983]
107. Demaria S, Pikarsky E, Karin M, Coussens LM, Chen YC, El-Omar EM, Trinchieri G, Dubinett SM, Mao JT, Szabo E, Krieg A, Weiner GJ, Fox BA, Coukos G, Wang E, Abraham RT, Carbone M, Lotze MT. Cancer and inflammation: promise for biologic therapy. *J Immunother.* 2010; 33:335–51. [PubMed: 20386472]
108. Liu S, Lizée G, Lou Y, Liu C, Overwijk WW, Wang G, Hwu P. IL-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. *Int Immunol.* 2007; 19:1213–21. [PubMed: 17898044]
109. Kumar R, Kamdar D, Madden L, Hills C, Crooks D, O'Brien D, Greenman J. Th1/Th2 cytokine imbalance in meningioma, anaplastic astrocytoma and glioblastoma multiforme patients. *Oncol Rep.* 2006; 15:1513–6. [PubMed: 16685388]
110. Roussel E, Gingras MC, Grimm EA, Bruner JM, Moser RP. Predominance of a type 2 intratumoural immune response in fresh tumour-infiltrating lymphocytes from human gliomas. *Clin Exp Immunol.* 1996; 105:344–52. [PubMed: 8706344]
111. Zisakis A, Piperi C, Themistocleous MS, Korkolopoulou P, Boviatsis EI, Sakas DE, Patsouris E, Lea RW, Kalofoutis A. Comparative analysis of peripheral and localised cytokine secretion in glioblastoma patients. *Cytokine.* 2007; 39:99–105. [PubMed: 17697783]
112. Gauthier T, Hamou MF, Monod L, Gallay P, Carrel S, de Tribolet N. Expression and release of interleukin-1 by human glioblastoma cells in vitro and in vivo. *Acta Neurochir (Wien).* 1993; 121:199–205. [PubMed: 8512018]

113. Paugh BS, Bryan L, Paugh SW, Wilczynska KM, Alvarez SM, Singh SK, Kapitonov D, Rokita H, Wright S, Griswold-Prenner I, Milstien S, Spiegel S, Kordula T. Interleukin-1 regulates the expression of sphingosine kinase 1 in glioblastoma cells. *J Biol Chem.* 2009; 284:3408–17. [PubMed: 19074142]
114. Ehtesham M, Black KL, Yu JS. Recent progress in immunotherapy for malignant glioma: treatment strategies and results from clinical trials. *Cancer Control.* 2004; 11:192–207. [PubMed: 15153843]
115. Jadus MR, Chen Y, Boldaji MT, Delgado C, Sanchez R, Douglass T, Al-Atar U, Schulz W, Lloyd C, Wepsic HT. Human U251MG glioma cells expressing the membrane form of macrophage colony-stimulating factor (mM-CSF) are killed by human monocytes in vitro and are rejected within immunodeficient mice via paraptosis that is associated with increased expression of three different heat shock proteins. *Cancer Gene Ther.* 2003; 10:411–20. [PubMed: 12719711]
116. Yu JJ, Sun X, Yuan X, Lee JW, Snyder EY, Yu JS. Immunomodulatory neural stem cells for brain tumour therapy. *Expert Opin Biol Ther.* 2006; 6:1255–62. [PubMed: 17223735]
117. Ghosh S, Tewari R, Dixit D, Sen E. TNF α induced oxidative stress dependent Akt signaling affects actin cytoskeletal organization in glioma cells. *Neurochem Int.* 2010; 56:194–201. [PubMed: 19836430]
118. Schwartzbaum JA, Ahlbom A, Lonn S, Malmer B, Wigertz A, Auvinen A, Brookes AJ, Collatz Christensen H, Henriksson R, Johansen C, Salminen T, Schoemaker MJ, Swerdlow AJ, Debinski W, Feychting M. An international case-control study of interleukin-4R α , interleukin-13, and cyclooxygenase-2 polymorphisms and glioblastoma risk. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:2448–54. [PubMed: 18006935]
119. Liu TF, Cai J, Gibo DM, Debinski W. Reoxygenation of hypoxic glioblastoma multiforme cells potentiates the killing effect of an interleukin-13-based cytotoxin. *Clin Cancer Res.* 2009; 15:160–8. [PubMed: 19118043]
120. Panek RB, Benveniste EN. Class II MHC gene expression in microglia. Regulation by the cytokines IFN- γ , TNF- α , and TGF- β . *J Immunol.* 1995; 154:2846–54. [PubMed: 7876554]
121. Van Gool S, Maes W, Ardon H, Verschuere T, Van Cauter S, De Vleeschouwer S. Dendritic cell therapy of high-grade gliomas. *Brain Pathol.* 2009; 19:694–712. [PubMed: 19744041]
122. Sondergaard H, Frederiksen KS, Thygesen P, Galsgaard ED, Skak K, Kristjansen PE, Odum N, Kragh M. Interleukin 21 therapy increases the density of tumor infiltrating CD8+ T cells and inhibits the growth of syngeneic tumors. *Cancer Immunol Immunother.* 2007; 56:1417–28. [PubMed: 17285290]
123. King C. New insights into the differentiation and function of T follicular helper cells. *Nat Rev Immunol.* 2009; 9:757–66. [PubMed: 19855402]
124. Sutherland CL, Rabinovich B, Chalupny NJ, Brawand P, Miller R, Cosman D. ULBPs, human ligands of the NKG2D receptor, stimulate tumor immunity with enhancement by IL-15. *Blood.* 2006; 108:1313–9. [PubMed: 16621962]
125. Wheeler CJ, Black KL. DCVax-Brain and DC vaccines in the treatment of GBM. *Expert Opin Investig Drugs.* 2009; 18:509–19.
126. Galley HF, Webster NR. The immuno-inflammatory cascade. *Br J Anaesth.* 1996; 77:11–6. [PubMed: 8703619]
127. Haque A, Das A, Hajiaghamohseni LM, Younger A, Banik NL, Ray SK. Induction of apoptosis and immune response by all-*trans* retinoic acid plus interferon-gamma in human malignant glioblastoma T98G and U87MG cells. *Cancer Immunol Immunother.* 2007; 56:615–25. [PubMed: 16947022]
128. Das A, Banik NL, Ray SK. Molecular mechanisms of the combination of retinoid and interferon-gamma for inducing differentiation and increasing apoptosis in human glioblastoma T98G and U87MG cells. *Neurochem Res.* 2009; 34:87–101. [PubMed: 18368485]
129. Karmakar S, Banik NL, Ray SK. Combination of all-*trans* retinoic acid and paclitaxel-induced differentiation and apoptosis in human glioblastoma U87MG xenografts in nude mice. *Cancer.* 2008; 112:596–607. [PubMed: 18098270]

130. Baj G, Arnulfo A, Deaglio S, Tibaldi E, Surico N, Malavasi F. All-trans retinoic acid inhibits the growth of breast cancer cells by up-regulating ICAM-1 expression. *J Biol Regul Homeost Agents*. 1999; 13:115–22. [PubMed: 10503735]
131. Jarnicki AG, Lysaght J, Todryk S, Mills KH. Suppression of antitumor immunity by IL-10 and TGF- β -producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. *J Immunol*. 2006; 177:896–904. [PubMed: 16818744]
132. Gajewski TF, Meng Y, Harlin H. Immune suppression in the tumor microenvironment. *J Immunother*. 2006; 29:233–40. [PubMed: 16699366]
133. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol*. 2006; 24:99–146. [PubMed: 16551245]
134. Ghiringhelli F, Puig PE, Roux S, Parcellier A, Schmitt E, Solary E, Kroemer G, Martin F, Chauffert B, Zitvogel L. Tumor cells convert immature myeloid dendritic cells into TGF- β -secreting cells inducing CD4+ CD25+ regulatory T cell proliferation. *J Exp Med*. 2005; 202:919–29. [PubMed: 16186184]
135. Bruna A, Darken RS, Rojo F, Ocana A, Penuelas S, Arias A, Paris R, Tortosa A, Mora J, Baselga J, Seoane J. High TGF β -Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell*. 2007; 11:147–60. [PubMed: 17292826]
136. Verneris MR, Karami M, Baker J, Jayaswal A, Negrin RS. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8+ T cells. *Blood*. 2004; 103:3065–72. [PubMed: 15070686]
137. Nakano Y, Kuroda E, Kito T, Yokota A, Yamashita U. Induction of macrophagic prostaglandin E2 synthesis by glioma cells. *J Neurosurg*. 2006; 104:574–82. [PubMed: 16619662]
138. Lalier L, Cartron PF, Pedelaborde F, Olivier C, Loussouarn D, Martin SA, Meflah K, Menanteau J, Vallette FM. Increase in PGE2 biosynthesis induces a Bax dependent apoptosis correlated to patients' survival in glioblastoma multiforme. *Oncogene*. 2007; 26:4999–5009. [PubMed: 17369862]
139. Guillemain GJ, Smythe G, Takikawa O, Brew BJ. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia*. 2005; 49:15–23. [PubMed: 15390107]
140. Macchiarulo A, Camaioni E, Nuti R, Pellicciari R. Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2,3-dioxygenase (IDO), a novel target in cancer disease. *Amino Acids*. 2009; 37:219–29. [PubMed: 18612775]
141. Banerjee T, Duhadaway JB, Gaspari P, Sutanto-Ward E, Munn DH, Mellor AL, Malachowski WP, Prendergast GC, Muller AJ. A key in vivo antitumor mechanism of action of natural product-based brassinins is inhibition of indoleamine 2,3-dioxygenase. *Oncogene*. 2008; 27:2851–7. [PubMed: 18026137]
142. Avril T, Saikali S, Vauleon E, Jary A, Hamlat A, De Tayrac M, Mosser J, Quillien V. Distinct effects of human glioblastoma immunoregulatory molecules programmed cell death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO) on tumour-specific T cell functions. *J Neuroimmunol*. 2010
143. Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. *Cancer Immunol Immunother*. 2005; 54:307–14. [PubMed: 15599732]
144. de Waal Malefyt R, Yssel H, de Vries JE. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. *J Immunol*. 1993; 150:4754–65. [PubMed: 7684412]
145. Stassi G, Todaro M, Zerilli M, Ricci-Vitiani L, Di Liberto D, Patti M, Florena A, Di Gaudio F, Di Gesu G, De Maria R. Thyroid cancer resistance to chemotherapeutic drugs via autocrine production of interleukin-4 and interleukin-10. *Cancer Res*. 2003; 63:6784–90. [PubMed: 14583474]

146. Salatino M, Croci DO, Bianco GA, Ilarregui JM, Toscano MA, Rabinovich GA. Galectin-1 as a potential therapeutic target in autoimmune disorders and cancer. *Expert Opin Biol Ther.* 2008; 8:45–57. [PubMed: 18081536]
147. Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. *Nat Rev Cancer.* 2005; 5:29–41. [PubMed: 15630413]
148. Roth P, Junker M, Tritschler I, Mittelbronn M, Dombrowski Y, Breit SN, Tabatabai G, Wick W, Weller M, Wischhusen J. GDF-15 Contributes to Proliferation and Immune Escape of Malignant Gliomas. *Clin Cancer Res.* 2010
149. Mertsch S, Schurgers LJ, Weber K, Paulus W, Senner V. Matrix gla protein (MGP): an overexpressed and migration-promoting mesenchymal component in glioblastoma. *BMC Cancer.* 2009; 9:302. [PubMed: 19712474]
150. Jenkinson SR, Williams NA, Morgan DJ. The role of intercellular adhesion molecule-1/LFA-1 interactions in the generation of tumor-specific CD8+ T cell responses. *J Immunol.* 2005; 174:3401–7. [PubMed: 15749873]
151. Serafini P, Mgebroff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res.* 2008; 68:5439–49. [PubMed: 18593947]
152. El Andaloussi A, Lesniak MS. An increase in CD4+ CD25+ FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro Oncol.* 2006; 8:234–43. [PubMed: 16723631]
153. El Andaloussi A, Han Y, Lesniak MS. Prolongation of survival following depletion of CD4+ CD25+ regulatory T cells in mice with experimental brain tumors. *J Neurosurg.* 2006; 105:430–7. [PubMed: 16961139]
154. Kren L, Muckova K, Lzicarova E, Sova M, Vybihal V, Svoboda T, Fadrus P, Smrcka M, Slaby O, Lakomy R, Vanhara P, Krenova Z, Michalek J. Production of immune-modulatory nonclassical molecules HLA-G and HLA-E by tumor infiltrating ameboid microglia/macrophages in glioblastomas: a role in innate immunity? *J Neuroimmunol.* 2010; 220:131–5. [PubMed: 20167379]
155. Rouas-Freiss N, Moreau P, Menier C, LeMaout J, Carosella ED. Expression of tolerogenic HLA-G molecules in cancer prevents antitumor responses. *Semin Cancer Biol.* 2007; 17:413–21. [PubMed: 17881247]
156. Zuber P, Kuppner MC, De Tribolet N. Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. *Eur J Immunol.* 1988; 18:1623–6. [PubMed: 3142781]

Table 1

Overview of reported gene alterations and frequency of mutations in glioblastoma

Genes	Changes	Frequencies (%)
LOH 10q	Loss	50–70
EGFR	Amplification	40–60
P16ink4a	Deletion	31
TP53	Mutation	50–60
PTEN	Mutation	60
MDM2	Polymorphism	40–60
MGMT	Hypermethylation	50–60
IDH1	Mutation	40–50
CDK4	Mutation	20–30
PDGFRA	Amplification	20–30

References: (6–10, 21–28)

Table 2

Potential glioblastoma antigens and biomarkers for the development of chemo-immunotherapeutics

Ags	Biomarkers
Aim-2, Art-1, Art-4, B-cyclin, EphA2, Ezh2, Fos11, Galt-3, GnT-V, Gp-100, Her-2/Neu, HNRPL, IL13R α 2, Mage-1, Mart-1, MRP-3, PTHrP, Sart-1, Sart-2, Sart-3, Sox-11, Survivin, hTert, Trp-1, Trp-2, Tyrosinase, Ube2V, Whsc2, YKL-40	AEG-1, ARF, ASCL1, Cav-1, CD44, CD133, EGFR, EGFRvIII, EW12, Gli1, HEY1, HIF-1, IDH2, IGF, LIFR, MDM2, MGMT, MMP, NGFR, PTEN, TEM1/endosialin, TP53, PROX1, Rad51, SSEA-1, VEGF

Reference: (19, 29, 33–39, 72)

Table 3

Tumor-associated molecules influencing immune recognition of malignant glioblastoma

Factors contributing to immune recognition of glioblastoma	Factors contributing to immune escape of glioblastoma
IFN- γ	Transforming growth factor-beta (TGF- β)
T cell growth factors: IL-2, IL-7, IL-12, IL-15	Prostaglandin E2 (PGE2)
Costimulatory molecules (CD80/CD86)	Indoleamine 2,3-dioxygenase (IDO)
CD40 agonists	Program death ligand (PD-L)
Anti-CTLA-4	Interleukin-10 (IL-10)
Anti-CD137 (anti-4-1BB)	Galectin-1 (Gal-1)
FLT3 ligand	Mayeloid-dereived supprespor cells (MDSC)
Vaccine adjuvants	Growth and differentiation factor 15(GDF-15)

References: (117–128, 133–156)