REVIEW

Malignant astrocytic glioma: genetics, biology, and paths to treatment

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Malignant astrocytic gliomas such as glioblastoma are the most common and lethal intracranial tumors. These cancers exhibit a relentless malignant progression characterized by widespread invasion throughout the brain, resistance to traditional and newer targeted therapeutic approaches, destruction of normal brain tissue, and certain death. The recent confluence of advances in stem cell biology, cell signaling, genome and computational science and genetic model systems have revolutionized our understanding of the mechanisms underlying the genetics, biology and clinical behavior of glioblastoma. This progress is fueling new opportunities for understanding the fundamental basis for development of this devastating disease and also novel therapies that, for the first time, portend meaningful clinical responses.

Malignant gliomas are classified and subtyped on the basis of histopathological features and clinical presentation (Fig. 1). The most common and biologically aggressive of these is glioblastoma (GBM), World Health Organization (WHO) grade IV, and is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, propensity for necrosis, robust angiogenesis, intense resistance to apoptosis, and rampant genomic instability. As reflected in the old moniker "multiforme," GBM presents with significant intratumoral heterogeneity on the cytopathological, transcriptional, and genomic levels. This complexity, combined with a putative cancer stem cell (CSC) subpopulation and an incomplete atlas of (epi)genetic lesions driving GBM pathogenesis, has conspired to make this cancer one of the most difficult to understand and to treat. Despite implementation of intensive therapeutic strategies and supportive care, the median survival of GBM has remained at 12 mo over the past decade.

In this review, we summarize current basic and translational challenges and highlight the striking scientific advances that promise to improve the clinical course of this lethal disease. These advances include a more comprehensive view of the altered genes and pathways in glioma and how such alterations drive the hallmark pathobiological features of the disease, the identification of new molecular subtypes in GBM, an improved understanding of the cellular origins of the disease and how CSCs may influence therapeutic responses, refined model systems for use in research and preclinical experimental therapeutics, and novel therapeutic strategies for targeting keystone genetic lesions and their pathways. For reasons of length, we have not discussed the advances in such important areas as tumor immunology, the blood-brain barrier, and tumor imaging. For the first time, there is a strong sentiment that meaningful therapeutic advances will soon flow from this explosion of new molecular and biological knowledge; the remarkable technological advances in genomics, proteomics,

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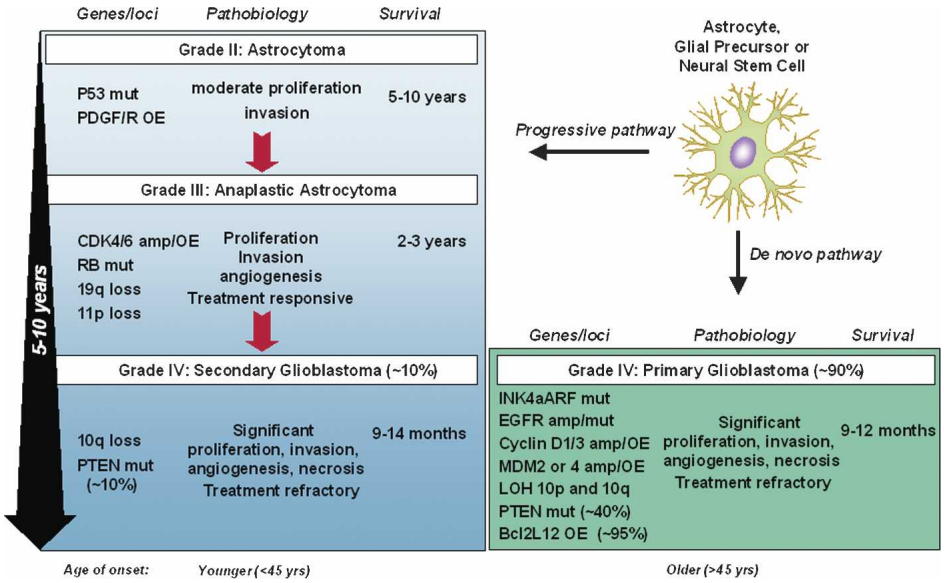

Figure 1. Chromosomal and genetic aberrations involved in the genesis of glioblastoma. Shown are the relationships between survival, pathobiology, and the molecular lesions that lead to the formation of primary (de novo) and secondary (progressive) glioblastomas. Although histologically indistinguishable, these grade IV gliomas occur in different age groups and present distinct genetic alterations affecting similar molecular pathways. For example, inactivation of p53 function occurs due to direct mutation in progressive GBMs or INK4aARF mutation/decrease in expression or MDM2 amplification in de novo GBMs. Similarly, activation of the PI3K pathway is achieved by several cooperative mechanisms, including EGFR amplification and mutation as well as PTEN mutation, although underexpression of PTEN in the absence of mutation is frequently seen as well. See the text and Figure 2 for details on the molecular function of implicated genes. (OE) Overexpressed; (amp) amplified; (mut) mutated.

and model systems; and the systematic and accurate development of small molecule drugs, therapeutic antibodies, and the entirely new class of RNA interference (RNAi)-based agents.

Classification and grading of glioma

The incidence of primary brain tumors worldwide is approximately seven per 100,000 individuals per year, accounting for ~2% of primary tumors and 7% of the years of life lost from cancer before the age of 70. The common gliomas affecting the cerebral hemispheres of adults are termed "diffuse" gliomas due to their propensity to infiltrate, early and extensively, throughout the brain parenchyma. These gliomas are classified histologically, immunohistochemically, and/or ultrastructurally as astrocytomas, oligodendrogliomas, or tumors with morphological features of both astrocytes and oligodendrocytes, termed oligoastrocytomas. Tumors are then graded on a WHO consensus-derived scale of I to IV according to their degree of malignancy as judged by various histological features accompanied by genetic alterations (Fig. 1; Louis et al. 2007). Grade I tumors are biologically benign and can be cured if they can be surgically resected; grade II tumors are low-grade malignancies that may follow long clinical courses, but early diffuse infiltration of the surrounding brain renders them incurable by surgery; grade III tumors exhibit increased anaplasia and proliferation over grade II tumors and are more rapidly fatal; grade IV tumors exhibit more advanced features of malignancy, including vascular proliferation and necrosis, and as they are recalcitrant to radio/chemotherapy they are generally lethal within 12 mo. This review focuses on tumors of the astrocytic series, emphasizing grade IV GBM.

On the basis of clinical presentation, GBMs have been further subdivided into the primary or secondary GBM subtypes. Primary GBMs account for the great majority of GBM cases in older patients, while secondary GBMs are quite rare and tend to occur in patients below the age of 45 yr. Primary GBM presents in an acute de novo manner with no evidence of a prior symptoms or antecedent lower grade pathology. In contrast, secondary GBM derives consistently from the progressive transformation of lower grade astrocytomas, with ~70% of grade II gliomas transforming into grade III/IV disease within 5–10 yr of diagnosis. Remarkably, despite their distinct clinical histories, primary and secondary GBMs are morphologically and clinically indistinguishable as reflected by an equally poor prognosis when adjusted for patient age. However, although these GBM subtypes achieve a common phenotypic endpoint, recent genomic profiles have revealed strikingly different transcriptional patterns and recurrent DNA copy number aberrations between primary and secondary GBM as well as new disease subclasses within each category (as discussed below; Maher et al. 2006; Phillips et al. 2006). These molecular distinctions make obvious the need to change the current standardized clinical management of these truly distinct diseases toward one of rational application

of targeted therapies to appropriate molecular subclasses.

Immunohistochemical markers are important and rapidly evolving tools in the classification and neuropathological diagnosis of malignant gliomas. Currently, the most clinically useful and specific of these markers for classification of gliomas are GFAP and OLIG2. GFAP is universally expressed in astrocytic and ependymal tumors and only rarely in oligodendroglial lineage tumors. OLIG2, a more recently discovered stem/progenitor and oligodendroglial marker, is CNS specific and is universally and abundantly expressed in all diffuse gliomas, but is rarely expressed at such high levels in other types of gliomas and CNS malignancies (Ligon et al. 2004; Rousseau et al. 2006). These markers thus serve as effective tools for unequivocal identification of gliomas and their distinction from non-CNS tumors while aiding the pathologist in distinction of different glioma classes.

A recently expanded collection of novel markers has emerged from numerous avenues of research and holds potential to be deployed to improve classification and inform the potential clinical course of glioma patients. Of particular interest are newly discovered stem and progenitor cell markers that, once clinically validated, may aid in the differential diagnosis of these tumors as well as monitoring their responses to therapy. Intensive research efforts are attempting to uncover agents that may target subpopulations of these cells with high tumorigenic potential and increased resistance to current therapies. Along these lines, the cell surface marker, CD133, and other markers of stem cells, such as Nestin and Musashi, have been shown to negatively correlate with outcome parameters. These newly discovered markers suggest that pathologists will soon have at their disposal highly useful tools for improved clinical diagnosis and classification of gliomas.

Immunohistochemical markers have also recently been shown to aid in prediction of the clinical course for certain classes of tumors. GBMs with intact expression of the PTEN (phosphatase and tensin homolog deleted on chromosome 10) and EGFRvIII proteins (for details, see next section) correlated with increased epidermal growth factor receptor (EGFR) inhibitor response and progression-free survival compared with those tumors expressing EGFRvIII but lacking PTEN (Mellinghoff et al. 2005). Also, patients with EGFR protein expression, mutant or wild-type, have been identified for the sake of targeting EGFR therapy to the appropriate patient population. Furthermore, a powerful and widely used molecular marker—combined loss of the short arm of chromosome 1 and the long arm of chromosome 19—is already widely used in the management of oligodendroglial gliomas, but its role in the evaluation of astrocytic gliomas such as GBM is not yet well defined (Reifenberger and Louis 2003; Louis et al. 2007). With the wealth of accumulating profiling and genomic data, an increase in confidence is merited that useful diagnostic, prognostic, and drug response biomarkers will be incorporated into routine clinical management of GBM in the near future.

Tumor biological processes and known underlying genetic alterations in astrocytic gliomas

The classical genetic alterations in glioma target pathways governing cellular proliferation, cellular survival (apoptosis and necrosis), invasion, and angiogenesis. The following subsections cover these hallmark biological processes and their links to specific genetic aberrations and associated signaling pathways (Figs. 1, 2).

Cell cycle dysregulation and enhanced glioma cell proliferation

Frequent mutations of cell cycle regulatory genes in glioma have underscored the importance of these genes in cellular proliferation and senescence. The RB and p53 pathways, which regulate the cell cycle primarily by governing the G1-to-S-phase transition, are major targets of inactivating mutations in GBM. The absence of these cell cycle guardians renders tumors particularly susceptible to inappropriate cell division driven by constitutively active mitogenic signaling effectors, such as phosphoinositide 3′-kinase (PI3K) and mitogen-activated protein kinase (MAPK).

The Rb pathway In quiescent cells, hypophosphorylated RB blocks proliferation by binding and sequestering the E2F family of transcription factors, which prevents the transactivation of genes essential for progression through the cell cycle (Sherr and McCormick 2002). Upon mitogenic stimulation, the activation of the MAPK cascade leads to the induction of cyclin D1 and its association with the cyclin-dependent kinases CDK4 and CDK6, as well as the degradation of the CDK2/cyclin E inhibitor, $p27^{Kip1}$ (Albanese et al. 1995; Lavoie et al. 1996; Aktas et al. 1997). These activated CDK complexes in turn phosphorylate RB, enabling E2F transactivation of its direct transcriptional targets governing S-phase entry and progression (Weinberg 1995; Frolov and Dyson 2004).

Gliomas circumvent RB-mediated cell cycle inhibition through any of several genetic alterations. The *Rb1* gene, which maps to chromosome 13q14, is mutated in ~25% of high-grade astrocytomas and the loss of 13q typifies the transition from low- to intermediate-grade gliomas (James et al. 1988; Henson et al. 1994). Moreover, amplification of the *CDK4* gene on chromosome 12q13-14 accounts for the functional inactivation of RB in ~15% high-grade gliomas, and *CDK6* is also amplified but at a lower frequency (Reifenberger et al. 1994; Costello et al. 1997). RB activity is also frequently lost through the inactivation of a critical negative regulator of both CDK4 and CDK6, $p16^{Ink4a}$ (Serrano et al. 1993). This gene is one of two transcripts generated at the *CDKN2A* locus on chromosome 9p21 (in addition to $p14^{ARF}$ [alternate reading frame p14]; see below), which is predominantly inactivated by allelic loss or hypermethylation in 50%–70% of high-grade gliomas and ~90% of cultured glioma cell lines (Jen et al. 1994; Schmidt et al. 1994; Merlo et al. 1995; Costello et al. 1996; Fueyo et al. 1996). Consistent with its role as an

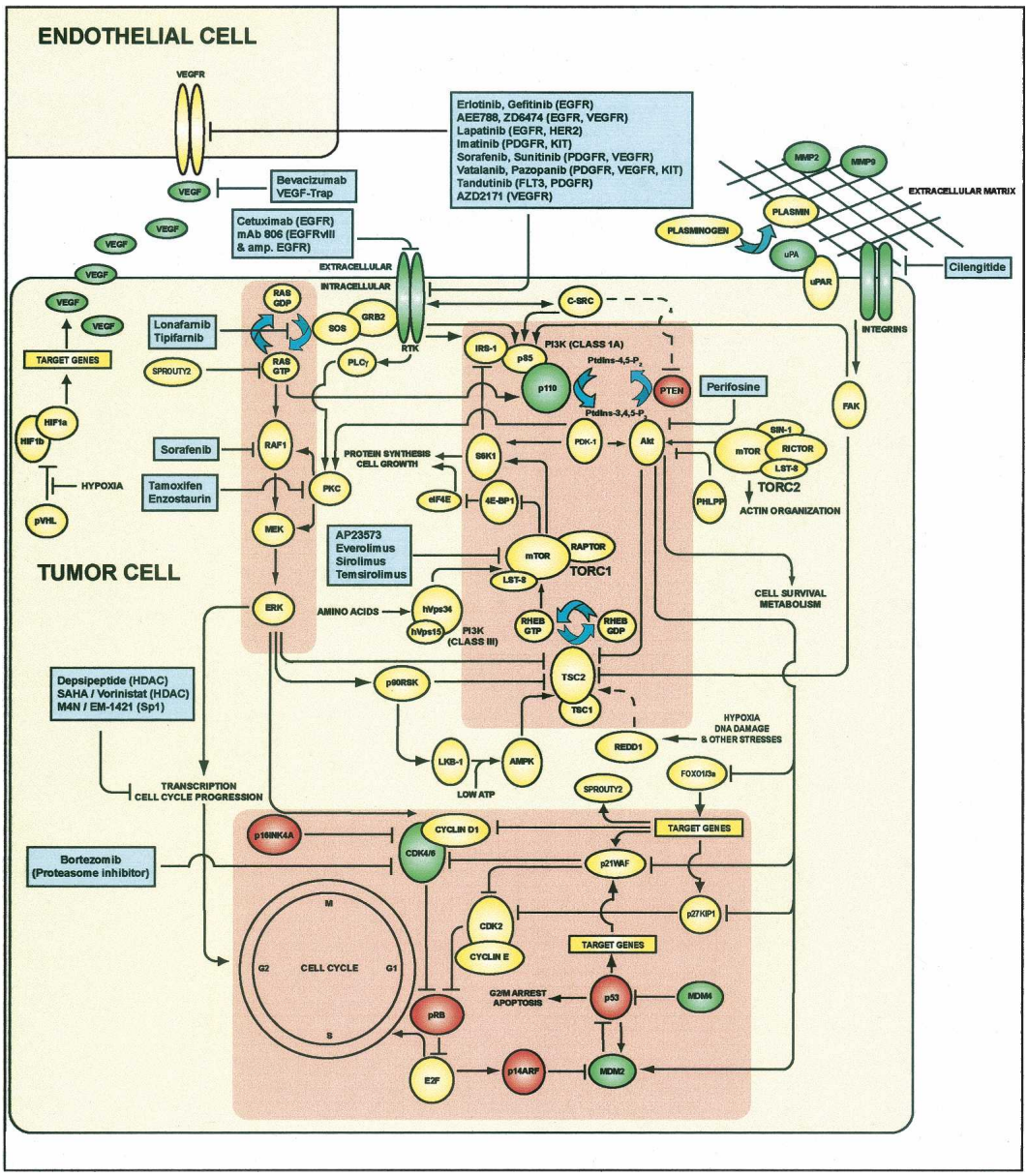

Figure 2. Genetic alterations characteristic of astrocytic glioma lead to aberrant activation of key pathways involved in mitogenic signaling and cell cycle control. Certain proto-oncogenes (shown in green) such as EGFR and PIK3CA (p110α) are activated by mutation, while other growth-promoting genes (also green) are commonly overexpressed. Tumor suppressor genes that are either lost or inactivated by mutation are shown in red. Knowledge of glioma genetics has driven the development of therapeutic agents (listed in blue boxes) that specifically target these pathways—both those intrinsic to the tumor cells and those that impact on the surrounding endothelium and extracellular matrix to direct glioma angiogenesis and invasion. Direct signaling connections, such as post-translational modification of target proteins, are shown in solid lines, while dashed lines represent indirect or uncharacterized interactions. The major mitogenic signaling modules downstream from RTKs (RAS-MAPK and PI3K-mTOR) and the cell cycle machinery are frequently dysregulated in glioma and are highlighted (see the text for details). (AKT) Murine thymoma viral oncogene homolog; (AMPK) AMP-dependent protein kinase; (c-src) sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; (ERK) extracellular signal-regulated kinase; (eIF4E) eukaryotic initiation factor 1; (4EBP1) eIF4E-binding protein 1; (HDAC) histone deacetylase; (mdm-2,4) murine double minute 2,4; (MEK) mitogen-activated protein kinase kinase; (mTOR) mammalian target of rapamycin; (p90RSK) p90 ribosomal protein S6 kinase; (PLCγ) phospholipase Cγ; (pRb) retinoblastoma protein; (RAF1) v-raf1 murine leukemia viral oncogene homolog 1; (RAS) rat sarcoma viral oncogene homolog; (REDD1) regulated in development and DNA damage responses; (RHEB) Ras homolog enriched in brain; (S6K1) p70 ribosomal protein S6 kinase 1; (TORC1,2) mTOR complex1,2.

important glioma tumor suppressor, $p16^{Ink4a}$ is also a critical inhibitor of progenitor cell renewal in the subventricular zone of aging mice (Molofsky et al. 2006). The importance of the inactivation of the RB pathway in glioma progression is evidenced by the near-universal and mutually exclusive alteration of RB pathway ef-

fectors and inhibitors in both primary and secondary GBM (Schmidt et al. 1994; Ueki et al. 1996). However, numerous in vitro and in vivo assays have demonstrated that the neutralization of this pathway alone is insufficient to abrogate cell cycle control to the extent needed for cellular transformation, suggesting that other important cell cycle regulation pathways complement its activities in preventing gliomagenesis (Holland et al. 1998a,b; Rich et al. 2001; Sonoda et al. 2001; Bachoo et al. 2002; Huang et al. 2002; Uhrbom et al. 2002, 2005; Xiao et al. 2002).

The p53 pathway The p53 tumor suppressor prevents the propagation of cells with unstable genomes, predominantly by halting the cell cycle in the G1 phase or instigating a program of apoptosis or proliferative arrest (Vousden and Lu 2002). P53 achieves these ends primarily through its function as a transcription factor: Upon being post-translationally modified by various genotoxic and cytotoxic stress-sensing agents, p53 is stabilized, then binds and transcriptionally regulates the promoters of >2500 potential effector genes (Hoh et al. 2002; Levine et al. 2006). The best characterized of these effectors is the transcriptional target *CDNK1A,* which encodes the protein for the CDK2 inhibitor p21 (El-Deiry et al. 1993; Harper et al. 1993). Although this gene has not been found to be genomically altered in gliomas, its expression is frequently abrogated by p53 functional inactivity as well as by mitogenic signaling through the PI3K and MAPK pathways.

The p53 pathway is nearly invariably altered in sporadic gliomas: Loss of p53, through either point mutations that prevent DNA binding or loss of chromosome 17p, is a frequent and early event in the pathological progression of secondary GBM (Louis 1994; Louis and Cavenee 1997). The importance of p53 in gliomagenesis is also underscored by the increased incidence of gliomas in Li-Fraumeni syndrome, a familial cancer-predisposition syndrome associated with germline *p53* mutations (Malkin et al. 1990; Srivastava et al. 1990). This genetic linkage has been reinforced by a glioma-prone condition in mice engineered with a commonly observed Li-Fraumeni *p53* mutation (Olive et al. 2004) as well as in $p19^{ARF}$-null mice, albeit at a low frequency (Kamijo et al. 1999).

The finding that a second promoter drives an alternatively spliced transcript at the *CDKN2A* locus prompted the discovery of an additional tumor suppressor gene that is inactivated at this locus (Quelle et al. 1995). The second protein encoded by *CDKN2A,* $p14^{ARF}$, was subsequently shown to be an important accessory to p53 activation under conditions of oncogenic stress due to its neutralization of the p53 ubiquitin ligase, MDM2 (Kamijo et al. 1998; Pomerantz et al. 1998; Stott et al. 1998; Honda and Yasuda 1999), an oncogene originally discovered amplified as double minute chromosomes in a spontaneously transformed murine cell line, and then later found to be a key negative regulator of p53 during normal development and in tumorigenesis (Fakharzadeh et al. 1991; Momand et al. 1992; Oliner et al. 1993; Jones et al. 1995; Montes de Oca Luna et al. 1995; Honda et al. 1997; Fang et al. 2000; Honda and Yasuda 2000). Concordantly, the chromosomal region containing *MDM2,* 12q14-15, is amplified in ~10% of primary GBM, the majority of which contain intact *p53* (Reifenberger et al. 1994). The discovery of the *MDM2*-related gene, *MDM4* (chromosome 1q32), which inhibits *p53* transcription and enhances the ubiquitin ligase activity of MDM2, prompted the finding that the p53 pathway is also inactivated by the amplification of MDM4 in 4% of GBM with neither *TP53* mutation nor *MDM2* amplification (Shvarts et al. 1996; Riemenschneider et al. 1999; Gu et al. 2002; Linares et al. 2003). Additionally, the recently discovered tumor suppressor gene *CHD5* (chromodomain helicase DNA-binding domain 5), which maps to chromosome 1p36 and is therefore frequently hemizygously deleted in those human gliomas that have 1p loss, has been shown to maintain p53 levels by facilitating expression of $p19^{Arf}$ (mouse $p14^{Arf}$ ortholog), and thus presents an additional mechanism for inactivation of this critical pathway (Bagchi et al. 2007).

Mitogenic signaling pathways Many mitogens and their specific membrane receptors are present in overactive form in gliomas. Proliferation of normal cells requires activation of mitogenic signaling pathways through diffusible growth factor binding, cell–cell adhesion, and/or contact with extracellular matrix (ECM) components. These signals are transduced intracellularly by transmembrane receptors that typically activate the PI3K and MAPK signaling pathways. In contrast, tumor cells acquire genomic alterations that greatly reduce their dependence on exogenous growth stimulation, enabling their inappropriate cell division, survival, and motility through the constitutive activation of these pathways. While gliomas overcome the normal impositions on the control of mitogenic signaling through multiple mechanisms, activation of receptor tyrosine kinases (RTKs), discussed in detail below, appears to be the predominant mechanism.

MAPK Proliferation signals can be transduced by the MAPK pathway by both integrins and RTKs. Integrins are membrane-bound ECM receptors that mediate the interaction between the ECM and the cytoskeleton. Upon adhesion to ECM, integrins bind cytoplasmic anchor proteins that coordinate the binding of integrins to actin filaments, thus creating a focal adhesion complex. Multiple molecules of focal adhesion kinase (FAK) cluster at these complexes and become activated by cross-phosphorylation, whereupon FAK activates a signal transduction cascade that leads to extracellular signal-regulated kinase (ERK) phosphorylation either through activation of Ras by the recruitment of the adaptor protein Grb2 and the Ras guanine nucleotide exchange factor SOS to phospho-FAK at the plasma membrane, or through Src-dependent phosphorylation of p130Cas (Schlaepfer et al. 1994, 1997; Schlaepfer and Hunter 1997). Ras-GTP in turn phosphorylates Raf kinase, which phosphorylates MEK, which phosphorylates ERK, which enters the nucleus and phosphorylates nuclear

transcription factors that induce the expression of genes promoting cell cycle progression, such as cyclin D1. RTKs activate the MAPK pathway when activated by growth factor signaling, mutation, or overexpression. As discussed in more detail below, RTK activation results in receptor dimerization and cross-phosphorylation, creating binding sites for adaptor protein complexes such as Grb2/SOS, which in turn activates Ras. While constitutively activated, mutated forms of Ras are found in ~50% of all human tumors, few Ras mutations have been found in gliomas. Despite this, high levels of active Ras-GTP are found in advanced astrocytomas (Guha et al. 1997), suggesting that a more relevant mechanism for MAPK-dependent mitogenic signaling in GBM is through inappropriate activation of RTKs and/or integrins.

PI3K/PTEN/AKT The class I PI3Ks catalyze the mitogen-stimulated phosphorylation of phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] to produce PtdIns(3,4,5)P_3. This creates docking sites for a multitude of signaling proteins containing domains capable of binding either to PtdIns(3,4,5)P_3 itself or to the 5-dephosphorylated product, PtdIns(3,4)P_2 (for reviews, see Vanhaesebroeck et al. 2001; Hawkins et al. 2006). The class IA PI3Ks are heterodimers that are recruited to activated RTKs and adaptor proteins via their regulatory subunit, of which there are five isoforms encoded by three genes: p85α, p55α, and p50α (*PIK3R1*); p85β (*PIKR2*); and p55γ (*PIKR3*).

Since the regulatory subunits appear thus far to be functionally equivalent, the class IA PI3Ks are currently defined by the catalytic isoform present: p110α, p110β, and p110δ, encoded by the *PIK3CA, PIK3CB,* and *PIK3CD* genes, respectively (Hawkins et al. 2006). Evidence for the importance of p110α in transformation derives from the discovery of a *vPIK3CA* oncogene in avian sarcoma virus with potent transforming activity in chicken embryo fibroblasts (CEFs) (Chang et al. 1997). *PIK3CA* gain-of-function point mutants have been detected in a variety of cancers, including malignant gliomas such as GBM, in which the frequency of mutation has been cited in some studies to be as high as 15% (Samuels et al. 2004; Gallia et al. 2006). Elevated expression of the *PIK3D* gene has also been reported in GBM (Knobbe and Reifenberger 2003; S. Kang et al. 2006).

In addition to p85 binding, the p110 subunits can also be activated by binding to GTP-bound Ras (Rodriguez-Viciana et al. 1994, 1996). Recently, the study of knock-in mice bearing a p110α point mutant that is unable to bind Ras has revealed that this interaction is essential both for normal development and for Ras-driven tumorigenesis, as assessed both by transformation of mouse embryonic fibroblasts (MEFs) by H-Ras and using a mouse model of K-ras-induced lung adenocarcinomas (Gupta et al. 2007).

The action of class I PI3K enzymes is directly antagonized by the PtdIns(3,4,5)P_3 3-phosphatase encoded by the PTEN gene located at 10q23.3 (Li et al. 1997; Steck et al. 1997; Maehama and Dixon 1998). PTEN is a major tumor suppressor that is inactivated in 50% of high-grade gliomas by mutations or epigenetic mechanisms, each resulting in uncontrolled PI3K signaling in these tumors (Knobbe and Reifenberger 2003; Ohgaki et al. 2004). In mouse models, brain-specific inactivation of PTEN caused overgrowth of the mouse brain and aberrant proliferation of astrocytes both in vivo and in vitro (Fraser et al. 2004). An elegant mouse model of astrocytoma has been developed in which the Rb family proteins are inactivated by GFAP-directed expression of SV40 T antigen (Xiao et al. 2002). In this model system, PTEN inactivation was associated with increased angiogenesis—a close parallel to the progression of high-grade disease in humans coincident with loss of *PTEN* (Xiao et al. 2002, 2005). While regulation of PI3K signaling is critical to controlling cell growth and survival, a number of recent studies have pointed to additional levels at which PTEN may act to suppress transformation and tumor progression. Differentiated and quiescent cells harbor high levels of nuclear PTEN, which appears to fulfill important roles in the maintenance of genomic integrity, through centromere stabilization and promotion of DNA repair (Shen et al. 2007). Importantly, a number of *PTEN* point mutations found in familial cancer predisposition syndromes have no effect on enzyme activity but instead lie within sequences important for regulating PTEN localization. Analysis of such mutants has confirmed that aberrant sequestration of PTEN into either the nucleus or the cytoplasm compromises its tumor suppressor function (Denning et al. 2007; Trotman et al. 2007).

Of the many signaling proteins that are recruited to the membrane and activated by binding to PtdIns(3,4,5)P_3, the phosphoinositide-dependent kinase (PDK1) and Akt/PKB (also the cellular homolog of a viral oncoprotein), are required for tumorigenesis in $PTEN^{+/-}$ mice and for growth of $PTEN^{-/-}$ embryonic stem (ES) cells as tumors in nude mice (Stiles et al. 2002; Bayascas et al. 2005; Chen et al. 2006). In response to PI3K activation, PDK1 and the mammalian target of rapamycin (mTOR, acting in the rapamycin-insensitive TORC2 complex) activate Akt via phosphorylation of two key residues, T308 and S473, respectively (Mora et al. 2004; Sarbassov et al. 2005). Assessment of the phosphorylation status of these residues is often the method of choice for monitoring PI3K pathway activity in cell lines and primary tumors, including GBM samples, 85% of which have been reported to display activated Akt (Wang et al. 2004). In addition to aberrant PI3K signaling, there are a number of other possible mechanisms by which Akt activation may become dysregulated in GBM. PHLPP (PH domain leucine-rich repeat protein phosphatase), which dephosphorylates S473, is expressed at very low levels in certain GBM cell lines, as is CTMP (C-terminal modulator protein), which binds to Akt and inhibits its phosphorylation (Maira et al. 2001; Knobbe et al. 2004; Gao et al. 2005). PIKE-A, a small GTPase highly expressed in GBMs and glioma cell lines, binds directly to phosphorylated Akt and enhances its anti-apoptotic function (Ahn et al. 2004; Knobbe et al. 2005).

Akt phosphorylates many proteins involved in the regulation of cell growth, proliferation, metabolism, and apoptosis. A recent study on v-H-ras-induced transformation of MEFs and skin carcinogenesis indicates that activation of mTOR in the rapamycin-sensitive TORC1 complex via inhibition of the TSC2 tumor suppressor is a key pro-oncogenic function of Akt (Skeen et al. 2006). Since mutant *H-ras* is seldom seen in human tumors, it will be important to determine whether Akt/TSC/TORC1 signaling is similarly required downstream from glioma-relevant perturbations, such as *EGFR* mutation and overexpression and/or *PTEN* loss. Evidence that this may indeed be the case is provided by the efficacy of PI-103, a small molecule inhibitor of both p110α and mTOR, which potently blocks the growth of glioma cell lines and of U87EGFRvIII xenografts following subcutaneous injection in nude mice, without discernable toxicity to the animals (Fan et al. 2006). The use of *TSC2*$^{-/-}$ cells, which display constitutive phosphorylation of the TORC1 substrates S6K1 and 4E-BP1, revealed the existence of a negative feedback loop, whereby inhibitory phosphorylation of the insulin receptor substrate (IRS-1) by S6K1 causes a reduction in Akt activation (Harrington et al. 2004; Shah et al. 2004; Riemenschneider et al. 2006; Shah and Hunter 2006). Treatment of glioma cells with TORC1-specific inhibitors, such as rapamycin, disrupts such feedback control, resulting in increased Akt activity (Fan et al. 2006). Dual inhibition of PI3K and TORC1 by PI-103 overcomes these problems and likely explains its increased efficacy.

In addition, phosphorylation of the FOXO transcription factors by Akt, which promotes their exclusion from the nucleus, reduces the expression of a number of important target genes, including the CDK inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ (both of which are also directly targeted by Akt) and the RB family member p130 (Medema et al. 2000; Kops et al. 2002; Seoane et al. 2004). Given the recent data illustrating context-specific actions of FOXO on various targets in different cell types and tissues, it may be prudent to validate these FOXO targets specifically in glioma (Paik et al. 2007).

PI3K–MAPK–p53–RB pathway interactions While the PI3K, MAPK, p53, and RB pathways are often considered as distinct entities, there is significant cross-talk among the pathways that serve to reinforce the inappropriate regulation of any single pathway perturbation. For example, because p53 enhances *PTEN* transcription and represses the expression of p110α (Stambolic et al. 2001; Singh et al. 2002), the loss of p53 in cells with constitutively active RTK signaling can further potentiate PI3K pathway activation. Therapies aimed at reactivating p53 in GBM may be compromised by MAPK and PI3K intervention in the activity of p53 and its effectors. MAPK signaling activates c-myc, which binds the miz-1 transcriptional repressor to block *p21* gene induction (Herold et al. 2002; Seoane et al. 2002), while Akt impacts on p53 function by phosphorylation of Mdm2 (Zhou et al. 2001; Shin et al. 2002; Feng et al. 2004) in addition to the direct inhibition of p21 discussed earlier. Moreover, these pathways can negate each other: p53 can inhibit activated FOXOs by inducing the expression of the kinase SGK1, which phosphorylates and exports FOXOs from the nucleus (You et al. 2004). Conversely, FOXOs can inhibit p53 transcriptional activity by increasing its association with nuclear export receptors that translocate it to the cytoplasm (You et al. 2006). The recent finding that Sprouty2, a gene involved in suppression of Ras signaling during oncogene-induced senescence, is also a direct transcriptional target of FoxO emphasizes the complexity of cross-talk that exists between the Ras/MAPK and PI3K pathways (Courtois-Cox et al. 2006; Paik et al. 2007). The complicated interplay among these critical molecules highlights the need for detailed dissection of the pathways that are aberrant in each tumor to accurately guide the choice of combination therapies that can simultaneously target multiple pathways.

RTKs Gliomas may activate receptor-driven pathways by different mechanisms: overexpression of both ligands and receptors leading to an autocrine loop, genomic amplification, and/or mutation of the receptor leading to constitutive activation in the absence of ligand. TheEGF and platelet-derived growth factor (PDGF) pathways play important roles in both CNS development and gliomagenesis, and targeted therapy against these potentially critical signaling pathways is currently under vigorous basic and clinical investigation.

EGFR *EGFR* gene amplification occurs in ~40% of all GBMs, and the amplified genes are frequently rearranged (Libermann et al. 1984, 1985; Ekstrand et al. 1991; Wong et al. 1992; Louis et al. 2007). An *EGFR* mutant allele with deletion of exons 2–7 (known variously as EGFRvIII, ΔEGFR, or EGFR*) occurs in 20–30% of all human GBM (and in 50%–60% of those that have amplified wild-type EGFR), making it the most common *EGFR* mutant (Sugawa et al. 1990; Frederick et al. 2000). EGFRvIII is a highly validated glioma target as evidenced by the capacity of activated EGFR mutants to enhance tumorigenic behavior of human GBM cells by reducing apoptosis and increasing proliferation (Nishikawa et al. 1994; Nagane et al. 1996; Huang et al. 1997; Narita et al. 2002) and to malignantly transform murine *Ink4a/Arf*-null neural stem cells (NSCs) or astrocytes in the mouse brain (Holland et al. 1998a; Bachoo et al. 2002). Thus, EGFR has been a prime target for therapeutic intervention in GBM with small molecule kinase inhibitors, antibody-based immunotherapy and immunotoxins (Lorimer et al. 1995; Mishima et al. 2001; Nagane et al. 2001; Jungbluth et al. 2003), and, more recently, small interfering RNA (siRNA)-directed neutralization of either wild-type *EGFR* or the unique junction present in the *EGFRvIII* allele (Fan and Weiss 2005; C.S. Kang et al. 2006).

Transcriptional profiles of GBM with EGFR overexpression have revealed distinct gene expression profiles that have enabled classification of molecular subgroups among phenotypically undistinguishable tumors (Mischel et al. 2003). Along similar lines, immunohistochemical studies have demonstrated that GBM could be

stratified according to PI3K pathway activation status and that these activation profiles are associated with EGFRvIII expression and *PTEN* loss (Choe et al. 2003). Such efforts to stratify patients appear to be important in the optimal deployment of small molecule EGFR inhibitors as only a small fraction of GBM patients show meaningful responses to such agents (Rich et al. 2004; Lassman et al. 2005). Thus far, in responsive cases, patients with coexpression of EGFRvIII (Mellinghoff et al. 2005) or wild-type EGFR (Haas-Kogan et al. 2005), together with PTEN presence or low Akt activation levels in their GBM cells, exhibited the most favorable outcomes to EGFR inhibitors. In accordance with findings of multiple activated pathways in GBM, addition of the mTOR inhibitor, rapamycin, has been shown to enhance the sensitivity of PTEN-deficient tumor cells to the EGFR kinase inhibitor, erlotinib (Fan et al. 2003; Goudar et al. 2005; Wang et al. 2006). Consistent with enhanced apoptosis resistance by EGFRvIII, activated EGFR has also been shown to confer radio- and chemo-resistance to GBM cells (Nagane et al. 1998; Chakravarti et al. 2002). These experimental observations and the capacity of EGFR inhibitors or dominant-negative EGFR-CD533 to sensitize GBM cells to radiation and chemotherapeutic agents (Nagane et al. 2001; Stea et al. 2003; Lammering et al. 2004; Sarkaria et al. 2006) predict that disruption of EGFR function at the time of ionizing radiation and subsequent chemotherapy, instead of at the time of recurrence, would improve therapeutic outcome (Nyati et al. 2006). These results, coupled with the recent identification of EGFR-activating ectodomain mutations in ~14% of GBMs that convey sensitivity toward erlotinib (Lee et al. 2006), are beginning to detail tumor molecular profiles and therapeutic regimens that will best benefit patients with EGF receptor and downstream pathway genetic lesions.

PDGF receptor (PDGFR) In addition to the EGFR signaling axis, PDGFRα and its ligands, PDGF-A and PDGF-B, are expressed in gliomas, particularly in high-grade tumors, while strong expression of PDGFRβ occurs in proliferating endothelial cells in GBM (Hermanson et al. 1992; Plate et al. 1992; Westermark et al. 1995; Di Rocco et al. 1998). PDGF-C and PDGF-D, which require proteolytic cleavage for activity, are also frequently expressed in glioma cell lines and in GBM tissues (Lokker et al. 2002). In contrast to *EGFR,* amplification or rearrangement of *PDGFR*α is much less common, and a relatively rare oncogenic deletion mutation of *PDGFR*α (loss of exons 8 and 9) has been described (Clarke and Dirks 2003) that, similar to EGFRvIII, is constitutively active and enhances tumorigenicity. Given the tumoral coexpression of PDGF and PDGFR, autocrine and paracrine loops may be the primary means by which this growth factor axis exerts its effects. Supportive evidence for a paracrine circuitry initiated by PDGF-B secretion that enhances glioma angiogenesis has been shown through stimulation of endothelial cells displaying PDGFRβ, in part, to express VEGF (Guo et al. 2003). Besides glial precursor cells, NSCs in the adult subventricular zone have been shown to express PDGFRα and PDGF could stimulate these NSCs to form glioma-like lesions in the mouse (Jackson et al. 2006). Furthermore, mice transgenic for neural progenitor PDGF-B expression resulted in the formation of oligodendrogliomas and forced elevation of PDGF-B levels increased overall tumor incidence (Dai et al. 2001; Shih et al. 2004), suggesting that targeted therapy against this pathway could have therapeutic potential (Shih and Holland 2006). To this end, an orally active kinase inhibitor of the 2-phenylaminopyrimidine class such as STI571 (imatinib mesylate, Gleevec) has been shown to be a potent inhibitor of these oncogenic loops (Kilic et al. 2000; Hagerstrand et al. 2006) and, when combined with hydroxyurea in a phase II study, has been shown to achieve durable anti-tumor activity in some patients with recurrent GBM (Reardon et al. 2005); in contrast, when used alone, imatinib has demonstrated minimal activity in malignant glioma (see below; Table 1; Wen et al. 2006).

RTK coactivation and cooperation One additional potential explanation for the failure of EGFR and PDGFR inhibitors to elicit significant clinical outcomes is that additional RTKs may cooperate to provide a signaling threshold that prevents the inhibition of mitogenic and survival signals through the inactivation of any single RTK. This hypothesis is supported by recent work that demonstrates that multiple RTKs in addition to EGFR and PDGFR are activated simultaneously in primary GBM patient samples (Stommel et al. 2007), and oncogenic signaling, survival, and anchorage-independent growth were not fully abrogated until cell lines with endogenous coactivation of RTKs were treated with pharmacological agents or siRNAs targeting at least three different receptors. Importantly, these effects were observed irrespective of *PTEN* status, indicating that the presence of this tumor suppressor may not be a critical determinant of therapeutic success as long as upstream signaling effectors are sufficiently inhibited. The discovery of receptor coactivation or cooperation suggests that tumor RTK profiling may be an important step in the development of a personalized GBM therapeutic regimen. Another study (Huang et al. 2007) showed that glioma cells engineered to overexpress EGFRvIII to levels observed in GBM caused increased c-MET phosphorylation that was dependent on the kinase activity and levels of this mutant EGFR. The cross-talk between the receptors could be targeted with specific inhibitors to both, resulting in enhanced cytotoxicity of EGFRvIII-expressing cells compared with either compound alone. It appears that the initially disappointing clinical trials using RTK-targeted agents in GBM should be reanalyzed with respect to the RTK activation profiles of the responders and nonresponders, and that future trials could take RTK coactivation into account when selecting combination inhibitor regimens.

Apoptosis

A hallmark feature of malignant glioma cells is an intense resistance to death-inducing stimuli such as radio-

Table 1. *Inhibitors being used in clinical trials and their targets*

	Inhibitor target	Mono-therapy	Combined therapy
RTK	EGFR	Erlotinib (Tarceva)	Erlotinib + radiation, erlotinib + temozolomide, erlotinib + temsirolimus, erlotinib + sorafenib
		Gefitinib (Iressa)	Gefitinib + everolimus
		Cetuximab (Erbitux)	Cetuximab + temozolomide + radiation
	EGFRvIII and amp wtEGFR	mAb 806	
	PDGFR	Imatinib (Gleevec) (PDGFR, c-Kit, Abl)	Imatinib + temozolomid, imatinib + vatalanib + hydroxyurea, imatinib + hydroxyurea
	VEGFR and multi-RTK	AZD2171 (VEGFR, PDGFR, c-Kit)	
		Vatalanib (VEGFR, PDGFR, c-Kit)	
		Sunitinib malate (PDGFR, VEGFR1/2, c-Kit)	
		AEE788 (EGFR, VEGFR1/2)	
		ZD6474 (EGFR, VEGFR2/3)	
		Lapatinib (EGFR, HER2)	
		Sorafenib (RAF, VEGFR2/3, PDGFR, c-Kit)	Sorafenib + temsirolimus, sorafenib + (Temsirolimus, Tipifarnib or Erlotinib)
		Pazopanib (VEGFR, PDGFR, Kit)	Pazopanib + lapatanib
		Tandutinib (FLT3, PDGFR)	
Ligand	VEGF	Bevacizumab (ligand)	Bevacizumab + irinotecan
		VEGF-Trap (ligand)	
Signal transduction	Akt	Perifosine	
	PKC	Tamoxifen	Tamoxifen + bortezomib
		Enzastaurin	
	mTOR	AP23573	
		Everolimus	Everolimus + temozolomide
		Sirolimus	
		Temsirolimus	Temsirolimus + temozolomide + radiation
Protein modification	HDAC	Suberoylanilide hydroxamic acid (SAHA, Vorinostat)	SAHA + temozolomide
	Farnesyltransferase	Tipifarnib	
		Lonafarnib	Lonafarnib + temozolomide, lonafarnib + temozolomide
		Depsipeptide	
Other	αvβ3 Integrin	Cilengitide	Cilengitide + radiation
	Steroid receptors	Synthetic retinoids (e.g., all-*trans* and 13-*cis* retinoic acid)	
	Proteosome	Bortezomib (Velcade)	
	Sp1 transcription factor	Tetra-O-methyl nordihydroguaiaretic acid	

Various drugable molecules and pathways implicated in glioma are being targeted by mono and combined therapeutic approaches. For detailed information, see the text and http://www.clinicaltrials.gov. (PKC) Protein kinase C; (HDAC) histone deacetylase.

therapy and chemotherapy. This biological property has been linked to genetic alterations of key regulatory molecules involved in mitogenic signaling, most prominently RTKs and the PI3K–PTEN–Akt signaling axis, as well as regulatory and effector molecules residing in classical cell death networks of both extrinsic (death receptor-mediated) and intrinsic (mitochondria-dependent) apoptosis signaling pathways.

The "death receptors" are cell surface molecules that, upon binding their cognate ligands, recruit adapter molecules to provide a molecular scaffold for the autoproteolytic processing and activation of caspases (for review, see Lavrik et al. 2005). The most important death receptor systems include TNFR1 (DR1/CD120a), TRAILR1 (DR4/APO-2), TRAILR2 (DR5/KILLER/TRICK2), and CD95 (DR2/Fas/APO-1). Several lines of evidence support important roles of these death receptors in glioma pathogenesis. First, various human glioma cell lines and primary glioma-derived cell cultures are sensitive to death ligand-mediated apoptosis in vitro and in xenograft

model systems in vivo (Weller et al. 1994; Roth et al. 1997; Shinoura et al. 1998; Nagane et al. 2000; Maleniak et al. 2001; Rohn et al. 2001). Second, expression levels of these death receptors and in particular of their corresponding (antagonistic) decoy receptors may correlate with susceptibility of glioma cells to death ligand-induced apoptosis. A prominent example is the decoy receptor for CD95 ligand (CD95L), soluble decoy receptor 3 (DcR3). It is expressed on malignant glioma cell lines, and its expression pattern correlates with the grade of malignancy in human glioma specimens (Roth et al. 2001). Interestingly, infiltration of $CD4^+$ and $CD8^+$ T cells and microglia/macrophages was significantly decreased in DcR3-driven xenografts, suggesting that glioma cells may escape CD95L-dependent immune-cytotoxic attack by expressing a decoy receptor that neutralizes CD95L by preventing its interaction with the receptor (Roth et al. 2001).

The TRAIL death receptor system in particular has gained considerable interest as a specific inducer of cancer cell apoptosis as its expression has been positively correlated with survival of patients with primary GBM (Kuijlen et al. 2006). In this regard, loco-regional administration of TRAIL inhibited growth of human glioma cell xenografts (Roth et al. 1999) and acted synergistically with chemotherapeutic drugs (Nagane et al. 2000; Rohn et al. 2001), in part through up-regulation of TRAIL-R2 and Bak protein and down-regulation of the caspase-8-specific inhibitor cFLIPs (LeBlanc et al. 2002; Arizono et al. 2003; J.H. Song et al. 2003). In addition, peptides derived from the second mitochondria-derived activator of caspases (Smac), a potent antagonist of members of the IAP family of caspase inhibitors, acted synergistically with TRAIL to induce tumor cell apoptosis in vitro and in vivo without demonstrable neurotoxicity (Fulda et al. 2002). Mechanistically, these peptides abrogate IAP-binding activity and, consequently inhibition of effector caspase-9, caspase-3, and caspase-7 activity downstream from mitochondrial membrane disintegration, underscoring the importance of post-mitochondrial caspase activation for apoptosis propagation in glioma cell lines and its validity as a therapeutic target (Fulda et al. 2002).

The role of the Bcl-2 family in gliomagenesis has also been extensively studied. On the mechanistic level, classical anti-apoptotic Bcl-2 family members (BAK, BAD, BID, BAX, BCL-X_L, MCL-1) modulate apoptosis signaling by preserving mitochondrial membrane integrity and the release of cytochrome c, which effects the caspase cascade and the apoptotic program (for review, see Green and Kroemer 2004). On the clinical level, there is a correlation between tumor grade and expression of several anti-apoptotic Bcl-2 proteins (BCL-2 and MCL-1) (Weller et al. 1995; Krajewski et al. 1997), and in general, this Bcl-2 "rheostat" is shifted toward an anti-apoptotic balance during the transition from initial to recurrent GBM (Strik et al. 1999). Additionally, Bcl-x_L is up-regulated by overexpression of EGFRvIII in glioma cells and this up-regulation confers resistance to the chemotherapeutic agent cisplatin (Nagane et al. 1998). In addition to their classical roles, Bcl2 family members may contribute to gliomagenesis through enhancement of migration and invasion by altering the expression of a set of metaloproteinases and their inhibitors (Wick et al. 1998, 2001, 2004). Due to their central role and importance in apoptosis signaling, neutralization of anti-apoptotic Bcl-2 proteins by antisense technology (Julien et al. 2000), small molecules that block BcL2 interactions with other families (Fesik 2005), or by viral-mediated delivery of select proapoptotic members (Naumann et al. 2003), may represent promising future avenues of therapeutic intervention.

Necrosis

While highly resistant to therapeutic apoptotic stimuli, GBM tumor cells exhibit the paradoxical propensity for extensive cellular necrosis. Indeed, necrosis is the most prominent form of spontaneous cell death in GBM, presented as foci of micronecrosis surrounded by broad hypercellular zones contiguous with normal tissue or by parenchymal infiltrates (Raza et al. 2002; Brat and Van Meir 2004). While limited blood supply and anoxia due to a microthrombotic process has been identified as an important cause of necrosis, the molecular basis for this necrotic phenotype, particularly in the context of intense apoptotic therapy resistance, has recently come into focus with the discovery and characterization of the Bcl2-like 12 (Bcl2L12) protein.

Bcl2L12 has been shown to be a potent inhibitor of post-mitochondrial apoptosis signal transduction that is significantly overexpressed in primary GBMs (Stegh et al. 2007). Bcl2L12 is a proline-rich protein characterized by a C-terminal 14-amino-acid sequence with significant homology with the BH (Bcl-2 Homology) 2 domain found in several members of the Bcl-2 protein family (Scorilas et al. 2001). Enforced expression of Bcl2L12 in primary cortical astrocytes inhibited apoptosis, and its RNAi-mediated knockdown sensitizes human glioma cell lines to drug-induced apoptosis and reduces tumor formation in an orthotopic transplant model in vivo (Stegh et al. 2007). The anti-apoptotic actions of Bcl2L12 relate significantly to its capacity to neutralize effector caspase activity downstream from mitochondrial dysfunction and apoptosome activity, likely through specific interaction with effector caspase-7 (Stegh et al. 2007). These activities of Bcl2L12 are highly relevant to the necrotic process in the light of studies showing that suppression of caspase activity downstream from mitochondria redirects the death program from apoptosis to necrosis (for review, see Nicotera and Melino 2004), indicating that post-mitochondrial caspase activation acts as a molecular switch between apoptotic and necrotic cell death paradigms (for review, see Nicotera and Melino 2004).

In support of this model, germline deletion of post-mitochondrial apoptosis signaling components, such as the caspase activator Apaf-1, or blockage of effector caspase maturation by pan-specific caspase inhibitors results in decreased apoptosis, yet causes an increase in necrosis (for review, see Nicotera and Melino 2004).

Mechanistically, oxidative phosphorylation and consequently intracellular ATP levels decrease due to extensive cytochrome c release and mitochondrial dysfunction, rendering cells unable to maintain ion homeostasis and provoking cellular edema, dissolution of organelles, and plasma membranes (for review, see Nicotera and Melino 2004). That apoptosis and necrosis signaling pathways are interconnected is evidenced by the ability of enforced Bcl2L12 expression to provoke necrotic cell morphology, as evidenced by substantial plasma membrane disintegration and enhanced nuclear and subcellular organelle swelling in apoptosis-primed astrocytes (Stegh et al. 2007). Therefore, up-regulation of Bcl2L12 as a novel regulator of the apoptosis/necrosis balance in glial cells may represent an important event in malignant glioma pathogenesis.

Angiogenesis

GBMs are among the most highly vascular of all solid tumors. Microvascular hyperplasia, the defining histopathological phenotype of both primary and secondary GBM, consists of proliferating endothelial cells that emerge from normal parent microvessels as tufted microaggregates (glomeruloid bodies) accompanied by stromal elements, including pericytes and basal lamina (Stiver et al. 2004). Microvascular density, a measure of microvascular proliferation, is an independent prognostic factor for adult gliomas (Leon et al. 1996; Birlik et al. 2006). The idea that angiogenesis is rate limiting for tumor growth, and therefore a rational therapeutic target, is strongly supported by animal studies that have shown that angiogenesis is vital for macroscopic solid tumor growth (Folkman 2007).

One common feature in the transition from low-grade or anaplastic astrocytomas to secondary GBM is a dramatic increase in microvascular proliferation. An equivalently robust microvasculature proliferation phenotype is observed in primary GBM. Since there are marked genomic differences between primary and secondary GBM (Maher et al. 2006), it is likely that different genetic programs converge on a final common angiogenesis pathway involving HIF and non-HIF-dependent downstream effectors that include positive (VEGF, PDGF, bFGF,IL-8, SDF-1) and negative (thrombospondin1, thrombospondin2, endostatin, tumstatin, interferons) regulators of this process (Nyberg et al. 2005). A comprehensive understanding of the molecular mechanisms driving angiogenesis in GBM will be necessary for the rational development and deployment of anti-angiogenesis therapies. Increasingly, it is becoming evident that tumor-associated angiogenesis is not simply a physiological adaptation to hypoxia as a result of an increasing tumor cell mass. Rather it appears to be the result of critical genetic mutations that activate a transcriptional program for angiogenesis with local tumor oxygen status further modifying this response. The relative contributions of these two mechanisms are not yet fully defined, but it is likely that both may operate to different extents in different tumors or even in different regions of the same tumor. Recently, a number of experimental studies have shown that key glioma-relevant mutations—including those in the *PTEN, EGFR,* and *CMYC* genes—may act as an "angiogenic switch" by stabilizing HIF-1α or one of its downstream targets, VEGF (Watnick et al. 2003; Blum et al. 2005; Phung et al. 2006; Shchors et al. 2006). The distinction between microvascular proliferation being an adaptive response to hypoxia or it being an epiphenomenon of critical genetic mutations that also activate a cascade of proangiogenesis pathways has clinical and therapeutic importance.

Another issue is the functional consequences of tumor angiogenesis, with respect to tissue perfusion (Vogel et al. 2004). Tumor microvessels are highly tortuous with sluggish flow and diminished gradient for oxygen delivery and increasing susceptibility to thrombosis and microhemorrhages (Kaur et al. 2004). Thus, the GBM microvasculature proliferation may provide little support in oxygen/nutrient delivery but rather paradoxically contribute to further exacerbating a metabolic mismatch between the "supply and demand," leading to progressive hypoxia and eventually necrosis. This scenario is supported by the recent experience with anti-angiogenesis drugs, where their limited clinical benefit seems to be the result of "pruning" immature vessel growth and allowing "normalization" of the pre-existing vasculature (see below; Horsman and Siemann 2006). In addition to the poor vascular architecture, endothelial cells associated with the tumor vasculature fail to form tight junctions and have few associated pericytes or astrocytic foot processes leaving the integrity of the BBB compromised, resulting in increased interstitial edema. Interstitial edema may further compromise regional blood flow and exacerbate tumor hypoxia leading to areas of necrosis. In addition to these maladapted biophysical properties of GBM microvasculature, specific genetic mutations in GBM likely contribute to compromised tumor bioenergetics, specifically the shift in energy reduction from oxidative phosphorylation to glycolysis (Elstrom et al. 2004; Fantin et al. 2006). These interrelated mechanisms lead to a level of metabolic demand that may exceed the ability of the cerebrovascular system to maintain adequate blood flow to prevent hypoxia and necrosis. The histological evidence of thrombosis and degenerating vessels with microhemorrhages are a common feature of GBM and likely reflect these biological processes.

Anti-angiogenesis therapies The hypothesis that interruption of blood supply to the tumor will lead to regression or dormancy of the tumor has led to the development of several drugs that target multiple steps in angiogenesis (Table 1; Fig. 2). Currently three approaches are in advanced stages of clinical testing that aim to target VEGF/VEGFR signaling pathways: (1) monoclonal antibodies directed against VEGF or its receptor(s) (Winkler et al. 2004; Vredenburgh et al. 2007), (2) small molecule inhibitors of VEGFR-2 tyrosine kinase activity (Batchelor et al. 2007), and (3) soluble decoy receptors created from VEFGR1 receptor that selectively inhibits VEGF (Folkman 2007). A fourth approach targeting αVβ3

and αVβ5 integrin receptors on endothelial cells (Nabors et al. 2007) is also in early clinical trials as an anti-angiogenesis therapy in GBM.

Clinical studies, in which anti-angiogenesis drugs have been used as "single" agents to treat GBM, have shown little efficacy. This may reflect the fact that these drugs have no direct effect on the pre-existing stable microvasculature that may be co-opted to support tumor growth especially at the infiltrating tumor edge. Recent data, however, suggest that anti-angiogenesis drugs may be more effective when combined with cytotoxic therapy (Table 1). Recently a single-arm phase II study of bevacizumab (Avastin; Genetech, Inc.) (Vredenburgh et al. 2007), a recombinant, humanized monoclonal antibody targeting VEGF, plus irinotecan (CPT-11) in patients with recurrent high-grade gliomas reported dramatic rates (63%) of radiographic response and a near doubling of 6 mo and median progression free survival (PFS) in the patients with GBM (30% and 20 wk, compared with historical controls of 15% and 9 wk). The therapeutic benefits in the setting of combination therapy (radiation and/or conventional chemotherapy) could be attributed to (1) improved drug delivery because of improved vascular flow, (2) improved drug penetration into the tumor because of reduced interstitial pressure, and/or (3) improved radiation/chemotherapy response as a result of reducing tumor hypoxia. Hypoxia is well known to create radiation resistance and reduce efficacy of chemotherapies (Semenza 2003). Overall, the early clinical data for the anti-angiogenic drugs when used in combination with radiation or conventional chemotherapies is encouraging. The possibility that anti-angiogenic drugs may enhance intratumoral concentration of conventional chemotherapeutics raises the intriguing possibility that these drugs may improve the efficacy profile of some of the currently available drugs. A possible mechanism for such synergy could be enhanced drug delivery, although off-target drug effects and/or poorly understood pharmacological mechanisms remain possibilities. The full benefit of anti-angiogenesis will derive from an improved understanding of the molecular basis of tumor angiogenesis process, how tumor cell metabolism drives angiogenesis versus cooptation of normal brain microvascular networks, and definition of those patients that are likely to benefit from various types of anti-angiogenic therapies operating on different levels of the process.

Tumor cell invasion

Infiltration throughout the brain is prominent feature of low- and high-grade malignant glioma (Lefranc et al. 2005) and is the principal basis for the lack of surgical cure. In >90% of cases, the recurrent tumor develops immediately adjacent to the resection margin or within several centimeters of the resection cavity. Invasion by glioma cells into regions of normal brain is driven by a multifactorial process involving cell interactions with the ECM and with adjacent cells, as well as accompanying biochemical processes supportive of proteolytic degradation of ECM and active cell movement. These processes bear a striking resemblance to the robust inherent migration potential of glial cells during embryogenesis (Hatten 1999).

The most frequent route of invasion of glial tumor cells is along white matter tracts and basement membranes of blood vessels. Whether this route offers a path of least resistance or there are biochemical substrates that mediate adhesion and promote migration, or both, is unclear. Invasion and migration of glial tumors differs from other tumors where local spread is very limited and dissemination occurs hematogenously or via the lymphatic system. In fact, glioma cells lack the ability to penetrate the basement membrane of blood vessels (Bernstein and Woodard 1995), and cells gaining access to the blood through a disrupted blood vessel within the tumor are unable to establish robust tumor growth outside the CNS. The molecular basis for this curious inability of glioma cells to metastasize outside of the CNS is not known and warrants further investigation.

Several genes involved in glioma invasiveness have been identified and include members of the family of metalloproteases (MMP) and their endogenous tissue inhibitors (TIMPs). Expression of MMP-2 and, to a lesser extent, MMP-9 correlate with invasiveness, proliferation and prognosis in astrocytomas (M. Wang et al. 2003). Other non-MMP proteases, including urokinase-type plasminogen activator (uPA) (Landau et al. 1994; Yamamoto et al. 1994a,b) and cysteine proteases (e.g., cathepsin B) (McCormick 1993), are elevated in high-grade malignant gliomas (for review, see Uhm et al. 1997). Despite these findings, the role of proteases in glioma invasion remains unclear since low-grade astrocytomas infiltrate diffusely throughout the brain, despite relatively normal levels of the proteases.

Integrins, especially αVβ3 complexes, are elevated in GBM and appear to be relevant to processes of glioma invasion and angiogenesis (Kanamori et al. 2004). Several studies have also reported potential novel glioma invasion genes. Invasion inhibitory protein 45 (*IIp45*), a potential tumor suppressor gene on chromosome 1p36, is frequently down-regulated in GBMs. Its product inhibits invasion through the binding of IGFBP2 (S.W. Song et al. 2003). In contrast, IGFBP2 promotes invasion in GBM by up-regulating a panel of genes involved in invasion, one of which is *MMP-2* (H. Wang et al. 2003). Other proteins are overexpressed in invasive areas of GBM, such as angiopoietin-2, which in addition to its involvement in angiogenesis also plays a role in inducing tumor cell infiltration by activating MMP-2 (Hu et al. 2003). Ephrin receptors and their ligands, the ephrins, mediate neurodevelopmental processes such as axon guidance and cell migration and in glioma have been shown to regulate migration and invasion. Compared with low-grade astrocytoma or normal brain, GBMs, in particular the migratory tumor cells, overexpress EphB2 (Hu et al. 2003). Intriguingly, EphA2 overexpression has been linked to poor survival in GBM (Liu et al. 2006).

Other novel invasion- and migration-associated genes have been identified using oligonucleotide microarray

technology (Demuth and Berens 2004; Tatenhorst et al. 2004) on RNA isolated by laser-captured microdissection of cryostat sections from human glioma biopsy tumor cores and invasive edges. These genes include *P311,* a 68-amino-acid polypeptide that has been described in embryonic neuronal migration (Studler et al. 1993); death-associated protein 3 (*DAP3*), which has been shown to confer protection from Fas-induced, ionizing radiation-induced, and streptonigrin-induced cell death (Kissil et al. 1999); and *FN14,* which encodes a cell surface receptor for the tumor necrosis factor superfamily member named TWEAK, all of which have functionally been shown to modulate glioma cell migration and apoptosis (Taylor et al. 2000; Mariani et al. 2001; Wiley and Winkles 2003).

Since migrating glioma cells show increased levels of phosphorylated Akt, PI3K inhibitors have been tested experimentally on these cells, resulting in a decrease in migration and an increase in apoptosis sensitivity (Joy et al. 2003). In conjunction with this, *PTEN* mutation has been implicated in an invasive phenotype, not only as contributing to deregulated PI3K signaling but also in its ability to stabilize E-cadherin and modulate cell matrix adhesion complexes (Kotelevets et al. 2001). These findings highlight the multitude of ways that gliomagenic lesions effect a broad spectrum of the tumor phenotypes ranging from aberrant cell proliferation to invasion and resistance to apoptosis.

Frontiers in glioma research and therapy

Genomic profiles of GBM

Copy number analysis Comparative genomic hybridization (CGH) analysis of astrocytic tumors has revealed numerous recurrent copy number alterations (CNAs), pointing to the existence of many additional oncogenes or tumor suppressor genes beyond the handful of classical GBM mutation targets described in the previous sections. Conventional and array-based CGH (aCGH) profiling have cataloged the multitude of recurrent CNAs, including gains/amplifications of 1p34-36, 1q32, 3q26-28, 5q, 7q31, 8q24, 11q, 12q13, 13q, 15p15, 17q22-25, 19q, 20p, and 20q and losses/deletions of 3q25-26, 4q, 6q26-27, 9p, 10p, 10q, 11p, 11q, 12q22, 13q, 14q13, 14q23-31, 15q13-21, 17p11-13, 18q22-23, 19q, and 22q (Reifenberger et al. 1994; Nishizaki et al. 2000; Hui et al. 2001; Burton et al. 2002; Nutt et al. 2003; Misra et al. 2005; Nigro et al. 2005; Phillips et al. 2006). These high-resolution studies also revealed new molecular markers of glioma that complement and extend histological (astrocytoma, oligodendroglioma, and GBM) (Kotliarov et al. 2006) and tumor grade classifiers (Nishizaki et al. 2000). This is particularly evident for primary and secondary GBMs, which are histopathologically indistinguishable yet show dramatically different patterns with the majority of recurrent CNAs being unique, rather than overlapping between the two entities. Analysis of these patterns using an unsupervised classification algorithm, termed genomic nonnegative matrix factorization (gNMF), showed that primary and secondary GBMs segregate distinctly into two classes, and that secondary GBM can be further stratified into two subgroups with different times to progression from low-grade to secondary GBM (Maher et al. 2006). Some of the recurrent genomic alterations have been shown to be prognostic—loss of 6q or 10q or gain of 19q is associated with shorter survival, while loss of 19q tracks with long-term survival (>3 yr) (Burton et al. 2002). Current efforts are now directed toward identifying the clinically relevant genes residing in these loci—efforts strongly motivated by the discovery of molecular signatures of drug response in the clinic (Haas-Kogan et al. 2005; Hegi et al. 2005; Mellinghoff et al. 2005).

Transcriptional profiling Gene expression profiling has proven to be a highly effective method to obtain global signatures reflecting the biological state of the tumor and underlying pathogenic mechanisms and providing markers for use in diagnosis and clinical management. Initial applications of transcriptional profiling to GBM confirmed that defined gene signatures could be used to classify different histological grades (Rickman et al. 2001; Godard et al. 2003; van den Boom et al. 2003). Indeed, among nonclassical lesions, classification by gene expression signatures more accurately predicted survival than standard pathological evaluation (Nutt et al. 2003). More recently, even among histologically indistinguishable GBMs, expression profiling was able to classify GBM into subgroups with different overall survival. Although further validation studies are needed to confirm that these signatures can be used prospectively, these studies suggest that gene expression profiling represents a useful approach in classifying categorize GBM (Liang et al. 2005).

In addition, given the observed intratumoral heterogeneity in GBM, these studies have provided important biological insights into the pathogenesis of GBM. When histologically distinct lesions from the same patient were compared, the gene signatures from these lesions more closely resembled each other than lesions from other patients, suggesting that they arise from a common precursor and share a common molecular life history (Liang et al. 2005). Such analyses have implicated angiogenesis, immune cell infiltration, and extracellular remodeling as drivers of differences between tumor subtypes (Godard et al. 2003; Liang et al. 2005). In a few cases, these studies have facilitated the identification of specific genes that predict survival, such as *FABP7, DLL,* and *ASPM* (Liang et al. 2005; Horvath et al. 2006; Phillips et al. 2006) and permit one to predict the clinical response to EGFR kinase inhibitors (Haas-Kogan et al. 2005; Mellinghoff et al. 2005). These studies suggest that further characterization, validation, and application of this technology will provide improved metrics for prognostication and choice of therapy.

Current status of targeted therapy in GBM

While surgery remains the primary intervention, several early-phase clinical trials of targeted therapies in high-

grade glioma have been completed or are underway either singly or in combination with standard chemotherapy and/or radiation therapy (for a detailed review, see Sathornsumetee et al. 2007 and references therein). A listing of such agents is presented in Table 1 (compiled from http://www.clinicaltrials.gov). The compendium of GBM agents reflects the prevalence of alterations in *EGFR,* such as the *EGFRvIII* deletion mutation (Scott et al. 2007) and PDGF signaling and modulators of PI3K signaling, as well as the prominence of biological processes such as angiogenesis and invasion. Clinical outcomes in these trials are often difficult to interpret and are best considered in the context of standard therapy. The mainstay of initial treatment for GBM has changed little over the last 25 yr and is based primarily on external beam radiation delivered conformally to the tumor volume, now commonly determined by both MRI contrast-enhancement and surrounding T2 signal hyperintensity (Walker et al. 1978, 1980). In conjunction with surgery and medical management, radiation therapy doubles median survival to 12 mo and extends 2-yr survival to 10%, with little added benefit from conventional chemotherapies (Shapiro et al. 1989; Fine et al. 1993; DeAngelis et al. 1998; Stewart 2002). More recently, a notable randomized prospective study demonstrated the first clear survival benefit for chemotherapy in the treatment of GBM: The oral alkylating agent temozolomide (TMZ), given concurrently with radiation and continued thereafter, was found to extend median survival to 15 mo and 2-yr survival to 26% (Stupp et al. 2005). Supporting the concept of molecularly informed clinical management, further analysis revealed that the survival benefit was largely restricted to those patients whose tumors showed epigenetic silencing of the DNA repair gene *MGMT:* Median survival was extended to nearly 2 yr when *MGMT* was methylated, whereas little benefit was seen in patients with tumors expressing MGMT (Hegi et al. 2005). It is tempting to speculate that MGMT-mediated DNA repair may itself be considered a potentially valuable therapeutic target for the 50% of patients that express the *MGMT* gene (Hegi et al. 2006).

Clinical trials of single-agent-targeted therapies typically recruit from the molecularly heterogeneous group of patients who have tumor relapse following radiation and other therapies. The difficulties in interpreting outcomes, whether radiographic response to treatment or overall survival, are well documented (Grant et al. 1997). Several studies have established the validity of 6-mo progression-free survival (PFS6), determined radiographically, as a meaningful endpoint in defining response to treatment: A PFS6 of 15% or less has been estimated as a benchmark for inactive therapy (Wong et al. 1999; Ballman et al. 2007). In comparison, TMZ given at first recurrence in GBM yields a PFS6 of 21% (Yung et al. 2000). In this challenging patient group, the initial results of the EGFR inhibitors erlotinib and gefitinib in single-agent trials have shown little activity overall, although a modest response may be seen in the subset of patients with intact *PTEN* (Prados et al. 2003; Rich et al. 2004; Mellinghoff et al. 2005). Interpretation may be complicated by variable EGFR inhibition in tumors treated by erlotinib and gefitinib, as measured by abundance of phosphorylated EGFR, Akt, and Erk (Lassman et al. 2005). Phase II single-agent trials for inhibitors of PDGFR (imatinib), RAS (tipifarnib), and mTor (temsirolimus) have shown minimal activity overall in GBM as well, although further analysis of tumor material and clinical parameters from sporadic responders may indicate prognostic features (Chang et al. 2005; Galanis et al. 2005; Cloughesy et al. 2006; Wen et al. 2006; Franceschi et al. 2007). VEGF/R inhibitors and multitarget tyrosine kinase inhibitors with anti-VEGFR potency are theoretically attractive agents for attacking GBM, particularly in combination with therapies that have direct tumor cytotoxicity. A recent trial of AZD2171, a multikinase inhibitor with pan-VEGFR, c-Kit, and PDGFR selectivity, demonstrated primary effects of tumor vasculature "normalization": namely, a reduction in contrast-enhancing tumor volume and surrounding edema that Batchelor et al. (2007) were able to link to vessel pruning and reconstitution of the blood-brain barrier through analysis of perfusion MRI and model systems. It remains to be seen whether these potent effects on tumor vasculature may be vividly improving the patient's MRI without impacting progression of the underlying tumor. Nonetheless, VEGF inhibition is likely to have a useful role in combination therapy (Vredenburgh et al. 2007). While targeted inhibitors have shown little durable effect as monotherapies, their specificity and generally modest side effect profiles facilitate combination together and with conventional cytotoxic agents. Table 1 lists currently active clinical trials investigating combined therapies. Although there is reason to believe that certain combinations will be effective in certain patients, the added complexity presents a challenge to clinical trial design, patient stratification and logistics.

Evidence for glioma origins

There is growing evidence that only a minor population of cells in solid tumors, including primary brain tumors (GBM, medulloblastoma, and ependymoma), are capable of forming a tumor when orthotopically transplanted into an immunocompromised mouse (Singh et al. 2004). The concept of the brain CSC (Reya et al. 2001) is based on the observation that only a small fraction of primary leukemic (AML) cells are capable of initiating and sustaining clonogenic growth and inducing leukemia in immunocompromised mice (Lapidot et al. 1994; Bonnet and Dick 1997). Importantly, these leukemic subclones shared the same cell surface markers ($CD43^+$, $CD38^-$) as "normal" human hematopoietic stem cells (HSCs), while the progeny of these leukemic clones, the blast cells, often expressed more differentiated lymphoid or myeloid lineage markers and were not capable of producing leukemic disease. At present it is unclear whether the CSC derives from a normal stem cell compartment or from a more differentiated progenitor that dedifferentiates into a stem cell-like state is not yet clear. The identification of the "cell of origin" remains an area of

active research for both hematological malignancies (Passegue et al. 2003) and solid tumors (Al-Hajj et al. 2003; Singh et al. 2004; Sanai et al. 2005; Taylor et al. 2005; Patrawala et al. 2006; Li et al. 2007; O'Brien et al. 2007; Prince et al. 2007).

The CSC hypothesis was independently proposed for GBM (Singh et al. 2003) and pediatric gliomas (Hemmati et al. 2003). There were two critical findings from these studies. First, only a minor population of cells identified in cell cultures, from a variety of primary CNS tumors (including GBM, medulloblastoma, ganglioglioma, ependymoma, and pilocytic astrocytomas) was able to self-renew and form clonogenic neurospheres. These self-renewing brain tumor cells were identified (Singh et al. 2003) by the expression of the cell surface marker, $CD133^+$ (1%–35% of total population). In contrast, the $CD133^-$ population failed to proliferate and remained as an adherent monolayer and expressed mature lineage-specific markers. Second, $CD133^+$ tumor neurospheres under NSC culture conditions expressed the stem cell marker Nestin and, upon exposure to serum, differentiated into a mixed population of neurons ($Tuj1^+$), astrocytes ($GFAP^+$), and oligodendrocytes ($PDGFR\alpha^+$), which mirrored the mixed cell types found in the original patient's tumor. These observations provide support for a hierarchical CSC hypothesis, suggesting that only $CD133^+$ brain tumor cells can self-renew and undergo lineage-specific differentiation.

Subsequently, substantial enrichment of the tumor-forming ability of FACS-sorted $CD133^+$ cells (as few as 100 implanted cells were able to produce orthotopic tumors) following in vitro expansion of these cells was reported (Singh et al. 2004). In contrast, $CD133^-$ cells failed to form tumors, even following injection of a much larger cell innoculum (10^5 per injection). The orthotopic tumors mirrored the original tumor heterogeneity, with $CD133^+$ cells forming a minor fraction and the $CD133^-$ cells failing to form tumors on serial transplantation. These data suggest that loss of CD133 expression reflects an "irreversible" loss of cellular ability to propagate a tumor. Whether $CD133^+$ cells are only important for tumor initiation and are less critical for tumor progression will require a genetic strategy, similar to that used to monitor skin stem cells in vivo using a doxycyline-inducible H2B-eGFP reporter tag that enabled selection of $CD133^+$ cells over time (Tumbar et al. 2004).

There is now substantial evidence for the enrichment of in vivo cancer-forming ability of $CD133^+$-expressing cells for GBM (Singh et al. 2004; Bao et al. 2006a; Piccirillo et al. 2006) and more recently in colon cancer (O'Brien et al. 2007; Ricci-Vitiani et al. 2007). There are, however, a number of reports that suggest a less clear distinction between the ability of $CD133^+$ and $CD133^-$ cells to form orthotopic tumors (Bao et al. 2006b; Sakariassen et al. 2006; Beier et al. 2007; Zheng et al. 2007). For example, it has been reported recently (Beier et al. 2007) that $CD133^-$ cells isolated from primary GBM tumors were equally capable of forming orthotopic tumors as the $CD133^+$ subpopulation, while under the same conditions, none of the secondary GBM tumors (zero of seven) produced viable neurosphere cultures. They also reported that for four out of 11 primary GBM tumors, $CD133^-$ cells grew as an adherent monolayer yet were able to produce orthotopic tumors. Similarly, $CD133^-$ primary GBM tumor cells, maintained as an adherent monolayer by addition of serum to stem cell culture media, were also able to produce highly infiltrative orthotopic tumors (Sakariassen et al. 2006). These data suggest that even brief ex vivo manipulations may alter the molecular and phenotypic properties of freshly isolated tumor cells, may complicate the conclusions that can be drawn from these sorts of experiments, and point at the need for studies using directly isolated tumor cells from fresh specimens and immediate implantation into immunocompromised mice. While the GBM-stem cell idea is in its infancy and many questions remain, its potential for our understanding of tumor development and therapy design and selection is exciting indeed.

Genetically engineered models of glioma

There is little debate of the importance of murine models in advancing our understanding of the complex biology of gliomas. Various types of in vivo model systems have been developed and utilized, including traditional orthotopic xenotransplants with established human glioma cell lines and, more recently, with primary human glioma cells enriched for surface expression of CD133 (Singh et al. 2004). There is great interest in the further development of the CD133 primary tumor model system as this appears to be superior in recapitulating well the diffuse infiltrative nature of the primary human disease. Whether the CD133 primary tumor system will prove to be a more accurate biological model or be more predictive in drug testing than xenotransplant models with established cell lines is an area of significant current investigation.

In recent years, important advances have been made in the construction of genetically engineered mouse (GEM) models harboring glioma-relevant mutations or combinations of mutations. In several cases, such GEMs predictably develop gliomas with many of the features of the human disease (Table 2; Weissenberger et al. 1997; Uhrbom et al. 1998; Kamijo et al. 1999; Holland et al. 2000; Reilly et al. 2000; Dai et al. 2001; Ding et al. 2001, 2003; Rich et al. 2001; Sonoda et al. 2001; Bachoo et al. 2002; Uhrbom et al. 2002; Xiao et al. 2002; Weiss et al. 2003; Holmen and Williams 2005; Zhu et al. 2005; Charest et al. 2006; Tchougounova et al. 2007). Given the experimentally tractable nature of the mouse, these glioma-prone GEM models are beginning to shed light on a number of key issues such as, for example, the glioma cell of origin (Zhu et al. 2005), the ordering of mutations and whether such events underlie various glioma subtypes (Hu et al. 2005), the cooperative and epistatic relationship of such mutations, and the complex heterotypic interactions between the evolving tumor cell and the host microenvironment, among other issues central to the problem of gliomagenesis. With further refinement, there is now increasing evidence that these GEM model systems will provide an additional vantage with

Table 2. *Mouse and human models of gliomagenesis based on genetic alterations found in astrocytic glioma*

	Tumor classification	Genetic pathway/method	Promoter	Study
Transgenic and knockout GEMs	Low-grade astrocytoma	*Ras/tg*	GFAP	Ding et al. 2001
		Src/tg	GFAP	Weissenberger et al. 1997
		Nf1 + p53/ko	—	Reilly et al. 2000
		floxNf1 + p53/ko	GFAP-Cre	Zhu et al. 2005
	Anaplastic astrocytoma	*Ras/tg*	GFAP	Ding et al. 2001
		Nf1 + p53/ko	—	Reilly et al. 2000
		Src/tg	GFAP	Weissenberger et al. 1997
		Rb/SV40 lg T *PTEN/ko*	GFAP	Xiao et al. 2002
		floxNf1 + p53/ko	GFAP-Cre	Zhu et al. 2005
	Glioblastoma	*Nf1 + p53/ko*	—	Reilly et al. 2000
		floxNf1 + p53/ko	GFAP-Cre	Zhu et al. 2005
		FIG-ROS + Ink4aArf ko	Ad-Cre	Charest et al. 2006
	Low-grade oligodendroglioma	*Arf/ko*	—	Kamijo et al. 1999
		v-erbB/tg	S100β	Weiss et al. 2003
		Ras + EGFRvIII/tg	GFAP	Ding et al. 2003
	High-grade oligodendroglioma	*v-erbB/tg + Inka/Arf ko*	S100β	Weiss et al. 2003
RCAS virus	Glioblastoma	*Ras + Akt*	Nestin	Holland et al. 2000
		Ink4aArf ko + Ras RCAS	GFAP/Nestin	Uhrbom et al. 2002
	Low-grade oligodendroglioma	*PDGFB*	Nestin	Dai et al. 2001
		Ink4a, Arf, Ink4aArf ko + PDGFB RCAS	GFAP/Nestin	Tchougounova et al. 2007
	Anaplastic oligodendroglioma	*Ink4aArf ko + PDGFB RCAS*	Nestin	Dai et al. 2001
		Ink4a, Arf, Ink4aArf ko + PDGFB RCAS	GFAP/Nestin	Tchougounova et al. 2007
	Mixed oligoastrocytoma	*Ink4aArf ko + PDGFB RCAS*	GFAP	Dai et al. 2001
	Glioblastoma	*Tet-off KRAS + Akt*	Nestin	Holmen and Williams 2005
Retroviral	Glioblastoma	PDGFB	Mixed	Uhrbom et al. 1998
Astrocyte and NSC transgenesis	High-grade gliomas	Inka/Arf ko/EGFRvIII retrovirus	GFAP and Nestin	Bachoo et al. 2002
NHA transformation	Anaplastic astrocytoma	*hTERT, H-ras, HPV E6 and E7*	—	Sonoda et al. 2001
	Anaplastic astrocytoma-glioblastoma	*hTERT, H-ras, SV40 T/t-Ag*	—	Rich et al. 2001

Temporal and compartmental transgene expression in somatic cells was achieved by nestin and S100β (glioneuronal progenitor cells) and GFAP (differentiated astrocytes) promoters. In general, the cell of tumor origin in knockout GEMs is unknown. (GEMs) Genetically engineered mice; (RCAS) replication-competent avian sarcoma-leukosis virus long terminal repeat (LTR) with a splice acceptor; (NHA) normal human astrocytes; (tg) transgene; (ko) knockout; (Nf1) neurofibromatosis 1; (floxNf1) LoxP-flanked Nf1 gene excised by Cre recombinase; (hTERT) human telomerase reverse transcriptase; (HPV E6 and E7) human papillomavirus oncoproteins; (SV40 T/t-Ag) simian virus 40 large and small T antigens; (Ad-Cre) adenovirus expressing Cre recombinase; (FIG-ROS) fused in glioblastoma-Ros oncogene.

which to test the timing, dosing, and combination of drugs in the pipeline and assist in the development of drug response biomarkers (Momota et al. 2005; Xiao et al. 2005).

Each of the GEM models in Table 2 offers distinct advantages and limitations for certain types of experimental inquiry. In particular, these models are ideal for investigation of biological mechanisms underlying tumorigenesis and for the functional validation of candidate genes identified through large-scale genomic analysis of tumor specimens. The need for accurate models is perhaps most acute in preclinical testing, where experimental data often determine the fate of a drug in development. Although additional study is needed, it is widely anticipated that refined GEM models of glioma should enable the identification of tumor maintenance genes and the testing of agents targeting such mission critical lesions, thereby identifying key targets, the best agent, and the right patient population (i.e., genotype) (for review, see Sharpless and Depinho 2006). Thus, GEM models may allow for culling of ineffective drugs and improved clinical trials design for those entering phase I/II clinical trials. In addition, the availability of refined GEM models that evolve through stages may help define the tumor grade where an agent or combination of agents may be most effective.

While current efforts are focused on the development of GEMs harboring signature mutations in human glioma, there remains a great utility for models engineered with nonstereotypical lesions that yet capture aspects of human disease behavior and appearance, including invasion, angiogenesis, necrosis, and tumor–ECM interactions. Novel therapies developed to block these biological pathways could be tested in such a model. Similarly, a model that recapitulates the genetics but lacks several of the clinical features of the tumor can be valuable. For example, a tumor driven by PDGF (Dai et al. 2001) could be used to study the downstream targets and the biological consequences of neutralization of the pathway. Finally, inducible and conditional models are gaining popularity as ideal systems for the somatic activation of genes in specific cell populations and for the assessment of genetic lesions in tumor progression and maintenance.

The recently developed glioma-prone GEM models have been notable for recapitulating most of the cardinal histological features of the human disease. That said, a fully accurate genocopy and phenocopy of the human disease has yet to be developed in which the most common mutations are engineered, genome instability is rampant, and orthologous acquired events are documented. Nevertheless, current models have provided important lessons for understanding the nature of gliomas: (1) Loss of a single tumor suppressor gene or overexpression of an oncogene is insufficient to induce high-grade gliomas with high penetrance; (2) modifying mutations are important in gliomagenesis; (3) cell-of-origin and the mutations or set of mutations in such cells plays a significant role in transformation; (4) dysregulating various family members of a pathway or regulatory machinery may have similar biological consequences; and (5) the mutation or combination of mutations has stark effects on a given state of differentiation.

Thus, while further refinement is needed, these GEM models have afforded opportunities to better understand many enigmatic aspects of human glioma development and therapy. Given the wealth of new data anticipated from The Cancer Genome Atlas (TCGA) (Hanauer et al. 2007), for which GBM is one of the select cancer types to be analyzed, a key challenge will be to assign the plethora of newly discovered cancer-associated genetic alterations with cancer relevance. Here, mouse models can serve two key roles: First, they can be used in comparative oncogenomics to identify loci/genes that are commonly targeted in cancer development across evolution, and second, they can serve as relevant model systems to validate genes as well as determine whether new genes cooperate (or not) with specifically engineered mutations—ultimately allowing for the placement of genetic lesions into certain pathways and the testing of drugs targeting these activities.

Future directions

The progress and depth of understanding of the biology and genetics of glioma, together with truly manipulable experimental models, now offer very real opportunities for the development of effective targeted therapy. Despite significant gaps in our understanding, a wealth of information now exists about the clinical and biological behavior of the tumors, the genetic pathways involved in gliomagenesis, and the nature and role of signature alterations in these pathways. The challenge now is to integrate all of this knowledge in an interdisciplinary way to fully understand this disease and how its signature heterogeneity contributes to its intractability. For example, the relatively poor response of GBM patients to EGFR inhibitors, together with emerging data showing that those who do respond have specific genetic combinations, suggests that a pathway targeting approach requires a more thorough understanding. Moreover, the fact that even those patients who do respond to these therapies eventually progress suggests that the evolution of therapeutic resistance is a hallmark feature in their effectiveness. This raises critical questions as to which genetic alterations should be targeted as drivers of tumor maintenance, which should be ignored because they are initially needed for tumor establishment, and which drive the glioma stem cell niche, thus providing a reservoir from which such therapeutic resistant cells can emerge (Bao et al. 2006a). These studies, along with new data that will emerge from the TCGA initiative, will likely transform our understanding of genetics underlying GBM.

To fully understand the relevance of this niche in driving therapeutic resistance (Bao et al. 2006a), many critical questions remain to be answered, including whether $CD133^+$ cells are equivalent to the actively proliferating tumor cells seen on routine histological analysis or represent a quiescent population that is activated by ex vivo manipulations. It is also not yet clear whether there is a prognostic correlation between $CD133^+$ and patient outcome, and if $CD133^+$ cells are selectively spared by radiation and chemotherapeutic drugs. Finally, it is not clear whether de novo $CD133^+$ cells are preferentially found in the neurovascular niche, as was recently proposed based on in vitro studies (Calabrese et al. 2007).

Beyond the stem cell issue is the emerging data noted above regarding RTK coactivation that provides a rational explanation for the feeble ability of RTK inhibitor monotherapy to effect durable clinical responses in GBM patients, in that the inhibition of a single RTK is insufficient to block signaling through critical growth and survival pathways (Huang et al. 2007; Stommel et al. 2007). This suggests that RTK profiling will be necessary to rationally determine an appropriate combination of inhibitors that will achieve a significant clinical outcome. Thus, a systematic study of combination RTK therapies in cancers harboring specific RTK coexpression patterns represents an important next step in the design of new clinical trials, and the secondary analysis of such tumor samples will yield valuable insight into mechanisms of response and resistance. Because FDA-approved RTK inhibitors already exist and additional novel drugs are under development, this treatment paradigm may be implemented in a relatively timely fashion for GBM and

other cancers that are currently highly refractory to virtually all existing therapies.

Our ability to isolate and culture neural and CSCs, astrocytes and oligodendrocytes and the creation of faithful models of this disease coupled to enormous advances in genomic characterization of gliomas and exquisite functional validation of causative mutations offer the very real prospect of rapid and thorough preclinical testing of compounds and other agents to directly answer these questions. By identifying the weaknesses of the tumor, useful treatments for patients with these devastating diseases will become a reality.

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