Chapter 13
Modeling and Preclinical Trials for Gliomas

Introduction

Glioma is the most common form of primary brain tumor. Although surgical resection of the tumor may be achieved, recurrence is almost inevitable because of the propensity of gliomas for infiltrative growth far into functionally intact brain tissue. Gliomas are classified into four clinical grades, with glioblastoma multiforme (GBM) being the most aggressive type (World Health Organization [WHO] Grade IV). GBMs develop de novo, as primary glioblastomas, or through progressive malignification of lower-grade gliomas, termed secondary glioblastomas. Gliosarcomas and giant cell glioblastomas are two rare variants that also sort into the GBM diagnosis. Diffuse gliomas (WHO Grade II) are categorized into astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas, depending on the cells' resemblance to astrocytes or oligodendrocytes. WHO Grade III gliomas constitute a more aggressive form and include anaplastic astrocytomas and anaplastic oligodendrogliomas (25).

Combined Ras and Akt pathway activation and disruption of cell-cycle arrest are common in GBM

Most GBMs contain mutations in genes encoding proteins in the G1 cell-cycle arrest pathways, such as the *INK4a-ARF* tumor suppressor locus, and mutations in the *p53* and in the amplified *cdk4* locus; or have lost the *Rb* gene (21). Additionally, GBMs display multiple mutations and gene expression alterations leading to increased signal transduction through pathways downstream of tyrosine kinase receptors.

Gliomas often produce growth factors, such as platelet-derived growth factor (PDGF) A and B, epidermal growth factor (EGF), transforming growth factor-a (TGF-a), and insulin-like growth factor I (IGF-I). The corresponding receptors, PDGFRa, PDGFRb, EGFR, and IGFR are also often overexpressed, sometimes because of amplification, making autocrine or paracrine receptor stimulation likely (9, 12, 16, 32, 41, 46, 51). Furthermore, in a subset of GBMs, the *EGFR* gene contains a deletion, generating a receptor that signals in the absence of ligand binding (10). Receptor tyrosine kinase activity results in activation of several downstream pathways, such as the RAS/RAF/mitogen-activated protein kinase (MAPK)/(extracellular signal-regulated kinase [ERK]) pathway, the phosphoinositide 3-kinase/AKT pathway, the phospholipase C-g/PKC pathway (*Fig. 13.1*)<fig.13.1>, and C-MYC. These pathways, in turn, control cell proliferation and differentiation, apoptosis, migratory response, cell shape, and metabolic processes (7, 42). Additionally, the tumor suppressor, phosphatase with homology to tensin (*PTEN*), is commonly mutated or deleted in GBMs (27, 50). The *PTEN* gene product negatively regulates AKT activation (*Fig. 13.1*), a protein with both anti-apoptotic and mitogenic functions (47). Signaling through the phosphoinositide 3-kinase/AKT pathway would, therefore, be greatly facilitated by *PTEN* deletion. Apart from these effects, components of the signaling pathways, such as AKT, also directly interact with proteins involved in cell-cycle regulation, such as MDM2 and cyclin D1 (30, 52).

Aside from the frequency of gene mutations that control signaling pathways in gliomas, the activities of those signaling pathways are also frequently affected in gliomas. Ras activity, as measured by Erk phosphorylation, is

elevated in all GBMs analyzed. In contrast, Akt activity is elevated in approximately 70% of GBMs, implying that combined Ras and Akt signaling characterizes the majority of human GBMs (20, 38).

SOMATIC CELL-TYPE SPECIFIC GENE TRANSFER VIA RCAS/TV-A

There are two important motives in modeling brain tumors in mice. The first is to identify causative genetic alterations, thereby distinguishing possible new targets for treatments, and ultimately, broadening the general understanding of brain tumor biology. The second reason is to construct a representative system in which to test potential therapies. The RCAS-/tv-a system allows retroviral-mediated cell-type–specific gene transfer in mice. This system uses RCAS viral vectors, derived from the avian retrovirus, ALV subgroup A (ALV-A), and mice transgenic for the expression of tv-a (the receptor for ALV-A) with tissue-specific promoters (*Fig. 13.2A*)<fig.13.2>. When RCAS vectors containing a gene, or combination of genes, are transferred or injected into the brains of transgenic mice expressing the tv-a receptor on target cells, relatively few are infected, reducing the chances for secondary mutations (*Fig. 13.2C*). One mouse line, Gtv-a, expresses tv-a from the astrocyte- specific promoter for the gene encoding glial fibrillary acidic protein (GFAP) (18). A second mouse line, Ntv-a, expresses tv-a from the nestin promoter that is active in glial progenitors (19) (*Fig. 13.2B*). The RCAS/tv-a system is, therefore, useful for studying the effects of single or multiple defined genetic changes sufficient to result in tumor formation.

COMBINED UNREGULATED RAS AND AKT SIGNALING IN CENTRAL NERVOUS SYSTEM (CNS) PROGENITORS CAUSES ASTROCYTIC GBM FORMATION IN MICE

The first model to produce GBMs used the RCAS/tv-a system to transfer mutant, constitutively active forms of K-Ras and Akt to nestin-expressing neuroglial progenitor cells (20). Activation of Ras or Akt alone did not lead to tumor formation, nor could the combination of Ras and Akt induce tumors in GFAP-expressing astrocytes. This demonstrates the importance of both the activation of certain signaling cascades and the differentiation status of the cell of origin in nestin-expressing, but not GFAP-expressing, cells.

RAS ALSO COOPERATES WITH LOSS OF ARF OR INK4A-ARF TO GENERATE GLIOMAS IN MICE

Disruption of the cell-cycle arrest pathways, most frequently by loss of *ink4a-arf*, is found in the majority of human malignant gliomas. However, animal model experimentation has shown that mice with targeted deletions in the *ink4a-arf* gene do not develop gliomas (21). Although the loss of *ink4a-arf* alone was insufficient for gliomagenesis, the mutation contributes to the formation of GBMs driven by Ras. Ras alone is capable of forming malignant gliomas of

sarcoma-like histology in a *ink4a-arf−/−* genetic background. This observation implies that Ras is capable of cooperating with either Akt or with the loss of *ink4a-arf* in the formation of gliomas of different histologies. A similar finding is found for mice with specific targeted deletions in *Arf* alone and much less so with specific deletions of *Ink4a*, implying that the majority of the effect of combined *ink4a-arf* loss is caused by loss of *Arf* (49).

OVEREXPRESSION OF BOTH PDGF AND PDGFR ARE COMMONLY FOUND IN HUMAN GLIOMAS

PDGF is a well-known mitogen for glial cells in culture. Previous studies have shown that not only does PDGF act to maintain glial precursors in an undifferentiated and proliferating state, but it also promotes the proliferation of CNS progenitors in vivo. Similar to EGF/EGFR, gliomas frequently overexpress the PDGF ligand and its receptor, suggesting that an autocrine loop may, in part, drive the proliferation of these tumors in vivo. Amplification of the PDGFR has been demonstrated in a few malignant gliomas, implying a selective advantage to high levels of signaling through this receptor.

Several lines of evidence implicate overactive signaling, especially through the PDGFR, in the genesis of these tumors. PDGFA and PDGFB are co-expressed with both PDGFRs (-a and -b) in almost all oligodendrogliomas (8) and, in approximately one-half of Grade II to III oligodendrogliomas, overexpression of the EGFR is also observed (38).

RCAS/TV-A–MEDIATED AUTOCRINE PDGF SIGNALING INDUCES OLIGODENDROGLIOMAS IN MICE

The RCAS/tv-a system has been used to investigate the functions of PDGF autocrine signaling in gliomagenesis by transferring the overexpression of PDGF-B into either nestin-expressing neural progenitors or GFAP-expressing astrocytes in both cell culture and in vivo. In cultured astrocytes, overexpression of PDGF-B causes a significant increase in the proliferation rate of both astrocytes and neural progenitors. Furthermore, PDGF gene transfer converts cultured astrocytes into cells with morphological and gene expression characteristics of glial precursors. In vivo, gene transfer of PDGF to neural progenitors induces the formation of oligodendrogliomas in 60% of mice by 12 weeks of age; PDGF transfer to astrocytes induces the formation of either oligodendrogliomas or mixed oligoastrocytomas in approximately 40% of mice in the same period. These data suggest that chronic autocrine PDGF signaling promotes a proliferating population of glial precursors and is potentially sufficient to induce gliomagenesis (5).

INCREASED PDGF EXPRESSION CORRELATES WITH HIGHER-GRADE GLIOMAS

There seem to be dose-dependent effects of PDGF-B on the histological grade of gliomas induced in the RCAS/tv-a system. In removing the 5¡ä-untranslated region translational inhibitory elements in the *PDGFB* messenger ribonucleic acid (mRNA), a substantial elevation of PDGF-B protein in tumor cells was observed. This increase in PDGF-B production resulted in tumors with shortened latency, increased cellularity, regions of necrosis, and general high-grade character. In addition, elevated PDGF-B in these tumors also mediated vascular smooth muscle cell recruitment that supported tumor angiogenesis. Treatment of high-grade PDGF-B tumors with a small molecule inhibitor of PDGFR resulted in reversion to a lower grade tumor histology, implying that PDGF-B signaling is required for the maintenance of the high-grade characteristics. This study indicated that PDGFR signaling quantitatively regulates glioma grade and is required to sustain these high-grade oligodendrogliomas in vivo (44).

BIOLUMINESCENCE IMAGING OF THE CELL CYCLE

Recently, use of in vivo bioluminescence imaging (BLI) has become an established method (3, 11, 28) that is more sensitive than other in vivo imaging methods presently available (2, 4). The Efluc transgenic construct (*Fig. 13.4A*)<fig.13.4>, consisting of the human E2F1 promoter controlling the expression of the firefly luciferase gene, was created to image the loss of RB pathway function in tumor cells in vivo. The E2F1 promoter is autoregulatory, containing four E2F binding sites. In vitro, the E2F1 promoter has a strict cell-cycle¨Cregulated expression with elevated activity during late G_1 to S phase (23) caused by E2F-mediated negative regulation, where E2F¨CRB inhibitory complexes bind to the promoter during early G_1 (*Fig. 13.4B*). In vivo, however, the E2F1 promoter mediates tumor-selective transgene expression unrelated to normal mitotic activity (33), probably caused by loss of RB pathway control in the tumor cells. Thus, bioluminescence from the Efluc transgenic mouse is expected to correlate not merely with the number of tumor cells, but also with the loss of Rb pathway necessary for transformation activity and the proliferative capacity of tumor cells. A reporter mouse line, known as Efluc, has been developed and has been used to report the findings with this tumor type.

EFLUC TRANSGENIC MICE ARE USED IN COMBINATION WITH RCAS/TV-A GLIOMA MODELING

To demonstrate that the Efluc mouse could be used for BLI of tumors, the Efluc transgenic mouse line was crossbred with the Ntv-a mouse strain, allowing BLI in the well-characterized RCAS/TV-A mouse model system of brain tumors (22). Double-transgenic Efluc/Ntv-a mice were infected with an RCAS-PDGFB vector to induce oligodendrogliomas (5). All mice with the Efluc transgene show a background light production, especially over the

regions of skin not covered by fur, whereas glioma-bearing mice emit additional light between the ears, and this emitted light can be quantified. The presence of gliomas in these mice is confirmed by histological analysis of the brains (*Fig. 13.4D*) after imaging (*Fig. 13.4C*). For tumors of the same proliferation index, the amount of light production roughly correlates with the size of the tumor. The Efluc model also allows measurement of glioma development over time. The time-dependent increase in light production represents both the sum of the tumor cells¡¯ capacity to proliferate and the overall size of the tumor.

SIGNAL TRANSDUCTION BLOCKADE RAPIDLY REDUCES EFLUC PHOTON OUTPUT IN GLIOMAS AND CORRELATES WITH PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA)

The PDGF-driven glioma model was used in two longitudinal preclinical studies aimed at determining the requirements for maintenance of tumor cell proliferation. BLI was used to follow PDGFB-induced glioma-bearing mice that were treated with an inhibitor of the PDGFR, PTK787/ZK222584 (5). Photon emission from the brain area of control buffer-treated mice increased during 5 days (*Fig. 13.5*)<fig.13.5>, whereas the mice treated with PTK787/ZK222584 showed a clear reduction in light emission (*Fig. 13.5*). Immunohistochemistry for proliferation markers, PCNA, Ki-67, and phospho histone H3 indicated that drug-treated tumors were arrested, whereas the buffer-treated (and untreated) tumors showed a high level of PCNA staining (>50% of tumor cells). In this particular case, there was minimal evidence of apoptosis, as analyzed by terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate nick end-labeling method (TUNEL) and caspase-3 staining, indicating that the light production from these tumors in an Efluc/Ntv-a transgenic background correlates with cell-cycle progression and proliferation.

SMALL MOLECULE BLOCKADE OF DOWNSTREAM SIGNALING COMPONENTS AFFECTS BOTH EFLUC PHOTONS AND PCNA

In the second preclinical study, the effect of mTOR inhibition, using the rapamycin analog, CCI-779, was tested in the PDGF-induced gliomas (*Fig. 13.1*) (35). Inhibition of mTOR by treatment with CCI-779 reduced the light production from PDGF-induced gliomas to an extent similar to that observed with PDGFR inhibition. Furthermore, when the brains from these mice were analyzed by immunohistochemistry for proliferation markers, the results showed essentially the same effect when inhibiting mTOR as observed for PDGFR inhibition. This is an example where the data suggests that the activities of both PDGFRs and mTOR are essential for the proliferation of PDGF-induced gliomas in mice.

The Efluc model makes it possible to investigate the importance of downstream signaling pathways in glioma maintenance. Previous results have shown that the Akt activity in PDGF-driven gliomas is quite low (6), and, therefore, one might have assumed that mTOR activity would be inconsequential for the proliferation of these tumor cells. However, present data demonstrates that this is not the case, although the mTOR dependence for these tumors may be trivial, having to do with the nutrient status of the cells (24). An alternative explanation for the substantial effect of mTOR inhibition would be that mTOR interferes with the recruitment of oncogenic mRNAs into polysomes. Recent data has shown that this is one of the mechanisms of Ras- and Akt-driven gliomagenesis (36). Assuming the effect is caused by mTOR inhibition, this data implies that pathways not activated by oncogenic stimulation may still be essential for tumor maintenance and, therefore, valid therapeutic targets. The development of the Efluc mouse can be of significant assistance in this process and will be a valuable tool in such in vivo explorations in the future.

USING MOUSE MODELS TO PROMOTE THE USE OF NOVEL DRUGS IN GLIOMAS

Thus, inhibition of mTOR results in G_1 arrest. Theoretically, blockade of Akt itself may be more efficacious; however, inhibition of Akt is not generally tolerated in vivo. The drug perifosine (PRF) is an oral Akt inhibitor, which exerts a marked cytotoxic effect on human tumor cell lines, and is currently being tested in several phase II trials for treatment of human cancers other than gliomas. Recent studies have suggested the molecular mechanism of action of PRF and the capacity of PRF to synergize with radiation or other anticancer drugs. PRF interferes with recruitment of Akt to the plasma membrane and inhibits Akt phosphorylation and activation (*Fig. 13.1*) (26). PRF also causes inhibition of MAP kinases and p21, resulting in cell-cycle arrest in G_1 and G_2 (34, 39, 40). However, the details of the mechanisms of action remain unclear. The Ras¨CMAP kinase signal transduction cascade is activated in nearly all GBMs (13, 29) and Akt is activated in approximately 70% of GBMs (1, 14, 20, 36). Moreover, evidence suggests that forced combined Ras and Akt activation in glial progenitors is sufficient to induce GBMs in mice (20).

AKT SIGNALING BLOCKADE SYNERGIZES WITH DEOXYRIBONUCLEIC ACID (DNA)-DAMAGING AGENTS IN CULTURE

Although the efficacy of PRF in human gliomas has yet to be established, Akt is activated in approximately 70% of human glioblastomas, focusing the studies on both the impact of PRF on glia in culture and on a mouse glioma model in vivo. It was shown that PRF strongly reduces phosphorylation levels of Akt and Erk 1/2, induces cell-cycle arrest in G_1 and G_2, and causes dose-dependent growth inhibition of mouse glial progenitors in which Akt or/and Ras-Erk 1/2 pathways are activated. Furthermore, because temozolomide (TMZ) is a common oral alkylating agent used in treatment of gliomas, the effect of PRF in combination with TMZ was analyzed. When both drugs were used in glial cell culture, an enhanced effect was observed. Given these results, a combination of PRF and TMZ was investigated as a treatment of PDGF-B¨Cdriven gliomas in mice.

Western blot analysis was used to analyze the effect of PRF on signaling pathways in modified glial progenitors. Three specific cell lines were investigated: PDGF¨Cdriven cells, because of their wild-type Akt and because they have relatively low Akt activity; RCAS-Akt infected cells, because these have a myristoylated and constitutively active Akt that is highly phosphorylated; and RCAS-LacZ infected cells, as a control. Each cell type showed a unique response to PRF over time; the Ntv-a/LacZ cells showed minimal effects of PRF on Akt or Erk 1/2 pathways, but showed some effect on 4E-BP1 phosphorylation at later time points; the Ntv-a/PDGF cells showed a strong blockade of Akt and S6K phosphorylation by 3 hours, with a decrease in 4E-BP1 and Erk 1/2 phosphorylation by 6 hours. The cells expressing the constitutively active Akt showed blockade of Akt phosphorylation by 3 hours, but a delayed effect of S6K phosphorylation. The effect on Erk 1/2 and 4E-BP1 was similar to the Ntv-a/PDGF cells.

PRF RESULTS IN MARKED REDUCTION IN CYCLING CELLS OF PDGF-DRIVEN GLIOMA CELLS

To investigate the impact of PRF on cell cycle, Cdk2 and Cdc2 kinase assays were performed. Cdk2 kinase activity was strongly inhibited in Ntv-a/PDGF cells after treatment with 45 mmol/L of PRF for 24 hours, whereas the effect on Cdc2 was milder. In contrast, the effects of PRF on Cdk2 in either cell type were not observed after 6 hours of treatment. At 6 hours, there was a strong effect on Akt, S6, 4E-BP1, and Erk 1/2 in the Ntv-a/PDGF cells. The data implies that the effects of PRF on Cdk2 are secondary to cell-cycle arrest induced by alterations in the signaling pathways rather than a direct effect of PRF on Cdk2. Flow cytometry cell-cycle analysis was performed on the analysis of Ntv-a/LacZ and Ntv-a/PDGF cells treated with various doses of PRF for 24 hours. At doses up to 75 mmol/L of PRF, little effect on the Ntv-a/LacZ cells was observed, but at 100 mmol/L, the cells developed a significant percent of sub-G_1 cells, consistent with cell death. In contrast, in the Ntv-a/PDGF cultures, PRF caused a dose-dependent loss of cells in S phase. At lower PRF concentrations (25 to 50 mmol/L), the cells arrested predominantly in G_1. At higher doses (75 to 100 mmol/L of PRF) in Ntv-a/PDGF cells, the cells arrested predominantly in G_2. The G_2 arrest observed at high PRF doses was observed in the other glial progenitors as well. Cells with 4N or greater DNA content could be either G_2 arrested or multinucleated. When the treated cells were stained with Giemsa and the percentage of multinuclear cells was calculated, it was found that only approximately 6% of all treated cells were found to be multinuclear; thus, the majority of the cells with 4N DNA content were arrested in G_2.

RELATIVE EFFECTS OF MOTOR INHIBITION AND PRF-MEDIATED AKT INHIBITION IN CULTURE

One effect of Akt blockade is a reduction of mTOR activity; however, PRF has several effects other than simple mTOR inhibition, such as affecting non-mTOR pathways downstream of Akt and MAPK. Several mTOR inhibitors are available in clinical trials. Given the central role of Akt activity in gliomas, blockade of the Akt pathway would ultimately be part of a combination of drugs used to treat gliomas. Therefore, the comparison of the effects of RPM and PRF as single agents and in combination with cytotoxic chemotherapy is currently used for gliomas in cell

cultures. First, the doses of RPM and PRF that equally inhibited phosphorylation of S6K were analyzed. Western blot analysis of cells treated with various concentrations of the drugs indicated that 0.1 nmol/L of RPM and 45mmol/L of PRF are doses that achieved equal blockade of S6K phosphorylation. At these doses, RPM showed a slight increase in phospho-Akt (P-Akt) levels, consistent with feedback effects, as has been reported previously (15, 43), and did not show inhibition of Erk 1/2 or 4E-BP1 phosphorylation. This limited effect of RPM contrasts with the effect of PRF on P-Akt, P-S6K, P-4E-BP1, and P-Erk 1/2, as noted above.

The effects of these individual drugs and combinations were all compared in culture. As single agents, PRF had a greater effect on Ntv-a/PDGF cells than RPM, but had minimal effects on Ntv-a/LacZ cells. Finally, whereas RPM had a minimal effect on cells treated with TMZ, PRF substantially enhanced the inhibitory effects of TMZ in Ntv-a/PDGF cells. Therefore, PRF results in blockade of several components of signal transduction and seems to cooperate with TMZ better than inhibition of mTOR alone with RPM.

Flow analysis of Ntv-a/PDGF cells with 300mmol/L of TMZ and 45mmol/L of PRF alone and in combination was used to better define the effects of the combination of these two drugs over time. The analysis indicated that, at these doses, the combination of these two drugs achieved a more complete arrest of the cell cycle than TMZ alone. The growth inhibitory effect was more pronounced when TMZ was used first, followed by PRF, rather than the reverse. Simultaneous treatment with the two drugs gave the most potent effect in these cells, compared with TMZ followed by PRF treatment.

COMBINATION THERAPY OF PRF AND TMZ REDUCES TUMOR PROLIFERATION IN VIVO

Preclinical trials in mice with gliomas demonstrated that PRF and TMZ combination therapy was more effective than TMZ treatment alone. The above data indicates that PRF results in blockade of several signaling pathways and achieves a cell-cycle arrest in culture that significantly enhances the effect of TMZ in culture. Given the enhanced effect of PRF over RPM in combination with TMZ, TMZ and PRF were tested as single agents and in combination against a PDGF-driven tumor in vivo. Mice with tumors were identified by BLI and treated with 100 mg/kg of TMZ, or 30 mg/kg of PRF, or a combination of 100 mg/kg of TMZ and 30 mg/kg of PRF (TMZ + PRF) for 3 to 5 days. The mice were killed and the tumors were analyzed histologically for cell proliferation by Ki-67 immunoreactivity. The baseline variability of untreated tumors in this model, established by a Ki-67 staining index (SI) of tumor cells in control mice, was 10.7±2.0% (n = 4). The measurement of Ki-67 SI in the mice treated with PRF, TMZ, and TMZ + PRF indicated that Ki-67 SI was significantly reduced in mice treated with either TMZ (Ki-67 SI = 5.5±1.2%) or PRF (Ki-67 SI = 3.2±1.1%), compared with controls, demonstrating the inhibitory effect on proliferation. Most importantly, the tumors treated with TMZ + PRF had the lowest Ki-67 SI (Ki-67 SI = 1.7±1.2%). The additional treatment with PRF resulted in a lower proliferation rate than TMZ alone. These results in the mouse models indicate that PRF is an effective drug in gliomas in which Akt and Ras-MAP kinase pathways are frequently activated, and that PRF may be a new candidate for glioma treatment in humans.

CONCLUSION

Genetic mouse models serve as a platform on which recapitulation of the genetics and histology of gliomas can be achieved, allowing us to better understand the biology of these tumors and identify targets for therapy. Efficient use of these models in preclinical trials require the development of imaging technology that provides noninvasive

longitudinal measurements of a tumor's biology and permits each mouse to serve as its own control. These models can be used to test the effects of many glioma therapies. In particular, small molecule inhibitors of signaling components can be targeted in these models, rendering the path to clinical trials in humans an optimistic one. Most likely, a combination of drugs will be required for tumor response, as observed in the models discussed in this chapter. These models may prove to be a critical tool in developing and testing such cocktails, in hopes that similar responses will occur in human clinical trials.

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FIG. 13.1 SIGNALING PATHWAYS DOWNSTREAM OF GROWTH FACTOR RECEPTORS CAUSALLY RELATED TO GLIOMA FORMATION. SMALL MOLECULE INHIBITORS OF SIGNALING COMPONENTS OF THESE PATHWAYS ARE ILLUSTRATED AND ARE POTENTIAL RATIONAL THERAPIES FOR TUMORS DRIVEN BY HYPERACTIVITY OF THESE PATHWAYS.

FIG. 13.2 The RCAS/tv-a system for somatic gene transfer in vivo. *A*, RCAS retroviral vectors can be designed to carry genes and will only infect mammalian cells if they are engineered to express the RCAS receptor, tv-a. *B*, transgenic mice are generated expressing tv-a from tissue specific promoters, such as nestin or GFAP. *C*, the mice are either infected with RCAS vectors in vivo or the brains of the transgenic mice are harvested and grown in culture and infected in vitro. *D*, gene transfer in vivo with RCAS vectors is dependent on expression of tv-a.

FIG. 13.3 Similarities between gliomas in mice generated by RCAS/tv-a and their human counterparts. *A*, *D*, hematoxylin and eosin stain demonstrating pseudopalisading necrosis. *B*, *C*, *E*, and *F*, contrast enhanced and T2-weighted MRI scans showing changes seen in gliomas of mice and humans. Figure courtesy of Andrew Lassman.

FIG. 13.4 BLI of the cell cycle in murine gliomas. *A*, the Efluc transgene that expresses firefly luciferase forms the E2F1 promoter that is involved in *B*, maintaining cells in the G_1 part of the cell cycle. *C*, the tumor can be imaged by BLI; and *D*, light production correlated with tumor size.

FIG. 13.5 MRI, bioluminescence, and histology effects of drug treatment of glioma models in vivo. Arrest of expanding tumor measured by T2-weighted MRI imaging in a PDGF-driven tumor treated with an inhibitor of PDGF signaling (*A*). A cohort of mice treated with the same inhibitor showing loss of light production from the Efluc transgene indicating arrest of proliferation or cell loss (*B*). Immunohistochemistry for the proliferation marker, PCNA, in the treated and untreated tumors (*C*).