Mouse Models of Brain Tumors and Their Applications in Preclinical Trials

Elena I. Fomchenko[1] and Eric C. Holland[1,2]

Abstract Primary brain tumors, including gliomas and medulloblastomas, often represent the most devastating and difficult-to-treat tumors, and are thought to arise from glial cells and/or their precursors or the external granule cell layer, respectively. The majority of genetic alterations characteristic of the human brain tumors are thought to occur in genes encoding proteins involved in signal transduction or cell cycle regulation. Accurate recapitulation of these genetic alterations using genetically engineered mouse models allows for *in vivo* modeling of brain tumors with similar histopathology, etiology, and biology. These mouse models, in turn, increase our understanding of brain tumor initiation, formation, progression, and metastasis, providing an experimental system to discover novel therapeutic targets and test various therapeutic agents.

Of all cancer types, brain tumors probably represent the most devastating and difficult-to-treat cancer. Although enormous advances in treating other solid cancers, such as lung and breast cancers, highlighted the last decade, median survival for glioblastoma multiforme (GBM; the most common and aggressive primary brain tumor) stayed nearly the same over the last 50 years, averaging ~ 1 year (1). Regardless of advances in surgical and imaging techniques, we still face multiple problems when treating brain tumors, some because of extensive infiltration of tumors cells, their invasion into normal brain parenchyma or other sites, and resistance to standard radiation and chemotherapy. However, our paramount inability to successfully treat brain cancer mostly stems from the lack of understanding of the underlying brain tumor biology. Another problem common to diagnosing and thus treating the disease results from difficulties in classifying brain tumors often defined by histologic rather than molecular criteria. For example, histologically similar primary GBMs arising *de novo* and secondary GBMs progressing from low- to high-grade tumors will be classified and treated the same regardless of the vast disparity in molecular characteristics (2, 3). Although curing brain cancer might be in the distant future, we have gained some understanding of genetic and molecular characteristics of human brain tumors (4). Recent advances in laboratory techniques and successful development of brain tumor models faithfully recapitulating the human disease shed further light on necessity and sufficiency of genetic alterations for brain tumor initiation, progression, and maintenance; help

identify relevant therapeutic targets; and provide an amenable field for testing novel therapeutic agents, translating into a better treatment for brain tumor patients.

Modeling Cancer for Drug Development

A multistep process of drug development is based on understanding the brain tumor characteristics by studying genomics, proteomics, genetics, and molecular epidemiology of the disease; preclinical studies are its making or breaking point (5). Preclinical studies culminate in patient clinical trials and translate to bedside upon success or are buried in dust otherwise, and, in turn, depend on our ability to accurately model the disease by using various research techniques and technologies. Approaches reiteratively used for cancer modeling during drug development are multidirectional and include a combination of *in vitro* cell culture experiments and *in vivo* use of animal models.

Cell culture. Cell culture experiments integral to drug development are commonly the first step in identifying therapeutic targets and addressing the effects of novel therapeutic agents (5). However, clinical trials have shown that these experiments often yield false-positive selections, because *in vitro* conditions do not recapitulate brain tumor environment, cause alterations in gene expression, acquisition of additional mutations, and differential selection resulting in clonal expansion of certain cell populations during culture times of primary normal or tumor cells. Primary tumor cultures rarely recapitulate cellular distribution within their tumor of origin; tumor cell lines cultured for decades certainly differ from tumors that gave rise to them (5–7). Although tumor-matrix interactions can be partially mimicked by providing cell cultures with matrix proteins and three-dimensional lattices, the full range of complex tumor-stromal interactions characterized by a combination of matrix proteins, serum and growth factors, hormones, and other substances is impossible to imitate. Likewise, many processes (tumor angiogenesis, invasiveness, and metastasis) dependent on the tumor-host interactions and essential for tumor initiation, formation, progression, and maintenance cannot be reproduced *in vitro*.

Authors' Affiliations: Departments of [1]Cancer Biology and Genetics, and [2]Surgery (Neurosurgery), Neurology, Memorial Sloan Kettering Cancer Center, New York, New York
Requests for reprints: Eric C. Holland, Departments of Surgery (Neurosurgery), Neurology, Memorial Sloan Kettering Cancer Center, 408 E. 69th Street, Z 1304, New York, NY 10021. Phone: 646-888-2053; Fax: 646-422-0231; E-mail: hollande@mskcc.org.

Animal models. *In vivo* modeling provides essential tumor-host interactions and is a more accurate means of modeling human cancer. Mouse models can be classified as xenograft tumor models or models of spontaneous tumor formation in genetically engineered mice (GEM), and help address issues of utmost importance in drug development: toxicity and *in vivo* antitumor effectiveness. Although the Food and Drug Administration does not require the latter before proceeding to clinical trials, *in vivo* modeling of drug efficacy is a gold standard required by a majority of pharmaceutical companies (5). Pharmacokinetic/pharmacodynamic models based on physiologic and anatomic representation of bodily organs and drug biochemistry are likely to supplement *in vivo* modeling during future drug development (8).

Xenograft tumor models. Xenograft tumor formation frequently used for *in vivo* cancer modeling refers to implantation or injection of primary tumor cells or cell lines s.c. (under the skin) or orthotopically (into native tumor site) of immunosuppressed, immunodeficient, or newborn immunonaive mice (9, 10). Xenograft tumors are characterized by synchrony and reproducibility of tumor formation, rapid tumor development, and high penetrance. Use of s.c. models allows for easy tumor visualization, making decisions of treatment initiation and drug application trivial. However, xenograft models lack stepwise genetic changes thought to occur during tumor progression; injected or implanted cells or cell lines have often been altered by culture or isolation conditions and lack their native tumor stroma. As a result, many xenograft tumors are well circumscribed, lack histologically accurate vascularization, and rarely recapitulate tumor-of-origin phenotype (11). Immunosuppressed or immunocompromised mice do not show antitumor immune effects and can produce false positives during drug trials. Some of these problems were addressed by reducing culture times, implanting human tumor specimens with underlying native benign tumor stroma, and using immunonaive animals. However, xenograft tumor models do not faithfully recapitulate etiology, pathobiology, and biochemistry of human tumors they were derived from, making it hard to recognize drug effects and separate them from the experimentally induced artifacts (12–14).

Spontaneous tumors in GEMs. In principle, strains of mice forming spontaneous tumors due to mutations in the genes characteristic of a human malignancy are appealing due to predictability of the tumor-initiating lesion(s), immunocompetence, and tumor development at the appropriate site. Such spontaneous tumor formation recapitulates complex processes of ever-changing tumor genetic makeup, angiogenesis, tumor-host interactions, metastasis to distant sites, and can provide invaluable insights onto efficacy of single or multiple drug treatment on the preformed tumor (14). In addition, use of faithful GEMs is essential for modeling less common cancers due to limited patient population. However, at least in brain tumor modeling, the use of mouse models forming spontaneous tumors is complicated by poor reproducibility, low tumor penetrance, prolonged tumor formation latency, and a need for advanced *in vivo* imaging techniques. More complications arise from the fact that mutant alleles are often expressed or deleted from the whole animal or tissue, compared with human tumors that are thought to arise from a single mutant cell or a small mutant cell population. In this aspect, GEMs are more representative of human cancer predisposition syndromes rather than random tumorigenesis: presence of tumor-initiating mutation(s) in a large cell population may result in tumors characterized by decreased cell heterogeneity (14). In addition, promoters used for gene targeting might affect cell population different from the one that normally gives rise to corresponding human tumors. Even mouse tumors that phenocopy human tumors might be different genetically and molecularly, translating into a vastly different response to therapeutic agents.

GEM models are derived either by germline modification strategies or somatic cell gene transfer. Germline modification strategies refer to gain-of-function, conditional gain-of-function, loss-of-function, chromosome engineering, and RNA interference (15). Gain-of-function models involve creating transgenic animals characterized by allele expression related to the normal gene, ubiquitous/constitutive or tissue/stage-specific, or the knock-in animals, in which endogenous genetic segment is replaced by an exogenous or mutant counterpart. The latter can result in embryonic lethality and spontaneous tumor formation at ectopic sites. Conditional gain-of-function models circumvent the problem by allowing oncogene expression activation by drug treatment (4-hydroxytamoxifen or tetracycline) or recombination (*Cre/Flp* induced or spontaneous; ref. 15). Loss-of-function approaches involve replacement of endogenous genetic segments with terminating elements (positive selection markers) or creating conditional knockout mice containing floxed/flirted alleles excised upon *Cre/Flp* –mediated recombination (15). Chromosome engineering allows modeling chromosomal translocations, deletions, and inversions by placing recombination target sequences in various orientations and/or at different chromosomal positions. By contrast, RNA interference strategies are based on sequence-specific mRNA silencing by short hairpin RNAs after the gene product has already been transcribed (15).

Somatic cell gene transfer differs from germline modification strategies because it is not heritable and presents a modified gene product to targeted cell population rather than to the whole host. Somatic cells genetically modified by viral vectors to become cancerous can pass on acquired mutation(s) to daughter cells. Most commonly used single-stranded RNA viruses are murine or avian. Although murine retroviruses can infect all previously uninfected cells upon a single round of replication, additional viral receptor expression is essential for infection with an avian retrovirus. Use of avian retroviruses has been successfully applied in the RCAS/*tv-a* system, which uses replication-deficient ALV-splice acceptor retroviral vector carrying an oncogene and mice genetically engineered to express *tv-a* receptor that binds RCAS in a certain cell population (16, 17). Both types of retroviruses can be delivered to cultured cells and secondarily transferred into mice (e.g., by primary *tv-a* cell infection and reinjection into the autologous or immunocompromised host), or used for direct *in vivo* infections (e.g., by i.p. or target-tissue injection of cell-free supernatant, or producer cell injection into the target tissue after birth). Nondividing cells can be infected using Moloney murine leukemia virus (MMLV) –based lentiviral systems, subject to compatibility between targeted cell type and vector envelope (18). Widely used viral transfer models include transplantation of fetal cells infected with engineered retroviruses, direct *in vivo* infection with MMLV-based systems, or direct gene transfer *in vivo* using RCAS vectors. Although MMLV-based lentiviral

systems allow infecting nondividing cells, RCAS/*tv-a* system is more specific and allows for lineage tracing from a limited cell-of-origin population of a certain differentiation stage, use of oncogene combinations, addressing the effects of a specific oncogene within the tumor environment, and use of bicistronic vectors encoding an oncogene and a modifying or a labeling gene (e.g., another oncogene, fluorescent reporter gene; refs. 16–18).

Brain Tumors: Gliomas and Medulloblastomas

Classification and histology. Normal brain is composed of a variety of cell types, including neurons, glia (astrocytes, oligodendrocytes, microglia, and ependymal cells), vascular epithelium, and meningeal cells. Primary brain tumors can be classified into gliomas, the most common adult brain tumors occurring in the brain parenchyma above the tentorium, and medulloblastomas, child or young adult cerebellar tumors occurring below the tentorium (9). Intracranial tumors arising from brain meninges (meningiomas) and tumor metastases from systemic cancers (lung, breast, colon) are not considered primary brain tumors and will not be discussed.

Depending on morphologic and histologic similarities to the normal cells, gliomas can be classified as astrocytic, oligodendrocytic, or mixed, and are proposed to arise from the respective cell type (9, 19). The WHO classification divides astrocytic tumors into grades 1 to 4, grade 4 GBM being the most aggressive and malignant primary glioma. Astrocytic tumors (including GBMs) are composed of glial fibrillary acidic protein (GFAP)–expressing cells with fibrillary cytoplasm and angular nuclei, whereas GFAP-negative oligodendroglial tumors present uniform cells with rounded nuclei. GBM features include microvascular proliferation, nuclear atypia, presence of giant cells, and pseudopalisading necrosis. Histologically indistinguishable but molecularly/genetically distinct primary and secondary GBMs develop as primary *de novo* tumors or progress from the diffuse grade 2 to anaplastic grade 3 astrocytomas and to secondary GBMs. Similar WHO classification subdivides oligodendrogliomas into grade 2 low-grade and grade 3 anaplastic oligodendrogliomas. Although patients with low-grade gliomas survive for 10 to 15 years, patients with grade 3 astrocytomas survive for only 2 to 3 years; the mean survival for patients with a GBM is ∼1 year (1, 19).

Medulloblastomas, "blue-cell tumors" composed of small cells with little cytoplasm, are locally invasive and express synaptophysin and NeuN. These cerebellar tumors are classified as desmoplastic, if they contain large-cytoplasm cell islands in the field of more typical medulloblastomas cells, or as large cell, if they contain large pleiomorphic cells. Medulloblastomas show good prognosis with a 65% to 85% cure rate and a high possibility of complete resection; yet, CSF tumor cell spreading and severe neuroaxis irradiation effects still pose a problem (9, 19).

Initial mouse brain tumor models. Initial spontaneous tumor models relied on direct or transplacental exposure to mutagenic DNA alkylating agents (e.g., nitrosoureas) causing point mutations (20). Resulting brain tumors contained multiple random genetic alterations and were histologically similar to human brain tumors, but tumor-initiating mutations and cell(s) of origin were obscured. Xenograft or allograft tumor models developed at the same time are still extensively used for testing novel therapeutic agents (10).

Genetic abnormalities in brain tumors. Frequently occurring somatic mutations and pathway abnormalities potentially implicated in human glioma initiation/progression have been identified in recent years. Most of them occur in genes implicated in signal transduction or cell cycle control, e.g., growth factor overexpression, gain-of-function mutations in receptor tyrosine kinases, and tumor suppressor loss (9). Genetic aberrations accumulate as tumors progress in grade and are sometimes indicative of the tumor formation process: primary GBMs normally contain epidermal growth factor receptor amplification, mutations or deletions of *PTEN*, and deletions of the *ink4a/arf* locus; secondary GBMs contain *p53* mutations. Genetic alterations in medulloblastomas and their importance in tumor initiation/progression are less well explored. However, in-depth understanding of Shh-dependent subset of medulloblastomas, which accounts for 20% of the human disease, represents one of the success stories in using animal models to study rare childhood cancers.

Activation of phosphatidylinositol 3-kinase (PI3K)/Akt, RAS/mitogen-activated protein kinase, phospholipase C/protein kinase C signal transduction pathways affecting cell proliferation, differentiation, apoptosis, and protein translation in human gliomas is frequently achieved by growth factor overexpression [e.g., fibroblast growth factor, ciliary neurotrophic factor, epidermal growth factor (EGF), platelet-derived growth factor (PDGF)] or activating mutations in corresponding receptors (Fig. 1A; ref. 9). *PTEN*, tumor suppressor negatively regulating Akt activity and frequently lost or mutated in primary GBMs, implicates Akt signaling in tumor formation. Deletion of the *ink4a/arf* locus in human gliomas causes cell cycle control disruption due to involvement of its gene products in two major cell cycle regulatory pathways: *Rb* and *p53* (Fig. 1A). *CDK4* amplifications are present in a small number of GBMs, *Rb* is affected in a third of anaplastic gliomas, and both *p53* mutations and *ink4a/arf* loss occur in the majority of anaplastic and a third of low-grade gliomas. Mutations of several components of the same pathway are rare in gliomas, but frequent presence of *p53* mutations with *CDK4* overexpression or *Rb* loss point to the importance of both pathways in gliomagenesis (9). Medulloblastomas, on the other hand, are often characterized by loss of heterozygosity of an unidentified tumor suppressor gene located on 17p distal to *p53*. An aggressive subset of medulloblastomas is characterized by *Myc* amplification/overexpression; some medulloblastomas show germline mutations in *Ptch* (receptor for Shh) and deregulation of Shh signaling that stimulates precursor cell proliferation (Fig. 1B). In addition, Notch signaling is essential for growth and survival of Shh-induced medulloblastomas (Fig. 1B; ref. 21).

GEM brain tumor models. Identification of genetic and molecular characteristics of human brain tumors raised questions of their necessity and sufficiency for different stages of tumorigenesis. Somatic cell gene transfer and germline modifications allow demonstrating causality; advances in mouse genetic techniques and understanding developmental biology make it possible to target oncogenic mutations to cells of various differentiation stages of the neural lineage tree, defining two variables in brain tumorigenesis—initiating mutation and tumor cell(s) of origin. Conditional mouse models and somatic gene transfer additionally allow time-dependent control over mutant allele expression and contribution of several oncogenes to brain tumor initiation.

Early mouse models of astrocytic tumors based on experiments in other species involved transgenic expression of *v-src* (v-src kinase mediating signaling from PDGF, EGF, and PI3K) from the GFAP promoter and resulted in astrocytomas in 15% to 50% of mice, depending on transgene copy number (Table 1; ref. 22). Although *Ras* mutations are uncommon in human gliomas, activation of Ras signaling often occurs as a result of upstream factors and can activate Raf/MEK/Erk, Rac/Jnkk/Jnk, MKK/p38, phospholipase C/protein kinase C, and PI3K/Akt pathways (Fig. 1A; ref. 23). Transgenic mice expressing $V^{12}H$-*Ras* from GFAP promoter developed astrocytomas in 95% cases by 4 to 6 months; mice homozygous and heterozygous for $V^{12}H$-*Ras* showed 50% survival at 3 and 12 weeks, respectively, implicating Ras in dose-dependent tumor initiation (Table 1; ref. 24). Molecular analysis of these tumors showed overexpression of CDK4, MDM2, and EGF receptor (EGFR), decreased levels of ink4a/arf and PTEN, and activation of signaling pathways stimulated by the active H-Ras. However, majority of Ras mutations in human gliomas are found in *K-Ras* and not *H-Ras*, and knockout *K-Ras*, *N-Ras*, and *H-Ras* mice show different phenotypes. Thus, specific roles of Ras isoforms in gliomagenesis are unknown (25).

Initially, oligodendrogliomas were found in ∼10% of *Arf* null mice (Table 1). Subsequent developments using RCAS/*tv-a* system showed that PDGF signaling is sufficient for tumor formation (Table 1; refs. 9, 26). Gene transfer into nestin-expressing progenitors resulted in low-grade oligodendroglioma formation in ∼60% mice by 12 weeks, whereas infection of GFAP-expressing cells resulted in oligodendroglial and mixed gliomas in ∼40% of mice. *Ink4a/arf* loss increased *PDGF*-induced tumor incidence and malignancy, and PDGF signaling showed dosage effect during gliomagenesis. *PDGF* 5′ untranslated region removal resulted in elevated PDGF expression and 97% incidence of high-grade oligodendrogliomas upon gene transfer into nestin-expressing progenitors. Although PDGF transiently activated PI3K, Ras, phospholipase Cγ, and signal transducers and activators of transcription, resulting oligodendrogliomas did not show elevated activity of PI3K/Akt or Ras/mitogen-activated protein kinase pathways (9, 26). Rho deregulation by p190RhoGAP (negative Rho regulator involved in oligodendrocyte differentiation) contributed to *PDGF*-induced gliomagenesis (26). *EGFR* abnormality frequent in human oligodendrogliomas resulted in a model expressing *v-erbB* (activated *EGFR*) from the S100β promoter active in both progenitors and differentiated astrocytes, showing 63% low-grade oligodendroglioma incidence at 1 year, and tumor grade, penetrance, and latency increased by additional *p53* or *ink4a/arf* loss (Table 1; ref. 27). Crossing GFAP-$V^{12}H$-*Ras* mice to *GFAP-EGFRvIII* transgenics with the mutation most common in malignant gliomas resulted in aggressive oligodendroglial or mixed gliomas in double but not single transgenics (Table 1; ref. 28).

All human GBMs present elevated Ras activity; increased Akt activity unrelated to activating *Akt* mutations is present in 75% of them (Fig. 1A; ref. 9). GBM modeling using RCAS/*tv-a* and constitutive expression of *K-Ras* and *Akt* in nestin-expressing progenitors implicated these genetic pathways as causal in GBM formation, resulting in 26% tumor incidence at 12 weeks (Table 1). Single or combined expression of activated *K-Ras* or *Akt* in GFAP-positive differentiated cells did not result in GBM formation using RCAS/*tv-a*, whereas high levels of H-Ras were sufficient to cause GBMs in *GFAP-$V^{12}H$-Ras* mice (9, 26). RCAS/*tv-a* and Cre/*loxP* modeling with *PTEN* loss in nestin-positive progenitors implicated combined *PTEN* loss and *Ras* activation in GBM formation comparable with *K-Ras* and *Akt* (26). Resulting GBMs exhibited similar histology, incidence, latency, and gene expression, proving that oncogenic effects of *PTEN* loss are mediated by increased Akt activity. GBM modeling by double heterozygous *p53/Nf1* loss exhibited variable numbers of secondary GBMs depending on loss of heterozygosity (Table 1; ref. 29). Conditional astrocyte-specific *Nf1*-knockout mice did not result in glioma formation, implying that GBMs either did not arise in astrocytes or depended on loss of both tumor suppressors. MMLV-based *PDGF* mouse model showed 40% tumor incidence, the majority being histologically similar to human GBMs and some resembling oligodendrogliomas or neuroectodermal tumors, potentially due to high infection and insertional mutagenesis rates or promiscuity of MMLV-based vectors, making it hard to identify tumor cells of origin (Table 1; ref. 18).

Shh pathway activation or equivalent loss of *Ptch* combined with *Akt* activation or cell cycle arrest disruption allows for medulloblastoma modeling (Fig. 1B; Table 1; refs. 9, 30). Mice heterozygous for *Ptch* showed 15% tumor incidence at 1 year, increasing to 95% at 12 weeks upon *p53* loss (Table 1; refs. 9, 30). MMLV-based and RCAS/*tv-a* models using viral vectors encoding Shh showed low tumor incidence; additional overexpression of *c-Myc* using RCAS/*tv-a* targeting nestin-positive progenitors enhanced tumor incidence to 30%. *Akt* activation or *IGF2* overexpression in the latter mouse model enhanced tumor incidence and shortened tumor latency (Table 1; ref. 31). Although *p53* and *Rb* are rarely mutated in human medulloblastomas, their combined loss in granule cell layer precursors resulted in 100% tumor incidence and histologic similarities to human medulloblastomas (Table 1; ref. 32). A *p53*-null mouse model with additional loss of poly(ADP)-ribose polymerase-1 (polymerase implicated in DNA repair) showed 50% tumor incidence with signaling abnormalities in the Shh pathway. *Lig4* (DNA repair enzyme) loss in the *p53*-null mice resulted in medulloblastoma formation as well (9). Recently developed GEMs overexpressing *Smo* in cerebellar granule layer precursor cells (*ND2:SmoA1*) showed 48% medulloblastoma incidence, characterized by high levels of Gli1, N-myc, Notch2, and HES5 (Table 1; ref. 34).

In vivo Imaging Technologies

Imaging modalities offer the possibility of adding *in vivo* monitoring of live animals for diagnostic purposes of tumor identification, observing tumor progression and longitudinal effects of drug action, each mouse being its own control. Magnetic resonance imaging, the representative image of which is shown in Fig. 2, can be used to quantify tumor volumes, evaluate tumor vasculature, and study tumor physiology and biochemistry of drug response *in vivo* (9, 34). In contrast, bioluminescence allows molecular imaging and monitoring of biological changes in cells and organisms using luciferase-expressing transgenic mice and sensitive photon detectors to collect light transmitted through mammalian tissues with a high signal-to-noise ratio and without the use of contrast-enhancing agents (Figs. 2 and 3; ref. 35).

A

CCR Focus
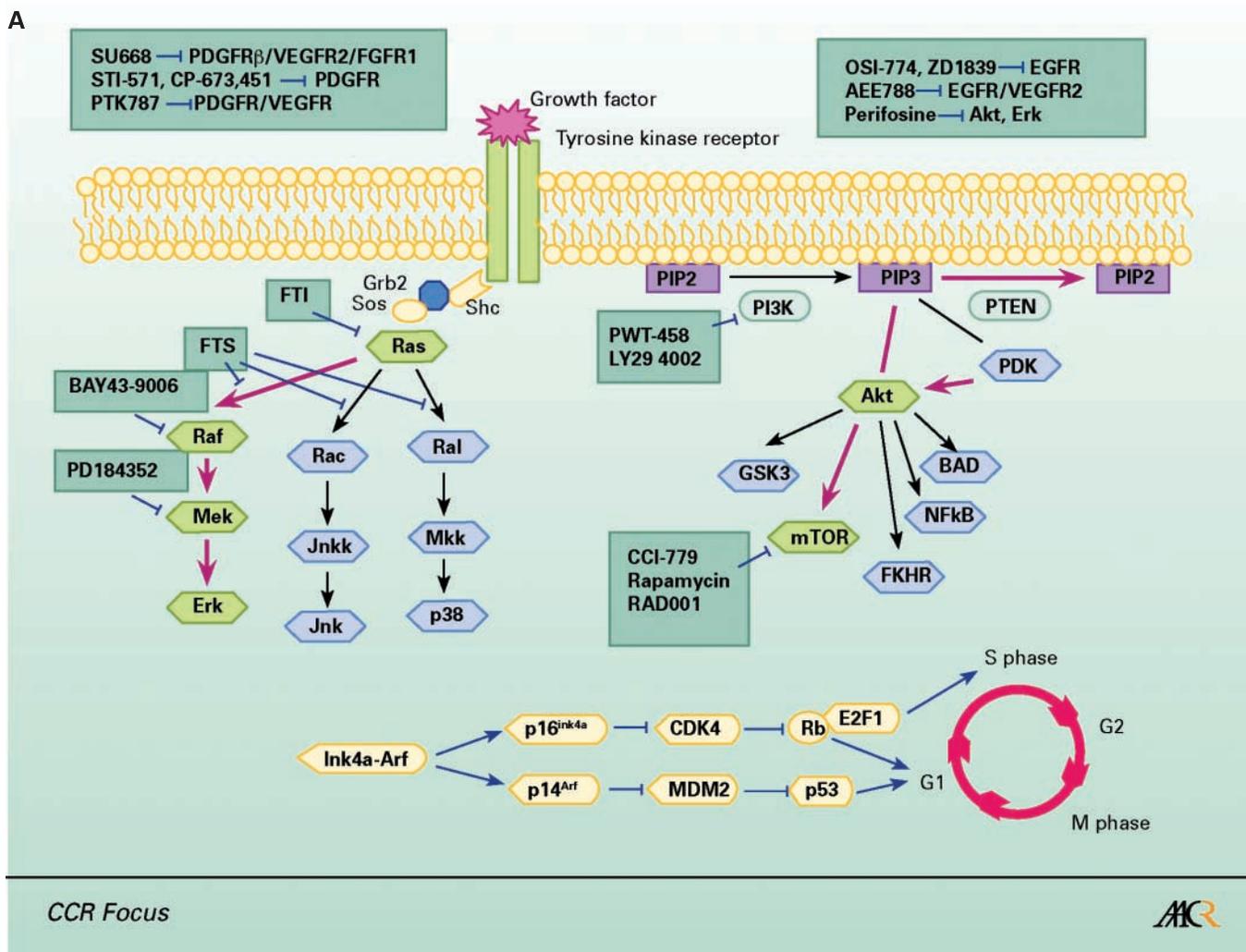
Fig. 1. Activation of signal transduction pathways and cell cycle deregulation in human and GEM brain tumors: Molecular targets and therapeutic agents. *A*, activation of Ras/mitogen-activated protein kinase and PI3K/Akt signal transduction pathways, and disruption of p53 and Rb cell cycle control pathways characteristic of human and GEM gliomas. Green box areas, molecular inhibitors for pathway components. Presumptive or known target inhibition is indicated on the chart.

Preclinical Trials in GEMs

GEMs closely recapitulate the human disease and are used to predict human response to a therapy, treatment or radiation schedule. Yet, GEM-tested pathway inhibitors often affect pathways used for brain tumor induction, based on the frequently failing assumption of continued need of the initiating lesion to maintain tumorigenesis (Figs. 1 and 3). However, during tumor progression, cancer cells may acquire additional mutations to become independent of the cancer-initiating lesions or resistant to a therapeutic agent, the former circumvented by inducible GEMs, which allow testing oncogene requirement for tumor maintenance and progression. In addition, human brain tumors are thought to result from accumulation of several genetic alterations, implying that targeting a single oncogenic pathway is likely to be insufficient for brain tumor treatment.

Current approaches in brain tumor management. Glioma and medulloblastoma treatments normally include tumor resection,

histologic examination, radiation therapy, and chemotherapy. The most common chemotherapeutic agents used for glioma treatment are DNA alkylating cytotoxic drugs such as carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea]; a triple combination of procarbazine, cisplatin, and vincristine; or the recently developed temozolomide, etoposide, or lomustine, whereas those used for medulloblastoma treatment are cisplatin, cyclophosphamide, or vincristine. Due to the time of incidence and its nature, medulloblastoma cure rate is 85% for children with nondisseminated tumors and 65% to 70% in high-risk patients (36). However, drugs listed above present serious side effects and eventually fail as patients develop resistance, whereas radiation and chemotherapy adversely affect neurocognitive and physical development (26, 37). Thus, although traditional brain tumor therapeutics relied on nonspecific cytotoxic agents, preventing cell proliferation and resulting in cell death due to DNA damage or disrupted cell division, current therapeutic agents are developed to avoid normal cell toxicity and target tumor
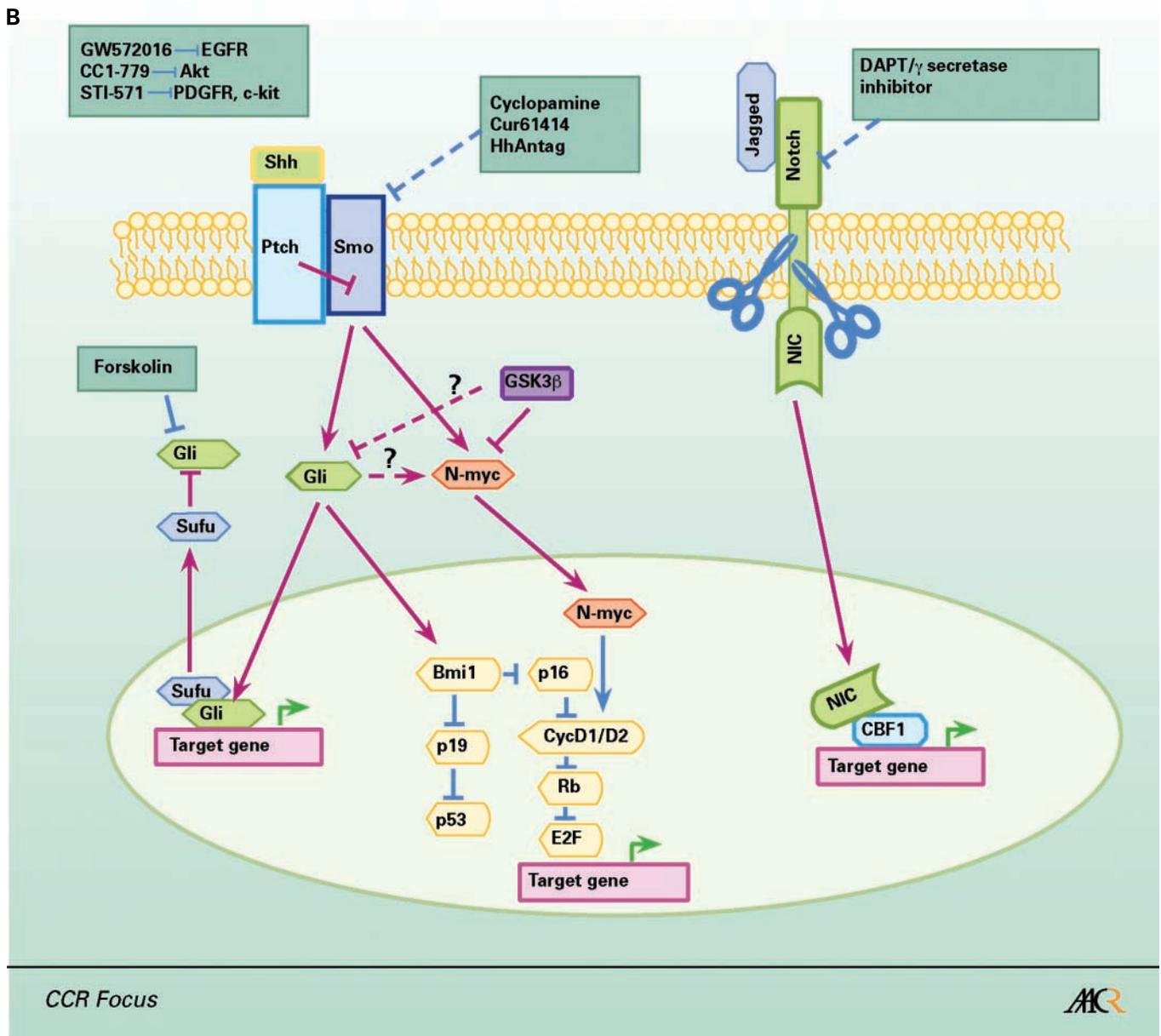
Fig. 1 *continued*. *B*, activation of Shh and Notch pathways typical for human and GEM medulloblastomas. Green box areas, molecular inhibitors of various pathway components. Their therapeutic targets are indicated adjacently.

cell–specific antigens and cell alterations responsible for growth, proliferation, invasion, and metastasis.

Therapeutic agents targeting signal transduction. Signaling abnormalities downstream of EGFR/PDGF receptor (PDGFR) and Ras/Akt pathways common in gliomas are frequently targeted with EGFR, PDGFR, mammalian target of rapamycin (mTOR), Akt, and Ras inhibitors (Fig. 1A; refs. 9, 26). EGFR inhibitors tested *in vitro* glioma cell lines and xenograft mouse tumor models during preclinical trials and currently in phase I/II clinical trials include Tarceva (erlotinib, OSI-774), Iressa (gefitinib, ZD1839), and an EGFR/vascular endothelial growth factor receptor 2 tyrosine kinase inhibitor AEE788 (38–40). GEMs useful for addressing roles of EGFR signaling in tumor maintenance and progression or testing novel inhibitors include *GFAP-V^{12}H-Ras*, *GFAP-EGFRvIII*, and *S100β-v-erbB* mouse models (Table 1; refs. 27, 28).

PDGFR signaling abnormalities are present in histologically unlike high-grade gliomas. Selective PDGFR/vascular endothelial growth factor receptor inhibitor PTK787/ZK222584 effective in converting high-grade oligodendrogliomas to low-grade tumors and inhibiting tumor proliferation without disrupting tumor vasculature in RCAS/*tv-a* GEM of PDGF-induced gliomagenesis is now in preclinical trials (Fig. 1; ref. 41). Other potentially useful PDGFR inhibitors tested in *in vitro* and *in vivo* xenograft models but not in GEMs include Gleevec (imatinib mesylate, STI-571), a PDGFR/c-kit/bcr-abl tyrosine

Table 1. Generation of primary brain tumors in GEM similar to and based on genetic alterations found in the human brain tumors

Tumor classification	Pathway/gene	Initial genetic alteration by vector	Tissue-specific promoter	Reference
Low-grade (WHO grade 2) astrocytoma	*Ras*	Gain	GFAP	24
	Nf1+p53	Loss	—	29
	Src	Gain	GFAP	22
Anaplastic (WHO grade 3) astrocytoma	*Ras*	Gain	GFAP	24
	Nf1 + p53	Loss	—	29
	Src	Gain	GFAP	22
	Rb inactivated by SV40 large T antigen	—	GFAP	62
GBM (WHO grade 4) astrocytoma	*Ras + Akt*	Gain	Nestin	37
	PDGFB	Gain	Mixed	18
	Nf1 + p53	Loss	—	29
	Ink4aArf + Ras	Loss/gain	GFAP/Nestin	63
Low-grade (WHO grade 2) oligodendroglioma	*Arf*	Loss	—	64
	PDGFB	Gain	Nestin	65
	v-erbB	Gain	S100β	27
	Ras + EGFRVIII	Gain	GFAP	28
Anaplastic (WHO grade 3) oligodendroglioma	*Ink4aArf + PDGFB*	Loss/gain	Nestin	65
Mixed glioma (oligoastrocytoma)	*Ink4aArf + PDGFB*	Loss/gain	GFAP	65
Medulloblastoma	*Ptch*	Loss	—	66
				67
				68
	Ptch + p53	Loss	—	70
	Rb + p53	Loss	GFAP	32
	Shh	Gain	Cerebellar progenitors	69
	Shh + myc	Gain	Nestin	71
	Lig4 + p53	Loss	—	72
	Parp + p53	Loss	—	73
	SmoA1	Gain	ND2	33
	Shh + Akt or IGF2	Gain	Nestin	31

NOTE: Tumor cells of origin are unknown in the knockout GEMs; promoters used for somatic cell gene transfer or limiting transgene expression are listed and correspond to glioneuronal progenitor cells (nestin, S100β), differentiated astrocytes (GFAP), or the external granule layer progenitor cells (ND2).

kinase inhibitor successful in treating chronic myelogenous leukemia and gastrointestinal stromal tumors, SU6668, PDGFRβ/vascular endothelial growth factor receptor 2/fibroblast growth factor receptor 1 tyrosine kinase inhibitor, and CP-673,451, PDGFRβ phosphorylation inhibitor (26, 42, 43).

Deregulated PI3K/Akt signaling proposed to account for tumor cell growth and resistance to radiation/chemotherapy is frequent in many cancers. Perifosine (orally administered compound causing Akt inhibition), now in preclinical trials, is cytotoxic to human tumor cell lines, causes dose-dependent growth inhibition, and induces G_1-G_2 cell cycle arrest in *Ras+Akt*–driven mouse progenitors. Perifosine synergizes with temozolomide (alkylating agent) *in vitro* cultures of mouse progenitors and in RCAS/*tv-a* GEM of *PDGF*-induced tumorigenesis (Fig. 1; ref. 44). Other PI3K/Akt signaling inhibitors effective *in vitro* and *in vivo* xenograft models but yet to be tested using GEMs include PWT-458 (pegylated-17-hydroxywortmannin, induces loss of Akt phosphorylation, and synergizes with

rapamycin), LY294002 (reverses PI3K-induced radiation resistance), and protein kinase Cβ–selective inhibitor enzastaurin (LY317615.HCl, suppresses Akt signaling; refs. 45–47).

Akt activation mainly targets mTOR, which can be inhibited by rapamycin (sirolimus), CCI-779 (temsirolimus), and RAD001 (everolimus; Figs. 1 and 3). Tumor cells of brain and other cancers show differential sensitivity to mTOR inhibitors. CCI-779 induces tumor cell death in a subset of cells at 48 hours of drug treatment using RCAS/*tv-a* GEM of *K-Ras+Akt*–induced GBM formation, endowing surviving tumor cells with histologic characteristics of oligodendroglial tumors with low Akt activity after 7-day treatment (26, 48). Thus, astrocytic GBM characteristics seem dependent on mTOR signaling. Use of mTOR inhibitors might be beneficial for patients with tumors characterized by *PTEN* loss, which has been shown equivalent to Akt activation by the RCAS/*tv-a* GEM.

Ras protein cycles from GTP-bound active to GDP-bound inactive state and is targeted to the membrane by cytosolic
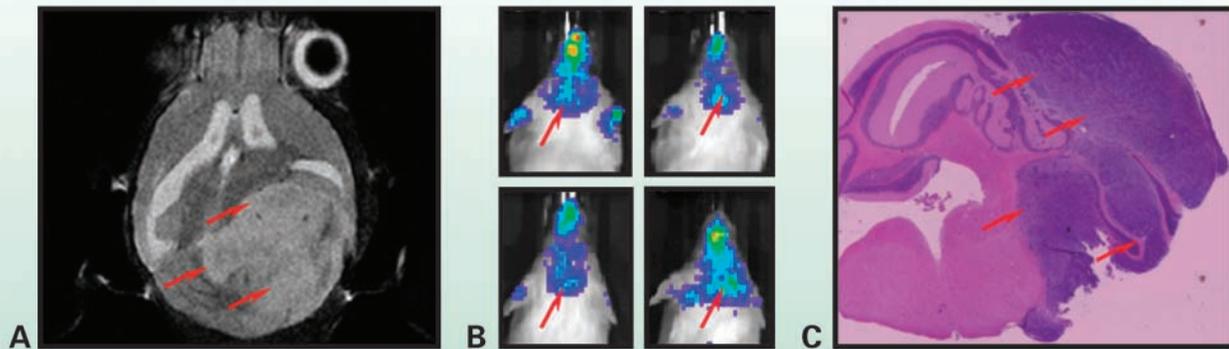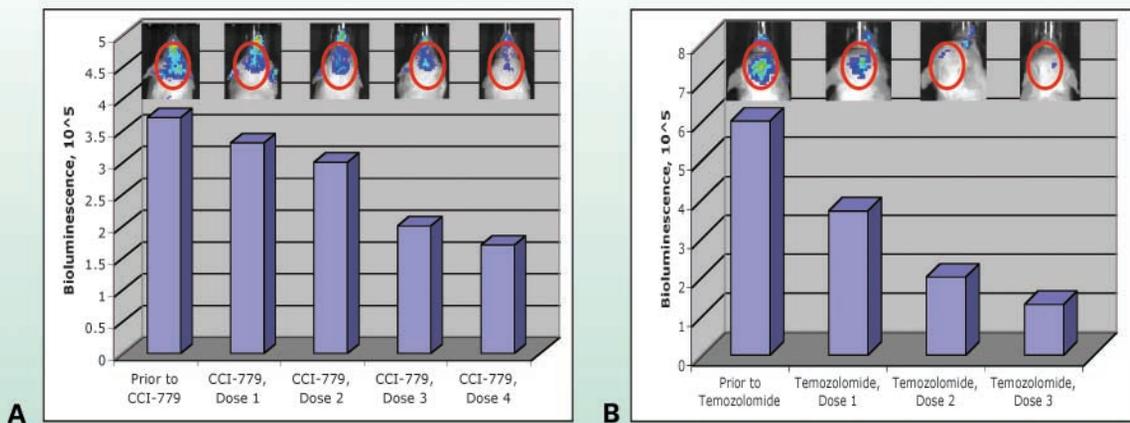
Fig. 2. *In vivo* imaging of brain tumorigenesis. *A*, T$_1$-weighted saggital plane magnetic resonance imaging of a 7-week-old *Ntv-a* mouse neonatally injected with RCAS-*Shh* and RCAS-*Akt*, without contrast enhancement. Magnetic resonance imaging image shows a large, slightly enhanced medulloblastoma (*red arrows*). (Images courtesy of J. Koutcher and O. Becher.) *B*, bioluminescent images produced by the *E2F-luc* transgenic *Ntv-a* mice neonatally injected with RCAS-*PDGF*, upon the retroorbital injection of 75 mg/kg D-luciferine and a 2-minute image acquisition by the Xenogen IVIS system. Red arrows, bioluminescence corresponding to presumptive brain tumorigenesis. (Images courtesy of E. Nerio.) *C*, whole-mount histologic analysis (H&E) of a 10% formalin-fixed, paraffin-embedded brain of an *Ntv-a* mouse with a medulloblastoma. Darker-staining cerebellar area with high cellular density (*red arrows*) corresponds to the tumor. (Image courtesy of O. Becher).

prenyltransferases adding geranylgeranyl or farnesyl groups at COOH-terminal Ras CAAX sequences. Ras activity elevated in human GBMs and frequent in lower-grade astrocytomas can be therapeutically targeted by small-molecule inhibitors acting on downstream effectors, or farnesyltransferase inhibitors and *S-trans*-transfarnesylthiosalicylic acid preventing Ras interactions with downstream components (Fig. 1). There is no published data describing the effects of these therapeutic agents

Fig. 3. Preclinical trials in the E2F-luc *Ntv-a* GEMs with PDGF-induced GBMs and oligodendrogliomas: applications of the RCAS/*tv-a* system and *in vivo* bioluminescence imaging (refer to Fig. 2 for imaging conditions). *A*, longitudinal imaging of a CCI-779–treated tumor-bearing mouse, indicating a 2.2-fold decrease in the amount of photon emission after a four-dose treatment. (Images courtesy of E. Nerio.) *B*, longitudinal imaging of a temozolomide-treated, tumor-bearing mouse, showing a 4.6-fold decrease in the amount of photon emission after a three-dose treatment. (Images courtesy of E. Nerio).

in GEMs, but some look promising by *in vitro* experiments, *in vivo* xenograft models, and effective treatments of other cancers. Small-molecule inhibitors include BAY43-9006 (Raf small-molecule inhibitor) and PD184352/CI-1040 (Mek small-molecule inhibitor); farnesyltransferase inhibitors include Zarnestra (tipifarnib, R115777), evaluated in phase I clinical trials for gliomas; Sarasar (lonafarnib, SCH66336) in clinical trials for other types of cancer; and prenylation inhibitors L-778,123 and BMS-214662 (26, 49). GEMs that form Ras-driven gliomas (e.g., *GFAP-V^{12}H-Ras* mice) could be useful for these preclinical trials (Table 1; ref. 28).

Use of single agents is rarely sufficient to halt tumor progression due to independence from the tumor-initiating mutation, cellular heterogeneity within tumors, and a variety of signaling patterns. Clinical and preclinical studies using drug combinations underscore the necessity of multilevel targeting, exhibiting better results in combination rather than single-agent use. For example, human GBM xenografts in athymic mice were better inhibited by a combination of EGFR/vascular endothelial growth factor receptor 2 tyrosine kinase inhibitor AEE788 and mTOR inhibitor RAD001, producing higher cell cycle arrest rates and reduced *in vitro* proliferation (50). Similarly, PDGF-induced gliomas in RCAS/*tv-a* GEMs showed *in vitro* and *in vivo* synergy between Akt inhibitor perifosine and alkylating agent temozolomide.

Some therapeutic glioma agents might be useful for medulloblastoma treatment due to its frequent overexpression of EGFR and/or its ligands, Akt, PDGFR, and c-kit, which can be targeted by tyrosine kinase small-molecule inhibitor lapatinib (GW572016), CCI-779, and Gleevec (imatinib mesylate, STI-571; Fig. 1; refs. 26, 52). Some of these agents were tested *in vitro* and *in vivo* xenograft tumors, but not in GEMs. Therapeutic agents tailored to medulloblastoma-specific signaling abnormalities include Shh-Gli, Notch, and γ-secretase inhibitors (Fig. 1B). Shh-Gli signaling inhibitors tested in *Ptc1*(+/−); *p53*(−/−) GEMs include cyclopamine (alkaloid found in *Veratrum californicum*) and HhAntag, both impairing activity of Smoothened (Fig. 1; refs. 52, 53). Cyclopamine administration resulted in reduced tumor burden, lower proliferation rates, and smaller numbers of Ptc1-expressing cells, whereas HhAntag caused inhibition of cell proliferation, extensive cell death, and tumor eradication at high doses (53, 54). Interestingly, *p53* loss did not make Shh-dependent tumors resistant to pathway inhibitors. Notch and γ-secretase inhibitor DAPT was shown to be effective in *in vitro* and *in vivo* xenograft tumors, but not in Shh-driven GEMs tumors (Fig. 1; ref. 33).

Non–signal transduction-based therapeutic agents. A number of other strategies yet to be tested in GEM models of brain tumorigenesis include histone deacetylase inhibitors (54), immune modulation (55, 56), angiogenetic therapy (58), monoclonal antibodies (59, 60), small interfering RNA approaches (52), and differentiation therapy (61). These therapies are interesting for their flexibility, which allows tailoring treatment to specific tumors and their environment by choosing antigens, antibodies, appropriate toxin or isotope conjugates, or administration methods.

Conclusions

Our increasing knowledge of underlying brain tumor biology suggests that brain tumor therapeutics is in its infancy—we still lack detailed understanding of mechanisms of drug actions, optimal concentrations, treatment schedules, and treatment course. Regardless of technological developments in the operating rooms, brain tumor patients still face a dim prognosis. Using *in vitro* systems and *in vivo* xenograft brain tumor modeling provides a quick and efficient way of testing novel therapeutic agents and targets, knowledge from which can be translated and tested in more sophisticated GEMs that faithfully recapitulate human brain tumors and will likely result in high-quality clinical trials with satisfactory treatment outcomes and reduced drug toxicities. Additional use of GEMs to establish causal links between the presence of various genetic alterations and brain tumor initiation or determining their necessity for tumor maintenance and/or progression provide us with a glimpse into other important aspects of brain tumor biology. Developing GEMs with multiple genetic abnormalities typical for human brain tumors and advancing imaging technologies will allow testing drug combinations, high-resolution mapping with an easy readout for the therapeutic efficacy, and longitudinal drug monitoring during preclinical trials. Thus, although curing brain tumors might still be in the distant future, we are advancing further in development of various therapies targeting different aspects of the enemy we have long sought to defeat.

Acknowledgments

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