Oligodendrogliomas Result from the Expression of an Activated Mutant Epidermal Growth Factor Receptor in a RAS Transgenic Mouse Astrocytoma Model

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ABSTRACT

A significant proportion of human malignant gliomas exhibit amplification, overexpression, or mutations of the epidermal growth factor receptor (EGFR). To define the functional role(s) of the EGFR in the pathogenesis of gliomas, we established transgenic mice that express both wild-type (wt) and mutant (EGFRvIII) EGFR molecules using the human glial fibrillary acidic protein (GFAP) promoter. Both GFAP-EGFRvIII and GFAP-EGFRvIII transgenic mice demonstrated increased numbers of astrocytes compared with control littermates, however, developed normally without formation of gliomas. To determine whether EGFR overexpression could modify the tumor phenotype in our previously reported GFAP-V12Ha-ras transgenic mouse astrocytoma model, mice expressing both activated RAS and EGFR were developed. GFAP-V12Ha-ras;GFAP-EGFRvIII, but not GFAP-V12Ha-ras;GFAP-EGFRvIII double transgenic mice, had decreased survival with fifty percent of the mice dead at 2–4 weeks from gliomas, compared with 12–16 weeks for the GFAP-V12Ha-ras mice. Furthermore, GFAP-V12Ha-ras;GFAP-EGFRvIII mice developed oligodendrogliomas and mixed oligoastrocytomas, instead of the fibrillary astrocytomas observed in GFAP-V12Ha-ras mice. In contrast to the fibrillary astrocytomas, the molecular pathogenesis of oligodendrogliomas is not as well understood, although similar molecular heterogeneity has been demonstrated (8). For example, a subset of oligodendrogliomas that typically are associated with improved survival and response to chemotherapy demonstrate loss of various regions of chromosome 1p and/or 19q (9). In contrast, another subset of oligodendrogliomas associated with poorer survival, despite radiation and chemotherapy, exhibit loss of p16 and harbor deletions in the regions of chromosome 10q and also have EGFR amplification and mutations (8). In addition, oligodendrogliomas developed in a transgenic mouse model of v-erbB under regulation of the S-100 promoter. This observation suggests that EGFR amplification and mutation may be a molecular marker of more aggressive oligodendrogliomas or mixed oligoastrocytomas, similar to that observed for highly malignant astrocytomas (GBMs).

INTRODUCTION

Diffuse gliomas are the most common primary malignancy of the human central nervous system. The WHO classifies gliomas according to their predominant cellular composition, with astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas as the most common (1). The most malignant and unfortunately the most common adult glioma is the GBM, with a median survival of 9–12 months (1). The glial lineage that gives rise to these heterogeneous GBMs is not known, although a large subset likely arises from type I fibillary astrocytes. However, as many as half of GBMs do not express the differentiated astrocytic GFAP marker, perhaps either as a result of cell de-differentiation or a common terminal transformation phenotype of several glial lineages (1).

The molecular progression of GBMs involves at least two or more pathways, although the tumors are pathologically indistinguishable. One pathway, involving progression from lower grade astrocytomas to secondary GBMs, is more prevalent in younger patients and is characterized by mutations in TP53 tumor suppressor. The second and more common primary or de novo pathway occurs mainly in older GBM patients. These GBMs lack TP53 mutations but harbor amplifications, overexpression, or mutations of the EGFR (3). The most common EGFR mutation is the EGFRvIII variant, involving an in-frame deletion of exons 2–7 in the extracellular domain of wt EGFR molecule, resulting in a constitutively phosphorylated and activated receptor (4). Overexpression of EGFRvIII provides a growth advantage to astrocytoma cell lines (5). In addition, EGFRvIII overexpression in younger patients with GBM has been shown to represent a negative clinical prognosticator and is associated with reduced patient survival (6, 7).

In contrast to the fibrillary astrocytomas, the molecular pathogenesis of oligodendrogliomas is not as well understood, although similar molecular heterogeneity has been demonstrated (8). For example, a subset of oligodendrogliomas that typically are associated with improved survival and response to chemotherapy demonstrate loss of various regions of chromosome 1p and/or 19q (9). In contrast, another subset of oligodendrogliomas associated with poorer survival, despite radiation and chemotherapy, exhibit loss of p16 and harbor deletions in the regions of chromosome 10q and also have EGFR amplification and mutations (8). In addition, oligodendrogliomas developed in a transgenic mouse model of v-erbB under regulation of the S-100 promoter. This observation suggests that EGFR amplification and mutation may be a molecular marker of more aggressive oligodendrogliomas or mixed oligoastrocytomas, similar to that observed for highly malignant astrocytomas (GBMs).

To explore the functional role(s) of EGFR proteins in gliomagenesis, we used ES cell-mediated transgenesis to express wt and mutant EGFR molecules in mouse glial cells in vivo. We found that overexpression of EGFRvIII or EGFRvIII in mouse astrocytes was not sufficient to cause astrocyte transformation and result in glioma formation. However, in mice harboring an activated oncogenic Ras in astrocytes sufficient for glioma formation (10), the additional expression of EGFRvIII, but not EGFRvIII, led to accelerated glioma formation. In addition, the brain tumors in these mice exhibited histopathological features of oligodendroglioma and mixed oligoastrocytoma tumors. These results support the observation from human molecular clinical-epidemiological data that alterations in EGFR expression are important in the progression, rather than the initiation of gliomas. Furthermore, the finding of oligodendroglioma and mixed oligoastrocytoma lineage tumors in the mice expressing both oncogenic Ras and activated EGFR molecules supports the idea that dysregulation of multiple genetic pathways are important determinants of the histological phenotype of gliomas.
MATERIALS AND METHODS

Plasmid Construction. The human EGFRwt and EGFRVIII cDNAs (provided by Dr. C. David James, Mayo Clinic) were ligated respectively to the human GFAP promoter (obtained from Dr. Michael Brenner, National Institutes of Neurological Disorders and Stroke) based on a cloning strategy as described previously (10). An IRESLacZ cassette in which LacZ gene was fused to a nuclear localization signal and an IRES sequence, was inserted in the above vector to form GFAP-EGFRwt (or EGFRVIII)-IRESLacZpolyA-loxP-neo-loxP (Fig. 1A).

ES Cell-mediated Transgenesis to Establish GFAP-EGFRwt and GFAP-EGFRVIII Transgenic Mice. We followed the procedure as described previously (11).

Genotyping. PCR and Southern blot analysis were applied for genotyping and analyzing transgene copy in the transgenic lines. PCR was performed with ear-punched DNA and a sense primer (5'-ACTCTCTTATAAGCCCTCG-3') located in the GFAP promoter, and an antisense primer (5'-GTG-GAGATCGCCACTGATGGA-3') located in the EGFR cDNA. PCR was performed for 35 cycles with denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min. Southern blot analysis was undertaken as per standard protocols.

IHC and in Situ Hybridization. Preparation of tissue sections, H&E staining, and IHC analysis were performed according to standard procedures. Primary antibodies used were rabbit anti-GFAP (dilution 1:300; DAKO), mouse monoclonal anti-Brdu (dilution 1:80; Boehinger), mouse monoclonal antilacZ (dilution 1:10,000; BioLab), rabbit anti-Oligo2 (dilution 1:200, developed by Dr. Takebuyashi Hirohide), rabbit anti-Nestin (dilution 1:1000; Chemicon), rabbit anti-Factor VIII (dilution 1:200; UBI), mouse anti-NeuN (dilution 1:200; Chemicon), and goat anti-MAG (dilution 1:200, Santa Cruz). terminal deoxynucleotidyl transferase-mediated nick end labeling assay for detecting apoptosis was done based on the commercial kit (Boehinger). RNA in situ analysis of frozen sections of mouse brains were performed according to established protocols (12), with sense and antisense digoxigenin-labeled RNA probes which were in vitro transcribed from the full-length mouse Olig1 and Olig2 coding sequence.

Quantitation of Astrocytes and Oligodendrocytes in Transgenic Mice. GFAP and APC IHC was used to identify differentiated astrocytes and oligodendrocytes respectively in 40 µm of free-floating sections (anti-GFAP: 1:10,000; Zymed and anti-APC (Ab-7); Oncogene Sciences). GFAP-immunoreactive astrocytes and APC-immunoreactive oligodendrocytes were counted in the CA1 region of the hippocampus of six consecutive serial sections obtained from four to six animals from each genotype, as described previously (13). The mean number of astrocytes and oligodendrocytes ± SD was analyzed with ANOVA followed by the Bonferroni t test with significance set at P < 0.05.

Derivative Astrocytes, Cell Proliferation, Cell Transfection, Ras Activity, and Xenografts in Nod-Scid Mice. Derivative astrocytes from newborn GFAP-EGFRwt and GFAP-EGFRVIII transgenics or normal ICR littermates and their proliferation measurement were performed as described previously (10). To determine the location of transgenes on cell membrane of transgenic astrocytes, FACS was performed with anti-EGFR and anti-EGFRVIII extra-
cellular domain (Zymed) based on standard protocol. Measurement of the activity of Akt in those established transgenic astrocytes was done by Western blot with anti-Akt and anti-phospho-Akt antibodies (Pharmagen). pCX-V12Ha-ras-IRESpuro in which the V12Ha-ras cDNA with HA at the 5′ was inserted downstream of β-actin promoter with a cytomegalovirus enhancer, was transfected into these derivative astrocytes with Lipofectamine (Life Technologies, Inc.), and pooled (>100) clones selected with puromycin (2.5 μg/ml; Sigma) selection. Expression of exogenous V12Ha-ras was measured using Western blot with anti-HA antibody (Sigma). Ras activity was measured as percentage of Ras-GTP/Ras-GDP, using a previously described luciferase-based enzymatic assay (14). These pooled transfecants were evaluated for tumorigenicity by stereotactic injection of 105 cells into the corpus striatum of the Nod-Scid mice.

RESULTS

Establishment of GFAP-EGFRvIII and GFAP-EGFRwt Transgenic Mice. The transgene constructs (Fig. 1A) were stably integrated into R1 ES cells. An IRESlacZ attachment 3′ to the wt- and EGFR-vIII-coding sequence allowed LacZ visualization of cells expressing the transgenes. Because the GFAP promotor is not active in ES cells, astrocytic expression of the transgene in transfected ES clones was screened by in vitro differentiation into astrocytic lineage using RA as described previously (15). ES clones that expressed LacZ upon RA differentiation were selected for ES cells ↔ embryo aggregation, and two chimeric lines from each transgenic construct were established. The transmitting chimeric males were crossed with 129S1 inbred and ICR outbred females to produce hemizygous-transgenic offspring.

To analyze the cell type-specific expression of the EGFRvIII or EGFRwt transgenes in mice, IHC analysis demonstrated LacZ expression in subsets of GFAP-positive astrocytes (Fig. 1B) but not in adjacent neurons or brains from control littermates. Derivative astrocyte cultures from these transgenic mice brain showed increased expression of EGFRwt and EGFRvIII protein by Western blot analysis, compared with nontransgenic littermates (Fig. 1C). Western blot analysis of the astrocytes expressing EGFRwt and EGFRvIII confirmed that both receptors were phosphorylated (Fig. 1D). FACS assay with specific anti-EGFR and anti-EGFRvIII extracellular domain antibodies demonstrated the EGFRwt and EGFRvIII protein were located on the cell membrane of transgenic astrocytes (Fig. 1E).

All established GFAP-EGFRwt and GFAP-EGFRvIII transgenic mice (hemizygous and homozygous on both 129S1 and ICR genetic background) were healthy and fertile and did not demonstrate any clinical abnormalities even at 24 months of age. To determine whether these transgenic mice harbored any abnormalities in glial cell numbers, we determined both astrocyte (GFAP+) and oligodendrocyte (APC+) numbers in the CA1 region of the hippocampus from 2-3-month-old transgenic and control ICR littermates. As shown in Fig. 1D, there was a 50–60% increase in the number of GFAP-immunoreactive cells in the brains from either the GFAP-EGFRwt or GFAP-EGFRvIII transgenic mice compared with control littermates. This increase in astrocyte number appears to be progressive as 5–6 week old GFAP-EGFRvIII mice exhibit 12–15% more astrocytes (data not shown). Such increased astrocytes was because of the cell proliferation, as demonstrated by an in vitro proliferation assay, which showed the transgenic astrocytes from GFAP-EGFRwt and GFAP-EGFRvIII mice had a 1.5-fold increased cell proliferation compared with the control normal mouse astrocytes (Fig. 6C). In contrast, no significant increases in APC-immunoreactive oligodendrocytes were observed at either age (Fig. 1D).

EGFRvIII Transgene Expression Accelerated the Development of Gliomas in GFAP-V12Ha-ras Transgenic Mice. We previously established a GFAP-V12Ha-ras transgenic mouse astrocytoma model using the GFAP promoter to express an activated RAS molecule in astrocytes (10) based on previous studies demonstrating increased levels of activated Ras-GTP in human astrocytoma cell lines and tumors (16). In GFAP-V12Ha-ras transgenic mice harboring a single copy of the RAS transgene (RasB8), 50% of the mice developed astrocytomas of varying grades and died by 12–16 weeks (10) and (Fig. 2A), with 95% of the mice dead with astrocytomas by 24 weeks. To determine whether alterations in EGFR expression and function in combination with RAS activation could modify mouse survival or tumor histopathological phenotype, we made double transgensics by crossing the GFAP-EGFRwt or GFAP-EGFRvIII mice that do not develop gliomas, with the RasB8 GFAP-V12Ha-ras mouse. The overall survival of the GFAP-EGFRwt;V12Ha-Ras double transgenic mice and the histopathology of the resulting astrocytomas were identical to the RasB8 mice. However, transgenic mice expressing both V12Ha-ras and activated EGFRvIII exhibited a dramatic reduction in survival, with 50% of these mice dying with gliomas at 2–4 weeks (Fig. 2).

GFAP-EGFRvIII;GFAP-V12Ha-ras Transgenic Mouse Developed Oligodendrogial Lineage Tumors. In addition to an effect on tumor latency, GFAP-EGFRvIII;GFAP-V12Ha-ras transgenic mice developed tumors with histopathological features of oligodendrogliomas, with a small subset resembling mixed oligoastrocytomas. This pathologic appearance was different from that observed in the GFAP- V12Ha-Ras (B8) transgenic mouse astrocytomas, which were largely composed of GFAP and Nestin immunoreactive malignant astrocytes (Fig. 3 G–I). Instead of fibrillary astrocytomas, we found tumors in the GFAP-EGFRvIII;GFAP-V12Ha-ras mice exhibited oligodendrogial features (Figs. 3 and 4). The tumor cells had the typical fried egg appearance on H&E staining (Fig. 3A) with majority being GFAP and Nestin immunoneagtive, Fig. 4). Instead of fibrillary astrocytomas, we found tumors in the GFAP-EGFRvIII;GFAP-V12Ha-ras mice exhibited oligodendrogial features (Figs. 3 and 4). The tumor cells had the typical fried egg appearance on H&E staining (Fig. 3) with majority being GFAP and Nestin immunoneagtive (Figs. 3, B and C). A small percentage of the tumors observed contained a subpopulation of transformed cells that were both GFAP and Nestin immunoreactive (Fig. 3, E and F), suggesting a mixed oligoastrocytoma tumor. In addition, the infiltrative multifocal tumors had regions of intratumoral hemorrhage were often clustered in periventricular regions with the chicken wire-like vascularity seen in human oligodendrogliomas (Fig. 4, A–C; Ref. 17). Additional IHC characterization demonstrated the neoplastic cells lacked expression of markers found in mature oligodendrocytes (MAC, Fig. 4D), which are also absent in the majority of human oligodendrogliomas (18). The tumor cells were not neurons (NeuN immunoneagtive, Fig. 4E) but infiltrated through them. Although there is no single pathogenic imhC marker of oligodendrogliomas, the tumor cells immunostained positively for PDGF-α (Fig. 4F) and expressed OLIG1 (Fig. 5, A and B) and OLIG2 (Fig. 5, C and D) mRNA as detected by in situ hybridization, which collectively are highly suggestive of oligodendrogial lineage tumor cells (19, 20).
To demonstrate that the cells in these tumors were neoplastic and not dysplastic cells, derivative primary low-passage glioma cell lines from GFAP-EGFRvIII:GFAP-V12 Ha-ras transgenic mice were generated and injected intracranially in Nod-Scid mice. Tumors developed in recipient mice within 1 month, and the resulting tumor cells were GFAP immunonegative (data not shown) and expressed OLIG2, similar to the parental tumors (Fig. 5E).

**EGFRvIII and V12 Ha-ras Cooperate to Transform Astrocytes in Xenograft Models.** To determine whether the genetic cooperativity between EGFRvIII and V12 Ha-ras observed in the transgenic mice in vivo could be recapitulated in cell culture and xenograft models, derivative astrocytes from postnatal day 1 (P1) GFAP-EGFRwt, GFAP-EGFRvIII, and nontransgenic littermate mice were established. P1 derivative astrocytes expressing either EGFRwt or EGFRvIII had a 1.5-fold increased in vitro proliferation rates as wt astrocytes (up to five passages evaluated; Fig. 6C) and did not grow in Nod-Scid mice (data not shown).

GFAP-EGFRwt and GFAP-EGFRvIII primary astrocytes were next transfected with pCX-HA:V12 Ha-ras-IRESpuro and multiple clones (>100) were pooled for additional characterization. Overexpression of V12 Ha-ras in wt astrocytes and EGFRwt transgenic mouse astrocytes resulted in cellular senescence, with complete cell death by 7 days after transfection. In contrast, EGFRvIII transgenic astrocytes expressing V12 Ha-ras survived and exhibited more rapid in vitro growth rates than GFAP-EGFRwt, GFAP-EGFRvIII, or wt astrocytes. These astrocytes also had an inhibition of apoptosis (<2% apoptotic cells) compared with the normal mouse astrocytes (30–40% apoptotic cells) or the transgenic astrocytes expressing EGFRwt and EGFRvIII (25% apoptotic cells; Fig. 6D). Expression of V12 Ha-ras protein in GFAP-EGFRvIII transgenic astrocytes was verified by Western blot (Fig. 6A). Ras-GTP levels in the V12 Ha-ras stably transfected GFAP-EGFRvIII transgenic astrocytes were 2–3-fold higher than in astrocytes from GFAP-EGFRvIII or GFAP-EGFRwt transgenic or nontransgenic wt mice (Fig. 6B). These V12 Ha-ras-expressing GFAP-EGFRvIII transgenic astrocytes exhibited a high saturation density and reduced serum requirements and anchorage-independent growth compared with parental cells (data not shown). In addition, the V12 Ha-ras-expressing GFAP-EGFRvIII transgenic astrocytes had a high level of phosphorylation of Akt compared with the undetectable pAkt in the normal astrocytes or transgenic astrocytes only expressing

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**Fig. 3.** Histopathological features of oligodendroglioma and oligoastrocytoma in the GFAP-EGFRvIII:GFAP-V12 Ha-ras double transgenic mice. A–C, majority of the double transgenics were oligodendrogliomas with H&E staining (A) showing the typical fried egg appearance with homogenous round nuclei and condensed chromatin and GFAP (B) and Nestin (C) immunonegativity. D–F, a minority of the double transgenics showed oligoastrocytoma features with transformed appearing astrocytes with irregular nuclei (H&E, A) which were GFAP (E) and Nestin (F) positive, among the predominant oligodendroglioma cells. G–I, in contrast to the double transgenic mice with activated Ras and EGFRvIII, the GFAP-V12 Ha-ras (RasB8) single transgenic mice had predominantly astrocytic differentiation with vesiculated irregular nuclei (G) with the transformed cells all being GFAP (H) and Nestin positive (I). All magnification, ×1000.
EGFR and EGFRvIII (Fig. 6E). Finally, the tumorigenicity of these cells was assessed by their ability to grow as s.c. explants in Nod-Scid mice. Inoculation of $1 \times 10^6$ V12 Ha-ras-expressing GFAP-EGFRvIII transgenic astrocytes resulted in tumor formation in Nod-Scid mice within 20 days. The resulting tumors also demonstrated features suggestive of oligodendroglioma and exhibited the fried egg appearance on H&E paraffin sections associated with increased BrdUrd labeling (Fig. 6F) and were also positive for OLIG2 (data not shown).

**DISCUSSION**

Amplifications, mutations, and overexpression of EGFR are common gain of function aberrations found in GBMs but not in low-grade gliomas of any lineage (3, 21). This has led to therapeutic strategies targeting this receptor or associated downstream signaling pathways in GBMs. Amplification of EGFR is also present in a subset of oligodendrogliomas and is not observed in tumors that carry a good prognosis and exhibit loss of 1p or 19q (8). In contrast, oligodendrogliomas with EGFR amplification more often have concurrent losses of p16 and 10q (not loss of PTEN/MMAC1) and have a poorer prognosis. Our double transgenic model of oligodendrogliomas with overexpression of EGFRvIII and V12 Ha-ras presented is more akin to this second group of human oligodendrogliomas, with increased mortality compared with our previously reported single transgenic GFAP-V12 Ha-ras astrocytoma model (10). These human pathological-molecular-epidemiological data suggest two hypotheses, which are supported by mouse models. First, aberrant EGFR expression and function represent progression-associated events, rather than initiation factors in gliomagenesis. Second, aberrations in EGFR expression and function are not exclusively associated with astrocytic GBM tumors but likely lead to gliomas of both astrocytic and oligodendroglial histopathological subtypes.

The role of EGFR and its mitogenic ligands in astroglial cell proliferation has been well established (22). These mitogenic growth factors include EGF, transforming growth factor-α, and amphiregulin, which have all been shown to promote astrocyte proliferation in vitro. In keeping with a growth stimulatory function for EGFR in glia, astrocytes from mice lacking EGFR expression (EGFR<sup>−/−</sup>) exhibit slower proliferation rates in vitro and 30–50% fewer astrocytes in vivo (23, 24).

Although EGFR activation promotes astroglial cell proliferation, previous studies have suggested that EGFRvIII overexpression alone is not sufficient for glioma development. Using the RCAS/TVA retroviral approach, EGFRvIII overexpression either in mice expressing the TVA viral receptor in Nestin+ (neuronal precursors) or GFAP+ (astrocytes) cells did not yield gliomas (25). Similarly, retroviral expression of wt or mutant (vIII) EGFR molecules into normal neural stem cells or GFAP-immunoreactive astrocytes failed to result in tumor formation when the cells were orthotopically implanted into Nod-Scid mice (2). In our experiments, we took a direct approach to evaluating the contribution of EGFR overexpression on tumor formation by establishing transgenic mice that specifically express either EGFR<sup>wt</sup> or mutant EGFRvIII in astrocytes. We observed an increase in astrocyte numbers of GFAP-EGFR<sup>wt</sup> and GFAP-EGFRvIII transgenic mice at 2–3 months of age, compared with normal littermates, but no glioma formation. In addition, primary astrocyte cultures from postnatal day 1 of the above transgenic mice had similar in vitro growth to normal murine astrocytes and did not grow in Nod-Scid mice. Collectively, these results suggest that overexpression of EGFR<sup>wt</sup> or EGFRvIII is not sufficient to initiate gliomagenesis.

The cooperativity observed between mutant EGFR and oncogenic RAS expression in the transgenic mice described in this study suggests that high-grade glioma formation is facilitated by additional genetic alterations associated with tumor progression. One of these progression factors is the activating mutant EGFRvIII, as we observed decreased tumor latency and increased tumor grade in mice doubly transgenic for EGFRvIII and oncogenic RAS overexpression. Similar results have been obtained using other approaches such as retroviral transgene delivery. In mice expressing the RCAS TVA receptor in Nestin+ neuronal precursors, EGFRvIII transgene expression in...
combination with Ink4a gene defects resulted in glioma formation (25). Cooperativity was also observed in this system with mutations in TP53. Using both astrocytes and neural stem cells, EGFRvIII overexpression induces high-grade glioma formation in cells lacking Ink4a (2). These results are consistent with our observations in the GFAP-EGFRvIII;GFAP-V12 Ha-ras mouse. Furthermore, high-grade gliomas in the RasB8 mouse exhibit a number of additional genetic alterations, including amplification of the mouse chromosomal region containing the EGFR and overexpression of EGFR protein (10). The expression of other growth regulatory genes was also affected in the RasB8 transgenic mouse astrocytomas, including loss of p53, Rb, p16, p19, or PTEN expression and overexpression of MDM2 or cdk4 (10). In contrast, we did not observe these changes in the GFAP-EGFRvIII;GFAP-V12 Ha-ras mouse tumors (data not shown), suggesting that expression of the activated EGFRvIII is a potent enough progression factor in mice harboring an oncogenic Ras initiating event, which additional genetic alterations are not required for gliomagenesis.

The surprising observation in this study was the development of tumors with features of oligodendroglioma in mice in which both the EGFRvIII and oncogenic Ras molecules were expressed under the GFAP promoter, with the transgenes expressed around embryonic day 14.5 (27). However, the in vivo administration of EGF to neural progenitors results in expansion of populations of cells with immature astrocyte or oligodendrocyte phenotypes (28). Whereas oncogenic RAS expression in astroglial progenitors might have resulted in primarily type 1 astrocyte lineage tumors, the expression of EGFRvIII, similar to EGF administration in vivo, could have altered the differentiation fate toward an oligodendroglial lineage, which in combination with oncogenic RAS expression, resulted in oligodendroglial tumors.

Normally, oligodendroglcyte proliferation and differentiation is regulated by another EGFR-like molecule, erbB2. erbB2-containing heterodimeric receptor complexes are responsive to neuregulin-1, which promotes the proliferation of O2A progenitor cells and maintains these cells as dividing precursors by inhibiting their progression from O4 to O1 oligodendroglia (29). In fact, expression of v-erbB under the control of S-100 promoter leaded to oligodendrogliomas.4 It is possible that aberrant expression of the EGFRvIII transgene in progenitor cells provided a permissive context for oligodendrocyte development. Inappropriate EGFR signaling could have resulted in abnormal progenitor cell survival and facilitated the development of oligodendrogliomas in the presence of a transforming oncogenic RAS molecule. In support of this notion, when a related mitogenic factor, PDGF-B, was introduced into GFAP+ or Nestin+ cells in vitro and in vivo using the RCAS/TVA system (30), oligodendroglioma tumors formed. These results suggested that aberrant PDGF autocrine stimulation was sufficient to alter astrocyte differentiation. We propose that inappropriate mitogenic growth factor signaling either conferred...
by PDGF or EGFRvIII overexpression results in phenotypic changes in astroglial cell differentiation and histopathological tumor appearance. Additional experiments using defined progenitor cell populations will be required to demonstrate changes in the differentiation potential of astroglial precursor cells expressing an activated EGFR.

In conclusion, we have demonstrated using transgenic mouse modeling that the mutated EGFRvIII molecule that is aberrantly activated and overexpressed in human GBMs and high-grade oligodendrogliomas is not sufficient by itself to initiate gliomagenesis. In the context of additional genetic alterations such as $V_{12}^{Ha-ras}$-ras expression, expression of EGFRvIII potentiates glioma formation and influences glioma histological phenotype. These mice provide a reproducible and spontaneous model of oligodendroglioma, which can be used to address the genetic and cell fate conditions critical for oligodendroglioma development and progression as well as to provide a tractable preclinical model for these currently incurable tumors.

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