VOLUME 28 · NUMBER 18 · JUNE 20 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Integrated Molecular Genetic Profiling of Pediatric High-Grade Gliomas Reveals Key Differences With the Adult Disease

Barbara S. Paugh, Chunxu Qu, Chris Jones, Zhaoli Liu, Martyna Adamowicz-Brice, Junyuan Zhang, Dorine A. Bax, Beth Coyle, Jennifer Barrow, Darren Hargrave, James Lowe, Amar Gajjar, Wei Zhao, Alberto Broniscer, David W. Ellison, Richard G. Grundy, and Suzanne J. Baker

See accompanying article on page 3054

A B S T R A C T

Purpose
To define copy number alterations and gene expression signatures underlying pediatric high-grade glioma (HGG).

Patients and Methods
We conducted a high-resolution analysis of genomic imbalances in 78 de novo pediatric HGGs, including seven diffuse intrinsic pontine gliomas, and 10 HGGs arising in children who received cranial irradiation for a previous cancer using single nucleotide polymorphism microarray analysis. Gene expression was analyzed with gene expression microarrays for 53 tumors. Results were compared with publicly available data from adult tumors.

Results
Significant differences in copy number alterations distinguish childhood and adult glioblastoma. *PDGFRA* was the predominant target of focal amplification in childhood HGG, including diffuse intrinsic pontine gliomas, and gene expression analyses supported an important role for deregulated PDGFRα signaling in pediatric HGG. No *IDH1* hotspot mutations were found in pediatric tumors, highlighting molecular differences with adult secondary glioblastoma. Pediatric and adult glioblastomas were clearly distinguished by frequent gain of chromosome 1q (30% *v* 9%, respectively) and lower frequency of chromosome 7 gain (13% *v* 74%, respectively) and 10q loss (35% *v* 80%, respectively). *PDGFRA* amplification and 1q gain occurred at significantly higher frequency in irradiation-induced tumors, suggesting that these are initiating events in childhood gliomagenesis. A subset of pediatric HGGs showed minimal copy number changes.

Conclusion
Integrated molecular profiling showed substantial differences in the molecular features underlying pediatric and adult HGG, indicating that findings in adult tumors cannot be simply extrapolated to younger patients. PDGFRα may be a useful target for pediatric HGG, including diffuse pontine gliomas.

J Clin Oncol 28:3061-3068. © 2010 by American Society of Clinical Oncology

From the St Jude Children's Research Hospital, Memphis, TN; Institute for Cancer Research; Royal Marsden National Health Service Foundation Trust, Surrey; and The Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, United Kingdom.

Submitted October 20, 2009; accepted March 10, 2010; published online ahead of print at www.jco.org on May 17, 2010.

Supported by grants from the Children's Brain Tumor Foundation (S.J.B.), Grant No. P01 CA096832 from the National Institutes of Health, The Sydney Schlobohm Chair of Research from the National Brain Tumor Society, the Ryan McGee Foundation, Musicians Against Childhood Cancer, the Noyes Brain Tumor Foundation, and American Lebanese Syrian Associated Charities. The Children's Brain Tumour Research Centre is supported by the Samantha Dickson Brain Tumour Trust, Air and Ground, The Connie and Albert Taylor Trust, and the Joe Foote Foundation. The Children's Cancer and Leukemia Group Tumour Bank is supported by Cancer Research UK. The Institute for Cancer Research is supported by Cancer Research UK and National Health Service funding to the National Institute for Health Research Biomedical Research Centre.

B.S.P., C.Q., and C.J. contributed equally to this work.

Presented in part at the Inaugural Pediatric Neuro-Oncology Basic and Translational Research Conference, Asheville, NC, October 2, 2009, and the American Association for Cancer Research Genetics and Biology of Brain Cancer Meeting, San Diego, CA, December 13-15, 2009.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Suzanne J. Baker, PhD, Department of Developmental Neurobiology, St Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105; e-mail: Suzanne.Baker@stjude.org.

0732-183X/10/2818-3061/$20.00

DOI: 10.1200/JCO.2009.26.7252

INTRODUCTION

High-grade gliomas (HGGs) comprise 15% to 20% of all childhood tumors of the CNS, and 70% to 90% of patients die within 2 years of diagnosis. Consequently, improved understanding of pediatric HGG to identify relevant therapeutic targets is essential.[1]

The frequency, anatomic location, and pathologic spectrum of gliomas differ in children and adults, suggesting that the representation of progenitor and mature cell types, as well as the microenvironment within the developing brain, may influence the disease process. Glioblastomas dominate adult disease, whereas juvenile pilocytic astrocytomas are the most common brain tumors in children. Pediatric glioblastomas often arise in brain regions that are rarely targeted in adult disease. In adults, most low-grade diffuse gliomas undergo anaplastic progression to a high-grade tumor over time, but progression of pediatric low-grade diffuse gliomas is rare.[2,3]

Array-based studies of adult glioblastoma identified common regions of genomic gain and loss and gene expression signatures, allowing molecular subclassification of tumors.[4-11] Comprehensive studies integrating copy number, gene expression, and mutation analyses reported that virtually all glioblastomas have disrupted the p53,

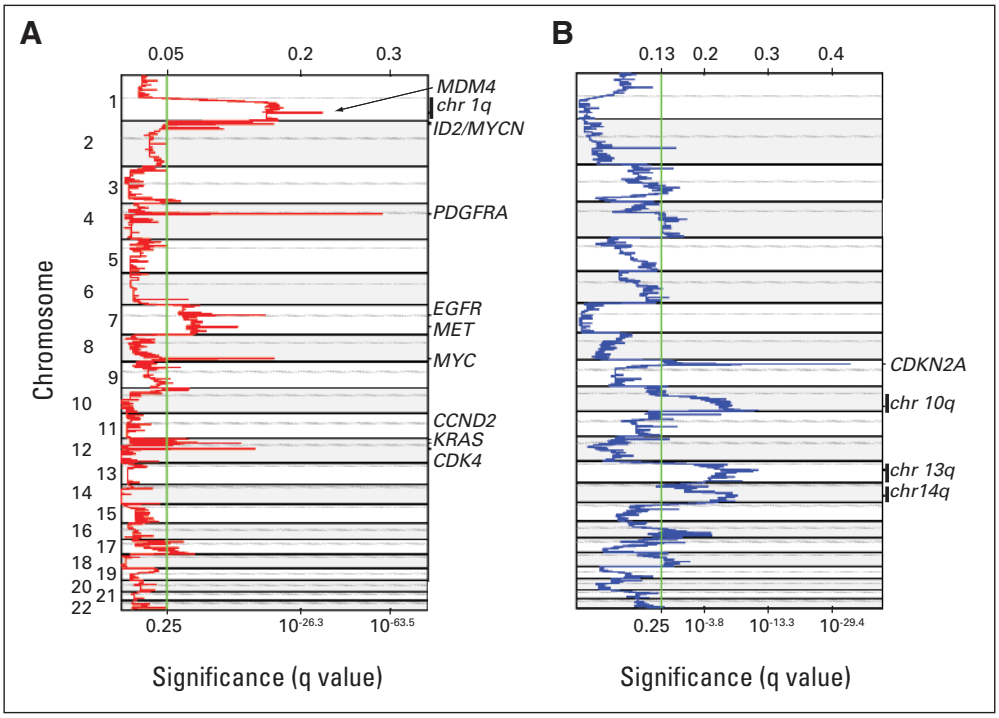

Fig 1. Most significant genomic alterations in pediatric high-grade glioma (HGG) identified by Genomic Identification of Significant Targets in Cancer (GISTIC). Significance of copy number (A) gains and (B) losses identified in 68 de novo pediatric HGGs by GISTIC is shown. Chromosome positions are displayed along the *y*-axis, calculated G-score is above the *x*-axis, and false discovery rate q values are along the lower *x*-axis. The green line indicates a q value threshold of 0.25.

PI3K/receptor tyrosine kinase (RTK), and RB pathways through various genetic mechanisms.[12,13]

By comparison, pediatric HGG is an understudied disease. Specific genetic alterations underlying pediatric HGG were defined primarily by directed analyses of genes that are mutated in the more common adult HGG. Mutations in *TP53*, *CDKN2A*, and *PIK3CA* are common in both adult and pediatric HGG.[14-16] *PTEN* mutations and *EGFR* amplifications, which are frequent in adult primary glioblastoma, are less common in pediatric HGGs, which also arise de novo.[15,17] Two disease subsets of pediatric glioblastoma with differential survival that were distinguishable from adult glioblastoma were identified based on expression signatures.[18] Array-based copy number studies of pediatric HGG using relatively small sample sizes supported a difference between childhood and adult tumors.[19,20]

Here, we provide, to our knowledge, the first report of a high-resolution unbiased analysis of genomic imbalances and gene expression signatures in a large collection of pediatric HGGs. We show that HGGs in children and adults are a related spectrum of disease driven by significantly different frequencies of genomic alterations.

PATIENTS AND METHODS

Samples and Nucleic Acid Extraction

We analyzed snap-frozen HGG specimens from 78 pediatric patients (< 23 years old) from St Jude Children's Research Hospital (Memphis, TN) and the Children's Cancer and Leukemia Group in the United Kingdom (Data Supplement). Ethical review committee approval was obtained from each institution/consortium. All tumors were collected before adjuvant therapy for the glioma including 10 gliomas that arose in patients who previously received irradiation (IR) for a different cancer (IR-induced tumors). Sections from matched formalin-fixed paraffin-embedded tissue were reviewed by neuropathologists (D.W.E. and J.L.). DNA extraction and, when sufficient material was available, RNA extraction and tissue smears were performed as described.[21]

Copy Number, mRNA Expression Profiling, and Statistical Analyses

DNA was labeled and hybridized to Affymetrix 500K GeneChips (Affymetrix, Santa Clara, CA). Fifty-three tumor samples with qualified RNA were profiled using Affymetrix Human Genome U133 Plus 2.0 arrays. Details of single nucleotide polymorphism data analyses, validation by quantitative polymerase chain reaction (Data Supplement) and FISH, and expression and statistical analyses are provided in the Appendix (online only). Array data are deposited at the Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/, accession No. GSE19578).

RESULTS

Comprehensive Mapping of Copy Number Changes in Pediatric HGG

We used Affymetrix 500K GeneChips to identify copy number imbalances in 58 WHO grade 4 tumors and 20 WHO grade 3 pediatric HGGs (Data Supplement). Genomic Identification of Significant Targets in Cancer (GISTIC)[22] was used to identify significant copy number aberrations (Fig 1). We excluded the 10 IR-induced tumors from the GISTIC analysis. To define candidate pediatric HGG cancer genes, we mapped regions of focal high-level amplification (copy number > five) or likely homozygous deletion (focal loss with copy number < 0.8), after exclusion for normal copy number variation (Data Supplement).

Recurrent broad low-amplitude gains of chromosome 1q and focal high-amplitude gains encompassing *PDGFRA* were observed at the highest frequency (29% and 12%, respectively; Fig 1A). The minimal common region of focal amplification was restricted to *PDGFRA*, which was consistently overexpressed when amplified (Fig 2, Data

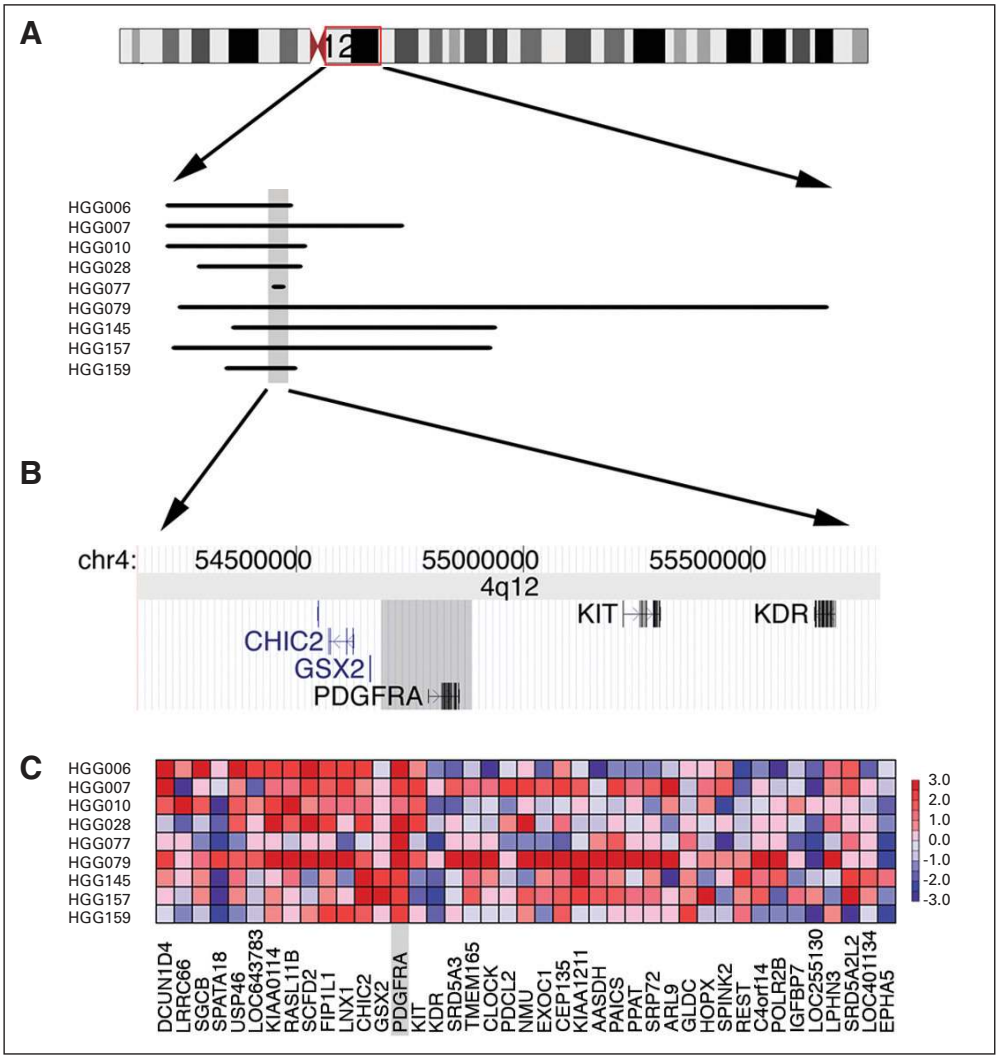

Fig 2. *PDGFRA* is the target of common amplifications. (A) The extent of amplification for each tumor is shown. (B) Genes within the minimal common region and 500 kilobases upstream and 900 kilobases downstream. (C) Expression of all probes within the maximal region of amplification. The minimal common region of amplification is shown with a gray bar in all panels. Only tumors with matched expression data are shown.

Supplement). Other significant gains were found in only 1% to 4% of tumors, with significance scores driven by high-level amplification (Fig 1A). Additional amplified genes included those encoding cell cycle progression proteins (*CCND2*, *CDK4*, *MYC*, and *MYCN*), RTKs and ligands (*EGFR*, *MET*, *PDGFB*, and *NRG1*), members of the PI3K pathway (*PIK3C2B*, *PIK3C2G*, *PIK3R5*, *KRAS*, *AKT1*, and *S6K1*), and p53 pathway regulation (*MDM4*; Data Supplement).

In contrast, most of the significant losses involved broad regions including chromosomes 10q (38%), 13q (34%), and 14q (29%; Fig 1B). The only narrow peak of high significance identified focal homozygous deletion encompassing *CDKN2A/CDKN2B* in 19% of tumors and was associated with absent expression of both genes (Data Supplement). Additional homozygous deletions of tumor suppressor genes of known importance in glioma included *CDKN2C*, *NF1*, *PTEN*, *RB1*, *TP53*, and *TP73*, reflecting abrogation of common signaling pathways,[12,13] and the tyrosine phosphatase *PTPRD*.[23,24] Further candidates included other tyrosine phosphatases (*PTPRE* and *PTPN2*), DNA repair genes (*ATR*, *TOPBP1*, and *KU80*), and members of the Notch pathway (*DLK1* and *NEDD4L*; Data Supplement).

Seven glioblastoma samples were diffuse intrinsic pontine gliomas, an understudied tumor type that is rarely biopsied.[1] Focal amplification of *PDGFRA* was observed in two (29%) of seven of these tumors. Copy number imbalances in this subgroup were not significantly different from other glioblastomas ($P > .1$ for gains or losses of all individual chromosome arms, *PDGFRA* amplification, and *CDKN2A* deletion; Data Supplement).

Overall, there was an average of 5.7 large regions of copy number imbalance per tumor (median, four regions; range, zero to 23 regions), with no difference between histologies or grade of tumor ($P > .3$; Data Supplement). The total numbers of large-scale gains and losses per tumor were not significantly different in IR-induced tumors ($P > .19$, nonparametric Wilcoxon test). However, specific gains of chromosome 1q and 9q and losses of 13q and 1p were significantly more frequent in IR-induced tumors compared with the rest of the HGGs (Table 1; $P = .03$, $P = .01$, $P = .04$, and $P = .01$, respectively). All other copy number imbalances for individual chromosomal arms were not significantly different between IR-induced tumors and other HGGs ($P > .1$, Fisher's exact test). One hundred ninety-six regions with focal aberrations were observed, comprising 66 amplifications and 130 deletions. Focal amplifications of *PDGFRA* and deletions of *CDKN2A/B* were significantly more common in IR-induced tumors (Table 1; $P = .01$ and $P = .05$, respectively).

Table 1. Copy Number Changes in Pediatric and Adult HGG

Region	IR-Induced HGG* (n = 10) %	Pediatric HGG† (n = 68) %	Pediatric GBM‡ (n = 46) %	Adult GBM§ (n = 189) %	P‖
	Percent				
Gains					
1q	70	29	30	9	.001
7	0	15	13	74	< .001
9q	40	7	9	8	1.00
Losses					
1p	50	12	9	2	.05
4q	20	21	22	2	< .001
9p	20	18	17	33	< .05
10q	20	38	35	80	< .001
13q	70	34	35	31	.7
14q	40	29	28	26	.9
16q	20	22	24	7	.003
22q	0	19	15	23	.13
Frequent focal changes					
PDGFRA	50	12	17	11¶	.2
EGFR	0	3	0	43¶	< .001
CDKN2A	50	19	20	55¶	< .001

Abbreviations: IR, irradiation; HGG, high-grade glioma; GBM, glioblastoma.
*Pediatric HGG arising in patients who previously received cranial irradiation for a different cancer.
†Includes all HGG tumor subtypes, except tumors with previous IR.
‡Pediatric GBMs only, excluding tumors with previous IR.
§Adult GBM copy number data were downloaded from The Cancer Genome Atlas (TCGA). Data for 189 samples analyzed on the SNP6 platform were available (February 2009) and used for large-scale gain and loss comparison. For focal gene aberrations, the data for 206 adult GBMs from TCGA were used.
‖Fisher's two-tailed test comparison of pediatric GBM and adult GBM.
¶Sample size was 206 tumors.

There was a significant association between 1q gain and decreased survival among patients with glioblastoma, excluding IR-induced tumors ($P = .04$; Data Supplement); however, we could not determine whether this effect was independent of treatment with the available sample size.

Pediatric HGG Genome Overlaps With, but Is Distinct From, Primary and Secondary Adult Glioblastoma

We compared imbalances in pediatric HGG with published findings on copy number changes in adult glioblastoma. We considered frequencies in all pediatric HGG and pediatric glioblastoma alone, excluding variant glioblastomas, for comparison with adult glioblastoma (Table 1 and Data Supplement). Pediatric glioblastomas were clearly distinguished from adult glioblastomas by frequent gain of chromosome 1q and the paucity of chromosome 7 gains and 10q losses. The most frequent focal amplifications differ, with *PDGFRA* and *EGFR* predominant in childhood and adult populations, respectively. In contrast, the frequencies of 13q and 14q loss were similar between pediatric and adult glioblastoma. Copy number imbalances were not significantly different between pediatric glioblastomas and all de novo pediatric HGG ($P > .2$; Table 1).

In adults, secondary glioblastomas show overexpression or amplification of *PDGFRA* but rarely contain *EGFR* amplification,[3] suggesting that pediatric HGG with *PDGFRA* amplification may be molecularly similar to adult secondary glioblastoma. *IDH1* mutations at codon 132 strongly distinguish adult secondary from primary glioblastoma, with frequencies of 85% compared with 5%, respectively.[12,25-28] We sequenced *IDH1* exon 4, containing codon 132, from 78 pediatric HGGs and 11 pediatric low-grade gliomas. No codon 132 mutations were detected, consistent with previous reports showing only rare *IDH1* mutations in smaller collections of pediatric HGGs.[26,28] The only *IDH1* alterations found in our pediatric collection were in HGG153, which contained two missense mutations in trans, encoding R49C and G97D, which were not found in the matched germline DNA (Data Supplement), and focal homozygous deletion encompassing *IDH1* in HGG10. The absence of hotspot mutations in *IDH1* strongly distinguished pediatric HGG from adult secondary glioblastoma.

Although the majority of pediatric HGGs showed multiple genomic imbalances, 15 tumors in our collection showed relatively stable genomes (Data Supplement). Tissue smears from the frozen sample used for DNA and RNA extraction were available for nine of 15 stable samples, and seven of nine smears showed greater than 75% tumor cells (reviewed by D.W.E.), strongly suggesting that the tumor samples were of sufficient purity to detect copy number imbalances. Normal tissue within primary tumor samples can mask detection of copy number imbalances, especially homozygous deletions.[29] Some of the stable cases showed conclusive evidence of minimal contaminating normal tissue by detection of homozygous deletions, loss of heterozygosity, and point mutations (Data Supplement). Thus, a subset of pediatric HGGs showed minimal copy number imbalances in relatively pure tumor samples.

Expression Profiling of Pediatric HGG Identifies Three Major Subclassifications

We analyzed gene expression profiles from 53 of the tumors in our collection. Unsupervised hierarchical clustering identified three main tumor subgroups (HC1 to HC3; Data Supplement). Gene ontology analysis of upregulated genes that most discriminate each subgroup from the others (Data Supplement) showed the most significantly overexpressed genes were associated with cell cycle regulation in HC1, with neuronal differentiation in HC2, and with extracellular matrix–receptor interactions and cell adhesion in HC3. We used gene set enrichment analysis to show that these pediatric subgroups significantly recapitulated subgroups previously defined in adult HGG, termed proliferative (Prolif), proneural (PN), and mesenchymal (Mes)[9] (Data Supplement).

We integrated genomic copy number imbalances with this expression subgroup classification (Fig 3). Most common abnormalities were distributed across subgroups. However, seven (88%) of eight of the amplifications targeting the PDGFR signaling cascade through *PDGFRA* and/or *PDGFB* were found in the Prolif/HC1 subgroup (association with this subgroup, odds ratio = 8.44, $P = .05$), implicating this pathway as a strong driver of proliferation in childhood tumors. Gain of 1q was found at high frequency in the Prolif/HC1 tumors (52%) and the PN/HC2 group (23%) but was significantly under-represented in the Mes/HC3 subclass (8%; odds ratio = 0.12; $P = .04$, Fisher's exact test).

Supervised comparison of glioblastomas to anaplastic astrocytomas showed significantly increased expression of genes associated with angiogenesis in glioblastoma, a strong molecular signature relating to the microvascular proliferation that is characteristic of these tumors (Data Supplement). Gene expression profiles were available

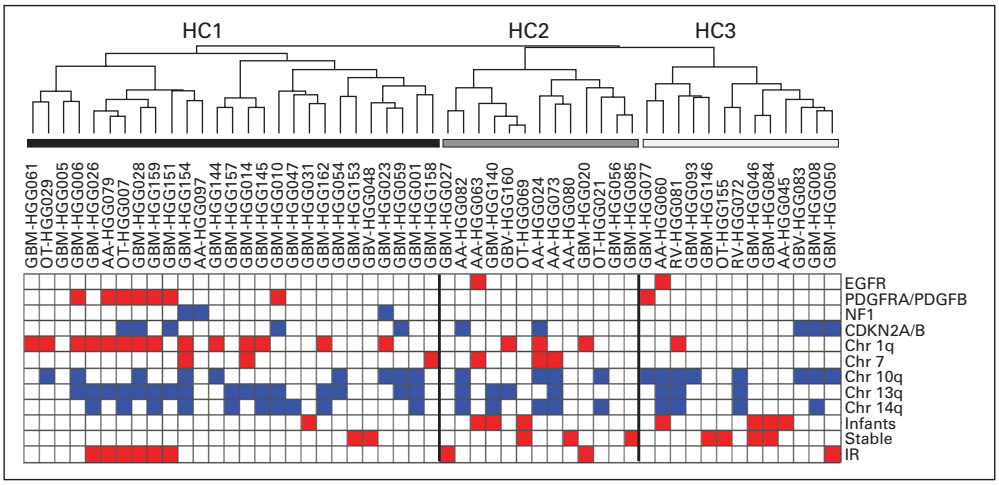

Fig 3. Integrated gene expression subgroups and copy number imbalances. Distribution of significant copy number features and age information among expression subgroups that were identified by unsupervised hierarchical clustering, with dendrogram and tumor identification numbers shown above. Copy number features include amplification of *EGFR*, *PDGFRA*, and *PDGFB* (red); homozygous deletion of *CDKN2A*; focal deletion or inherited mutation of *NF1* (blue); and broad regions of gain (red, chr1q and chr7) and loss (blue, chr10q, chr13q, and chr14q). "Infants" are patients younger than age 3 years at diagnosis. "Stable" indicates tumors that did not have large-scale genomic changes. IR, irradiation induced.

from nine of 15 tumors showing overall genomic stability, and they were represented in each expression subgroup (Fig 3). As a group, tumors with a stable genome showed decreased expression of genes associated with cell cycle and DNA repair (Data Supplement).

Patients with HGG younger than age 3 years have a better prognosis than older children.[30] None of the 11 infant HGGs showed chromosome 1q gain, a significant difference compared with HGGs from older children ($P = .03$). At the gene expression level, infant tumor gene expression profiles were heterogeneous, distributing among all three expression subclasses (Fig 3). Infant HGGs overexpressed genes involved in nervous system development and calcium ion binding and showed coordinated underexpression of multiple *HOX* genes when compared with HGGs from older children (Data Supplement).

As a group, IR-induced tumors significantly overexpressed genes relating to control of gene expression and metabolic processes compared with other pediatric HGGs (Data Supplement). There was significant over-representation of genes that mapped to chromosome 1q ($P < .001$) and the region of amplification encompassing *PDGFRA* ($P < .001$), reflecting the higher incidence of these copy number gains in IR-induced tumors.

Differential Gene Expression Signature Drivers in Pediatric Versus Adult HGG

We performed a principal component analysis (PCA) using data from our pediatric glioblastomas and adult glioblastomas that were analyzed on the same platform (n = 32,[22,31] Fig 4). The first component of the PCA is predominantly associated with differences between the PN, Prolif, and Mes subgroups found in both pediatric and adult tumors. However, the second principal component shows a trend separating pediatric and adult tumors. Consistent with differences in frequencies of amplification, *PDGFRA* was significantly overexpressed (q = 0.000002) and *EGFR* was significantly repressed in pediatric tumors (q = 0.0003; Data Supplement). Gene ontology analysis highlighted over-represented pathways among the differentially expressed genes, including immune response, response to extracellular stimulus, cell adhesion, cytokine- and chemokine-mediated signaling pathways, and calcium-mediated pathways.

To determine whether gene expression signatures of pediatric HGG more closely resemble the smaller subset of adult glioblastomas with *PDGFRA* amplification, we identified gene sets distinguishing adult glioblastomas with *PDGFRA* amplification from those with *EGFR* amplification using published data[13] (Data Supplement) and applied gene set enrichment analysis to the combined data from our pediatric collection and the adult glioblastoma data set used in Figure 4. Pediatric glioblastomas, regardless of *PDGFRA* amplification status, show significantly increased expression of the gene set upregulated in adult *PDGFRA*-amplified tumors (Fig 5A), whereas the gene set that is upregulated in adult *EGFR*-amplified tumors is downregulated in pediatric HGG as well as the adult PN subclass (Fig 5B). The adult PN subclass also shows overexpression of certain genes within the adult *PDGFRA*-amplified gene set, although these genes comprise a distinct subset compared with those upregulated in pediatric HGG (Fig 5A; Data Supplement). Notably, one adult tumor from a 53-year-old patient was similar to pediatric tumors in expression of the TCGA *PDGFRA* gene set and showed similar positioning to pediatric tumors in the PCA analysis (Figs 4 and 5). The subset of the *PDGFRA*-amplified gene set that showed the greatest enrichment in pediatric

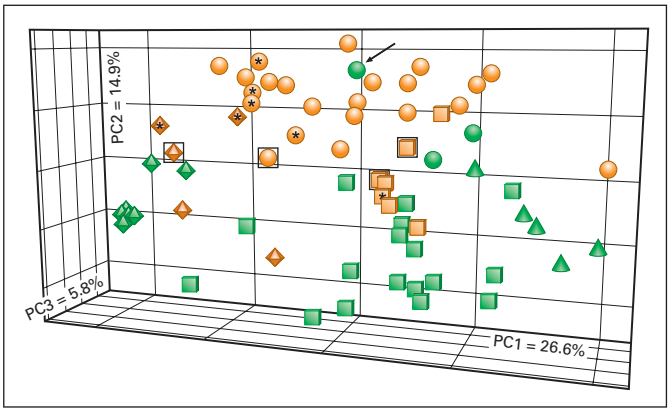

Fig 4. Principal component analysis (PCA) shows differences and similarities between pediatric and adult glioblastoma. PCA was generated using the 1,000 most variable probes and glioblastomas from our pediatric cohort and from published data from adult glioblastoma.[22,31] Pediatric and adult tumors are shown in orange and green, respectively. Tumor subgroups are indicated by shape as follows: proneural (diamonds), proliferative (Prolif; spheres), mesenchymal (Mes; cubes), and Prolif/Mes (cones). Adult tumor subgroups are based on Lee et al.[31] Asterisks indicate irradiation-induced tumors. Arrow indicates tumor from 53-year-old patient. Black boxes indicate tumors from infants.

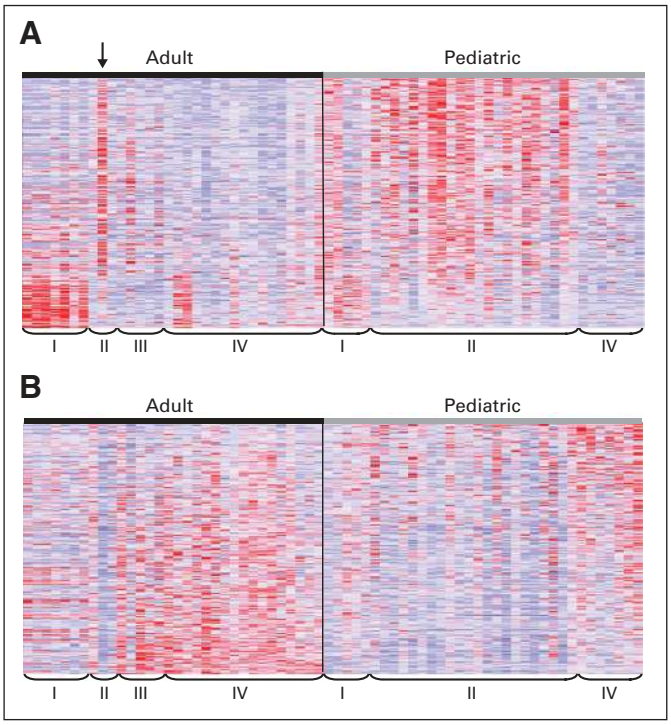

Fig 5. Differential expression of *PDGFRA* and *EGFR* gene signatures in pediatric versus adult glioblastoma. Gene sets that are either upregulated in *PDGFRA*-amplified tumors (The Cancer Genome Atlas [TCGA] *PDGFRA* set) or upregulated in *EGFR*-amplified tumors (TCGA *EGFR* set) were identified using TCGA data from adult glioblastomas and used for gene set enrichment analysis (GSEA) to compare our pediatric glioblastoma data to published adult glioblastoma data.[22,31] Expression heat maps of (A) the TCGA *PDGFRA* set genes and (B) the TCGA *EGFR* set genes in adult and pediatric glioblastomas are shown. Tumor subgroups are indicated by proneural (PN; I), proliferative (Prolif; II), Prolif/mesenchymal (Prolif/Mes; III), and Mes (IV). Adult tumor subgroups are based on Lee et al,[31] and pediatric tumor subgroups are based on unsupervised hierarchical clustering. Arrow indicates tumor from a 53-year-old patient. GSEA showed significantly increased expression of the TCGA *PDGFRA* set (nominal $P = .116$, false discovery rate [FDR] = 0.121), whereas the TCGA *EGFR* set is downregulated in pediatric high-grade gliomas (nominal $P = .229$, FDR = 0.238) as well as the adult PN subclass. Overexpression of the TCGA *PDGFRA* set and underexpression of the TCGA *EGFR* set shows even greater enrichment in pediatric glioblastoma if pediatric tumors in the Mes subgroup are removed from the analysis (upregulation of *PDGFRA* gene set; nominal $P = .048$, FDR = 0.108 and downregulation of *EGFR* gene set, nominal $P = .108$, FDR = 0.108).

compared with adult glioblastoma was associated with cell cycle regulation and multicellular organismal development.

DISCUSSION

The high-resolution analysis of copy number and gene expression signatures reported here demonstrates that pediatric and adult HGGs represent a related spectrum of disease distinguished by differences in the frequency of copy number changes, in specific gene expression signatures, and by the absence of *IDH1* hotspot mutations. In pediatric HGG, numerous genes within the p53, PI3K/RTK, and RB pathways are targeted by focal gain or loss (Data Supplement), but with the exception of *PDGFRA* and *CDKN2A*, other alterations were found only at low frequency.

Although the majority of pediatric HGGs in our series showed multiple genomic imbalances, a subgroup of tumors (15 of 78 tumors, 19%) lacked large-scale copy number changes. Other childhood tumors show subsets with balanced genomic profiles including ependymoma and CNS supratentorial primitive neuroectodermal tumors,[32-34] Ewing sarcoma,[35] and Wilms tumor.[36] Tumors with balanced genomes may possess an inherited or acquired predisposition for generating copy neutral mutations, such as the subset of colorectal cancers that arise in the context of DNA mismatch repair.[37] Alternatively, relatively fewer mutations may be required to drive the disease, as in pediatric acute myeloid leukemias, which show low frequency copy number imbalances and sequence alterations.[38]

Frequent gain of chromosome 1q clearly distinguished childhood from adult HGG and showed corresponding upregulation of gene expression involving the whole chromosomal arm, precluding identification of a focal target. A similar pattern of differential gain of 1q in childhood compared with adult brain tumors is seen in ependymoma,[32,33,39] and gain of 1q is common in other pediatric malignancies.[40-43]

PDGFRA is the predominant target of focal amplification in pediatric HGG, in contrast to adult glioblastoma, where *EGFR* is the most common target. Previous studies have suggested that overexpression may be an alternative mechanism of activating EGFR in childhood glioblastoma.[44-46] However, we found that *EGFR* was significantly underexpressed in pediatric compared with adult glioblastoma. The gene expression signature of adult tumors associated with *EGFR* amplification was relatively underexpressed in pediatric glioblastoma, whereas the gene expression signature associated with *PDGFRA* amplification was significantly overexpressed in pediatric glioblastomas, even in tumors that did not show amplification of the gene. Overall, both the copy number and gene expression analyses suggest that PDGFRα may be an important therapeutic target for pediatric HGG, including diffuse intrinsic pontine gliomas. A small subset of pediatric glioblastomas within the Mes subgroup appeared similar to adult tumors of the same subgroup when evaluated by PCA (Fig 4). The median age of onset for these tumors was 11.6 years, and one tumor was from an infant, so this similarity is independent of age. Interestingly, the gene expression signatures in this subset of pediatric tumors also showed the greatest similarity to adult glioblastomas with *EGFR* amplification (Fig 5B) and may indicate a small pediatric subgroup in which EGFR inhibitors may have a greater effect.

The preferential targeting of *PDGFRA* and *EGFR* in pediatric and adult HGG, respectively, suggests that the developing brain is more susceptible to oncogenic transformation triggered by aberrant PDGFR signaling. These differences may reflect changes in the populations of cell types expressing the growth factor receptors and their ligands during development and differentiation and complex regulation causing different cellular responses to activated signaling of the receptors. Both growth factor receptors play important roles in nervous system development and lineage commitment.[47-49] Mouse models suggest that aberrant PDGF signaling, but not EGFR activation, was sufficient to trigger glioma formation.[50-54] This is consistent with observations in human tumors where *EGFR* amplifications are rare in lower grade gliomas, whereas PDGF and PDGFR overexpression/amplification are found in both low-grade and high-grade astrocytomas, suggesting that activated PDGFR signaling is an early event in gliomagenesis.[3]

Mutations that cause tumor initiation will lead to tumor formation more rapidly by expanding the pool of available cells that may acquire additional mutations. This may be particularly relevant in

early disease onset in children. Ten tumors in our study arose in patients previously treated for another cancer with cranial IR (IR induced), a mutagenic exposure that increases the risk of brain tumors.[55] IR-induced tumors were similar to other pediatric HGGs, with gene expression profiles distinguishing them from adult glioblastomas by PCA (Fig 4) and in histopathologic features. The increased incidence of chromosome 1q gain and *PDGFRA* amplifications seen in IR-induced HGG may reflect radiation-induced initiating mutations that greatly increase the likelihood of developing childhood HGG. The same mutations also confer a strong selective advantage in pediatric HGG tumors arising spontaneously. Many pediatric HGG that lack amplification of *PDGFRA* or *PDGFB* still show strong expression of the gene signatures associated with *PDGFRA* amplification (Fig 5A), supporting the hypothesis that this pathway plays a central role in pediatric HGG and may be activated by multiple mechanisms.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Barbara S. Paugh, Chunxu Qu, Zhaoli Liu, David W. Ellison, Richard G. Grundy, Suzanne J. Baker
Financial support: Amar Gajjar, Richard G. Grundy, Suzanne J. Baker
Administrative support: Amar Gajjar
Provision of study materials or patients: Chris Jones, Darren Hargrave, Amar Gajjar, Alberto Broniscer, David W. Ellison, Richard G. Grundy
Collection and assembly of data: Barbara S. Paugh, Zhaoli Liu, Martyna Adamowicz-Brice, Junyuan Zhang, Dorine A. Bax, Darren Hargrave, James Lowe, Alberto Broniscer, David W. Ellison, Richard G. Grundy
Data analysis and interpretation: Barbara S. Paugh, Chunxu Qu, Chris Jones, Zhaoli Liu, Martyna Adamowicz-Brice, Dorine A. Bax, Beth Coyle, Jennifer Barrow, James Lowe, Wei Zhao, David W. Ellison, Richard G. Grundy, Suzanne J. Baker
Manuscript writing: Barbara S. Paugh, Chunxu Qu, Chris Jones, David W. Ellison, Richard G. Grundy, Suzanne J. Baker
Final approval of manuscript: Barbara S. Paugh, Chunxu Qu, Chris Jones, Zhaoli Liu, Martyna Adamowicz-Brice, Junyuan Zhang, Dorine A. Bax, Beth Coyle, Jennifer Barrow, Darren Hargrave, James Lowe, Amar Gajjar, Wei Zhao, Alberto Broniscer, David W. Ellison, Richard G. Grundy, Suzanne J. Baker

REFERENCES

1. Broniscer A, Gajjar A: Supratentorial high-grade astrocytoma and diffuse brainstem glioma: Two challenges for the pediatric oncologist. Oncologist 9:197-206, 2004
2. Broniscer A, Baker SJ, West AN, et al: Clinical and molecular characteristics of malignant transformation of low-grade glioma in children. J Clin Oncol 25:682-689, 2007
3. Furnari FB, Fenton T, Bachoo RM, et al: Malignant astrocytic glioma: Genetics, biology, and paths to treatment. Genes Dev 21:2683-2710, 2007
4. Bredel M, Bredel C, Juric D, et al: High-resolution genome-wide mapping of genetic alterations in human glial brain tumors. Cancer Res 65:4088-4096, 2005
5. Kotliarov Y, Steed ME, Christopher N, et al: High-resolution global genomic survey of 178 gliomas reveals novel regions of copy number alteration and allelic imbalances. Cancer Res 66:9428-9436, 2006
6. Maher EA, Brennan C, Wen PY, et al: Marked genomic differences characterize primary and secondary glioblastoma subtypes and identify two distinct molecular and clinical secondary glioblastoma entities. Cancer Res 66:11502-11513, 2006
7. Nigro JM, Misra A, Zhang L, et al: Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. Cancer Res 65:1678-1686, 2005
8. Liang Y, Diehn M, Watson N, et al: Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. Proc Natl Acad Sci U S A 102:5814-5819, 2005
9. Phillips HS, Kharbanda S, Chen R, et al: Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9:157-173, 2006
10. Rich JN, Hans C, Jones B, et al: Gene expression profiling and genetic markers in glioblastoma survival. Cancer Res 65:4051-4058, 2005
11. Ruano Y, Mollejo M, Ribalta T, et al: Identification of novel candidate target genes in amplicons of glioblastoma multiforme tumors detected by expression and CGH microarray profiling. Mol Cancer 5:39, 2006
12. Parsons DW, Jones S, Zhang X, et al: An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807-1812, 2008
13. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061-1068, 2008
14. Pollack IF, Finkelstein SD, Burnham J, et al: Age and TP53 mutation frequency in childhood malignant gliomas: Results in a multi-institutional cohort. Cancer Res 61:7404-7407, 2001
15. Newcomb EW, Alonso M, Sung T, et al: Incidence of p14ARF gene deletion in high-grade adult and pediatric astrocytomas. Hum Pathol 31:115-119, 2000
16. Gallia GL, Rand V, Siu IM, et al: PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. Mol Cancer Res 4:709-714, 2006
17. Pollack IF, Hamilton RL, James CD, et al: Rarity of PTEN deletions and EGFR amplification in malignant gliomas of childhood: Results from the Children's Cancer Group 945 cohort. J Neurosurg 105:418-424, 2006
18. Faury D, Nantel A, Dunn SE, et al: Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. J Clin Oncol 25:1196-1208, 2007
19. Rickert CH, Strater R, Kaatsch P, et al: Pediatric high-grade astrocytomas show chromosomal imbalances distinct from adult cases. Am J Pathol 158:1525-1532, 2001
20. Wong KK, Tsang YT, Chang YM, et al: Genome-wide allelic imbalance analysis of pediatric gliomas by single nucleotide polymorphic allele array. Cancer Res 66:11172-11178, 2006
21. Torchia EC, Boyd K, Rehg JE, et al: EWS/FLI-1 induces rapid onset of myeloid/erythroid leukemia in mice. Mol Cell Biol 27:7918-7934, 2007
22. Beroukhim R, Getz G, Nghiemphu L, et al: Assessing the significance of chromosomal aberrations in cancer: Methodology and application to glioma. Proc Natl Acad Sci U S A 104:20007-20012, 2007
23. Veeriah S, Brennan C, Meng S, et al: The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. Proc Natl Acad Sci U S A 106:9435-9440, 2009
24. Solomon DA, Kim JS, Cronin JC, et al: Mutational inactivation of PTPRD in glioblastoma multiforme and malignant melanoma. Cancer Res 68:10300-10306, 2008
25. Ichimura K, Pearson DM, Kocialkowski S, et al: IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 11:341-347, 2009
26. Balss J, Meyer J, Mueller W, et al: Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597-602, 2008
27. Watanabe T, Nobusawa S, Kleihues P, et al: IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149-1153, 2009
28. Yan H, Parsons DW, Jin G, et al: IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765-773, 2009
29. Solomon DA, Kim JS, Ressom HW, et al: Sample type bias in the analysis of cancer genomes. Cancer Res 69:5630-5633, 2009
30. Sanders RP, Kocak M, Burger PC, et al: High-grade astrocytoma in very young children. Pediatr Blood Cancer 49:888-893, 2007
31. Lee Y, Scheck AC, Cloughesy TF, et al: Gene expression analysis of glioblastomas identifies the major molecular basis for the prognostic benefit of younger age. BMC Med Genomics 1:52, 2008
32. Dyer S, Prebble E, Davison V, et al: Genomic imbalances in pediatric intracranial ependymomas define clinically relevant groups. Am J Pathol 161:2133-2141, 2002
33. Carter M, Nicholson J, Ross F, et al: Genetic abnormalities detected in ependymomas by comparative genomic hybridisation. Br J Cancer 86:929-939, 2002
34. Li M, Lee KF, Lu Y, et al: Frequent amplification of a chr19q13.41 microRNA polycistron in aggressive primitive neuroectodermal brain tumors. Cancer Cell 16:533-546, 2009

35. Ferreira BI, Alonso J, Carrillo J, et al: Array CGH and gene-expression profiling reveals distinct genomic instability patterns associated with DNA repair and cell-cycle checkpoint pathways in Ewing's sarcoma. Oncogene 27:2084-2090, 2008

36. Natrajan R, Williams RD, Hing SN, et al: Array CGH profiling of favourable histology Wilms tumours reveals novel gains and losses associated with relapse. J Pathol 210:49-58, 2006

37. Bardelli A, Cahill DP, Lederer G, et al: Carcinogen-specific induction of genetic instability. Proc Natl Acad Sci U S A 98:5770-5775, 2001

38. Radtke I, Mullighan CG, Ishii M, et al: Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. Proc Natl Acad Sci U S A 106:12944-12949, 2009

39. Mendrzyk F, Korshunov A, Benner A, et al: Identification of gains on 1q and epidermal growth factor receptor overexpression as independent prognostic markers in intracranial ependymoma. Clin Cancer Res 12:2070-2079, 2006

40. Roberts P, Burchill SA, Brownhill S, et al: Ploidy and karyotype complexity are powerful prognostic indicators in the Ewing's sarcoma family of tumors: A study by the United Kingdom Cancer Cytogenetics and the Children's Cancer and Leukaemia Group. Genes Chromosomes Cancer 47:207-220, 2008

41. Hattinger CM, Potschger U, Tarkkanen M, et al: Prognostic impact of chromosomal aberrations in Ewing tumours. Br J Cancer 86:1763-1769, 2002

42. Hing S, Lu YJ, Summersgill B, et al: Gain of 1q is associated with adverse outcome in favorable histology Wilms' tumors. Am J Pathol 158:393-398, 2001

43. Clifford SC, O'Toole K, Ellison DW: Chromosome 1q gain is not associated with a poor outcome in childhood medulloblastoma: Requirements for the validation of potential prognostic biomarkers. Cell Cycle 8:787, 2009

44. Thorarinsdottir HK, Santi M, McCarter R, et al: Protein expression of platelet-derived growth factor receptor correlates with malignant histology and PTEN with survival in childhood gliomas. Clin Cancer Res 14:3386-3394, 2008

45. Bredel M, Pollack IF, Hamilton RL, et al: Epidermal growth factor receptor expression and gene amplification in high-grade non-brainstem gliomas of childhood. Clin Cancer Res 5:1786-1792, 1999

46. Khatua S, Peterson KM, Brown KM, et al: Overexpression of the EGFR/FKBP12/HIF-2alpha pathway identified in childhood astrocytomas by angiogenesis gene profiling. Cancer Res 63:1865-1870, 2003

47. Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, et al: PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. Neuron 51:187-199, 2006

48. Burrows RC, Wancio D, Levitt P, et al: Response diversity and the timing of progenitor cell maturation are regulated by developmental changes in EGFR expression in the cortex. Neuron 19:251-267, 1997

49. Sun Y, Goderie SK, Temple S: Asymmetric distribution of EGFR receptor during mitosis generates diverse CNS progenitor cells. Neuron 45:873-886, 2005

50. Tchougounova E, Kastemar M, Brasater D, et al: Loss of Arf causes tumor progression of PDGFB-induced oligodendroglioma. Oncogene 26:6289-6296, 2007

51. Dai C, Celestino JC, Okada Y, et al: PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev 15:1913-1925, 2001

52. Lindberg N, Kastemar M, Olofsson T, et al: Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. Oncogene 28:2266-2275, 2009

53. Ding H, Shannon P, Lau N, et al: Oligodendrogliomas result from the expression of an activated mutant epidermal growth factor receptor in a RAS transgenic mouse astrocytoma model. Cancer Res 63:1106-1113, 2003

54. Holland EC, Hively WP, DePinho RA, et al: A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev 12:3675-3685, 1998

55. Walter AW, Hancock ML, Pui CH, et al: Secondary brain tumors in children treated for acute lymphoblastic leukemia at St Jude Children's Research Hospital. J Clin Oncol 16:3761-3767, 1998

