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Advances in Brief

Age and *TP53* Mutation Frequency in Childhood Malignant Gliomas: Results in a Multi-institutional Cohort[1]

Ian F. Pollack,[2] Sydney D. Finkelstein, Judith Burnham, Emiko J. Holmes, Ronald L. Hamilton, Allan J. Yates, Jonathan L. Finlay, and Richard Sposto, for the Children's Cancer Group

Departments of Neurosurgery [I. F. P.] and Pathology [S. D. F., J. B., R. L. H.], University of Pittsburgh Medical Center and the Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15213; Ohio State University, Columbus, Ohio 43210 [A. J. Y.]; Department of Pediatrics, New York University Medical Center, New York, New York 10016 [J. L. F.]; Children's Oncology Group, Arcadia, California 91006 [E. J. H.]; and Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California 90033 [R. S.]

Abstract

Malignant astrocytoma is one of the most deadly primary central nervous system tumors. Although significant progress has been made in understanding the molecular pathways that lead to the development of these tumors in adults, comparatively little analysis has been done in childhood astrocytomas, which are less common and have a more favorable prognosis. Our previous studies of an institutional cohort of children with malignant gliomas suggested the existence of distinct molecular pathways of tumorigenesis in younger *versus* older children, based on the finding of a high frequency of *TP53* mutations in tumors from children >3 years of age at diagnosis, compared with those from younger children. In the current study, the association between *TP53* mutations and age was examined in greater detail using the multi-institutional group of children enrolled in Children's Cancer Group Study 945, the largest cohort of childhood high-grade gliomas analyzed to date. Seventy-seven tumors with centrally reviewed diagnoses of anaplastic astrocytoma or glioblastoma multiforme had sufficient archival histopathological material for microdissection-based genotyping. Sections were examined histologically, and topographic targets that contained malignant tissue were isolated by microdissection and subjected to PCR-based amplification and sequencing of *TP53* exons 5–8. Twenty-six tumors (33.8%) had mutations in those exons. Mutations were observed in 2 of 17 tumors (11.8%) from children <3 years of age at diagnosis *versus* 24 of 60 tumors (40%) from older children, a difference that was statistically significant ($P = 0.04$), in agreement with our previous results. Whereas malignant gliomas in older children have a frequency of mutations comparable to tumors that arise in young adults, those from children <3 years old do not. The association between age and frequency of *TP53* mutations among pediatric malignant gliomas indicates the probable existence of two distinct pathways of molecular tumorigenesis in younger *versus* older children.

Introduction

High-grade astrocytomas are the most common primary central nervous system tumors in adults, but they are less common in children (1, 2). Although they generally respond poorly to conventional therapy with surgery, radiotherapy, and chemotherapy, numerous studies have indicated that malignant gliomas in children and young adults, as a group, have a better prognosis than those that occur in older patients and that young patients account for a disproportionate percentage of long-term survivors (3–7). Although this observation implies age-related differences in tumor biology or host-tumor interactions (8), a systematic comparison of pediatric and adult malignant gliomas on a molecular rather than histological basis has yet to be done.

In that context, it has been recognized for some time that malignant gliomas in adults can arise by at least two distinct molecular pathways. One group includes the so-called primary or *de novo* tumors, which are histologically malignant at diagnosis and characteristically manifest in older adult patients. These lesions commonly exhibit amplification of *EGFR* (9–14). A second group includes the so-called secondary gliomas, which generally occur in adults <40 years of age, evolving in some cases from previously detected lower-grade gliomas and therefore with longer overall natural histories than the primary tumors. These lesions have a high frequency of *TP53* mutations but a relatively low frequency of *EGFR* amplification (9–14). Apart from those differences, both groups of tumors share many common genetic alterations, including frequent abnormalities in genes that control G_1-S cell cycle progression, such as mutation or homozygous deletion of *RB1*, *CDKN2A*, or *CDKN2B* or amplification of *CDK4* (15), and a high frequency of deletions involving chromosome 10 (16–19), in many cases incorporating the *PTEN/MMAC1* locus (17, 20–22).

Compared with the extensive work that has been done to characterize the molecular features of adult high-grade gliomas (9, 13, 15, 22), relatively little data have been collected on pediatric tumors, in part reflecting the fact that these lesions are less common. However, institutional pilot studies from our group (23, 24) and others (25–27) have suggested that *de novo* pediatric malignant gliomas rarely show amplification of the *EGFR* gene and more commonly exhibit mutations of the *TP53* gene, similar to secondary gliomas in young adults. Because these studies have all incorporated relatively small cohorts of patients, it has been difficult to evaluate the potential existence of distinct pathways of tumorigenesis among pediatric malignant gliomas. In support of this possibility, our previous studies noted that *TP53* mutations were distinctly less common in tumors from children diagnosed before 3 years of age than in older children (24), raising the possibility that malignant gliomas in young children may arise from molecular pathways distinct from those in older children.

To address this issue in greater detail, we initiated a more extensive analysis of the molecular features of pediatric high-grade gliomas by incorporating the multi-institutional cohort of children of CCG[3] study CCG-945, the largest group of pediatric high-grade gliomas accrued to date (28). The availability of centralized neuropathological review, coupled with the large size of cohort available for correlative biological analysis, provided a unique opportunity to address issues of molecular etiology. The results reported here indicate that malignant gliomas in young children show a significantly lower frequency of *TP53* mutations than those in older children, confirming the existence

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[2] To whom requests for reprints should be addressed, at Children's Oncology Group, P. O. Box 60012, Arcadia, CA 91066-6012. Phone: (626) 447-0064, ext. 122; Fax: (626) 445-4334; E-mail: Pollaci@chplink.chp.edu.

[3] The abbreviations used are: CCG, Children's Cancer Group; AA, anaplastic astrocytoma; GBM, glioblastoma multiforme.

of at least two distinct pathways of tumorigenesis among pediatric malignant gliomas, a finding that may have important therapeutic implications.

Patients and Methods

Patient Population. The cohort examined in these studies consisted of the patients included in the CCG non-brainstem high-grade glioma study, CCG-945 (28), who had centrally reviewed diagnoses of AA or GBM. Patients were treated with a combination of surgery, radiotherapy (5400 cGy in 180-cGy fractions), and chemotherapy with adjuvant prednisone, lomustine, and vincristine, or the "8-drugs-in-1-day" (eight-in-one) regimen administered for two cycles before irradiation and continued after irradiation. Patients younger than 24 or 36 months, depending on the year of study entry, and those with spinal cord malignant gliomas were nonrandomly assigned to receive the eight-in-one regimen, whereas older children with intracranial high-grade gliomas were randomly assigned between the eight-in-one and prednisone, lomustine, and vincristine regimens (29, 30). In all patients, histological and clinical factors were monitored rigorously, and long-term follow-up was achieved. No significant difference in survival was noted between the two treatment arms (28).

Tissue accrual for the current study was initiated by the Pediatric Branch of the Cooperative Human Tissue Network in the context of a CCG-endorsed biopathology study, CCG-B975, also approved by the Children's Hospital of Pittsburgh Human Rights Committee. The original clinical cohort included a sizeable subgroup of tumors that were later found to be ineligible (*e.g.*, atypical low-grade gliomas) on central review (28). Therefore, the current molecular analysis was restricted to tumors in which the neuropathologist who performed central review for the CCG-945 clinical study (A. J. Y.) confirmed diagnoses of AA or GBM in a recent blinded re-review, using contemporary WHO criteria (31), and in which sufficient histopathological material was available from the tumor specimen for microdissection-based genotyping. After prescreening, 77 specimens were eligible for inclusion in the studies reported here.

***TP53* Mutation Analysis.** Paraffin-embedded specimens were used for all aspects of the analysis. Samples were provided in a coded format to "blind" investigators to clinical, histological, and outcome results. Tumor slides were reviewed, and blocks that contained malignant glioma were sectioned at a thickness of 4 μm. Sections were stained with H&E to confirm that characteristic tissue had been obtained. Adjacent sections were subjected to genotyping analyses. For assessment of *TP53* mutations, exons 5–8 were specifically examined because those regions encompass most *TP53* mutations that have been detected in astrocytic (32) and nonastrocytic (33) tumors.

Tissue targets from regions of highest anaplasia were removed directly from the tumor sections, using microdissection-based techniques as described previously (24, 34–36). For larger targets, manual microdissection was performed under stereomicroscopic viewing, whereas laser capture microdissection (PixCell II; Arcturus) was used for smaller targets. Microdissected tissue was collected in 100 μl of dilute Tris buffer with 1% SDS. After phenol/chloroform extraction, sample DNA was precipitated with 100% ethanol, washed in 70% ethanol, resuspended in Tris buffer, and stored at -20°C for subsequent nucleic acid amplification.

Individual exons were amplified independently using the following primer pairs: (*a*) exon 5, (sense) GCAGTACTCCCCTGCCTCAA and (antisense) GCCCCAGCTGCTCACCATCGC; (*b*) exon 6, (sense) GGGTCCCCAGGCCTCTGATT and (antisense) CCTCCCAGAGACCCCAGTT; (*c*) exon 7, (sense) CTTGCCACAGGTCTCCCCAAG and (antisense) GCAGGCCAGTGTGCAGGGTGG; and (*d*) exon 8, (sense) TTTTCCTATCCTGAGTAGTGG; and (antisense) GGTCTCCTCCACCGCTTCTTG as reported previously (24, 36). PCR products were then isolated and directly sequenced by dideoxy chain termination using S-35 dATP (Sequenase; United States Biochemical Corp., Cleveland, OH) as described previously (24, 36), using the above-mentioned primers. Sequences were read from overnight-exposed autoradiograms of 6% polyacrylamide gels.

Statistical Analysis. To assess the association between *TP53* mutations and both patient age and tumor histology in the current study, mutation status and clinical and histological information were provided in a blinded fashion. Association between each of these parameters was assessed using Fisher's exact test.

Results

Patient Characteristics. Seventy-seven patients were identified with non-brain stem malignant gliomas that met the criteria for the current study. Thirty-five tumors had review diagnoses of AA, and 42 tumors had review diagnoses of GBM. Seventeen patients were <3 years of age at diagnosis, and 60 patients ranged in age from 3–18 years. Five-year event-free survival in the group of 77 review-confirmed malignant gliomas (16.9 $\pm$ 4.3%) was comparable with that of the group of 71 tumors with eligible histologies in which adequate tissue was not available for *TP53* genotyping (19.7 $\pm$ 4.7%; $P = 0.31$, log-rank test).

***TP53* Mutation Analysis.** Table 1 summarizes data on *TP53* mutations, age, and tumor histologies of the 77 eligible patients. In the overall group, 26 tumors (33.8%) had mutations within *TP53* exons 5–8. A significant difference was apparent in the frequency of mutations in tumors from children <3 years of age at diagnosis (2 of 17 tumors, 11.8%) *versus* that in children between 3 and 18 years old (24 of 60 tumors, 40%; $P = 0.04$). Among the latter group, the frequency of mutations did not vary significantly in different age subgroups. There were mutations in 12 of 27 tumors (44.4%) from children diagnosed between 3 and 10 years old *versus* 12 of 33 tumors (36.4%) from children between 10 and 18 years old at diagnosis.

Although there was a trend between frequency of mutations and increasing histopathological grade, it did not reach statistical significance. Mutations were observed in 9 of 35 tumors (25.7%) classified as AA (grade III astrocytoma) *versus* 17 of 42 (40.4%) tumors classified as GBM (grade IV astrocytoma; $P = 0.23$). Differences in distribution of tumor histology in younger and older age groups did not account for age-related differences in the frequency of *TP53* mutations. Mutations were observed in 1 of 10 (10%) AAs from children <3 years of age at diagnosis *versus* 8 of 25 (32.0%) AAs from older children. Mutations were observed in only 1 of 7 (14.3%) GBMs of children <3 years of age *versus* 16 of 35 (45.7%) GBMs in older children.

Discussion

Although initial studies that involved childhood malignant gliomas suggested that these tumors rarely exhibited *TP53* mutations (37–39),

Table 1 *Histopathological and clinical characteristics of 26 pediatric high-grade gliomas with TP53 mutations*

Age (yrs)	Tumor location	Review pathology diagnosis	Mutation type
0.5	Frontal	GBM	208 DN
0.6	Frontal	AA	175 RH
3.1	Frontal	GBM	245 GV
3.6	Temporal	GBM	175 RC
3.9	Temporal	AA	Exon 5 deletion
4.7	Frontal	GBM	Exon 6 deletion
5.5	Lateral ventricle	GBM	248 RW
5.8	Temporal	GBM	273 RH
5.8	Temporal	GBM	298 EV
6.1	Thalamus	AA	161AT
6.3	Parietal	GBM	Exon 5 deletion
7.8	Occipital	GBM	282 RW
9.8	Parietal	AA	275 CY
9.9	Thalamus	GBM	Exon 5 insertion
11.1	Thalamus	AA	286 EQ
12.1	Occipital	AA	269 RS
12.8	Temporal	GBM	248 RW
13.5	Parietal	GBM	241 SF
13.5	Frontal	GBM	283 RP
14.5	Thalamus	AA	155 TA
15.6	Frontal	GBM	220 YC
16	Occipital	AA	282 RW
16.1	Frontal	GBM	271 EK
16.3	Parietal	GBM	213 RS
16.4	Frontal	GBM	275 CY
18	Temporal	AA	272 VL

recent reports, which have incorporated more rigorous histological criteria, have suggested that a subgroup of AAs and glioblastomas of childhood exhibits such mutations (24–27). The importance of this observation is that tumors with *TP53* mutations may be resistant to p53-dependent apoptotic pathways, which help mediate the cytotoxic effects of conventional chemotherapy and radiotherapy (40–43). In other tumor types, lesions with intact p53 pathways have been observed to be more susceptible to the therapeutic effects of those modalities than lesions with p53 mutations (45–48). In preclinical models that involved tumors with mutated *TP53*, transfer of wild-type *TP53* constructs enhanced therapeutic responsiveness (48–50).

In view of the influence of tumor *TP53* mutation status on responsiveness to adjuvant therapies, the present study yielded several potentially important observations. First, the frequency of *TP53* mutations in this centrally reviewed multi-institutional series of pediatric malignant gliomas (26 of 77 tumors, 33.8%) was greater than that seen in previous childhood studies (37–39) and similar to frequencies observed in secondary malignant gliomas typical in young adults (14, 15, 38). Both groups of tumors commonly exhibit *TP53* mutations but rarely show *EGFR* amplification (23, 26), unlike *de novo* malignant gliomas that generally occur in older adults (9, 13, 20, 22), which suggests that the biological behavior and molecular pathogenesis of most primary childhood high-grade gliomas may be more similar to those in young adults than those in older adults. The greater incidence of *TP53* mutations in the current study compared with earlier reports (37–39) may largely reflect that previous studies of childhood gliomas likely included significant percentages of atypical pilocytic or other unusual low-grade astrocytoma variants, such as pleomorphic xanthoastrocytoma, which can exhibit some features of malignant gliomas and present a diagnostic challenge. These lesions are clearly distinguished from truly high-grade (i.e., grade III or IV) gliomas in contemporary classification schemes (31). In contrast, our previous institutional pilot study, which included only high-grade gliomas in which the diagnosis was confirmed by central review of the histopathological material, noted a frequency of *TP53* mutations comparable with the current series (24).

Second, the observation that a substantial percentage of malignant gliomas from children >3 years of age at diagnosis had *TP53* mutations but that such mutations were rare in tumors from younger children suggests that infant malignant gliomas may differ on a biological and molecular pathogenetic basis from similar lesions in older children. Although it was not possible to assess age-related differences in treatment response in the current cohort because young children were treated differently than older children [in many cases, younger children did not receive radiotherapy after eight-in-one chemotherapy (28, 51)], more recent clinical studies suggest that infant malignant gliomas may be more likely to respond favorably to therapy than histologically similar lesions in older patients. For example, regimens that incorporate intensive chemotherapy in an effort to delay radiotherapy and thereby minimize the severity of radiation-related cognitive deterioration have achieved objective response rates of more than 50% and 5-year survival rates of up to 50% in infants with malignant gliomas (52, 53), often without irradiation. These observations contrast with the generally discouraging results that have been obtained in older patients, despite the use of irradiation and either before or after irradiation chemotherapy (28, 29).

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