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


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 G1-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 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...
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 samples 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) GCAGTACTCCCTGGCTTCAA and (antisense) GCCCCAGCTGCTCACCCTCCG; (b) exon 6, (sense) GGTTCCCCAGGCTCCTGATT and (antisense) CTCCTCCAGAGCCCCCTTGT; (c) exon 7, (sense) CCTGCCCACAGGCTTCCCAAG and (antisense) CAAGGCGCTGTTCCAGGGG; and (d) exon 8, (sense) TTTCTCATCCTCCAGTATTGTG and (antisense) GTGCTTCCCTACCCCGCTTTGTG, 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 ± 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 ± 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>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Tumor location</th>
<th>Review pathology diagnosis</th>
<th>Mutation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Frontal</td>
<td>GBM</td>
<td>208 DN</td>
</tr>
<tr>
<td>0.6</td>
<td>Frontal</td>
<td>AA</td>
<td>175 RH</td>
</tr>
<tr>
<td>3.1</td>
<td>Frontal</td>
<td>GBM</td>
<td>245 GV</td>
</tr>
<tr>
<td>3.6</td>
<td>Temporal</td>
<td>GBM</td>
<td>175 RC</td>
</tr>
<tr>
<td>3.9</td>
<td>Temporal</td>
<td>AA</td>
<td>Exon 5 deletion</td>
</tr>
<tr>
<td>4.7</td>
<td>Frontal</td>
<td>GBM</td>
<td>Exon 6 deletion</td>
</tr>
<tr>
<td>5.5</td>
<td>Lateral ventricle</td>
<td>GBM</td>
<td>248 RW</td>
</tr>
<tr>
<td>5.8</td>
<td>Temporal</td>
<td>GBM</td>
<td>273 RH</td>
</tr>
<tr>
<td>5.8</td>
<td>Temporal</td>
<td>GBM</td>
<td>298 EV</td>
</tr>
<tr>
<td>6.1</td>
<td>Thalamus</td>
<td>AA</td>
<td>161 AT</td>
</tr>
<tr>
<td>6.3</td>
<td>Parietal</td>
<td>AA</td>
<td>Exon 5 deletion</td>
</tr>
<tr>
<td>7.8</td>
<td>Occipital</td>
<td>GBM</td>
<td>282 RW</td>
</tr>
<tr>
<td>9.8</td>
<td>Parietal</td>
<td>AA</td>
<td>275 CY</td>
</tr>
<tr>
<td>9.9</td>
<td>Thalamus</td>
<td>GBM</td>
<td>Exon 5 insertion</td>
</tr>
<tr>
<td>11.1</td>
<td>Thalamus</td>
<td>AA</td>
<td>286 EQ</td>
</tr>
<tr>
<td>12.1</td>
<td>Occipital</td>
<td>AA</td>
<td>269 RS</td>
</tr>
<tr>
<td>12.8</td>
<td>Temporal</td>
<td>GBM</td>
<td>248 RW</td>
</tr>
<tr>
<td>13.5</td>
<td>Parietal</td>
<td>GBM</td>
<td>241 SF</td>
</tr>
<tr>
<td>13.5</td>
<td>Frontal</td>
<td>GBM</td>
<td>283 RP</td>
</tr>
<tr>
<td>14.5</td>
<td>Thalamus</td>
<td>AA</td>
<td>155 TA</td>
</tr>
<tr>
<td>15.6</td>
<td>Frontal</td>
<td>AA</td>
<td>220 YC</td>
</tr>
<tr>
<td>16</td>
<td>Occipital</td>
<td>AA</td>
<td>282 RW</td>
</tr>
<tr>
<td>16.1</td>
<td>Frontal</td>
<td>GBM</td>
<td>271 EK</td>
</tr>
<tr>
<td>16.3</td>
<td>Parietal</td>
<td>GBM</td>
<td>213 RS</td>
</tr>
<tr>
<td>16.4</td>
<td>Frontal</td>
<td>GBM</td>
<td>275 CY</td>
</tr>
<tr>
<td>18</td>
<td>Temporal</td>
<td>AA</td>
<td>272 VL</td>
</tr>
</tbody>
</table>
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 rarely show EGFR amplification (23, 26), unlike de novo malignant gliomas that generally occur in older adults (9, 13, 20, 22), which rarely show EGFR amplification.”

References


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


*Cancer Res* 2001;61:7404-7407.

Updated version: Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/61/20/7404

Cited articles: This article cites 51 articles, 20 of which you can access for free at: http://cancerres.aacrjournals.org/content/61/20/7404.full.html#ref-list-1

Citing articles: This article has been cited by 17 HighWire-hosted articles. Access the articles at: /content/61/20/7404.full.html#related-urls

E-mail alerts: Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions: To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions: To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.