Prognostic Significance of p53 Immunoreactivity in Patients with Glioma

Athanassios P. Kyritsis,1,2 Melissa L. Bondy, Kenneth R. Hess, Joan E. Cunningham, Dakai Zhu, Christopher J. Amos, W. K. A. Yung, Victor A. Levin, and Janet M. Bruner


ABSTRACT

Abnormal p53 as revealed by immunostaining has been shown to be a predictor of poor outcome in a variety of malignant tumors. This study examines the relationship of p53 immunostaining and survival in 182 adult patients with gliomas. Tumor tissues obtained from patients with glioma within 4 months of initial diagnosis were investigated by immunohistochemical analysis for detection of p53 protein abnormalities using the monoclonal antibody PAb 1801. There were 122 patients with glioblastoma multiforme, 48 patients with anaplastic glioma, and 12 patients with low-grade glioma. Among these patients, 73 of those with glioblastoma multiforme, 35 with anaplastic glioma, and 6 with low-grade glioma had positive p53 immunoreactivity. Kaplan-Meier survival plots (log rank test) showed that the patients with anaplastic astrocytoma or low-grade glioma and p53-positive tumors had longer survival times compared to the patients with p53-negative tumors. No differences in survival were detected among the glioblastoma patients. Cox proportional hazards regression analysis, adjusted for age at diagnosis, showed that the p53 positivity was a significant predictor of longer survival (relative risk = 0.56; 95% confidence intervals = 0.35, 0.90; P = 0.015) in anaplastic astrocytoma patients, but not in glioblastoma patients (relative risk = 1.03; 95% confidence intervals = 0.82, 1.29; P = 0.80). These results suggest that anaplastic glioma patients with p53 protein alterations may have a better response to chemoradiation, possibly because the malignant cells cannot arrest in G1, to correct lethal damage induced by chemotherapy or radiotherapy.

INTRODUCTION

Glioblastoma multiforme is the most common primary brain tumor and is highly malignant, with a median survival of only 1–1.5 years, even with the most aggressive treatment. This tumor is notorious for its phenotypic and biological heterogeneity, which is probably related to an accumulation of multiple genetic abnormalities during the transformation of astrocytic cells. The only known prognostic indicators for malignant gliomas are age, performance status, and tumor grade (1). However, it is well known that the majority of anaplastic astrocytomas and a small percentage of glioblastomas respond initially to radiotherapy and chemotherapy before they eventually become resistant to any therapeutic modality. In addition, a small subgroup of the responding glioblastoma and anaplastic glioma patients has a long survival. Detection of prognostic factors will be very important in planning and selecting treatment for patients with these primary brain neoplasms.

The tumor suppressor gene p53 and its protein product have been widely investigated in many human tumors, including gliomas. Previous studies of human brain tumors have revealed a loss of heterozygosity of chromosome 17 in up to 60% of patients with astrocytomas (2). The p53 gene, localized on the short arm of chromosome 17, encodes for a nuclear protein involved in the regulation of the cell cycle and in cellular proliferation (3). Because the wild-type protein has a short half-life, it cannot be detected by immunohistochemistry (4). The monoclonal antibody PAb 1801 to the amino-terminal portion of the p53 protein is human specific but labels both wild-type and mutant protein (5–7). However, since the wild-type protein has a short half-life, PAb 1801 is a very useful antibody for detecting mutant or abnormally stabilized p53. In the present study, we examined the prognostic significance of p53 abnormalities as detected by immunohistochemistry in 182 adult patients with supratentorial glioma tumors.

PATIENTS AND METHODS

Patients. Tumor tissues from 182 adult patients with supratentorial gliomas were used in this study. All of the patients were operated on within 4 months of the initial diagnosis at the University of Texas M. D. Anderson Cancer Center. Another group of 92 patients who had their operation and p53 immunostaining only at recurrence at the University of Texas M. D. Anderson Cancer Center were excluded from this study. The tumor grade at the time of surgery was used in our survival analysis, and the reference and end points for each case were the date of the surgery and the date of death, respectively. Gliomas were classified and graded according to a three-tiered system that has been shown to be prognostically relevant (8). Grades used were low-grade glioma, anaplastic glioma, and glioblastoma multiforme. The low-grade and anaplastic glioma groups included tumors of pure astrocytic and mixed oligoastrocytic origin. Pure oligodendrogliomas were excluded from this study.
All patients were treated with postoperative radiotherapy and the patients with either glioblastoma multiforme or anaplastic glioma were treated with additional adjuvant nitrosourea-based chemotherapy.

**Tissue Handling.** Gross tissue specimens were examined and divided by a neuropathologist. Paraffin-embedded tissue was used for final diagnosis and grading. This tissue was fixed for 6 to 20 h in neutral 10% formalin solution, then routinely processed to paraffin blocks. Appropriate tumor sections were chosen after paraffin-embedded tissue was stained with hematoxylin and eosin to ensure the presence of the desired tissue in the sections. Sequential sections were used for immunocytochemistry studies.

**Protein Expression/Immunohistochemistry.** Protein expression was detected in paraffin-embedded sections of brain tumor using the unlabeled antibody immunoperoxidase method. This method has been described in detail elsewhere (5, 6).

As negative controls, sequential sections were subjected to the identical procedure with omission of the primary antibody. Positive controls were run for each section using an anti-glial fibrillary acidic protein monoclonal antibody. Known positive tissue controls (paraffin-embedded U251 glioma cells with known codon 273 p53 gene mutation and human glioma tumors with sequencing verified p53 gene mutations) were also included for each individual procedure.

In most cases, the types of cells labeled by p53 immunohistochemical methods and a semiquantitation of general numbers of tumor cells labeled were recorded (0, no labeling; 1, fewer than 5% labeled; 2, 5–30% labeled; 3, >30% labeled). Each section was individually coded according to the patient specimen, and the distribution and cellular location of the positive reaction was specified.

**Statistical Methods.** Survival analysis from the date of surgery was performed using Kaplan-Meier plots, Cox-Mantel log rank tests, and Cox proportional hazards regression analysis. Relative risk estimates were computed from the Cox regression coefficients, and median survival time estimates were computed from the Kaplan-Meier estimates. Confidence intervals are also given for the relative risk and median survival time estimates. S-PLUS software (Statistical Sciences, Inc., Seattle, WA) was used for analyses and graphics. Confidence intervals for the median survival time were computed using the method of Elashoff et al. (9).

### RESULTS

Among the 182 patients, 122 (67%) had glioblastoma multiforme, 48 (26%) had anaplastic glioma, and 12 (7%) had low-grade glioma. The patient characteristics of these groups are shown in Table 1. There were 105 male and 77 female patients. The median age of patients with glioblastoma multiforme was 56 years, whereas it was 37 years for patients with anaplastic gliomas and 33 years for those with low-grade glioma. The median age by diagnosis and p53 status is shown in Table 1.

<table>
<thead>
<tr>
<th>p53 grade</th>
<th>Glioblastoma</th>
<th>Anaplastic glioma</th>
<th>Low-grade glioma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49 (40%)</td>
<td>13 (27%)</td>
<td>6 (50%)</td>
<td>68 (37%)</td>
</tr>
<tr>
<td>1</td>
<td>38 (31%)</td>
<td>6 (12%)</td>
<td>2 (17%)</td>
<td>46 (25%)</td>
</tr>
<tr>
<td>2</td>
<td>22 (18%)</td>
<td>20 (42%)</td>
<td>3 (25%)</td>
<td>45 (25%)</td>
</tr>
<tr>
<td>3</td>
<td>13 (11%)</td>
<td>9 (19%)</td>
<td>1 (8%)</td>
<td>23 (13%)</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>48</td>
<td>12</td>
<td>182</td>
</tr>
</tbody>
</table>

*F, female; M, male.

The Kaplan-Meier survival curves for patients with p53-positive and -negative glioblastomas are shown in Fig. 1. Median survival time (with 95% confidence intervals) was 49 (range, 34–61) weeks for patients with p53-positive tumors and 51 (range, 41–61) weeks for those with p53-negative tumors. There were no differences in survival curves between the two subgroups (log rank test, \( P = 0.438 \)). In addition, there were no statistical differences in survival between the patients with the various p53 immunohistochemistry grades (data not shown). In the anaplastic glioma group, the median survival times of patients with positive or negative p53 immunostaining were 213 (range, 82, not estimable) and 63 (range, 26–142) weeks, respectively (Fig. 2). In this group, the p53-positive patients survived significantly longer than the p53-negative patients (log rank test, \( P = 0.003 \)). There were no sufficient numbers of anaplastic glioma patients to accurately assess each p53 subcategory separately in relation to prognosis. In the low-grade glioma group, there were too few patients to perform any valid statistical comparisons in relation to p53 status (Fig. 3). However, the trend was for better survival in patients with p53-positive tumors. The median survival time for all patients with low-grade gliomas was 340 (range, 115–451) weeks, 402 for positive, and 192 for negative.

Multivariate analysis according to the Cox proportional hazards regression model revealed that the p53 positivity was a significant predictor of longer survival, after adjusting for age, for patients with anaplastic glioma but not for patients with glioblastomas. Adjusting for age, the relative risk for p53 (pos-
Fig. 1 Kaplan-Meier survival curves in glioblastomas according to p53 status. The differences in survival curves were not significant (log rank test, $P = 0.438$). —— patients with p53-positive tumors; --- patients with p53-negative tumors.

Fig. 2 Kaplan-Meier survival curves in anaplastic gliomas according to p53 status. The differences in survival were significant (log rank test, $P = 0.003$). —— patients with p53-positive tumors; --- patients with p53-negative tumors.

Positive versus negative) was 0.56 (95% confidence intervals = 0.35, 0.90; $P = 0.015$) for anaplastic gliomas and 1.03 (95% confidence intervals = 0.82, 1.29; $P = 0.80$) for glioblastomas (Table 3). Among the other variables tested, age and histological grade significantly correlated with survival.

**DISCUSSION**

The present study examined the abnormalities in p53 expression in the largest reported cohort of patients with glioma. Positive immunoreactivity for p53 was detected in the majority of the glioma tumors (63% for all grades). There were no
significant differences in the p53 immunoreactivity among the various grades of tumors (60%, 73%, and 50%). In the anaplastic glioma group, patients with p53-positive tumors had significantly improved survival compared to the patients with p53-negative tumors. No differences in survival were observed in the glioblastoma group between immunopositive and immunonegative tumors. Multivariate analysis also clearly demonstrated that the longer survival for positive p53 tumor immunoreactivity is independent of age in patients with anaplastic gliomas.

A recent study of 149 patients with gliomas using univariate and multivariate analyses failed to show any association between p53 overexpression as detected by immunohistochemical methods and survival (10). However, in that study, there was a trend toward longer survival in the patients with grade III/IV tumors and p53-positive status compared to patients with grade III/IV tumors and negative p53 immunoreactivity. They also reported a trend toward poorer survival with p53-positive versus negative status in 24 patients with low-grade gliomas, although the differences did not reach statistical significance. In our study, no conclusions could be drawn regarding the low-grade glioma group because it included only 12 patients; however, it appeared that patients with p53-positive tumors had better outcome. Chozick et al. (10) reported that the median survival time of patients with p53-positive low-grade gliomas was approximately 37 months, much lower than the 102 months observed in our study. Because of the small number of patients with low-grade gliomas in both studies, any conclusions or even trends of the effect of p53 immunoreactivity on survival in this histological group should be viewed with reservation. Another study in 66 patients with low-grade gliomas demonstrated no statistical differences in survival between patients with p53 immunopositive and immunonegative tumors (11).

In a study by Montine et al. (5) of 95 patients with astrocytic neoplasms, including 50 glioblastomas, 19 anaplastic astrocytomas, and 26 low-grade astrocytomas, there was an overall trend for longer survival of patients with p53-immunopositive tumors but no statistical correlation between p53 immunoreactivity and survival in any histological grade. A study of 43 patients with astrocytic tumors found poor survival in patients with p53 immunoreactivity, but this was not significant in the multivariate analysis (12).

Correlative studies of p53 gene mutations and p53 immunostaining in gliomas have demonstrated that, although in most cases there was reliable correlation, in some cases there was a discrepancy between the molecular biological and immunohistochemical data (13–15). Earlier studies have shown mutations in approximately 30% of various grades of gliomas (2, 14–19). However, immunohistochemical techniques have demonstrated much higher positive p53 immunoreactivity ranging from 50 to

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**Table 3** Multivariate analysis of p53-positive versus p53-negative tumors in patients with glioma

<table>
<thead>
<tr>
<th>Adjusting factor vs. patient population</th>
<th>Relative risk* (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None vs. all patients</td>
<td>0.88 (0.72–1.06)</td>
<td>0.18</td>
</tr>
<tr>
<td>Age vs. all patients</td>
<td>0.86 (0.70–1.04)</td>
<td>0.12</td>
</tr>
<tr>
<td>Age and histology vs. all patients</td>
<td>0.89 (0.73–1.09)</td>
<td>0.25</td>
</tr>
<tr>
<td>Age vs. AG* patients</td>
<td>0.56 (0.35–0.90)</td>
<td>0.015</td>
</tr>
<tr>
<td>Age vs. GBM patients</td>
<td>1.03 (0.82–1.29)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* Relative hazard rate computed using Cox proportional hazards regression.

AG, anaplastic glioma; GBM, glioblastoma multiforme.

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70% (5, 20, 21). We have examined the relationship of p53 immunostaining and direct sequencing of PCR-amplified p53 cDNA in 61 glioma tumors.3 Our results from this analysis indicated that there was very good correlation (70%) of p53 immunostaining and sequencing data when the percentage of abnormal cells within the tumor was >5% (p53 staining grades 2 and 3), but poor correlation (6–10%) when the percentage of abnormal cells fell below 5% (p53 staining grade 1) due to decreased sensitivity of the molecular biological techniques in such cases. Thus, p53 immunostaining may be a more accurate technique in detecting p53 alterations when the percentage of abnormal cells is small.3

The present study demonstrated that patients with the intermediate glioma grade, anaplastic glioma, had a significantly longer survival if their tumors were immunopositive for p53. The mechanism for this protective role of the mutant p53 protein expression is currently unknown and speculative. The p53 protein mediates its effect on cellular growth through two main pathogenetic mechanisms: one through induction of apoptosis or programmed cell death (22) and the second through a transient cellular arrest at the G1, which permits repair of DNA damage before each mitotic cycle, an active cellular process that enhances cell survival, and limits the propagation of genetic abnormalities (22–25). It is possible that in anaplastic glioma patients, the altered p53 protein fails to produce G1 arrest, and thus the tumor cells cannot repair the chemotherapy- or radiotherapy-induced lethal damage. The fact that abnormal p53 expression has no effect on survival in patients with glioblastomas suggests that other downstream or additional abnormalities may have supervised in that highly malignant glioma. It is interesting that a recent study of 21 glioma xenografts treated with procarbazine described increased sensitivity to chemotherapy in tumors with p53 mutations (26). In addition, two other reports in breast cancer patients demonstrated increased chemosensitivity when the tumors expressed mutant p53 protein (27, 28). Another possibility, at least for the p53 immunopositive low-grade gliomas, is that they may recur as the less aggressive form, the anaplastic glioma, and the p53 immunonegative tumors may recur directly as glioblastomas, resulting in a poorer prognosis (29).

Our results in 182 patients with gliomas demonstrated that immunohistochemically detectable abnormal p53 protein confers an advantage for survival in anaplastic glioma but not glioblastoma patients. The benefit of the abnormal p53 expression could be due to failure of the altered cells to stop at G1 after treatment with DNA-damaging agents, such as radiation and chemotherapy, and thus inability to repair lethal damage. However, since the function of p53 on growth arrest is multifactorial, the protective role of abnormal p53 expression in this subgroup may be more complex and involve a combination of factors. Correlation of the p53 data with other variables such as family history of cancer and cell regulatory proteins, such as p16 or retinoblastoma expression abnormalities, may aid in defining such subgroups and guide the clinician during treatment decisions.

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