Apurinic Endonuclease Activity in Adult Gliomas and Time to Tumor Progression after Alkylating Agent-Based Chemotherapy and after Radiotherapy

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ABSTRACT

Purpose: Apurinic/apyrimidinic endonuclease (Ap endo) is a key DNA repair enzyme that cleaves DNA at cytotoxic abasic sites caused by alkylating agents and radiation. We have observed that human glioma cells deficient in Ap endo activity are hypersensitive to clinically used alkylators (Silber et al., Clin Cancer Res 2002;8:3008). Here we examine the association of glioma Ap endo activity with clinical response after alkylating agent-based chemotherapy or after radiotherapy.

Experimental Design: Cox proportional hazards regression models were used to analyze the relationship of Ap endo activity with time to tumor progression (TTP).

Results: In a univariate model with Ap endo activity entered as a continuous variable, the hazard ratio (HR) for progression after alkylator therapy in 30 grade III gliomas increased by a factor of 1.061 for every 0.01 increase in activity (P = 0.013). Adjusting for age, gender, extent of resection, and prior treatment strengthened slightly the association (HR = 1.094; P = 0.003). Similarly, the HR for progression after radiotherapy in 44 grade II and III tumors increased by a factor of 1.069 (P = 0.008). Adjusting for the aforementioned variables had little effect on the association. In contrast, we observed no association between activity and TTP in grade IV gliomas after either alkylator therapy in 34 tumors or radiotherapy in 26 tumors.

Conclusions: Our data suggest that Ap endo activity mediates resistance to alkylating agents and radiation and may be a useful predictor of progression after adjuvant therapy in a subset of gliomas.

INTRODUCTION

Adult gliomas comprise a histologically and genetically diverse group of tumors that are among the most deadly of human cancers (1, 2). Low-grade gliomas (grade II in the World Health Organization classification) include astrocytoma, oligodendroglioma, and mixed oligodendroglioma-astrocytoma (3); because these tumors are diffusely infiltrative and difficult to resect completely, they eventually recur, frequently as higher-grade tumors (4). High-grade gliomas include grades III (anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic mixed glioma) and grade IV (glioblastoma multiforme); these tumors are distinguished from low-grade gliomas by the presence of mitoses, pronounced nuclear and cellular atypia, and microvascular proliferation (3). In general, the presence of intratumoral necrosis distinguishes grade IV from grade III. Grade III and IV tumors account for approximately 25 and 50% of new diagnoses, respectively. The prognosis for gliomas closely reflects grade, with median survival after surgery and adjuvant therapy ranging from 5 to 10 years for low-grade gliomas to 9 to 12 months for grade IV (2, 4).

Treatment for all diagnostic categories includes surgical resection to the maximal extent consistent with preservation of function. Contemporary postoperative care for high-grade gliomas usually includes radiation therapy (RT) followed by alkylating agent-based chemotherapy. The chloroethylators 1,3-bis(2-chloroethyl)-1-nitosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitosourea (CCNU) and the methylators procarbazine and temozolomide, used singly (e.g., 5–7) or in combination (e.g., 8–10), are the mainstays of adjuvant chemotherapy for newly diagnosed and recurrent gliomas. Clinical trials have shown that radiation and alkylators significantly increase survival for glioblastoma and anaplastic astrocytoma, the most common high-grade gliomas (5, 11) and for anaplastic oligodendroglioma (12). However, 10 to 15% of high-grade gliomas progress during RT (13) and 50% are unresponsive to alkylator-based chemotherapy (14). Clinical trials have also shown the efficacy of postoperative RT in providing symptomatic relief (15) and prolonging progression-free survival (16) in low-grade gliomas. The role of alkylating agents in the treatment of grade II gliomas is under active investigation. Although early studies indicated that alkylating agents provided no survival benefit (17), recent trials have shown that temozolomide can increase TTP and enhance overall survival in recurrent low-grade gliomas (18).
Because of the unresponsiveness of many gliomas to RT and alkylators, and the toxicity of these adjuvant therapies (e.g., radiation-induced necrosis, alkyla
tion-induced bone marrow suppression), prognostic indicators of response are urgently needed. The tumor
cidal activity of alkylating agents and ionizing radiation is
presumptively attributable in large measure to DNA damage.
Therefore, it is reasonable to assume that glioma response to
adjuvant treatment reflects, at least in part, capacity to repair
cytotoxic DNA lesions. The protein O6-methylguanine-DNA
methyltransferase (MGMT) repairs O6-alkylguanine in DNA (19)
and is an important mechanism of resistance to alkylating agents.
MGMT potentiates alkylator cytotoxicity in human glioma cell
lines (20) and tumor xenografts (21); moreover, some (22–24),
although not all (25), analyses have associated low levels or inac-
tivation of MGMT with better outcome in grade III and IV gliomas.
We have shown recently that the DNA repair enzyme Ape1/Ref-1,
which accounts for 95% of apurinic/apyrimidinic endonuclease
(Ap endo) activity in mammalian cells (26), also contributes to the
resistance of human glioma cells to clinically used alkylators (27).
Ape1/Ref-1 initiates repair of potentially lethal abasic sites caused
by alkylating agents and radiation (26, 28). We have also found that
Ap endo activity is almost invariably elevated in gliomas relative to
adjacent histologically normal brain (29).

Here, we examined the association of glioma Ap endo
activity with time to tumor progression (TTP) after alkyla
ing agent-based chemotherapy. We observed a strong, inverse
correlation between Ap endo in grade III gliomas obtained before
alkylator treatment and TTP after alkylator therapy; thus, greater
Ap endo activity was associated with shorter TTP. We also
examined the association of glioma Ap endo activity with TTP
after RT. In accord with the result for alkylators, we observed a
strong, inverse correlation between Ap endo activity in a sample
of grade II and III gliomas obtained before radiation and TTP
after RT. Our findings suggest that glioma Ap endo activity may
promote resistance to alkylating agents and radiation and sug-
gest a role for Ap endo activity in predicting response to
adjuvant therapy in a subset of gliomas.

MATERIALS AND METHODS

Tissue. Ninety-six gliomas from 96 different patients
were obtained at the University of Washington Medical Center
from 1992 to 2000. All tumors were reviewed by a panel of
neuropathologists, including one of the authors (A. M. S) and
diagnosis was obtained from the final neuropathology report.
Sixty-four gliomas were included in the analysis of alkyla
ting agent-based chemotherapy. We observed a strong, inverse cor-
relation between Ap endo in grade III gliomas obtained before
alkylator treatment and TTP after alkylator therapy; thus, greater
Ap endo activity was associated with shorter TTP. We also
examined the association of glioma Ap endo activity with TTP
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promote resistance to alkylating agents and radiation and sug-
gest a role for Ap endo activity in predicting response to
adjuvant therapy in a subset of gliomas.

Alkylating Agent-Based Chemotherapy. The associa-
tion of Ap endo activity with TTP after alkylating agent-based
chemotherapy was examined in 64 gliomas. All received prior
RT (54–62 Gy). Alkylating agent regimens were as follows:
BCNU (200 mg/m2 every 6–8 weeks for up to 6 cycles); BCNU
with cisplatin (80 mg/m2 BCNU and 33 mg/m2 cisplatin daily
for 3 days every 6 weeks for 3 cycles followed by 80 mg/m2
BCNU daily for 3 days every 6 weeks for 2 cycles); CCNU (110
mg/m2 repeated every 6 to 8 weeks for up to 8 cycles);
procarbazine, CCNU, and vincristine (PCV), given as described
previously (8); PCV plus 6-thioguanine, dibromodulcitol, 5-
fluouracil and hydroxyurea using the doses and schedules
previously described (9, 10); temozolomide (200 mg/m2 daily
for the first 5 days of a 28 day cycle, the cycle repeated until
tumor progression). Chemotherapy was terminated upon radio-
logical evidence of tumor progression (see below). All patients
had a Karnofsky performance score ≥70 at the initiation of
chemotherapy as part of the inclusion criteria for this study.

Radiation Therapy. The association of Ap endo activity
with TTP after RT was examined in 70 gliomas. Tumors received
standard fractionated, conformal radiotherapy (54–55.8 Gy for
grade II, and 59.4–62 Gy for grade III and IV) over approximately
6 weeks. All patients had a Karnofsky performance score ≥70 at
the initiation of RT as part of the inclusion criteria for this study.

Ap Endonuclease Assay. Ap endo activity was assayed in
high-speed supernatants of intact tissue sonicates by measuring the
conversion of plasmid DNA from supercoiled to relaxed form
caused by incision at an abasic site (31). Activity (fmol abasic sites
incised/cell/minute, abbreviated to fmol/cell/minute) is the mean of
at least three separate determinations that differed, in general, by
<15%, and in all cases by <30%. Each determination comprised
an assay of increasing amounts of sample and yielded activity
calculated by regression analysis of points on the linear portion of
the curve, as we have described previously (27, 29). Illustrative
assays of extracts of glioma and adjacent histologically normal
brain, showing linearity with added extract, and including resolu-
tion of supercoiled and nicked DNA by agarose gel electrophoresis,
can be found in Fig. 1 of reference 29. Validation of the assay for
glioma extracts, and controls indicating that the observed activities
are unlikely to reflect degradation of enzyme and/or substrate
during extraction and assay, or a diffusible inhibitor in extracts,
have also been described (29).

Statistical Analysis. The outcome variable was TTP as-
essed by radiological imaging. Tumor progression was defined
as follows: in the case of gross total resection, appearance of
tumor growth; in the case of sub-total resection, increase in the
largest dimension of residual tumor by at least 25% and/or
tumor growth at a different site. Observations were censored at
the last documented follow-up time if progression was not yet
observed. For the analyses of TTP after RT, observations were
censored at the time alkylating agent therapy was initiated in
cases where alkylator therapy was begun before progression.
Mean TTP was determined by the method of Kaplan-Meier.
We calculated the hazard ratio (HR) for tumor progression as a
function of Ap endo activity using Cox regression analysis.
Ap endo was entered into the regression models as a continuous
variable and scaled (i.e., multiplied by 100) so that the tabulated
HR represents the relative change in hazard for a 0.01 unit
change in measured Ap endo activity. We examined possible
confounding by age, sex, extent of resection (assessed by mag-
netic resonance imaging), prior treatment, grade, and diagnosis
of grade II and III gliomas obtained before radiation and TTP
after RT. Our findings suggest that glioma Ap endo activity may
promote resistance to alkylating agents and radiation and sug-
gest a role for Ap endo activity in predicting response to
adjuvant therapy in a subset of gliomas.
using multivariate Cox regression analysis. For graphical display of the association between Ap endo activity and TTP, activity was categorized by quartiles, and progression curves were estimated for the four subgroups by the Kaplan-Meier method. Analyses were performed using the statistics program Stata (Stata Corporation, College Station, TX).

RESULTS

Ap Endo Activity Is Inversely Associated with TTP after Alkylating Agent-Based Chemotherapy in Grade III Gliomas. We analyzed the association of Ap endo activity with TTP after alkylator therapy in 30 grade III gliomas. All tumors received RT therapy before alkylating agents in accord with common practice. In every case, activity was determined in tumors obtained before initiation of alkylator treatment. As shown in Table 1, mean patient age was 40 ± 9 years, and the male/female ratio was 1.5. Gliomas were divided roughly equally among the three major diagnostic categories. Gross total resection (excision of ≥95% of tumor as assessed by magnetic resonance imaging) was achieved in about 60% of cases. Mean Ap endo activity in the 30 tumors was 0.069 ± 0.093 fmol/cell/minutes (range 0.000016 to 0.4), and did not differ significantly between the diagnostic categories, in accord with previous data (29). Activity did not differ between 13 newly operated tumors and 17 that were reoperated after either prior surgery/biopsy or prior surgery/biopsy and RT, a result consistent with our previous data.

Table 1 Characteristics of patients receiving alkylating agent-based chemotherapy

<table>
<thead>
<tr>
<th>Grade III</th>
<th>Grade IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30 *</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>Male/female</td>
<td>1.5</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Oligodendrogioma</td>
<td>8 (27%)</td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>10 (33%)</td>
</tr>
<tr>
<td>Extent of resection</td>
<td></td>
</tr>
<tr>
<td>Gross total †</td>
<td>17 (57%)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>13 (43%)</td>
</tr>
<tr>
<td>Ap endo activity ‡</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.069 ± 0.093</td>
</tr>
<tr>
<td></td>
<td>(0.00016–0.40) §</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>0.072 ± 0.11</td>
</tr>
<tr>
<td>Oligodendrogioma</td>
<td>0.078 ± 0.091</td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>0.059 ± 0.076</td>
</tr>
<tr>
<td>Newly operated</td>
<td>0.082 ± 0.12</td>
</tr>
<tr>
<td>Reoperated</td>
<td>(n = 13)*</td>
</tr>
<tr>
<td>Prior surgery/biopsy</td>
<td>0.049 ± 0.098</td>
</tr>
<tr>
<td>Prior surgery/biopsy + radioactive therapy</td>
<td>0.061 ± 0.059</td>
</tr>
<tr>
<td>Alkylator therapy</td>
<td></td>
</tr>
<tr>
<td>BCNU or BCNU + cisplatin</td>
<td>0</td>
</tr>
<tr>
<td>CCNU</td>
<td>1</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>0</td>
</tr>
<tr>
<td>PCV or PCV + 4 drugs</td>
<td>29</td>
</tr>
<tr>
<td>Number progressed</td>
<td>22 (73%)</td>
</tr>
<tr>
<td>Mean TTP</td>
<td>22 months</td>
</tr>
</tbody>
</table>

* Seven tumors were obtained at initial resection from patients who subsequently progressed but did not undergo a second surgery. These recurrent tumors, which were grade II initially, were presumptively grade III at the time of alkylator therapy, in accord with contrast enhancement in magnetic resonance scans and clinical assessment. The association between Ap endo activity and TTP was unchanged when these seven tumors were excluded (HR = 1.074; CI = [1.019, 1.133]; P = 0.008).
† ≥95% excision of tumor.
‡ fmol abasic sites incised/cell/minute.
§ Range.
¶ Five tumors were treated with alkylators 2 to 4 weeks after completion of RT and 8 upon recurrence following RT (TTP = 18 ± 26 months post-RT).
|| Three tumors were treated with alkylators 2 to 4 weeks after completion of RT and 12 upon recurrence following RT (TTP = 8 ± 7 months post-RT).
** Two tumors were treated with alkylators 2 to 4 weeks after completion of RT and 5 upon recurrence following RT (TTP = 18 ± 17 months post-RT).
†† One tumor was treated with alkylators 2 to 4 weeks after completion of RT therapy and 2 upon recurrence following RT (TTP = 4 months post-RT).
Ap Endo Is Not Associated with TTP after Alkylator Therapy in Grade IV Gliomas. As indicated in Table 1, the 34 patients in this group were significantly older than the grade III patients ($P = 0.011$), as observed previously (1). The male/female ratio was 1.1. As for the grade III tumors, gross total resection was achieved in about 60% of cases. Mean Ap endo activity in the grade IV tumors was 0.052 ± 0.086 fmol/cell/minute (range 0.0011–0.42) and did not differ between 15 newly diagnosed tumors and 19 that had recurred after previous surgery/biopsy or surgery/biopsy followed by radiation therapy.

Of the 34 grade IV tumors, treated with alkylators as described in Table 1, progression was observed in 32, two observations were censored, and the mean TTP was 5 months, estimated by the Kaplan-Meier method. In contrast to the results for grade III tumors, TTP was not associated significantly with Ap endo activity ($HR = 0.996; CI = [0.962, 1.030]; P = 0.80$). The association between Ap endo activity and TTP remained insignificant after adjusting for age, gender, extent of resection, and prior treatment in a multivariate model (Table 3). In accord with previous findings (33, 34), TTP was associated with age when entered as a continuous variable in a univariate Cox model ($P = 0.046$); the HR for progression increased by a factor of 1.034 for every 1-year increase in age ($CI = [1.001, 1.068]$).

About half of grade IV tumors (18 of 34) versus almost all grade III tumors (29 of 30) were treated with PCV (Table 1), raising the possibility that the observed difference between grades in the association Ap endo activity with TTP might reflect an association with PCV therapy rather than with tumor quartiles would prove statistically significant with larger sample size.

Age (1, 2), gender (32, 33), and extent of resection (34, 35) have been associated with response to alkylator therapy and/or overall survival. Neither these variables, nor prior therapy, were significantly associated with TTP after alkylator therapy in this study (Table 3). Adjusting for age, gender, extent of resection, and prior treatment in a multivariate analysis slightly increased the estimated association between Ap endo activity and TTP ($HR = 1.098; P = 0.003$; Table 3), showing that the strong association between activity and TTP is not an artifact of confounding by any of these variables. Oligodendrogliomas, particularly those with deletions in chromosomes 1p and 19q, have been observed to be more responsive to adjuvant therapy than astrocytomas or mixed oligodendroglioma-astrocytomas (12). However, we found that adjusting for diagnosis in a multivariate model did not significantly affect the association between Ap endo activity and TTP ($HR = 1.054; P = 0.031$; [CI = 1.005, 1.106]).

Table 2: Hazard ratio for progression for each quartile of Ap endo activity in grade III gliomas treated with alkylating agents

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap endo (2nd quartile versus 1st)</td>
<td>1.6</td>
<td>[0.4, 6.6]</td>
<td>0.49</td>
</tr>
<tr>
<td>Ap endo (3rd quartile versus 1st)</td>
<td>4.4</td>
<td>[1.1, 18]</td>
<td>0.04</td>
</tr>
<tr>
<td>Ap endo (4th quartile versus 1st)</td>
<td>6.1</td>
<td>[1.4, 27]</td>
<td>0.02</td>
</tr>
</tbody>
</table>

NOTE. See legend to Fig. 1 for details. * Cutpoints for quartiles: activity ≤ 0.0043, ≤ 0.026 and ≤ 0.156 fmol/cell/minute.
grade directly. To address this possibility, we separately analyzed the relationship of Ap endo activity with TTP in the 18 grade IV tumors treated with PCV and the 16 treated with BCNU or temozolomide. In a univariate Cox model, the increase in HR for every 0.01 increase in activity was 1.00 for PCV ($P = 0.88$) and 1.05 ($P = 0.35$) for BCNU or temozolomide, and the difference in HRs was not significant, providing no evidence for a difference in the association of Ap endo activity and TTP for these therapies.

### Ap endo Activity Is Inversely Associated with TTP after Radiation Therapy in Grades II and III but not in Grade IV Gliomas.

We also examined the association between Ap endo activity and TTP after RT in 70 gliomas including 25 grade II, 19 grade III, and 26 grade IV tumors (Table 4). Mean patient age for grade IV tumors was significantly greater than that for grades II and III (41 ± 10 versus 52 ± 15 years; $P = 0.0012$). Gross total resection was obtained for about a third of each group. Mean Ap endo activity did not differ significantly between the groups. However, the distribution of Ap endo activity was significantly different between the grades, with grade IV tumors having lower activity than grades II and III ($P = 0.0012$).

Table 4  Characteristics of patients receiving radiation therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>25</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>40 ± 9</td>
<td>43 ± 12</td>
<td>52 ± 15</td>
</tr>
<tr>
<td>Male/female</td>
<td>1.1</td>
<td>5.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>3 (12%)</td>
<td>10 (53%)</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>13 (52%)</td>
<td>3 (16%)</td>
<td></td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>9 (36%)</td>
<td>6 (31%)</td>
<td></td>
</tr>
<tr>
<td>Extent of resection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross total *</td>
<td>9 (36%)</td>
<td>6 (32%)</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>16 (64%)</td>
<td>13 (68%)</td>
<td>17 (65%)</td>
</tr>
<tr>
<td>Ap endo activity †</td>
<td>0.036 ± 0.059</td>
<td>0.070 ± 0.10</td>
<td>0.055 ± 0.071</td>
</tr>
<tr>
<td>(0.00016–0.22) ‡</td>
<td>(0.00051–0.40) (0.00011–0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>0.073 ± 0.051</td>
<td>0.072 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>(0.032–0.13) ‡</td>
<td>(0.00051–0.40) (0.00011–0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>0.024 ± 0.054</td>
<td>0.083 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>(0.00016–0.20) ‡</td>
<td>(0.0092–0.20) (0.00016–0.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>0.040 ± 0.069</td>
<td>0.061 ± 0.085</td>
<td></td>
</tr>
<tr>
<td>(0.0034–0.22) ‡</td>
<td>(0.0082–0.17) (0.0008–0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly operated</td>
<td>0.027 ± 0.048</td>
<td>0.066 ± 0.11</td>
<td>0.049 ± 0.066</td>
</tr>
<tr>
<td>(n = 16; 0.0013–0.20)</td>
<td>(n = 14; 0.00051–0.40) (n = 20; 0.0018–0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior surgery/biopsy</td>
<td>0.051 ± 0.076</td>
<td>0.083 ± 0.094</td>
<td>0.077 ± 0.088</td>
</tr>
<tr>
<td>(n = 9; 0.00016–0.22)</td>
<td>(n = 5; 0.0014–0.20) (n = 6; 0.0011–0.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number progressed</td>
<td>12 (48%)</td>
<td>9 (47%)</td>
<td>22 (85%)</td>
</tr>
<tr>
<td>Mean TTP</td>
<td>40 months</td>
<td>13 months</td>
<td>4 months</td>
</tr>
</tbody>
</table>

* >95% excision of tumor.
† fmol abasic sites incised/cell/minute.
‡ Range.
tween the three diagnostic categories, either within grades II and III tumors separately (Table 4) or in both groups combined (data not shown). Activity did not differ between the 50 newly operated tumors and the 20 that were reoperated after prior surgery/biopsy (0.046 ± 0.077 versus 0.067 ± 0.081 fmol/cell/minutes), as also observed within each grade.

For grade II tumors, 12 progressed, 13 observations were censored, and mean TTP was 40 months. For grade III, 9 progressed, 10 observations were censored, and mean TTP was 13 months. For grade IV, 22 progressed, 4 observations were censored, and mean TTP was 4 months. The declining TTP with grade is in agreement with previous studies (2, 34). A univariate Cox regression model was used to estimate the association between Ap endo activity and TTP after radiation therapy for grade II and grade III tumors separately. The estimate showed that for every 0.01 increase in Ap endo activity the HR increased by a factor of 1.087 for grade II (P = 0.29; CI = [0.933, 1.266]) and by a factor of 1.046 for grade III (P = 0.09; CI = [0.993, 1.101]). However, the small number of grade II and III tumors that progressed limited the ability to precisely estimate the effect of activity on TTP or to detect a significant effect within each grade alone. Hence, both grades were combined in a univariate Cox model that revealed an estimated HR = 1.069 for every 0.01 increase in activity (P = 0.008; CI = [1.018, 1.123]). As shown in Table 5, age, gender, extent of resection and prior surgery/biopsy were not significantly associated with TTP, although a strong association with grade was observed, consistent with the better prognosis for grade II versus III gliomas (2, 34). Adjustment for potential confounding by these variables in a multivariate analysis had little effect on the association of Ap endo activity with TTP after RT (HR = 1.065; P = 0.030; CI = [1.009, 1.125]).

When the univariate analysis was done for grade IV tumors, no association between Ap endo activity and TTP was observed: i.e., the estimated HR was 0.996 for each 0.01 increase in activity (P = 0.90; CI = [0.937, 1.059]; Table 5). Similarly, there was no significant association between activity and TTP after adjusting for age, gender, prior surgery or biopsy, and extent of resection. As in the foregoing analysis of grade IV tumors, a significant association of TTP with age was observed (P = 0.007); when entered as a continuous variable in a univariate model, the HR for progression increased by a factor of 1.060 for every 1-year increase in age (CI = [1.016, 1.105]). A very similar association with age was also observed in the multivariate model (Table 5).

**DISCUSSION**

Despite advances in surgery and intensive investigation into novel therapies, the overall 2-year survival rate for malignant gliomas (i.e., grades III and IV) remains <20% (1, 14). For the most common diagnostic types, adjuvant therapy with radiation and alkylating agent-based chemotherapy modestly increases response rates and survival times (5, 11). However, adjuvant therapy provides no benefit to a sizeable fraction of patients and seldom produces long term remission. In addition, the modest benefit conferred depends greatly on patient and clinical variables, including age, neurological status, and histology (1, 2, 34). Intrinsic and acquired resistance to alkylators and radiation are major factors affecting outcome. Hence, characterization of mechanisms underlying resistance is essential to identify potential targets for antiresistance therapies and to develop potential prognostic markers to guide adjuvant therapies. Our results suggest that Ap endo activity promotes resistance to alkylating and radiation in grade II and III gliomas and may be useful in predicting TTP.

It is likely that the association we observed between Ap endo activity and TTP after either alkylating agent-based

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**Table 5** Hazard ratio estimates for the association between Ap endo activity and TTP in gliomas treated with radiation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade II and III</th>
<th>Grade IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Ap endo *</td>
<td>1.069</td>
<td>[1.018, 1.123]</td>
</tr>
<tr>
<td>Grade</td>
<td>4.8</td>
<td>[1.8, 13]</td>
</tr>
<tr>
<td>Age †</td>
<td>1.009</td>
<td>[0.968, 1.051]</td>
</tr>
<tr>
<td>Male versus female ‡</td>
<td>0.6</td>
<td>[0.3, 1.6]</td>
</tr>
<tr>
<td>Total versus subtotal resection §</td>
<td>1.1</td>
<td>[0.4, 2.8]</td>
</tr>
<tr>
<td>Prior versus no prior treatment ¶</td>
<td>2.0</td>
<td>[0.8, 5.3]</td>
</tr>
<tr>
<td>Ap endo *</td>
<td>1.072</td>
<td>[1.011, 1.134]</td>
</tr>
<tr>
<td>Grade</td>
<td>11</td>
<td>[2.7, 42]</td>
</tr>
<tr>
<td>Age †</td>
<td>1.004</td>
<td>[0.948, 1.064]</td>
</tr>
<tr>
<td>Male versus female ‡</td>
<td>0.3</td>
<td>[0.1, 1.1]</td>
</tr>
<tr>
<td>Total versus subtotal resection §</td>
<td>0.7</td>
<td>[0.2, 2.5]</td>
</tr>
<tr>
<td>Prior versus no prior treatment ¶</td>
<td>3.0</td>
<td>[0.8, 11]</td>
</tr>
</tbody>
</table>

* Per 0.01 increase in activity.
† Per 1 year increase.
‡ Male = 1, female = 0.
§ Gross total resection = 1, subtotal = 0.
¶ Prior treatment = 1; no prior treatment = 0.
chemotherapy or RT reflects, at least in part, repair of abasic sites. Both alkylators and radiation produce this cytotoxic DNA lesion (26, 28). The majority (50–80%) of damaged DNA bases caused by therapeutic alkylating agents are N-alkyl purines (36, 37), which can be converted to abasic sites either by DNA glycosylase-mediated alkylation (38) or by spontaneous hydrolysis of the glycosyl linkage between deoxyribose and the altered base (39). Ionizing radiation also generates abasic sites, either directly by free radical attack at deoxyribose (28) or by formation of oxidized bases that are substrates for DNA glycosylases (40). Abasic sites block DNA synthesis (41–43), suggesting a mechanism for their cytotoxicity. The contribution of abasic sites to the lethality of both alkylating agents and ionizing radiation is evidenced by the hypersensitivity of bacterial, yeast, and mammalian cells that are deficient in repair of this lesion (reviewed in ref. 26, 28). Suppression of Ap endo activity (26) in rat glioma cells increases sensitivity to the methylating agent methyl methanesulfonate and the oxidizing agent H2O2 (44). We have found that suppressing Ape1/Ref-1 in a human glioma line results in decreased Ap endo activity, increased abasic site content, and increased sensitivity to BCNU and temozolomide. Conversely, we observed that elevation of Ap endo activity and Ape1/Ref-1 level is accompanied by decreased abasic site abundance and decreased sensitivity to BCNU and temozolomide (27). Another recent finding supports a contribution of the abasic site cleavage activity of Ape1/Ref-1 to the observed association of Ap endo activity with response to alkylators; i.e., a chimeric protein possessing Ap endo and MGMT activities, but no Ref-1 (see below), conferred greater BCNU resistance to human leukemia cells than MGMT alone (45).

The associations between Ap endo activity and TTP we observed here could reflect mechanisms in addition to, or other than, repair of abasic sites. Ape1/Ref-1 exhibits multiple catalytic activities in vitro, including 3'-phosphodiesterase, 3'-phosphatase, and 3'-exonuclease (26, 28), which are apparently mediated at the same active site as abasic site incision and which may promote resistance to oxidative DNA damage. Ape1/Ref-1 also contains the reduction-oxidation protein Ref-1, located at the amino terminus, that participates in cellular processes including the response to oxidative stress, cell cycle control, and apoptosis (26). Ape1/Ref-1 has been shown to regulate the transactivation and proapoptotic activities of p53 in vivo; stimulation of p53 transcriptional activity is modulated by altering Ape1/Ref-1 levels, suggesting a mechanism by which Ape1/Ref-1 may determine response to adjuvant therapy (46). However, the high frequency of loss of p53 function observed in grade II and III gliomas (47) suggests that our results are unlikely to reflect this mechanism alone.

To our knowledge, this is the first report of an association between Ap endo activity in tumor tissue and treatment outcome. However, the subcellular localization of Ape1/Ref-1 has been associated with outcome in tumors other than gliomas. For example, predominantly cytoplasmic expression of Ape1/Ref-1 is associated with poorer survival in squamous cell cancers and adenocarcinomas of the lung (48, 49). In contrast, predominantly nuclear expression of Ape1/Ref-1 is correlated with poor prognosis after combined chemotherapy and RT in head and neck cancers (50), and mixed nuclear-cytoplasmic localization is associated with significantly poorer survival in stage I to III breast cancer (51). With respect to the present work, the Ap endo activity we measured was extracted from whole cells and could be nuclear and/or cytoplasmic. Ape1/Ref-1 has been localized to mitochondria (26) where it may participate in the base excision repair of alkylation (52) and oxidative (53) damage.

Interestingly, we did not observe an association between Ap endo activity and TTP in grade IV gliomas. It is possible that the limited range of progression-free survival after adjuvant therapy in our glioblastoma sample may have obscured an association. This limitation has been cited in other studies seeking genetic or molecular markers of clinical response in glioblastoma. Segregation of glioblastomas into long-term and short-term survivors was required to reveal association of certain prognostic markers with clinical outcome (54), and a similar segregation may reveal an association of Ap endo activity with TTP in a larger sample. On the other hand, Ap endo activity, and/or one of the other functions of Ape1/Ref-1, may not be limiting for TTP after adjuvant therapy in grade IV gliomas. For example, these tumors may be better able to tolerate persistent abasic sites or to repair secondary lesions arising from them, such as double-strand breaks that can occur at blocked replication forks. Possibly repair of DNA lesions other than abasic sites is limiting for survival in glioblastomas, or a mechanism(s) other than DNA repair is a primary determinant of progression.

Our findings suggest that Ap endo activity may be useful in guiding alkylator-based chemotherapy in grade III gliomas. For example, high-activity tumors could be treated with potentially more effective regimens that do not include alkylating agents, sparing patients unnecessary alkylation-induced systemic toxicity. The results, taken together with our previous findings for cultured glioma cells (27), also suggest that Ap endo activity is an attractive target for resistance therapies. Low molecular weight compounds have been described that inhibit incision at abasic sites (39, 55). Combinations of inhibitors of Ap endo activity and MGMT (e.g., O6-benzylguanine; ref. 56) may be particularly effective.

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Apurinic Endonuclease Activity in Adult Gliomas and Time to Tumor Progression after Alkylating Agent-Based Chemotherapy and after Radiotherapy

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