Apurinic/Apyrimidinic Endonuclease Activity Is Elevated in Human Adult Gliomas

Michael S. Bobola, A. Blank, Mitchel S. Berger, Bobby A. Stevens, and John R. Silber

ABSTRACT

Apurinic/apyrimidinic endonuclease (Ap endo) is a key DNA repair activity that confers resistance to ionizing radiation and alkylating agents in human cell lines. The major Ap endo in human cells is Ape1, an abundant multi-functional protein also known as Ref-1, Hap-1, and Apex. In this work, we assayed Ap endo activity in human adult gliomas to establish correlates with tumor characteristics, and in histologically normal brain adjacent to tumors to characterize changes in activity accompanying neurocarcinogenesis. To our knowledge, this is the first available analysis of Ap endo activity in human brain tumors. Mean activity in 84 gliomas of different diagnostic types and grades was 0.072 ± 0.095 fmol abasic sites incised/cell/min, ranging ~550-fold from 0.00077 to 0.42. The mean for high-grade gliomas was 3.5-fold greater than for low-grade tumors (P < 4.0 × 10^-5), a difference observed within all diagnostic types. Activity was correlated with the fraction of S-phase cells in diploid gliomas (P < 0.02), suggesting that proliferation could be a determinant of activity in these tumors. Activity was also correlated with S-phase fraction in the majority of aneuploid gliomas (P < 0.03). Moreover, within the aneuploid tumors, there was a significant relationship between activity and the fraction of aneuploid cells (P < 4.0 × 10^-4). In the 58 cases analyzed, mean activity was 7.3-fold higher in gliomas than in adjacent histologically normal brain (0.070 ± 0.10 versus 0.0096 ± 0.012 fmol/cell/min; P ≤ 3.0 × 10^-5). Increased tumor activity was observed in 93% of tumor/normal pairs, indicating that elevation of Ap endo activity is characteristic of human gliomagenesis. The elevation was large within most pairs, being 13-fold on average and ≥ 10-fold in 43% of cases. A concomitant increase in Ape1 protein was observed by Western blotting in the subset of tumor/normal pairs examined. A clinically important consequence of the increase in Ap endo activity that accompanies neurocarcinogenesis may be enhanced resistance to the radiotherapy and alkylating agent-based chemotherapy that are mainstays of adjuvant therapy for malignant gliomas.

INTRODUCTION

The major mammalian Ap endo, Ape1 (also known as Ref-1, Hap-1, and Apex), is a multifunctional enzyme that mediates repair of ionizing radiation and alkylating agent-induced DNA damage (1, 2). Ape1 is abundant in human cells and accounts for nearly all of the abasic site cleavage activity observed in cellular extracts (3). Ape1 possesses a strong, Mg^2⁺-dependent endonuclease activity that hydrolyzes the phosphodiester bond 5’ to potentially cytotoxic abasic sites, leaving a 3’-OH and a 5’-deoxyribose phosphate (1, 2, 4). Ape1 acts in base excision repair pathways to hydrolyze abasic sites arising from enzymatic removal of damaged purine and pyrimidine bases, and also cleaves at abasic sites arising from spontaneous hydrolysis of damaged bases (5). In addition, Ape1 has a 3’-phosphodiesterase activity that excises deoxyribose fragments and phosphate groups at the 3’ terminus of DNA strand breaks caused by ionizing radiation (6, 7), yielding a 3’-OH substrate for DNA repair synthesis. Ape1 possesses 3’-exonuclease activity as well (1, 2).

Ape1 participates in other crucial cellular processes including the response to oxidative stress, regulation of transcription factors, cell cycle control and apoptosis. Ape1, as the reduction-oxidation protein Ref-1 (1), can reduce a conserved cysteine residue in members of the Jun/Fos and related ATF/CREB families of proteins, facilitating formation of hetero- and homodimers that bind to transcriptional regulatory elements containing activator protein-1 (AP-1) and cyclic AMP (CRE) motifs (8). Notably, Jun/Fos family members participate in signal transduction cascades induced by oxidative stress (9, 10). In addition, Ape1 stimulates the DNA binding of other transcription factors, including HIF-1α, NF-κ B, Myb, Pax5 and Pax8 (reviewed in Ref. 1). Ape1/Ref-1 has also been implicated in regulating the transactivation and pro-apoptotic activities of p53 (11). The biological importance of Ape1 is evidenced by its essentiality for early embryonic development: homozygous null APE1/- APE1-/- mice die shortly after blastocyst formation (12, 13). The potential roles and significance of Ape1 in the development and progression of diverse human cancers are currently...
being explored (1). A second human Ap endo, Ape2, has been discovered recently. Unlike Ape1, recombinant Ape2 exhibits weak abasic site cleavage activity, and endogenous Ape2 has been difficult to detect in extracts by Western blotting (14).

Substantial evidence documents the participation of Ape1 in protecting mammalian cells against the lethality of ionizing radiation and alkylating agents (1). Reduction of Ape1 activity by stable expression of antisense mRNA increases the sensitivity of mammalian cell lines to agents, including X-rays, that generate oxidative free radicals and to the methylation agent methyl methanesulfonate (15–17). In accord, explanted APE1–/APE1– mouse blastocysts display hypersensitivity to ionizing radiation (13). Ape1 activity is transiently elevated in human and rodent cell lines exposed to minimally toxic levels of agents that generate oxidative free radicals (18, 19). Increased resistance to the cytotoxicity of γ-rays and methyl methanesulfonate accompanies the elevation, suggesting a role for Ape1 in the transient adaptive response of mammalian cells to oxidative insult (20).

Malignant gliomas are the most common primary, intracranial tumor in adults and are among the least curable of human cancers. Clinical trials have demonstrated a significant, but limited, benefit of radiation therapy in conjunction with alkylating agent-based chemotherapy in prolonging progression-free survival (21, 22). Although little is known of the factors that limit the efficacy of radiation and alkylator-based therapies for glioma, the biochemical and biological functions of Ape1 suggest a role in resistance to these treatment modalities. For example, a significant correlation between Ap endo activity and resistance to methyl methanesulfonate has been observed in 10 human glioma cell lines (23). In addition, the radiosensitivity of primary cultures of human cervical cancer was significantly correlated with Ape1 immunopositivity (24). More recently, it has been shown that overexpression of Ape1 activity in a human germ cell tumor line was accompanied by increased resistance to bleomycin and ionizing radiation (25).

To provide information relevant to the hypothesis that Ap endo activity contributes to radiation and alkylating agent resistance of primary human brain tumors, we describe here a survey of Ap endo activity in 84 adult gliomas and, in 58 cases, in adjacent, histologically normal brain. To our knowledge, this is the only available analysis of Ap endo activity in human glial tumors. Our findings may be significant for understanding the clinical response to adjuvant treatment with radiation and alkylating agents.

**MATERIALS AND METHODS**

**Tissue.** Tumors were resected at the University of Washington Medical Center and samples were transported to the laboratory within minutes; immediately adjacent samples were sent for tumor diagnosis. Measures taken to preserve tissue viability and enzymatic activities during transport, and determination of cell number, are described elsewhere (26). Diagnosis was obtained from the final neuropathology report; no areas of normal histology were noted. Demographic information together with course of therapy was obtained from medical records. S-phase and aneuploid cell fraction of an unselected subset of tumors were determined by flow cytometry. Subcortical normal brain adjacent to tumor, obtained in two-thirds of cases, was microscopically free of hypercellularity, infiltrating tumor, endothelial proliferation, edema and gliosis; care was taken to ensure that normal brain specimens analyzed in this study were distant from the tumor margin.

**Preparation of Extracts.** Extracts were prepared from ~100 mg of tissue that was homogenized with a Polytron in 1.0 ml of 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride and 1 μg/ml each of aprotinin, leupeptin, and pepstatin. The homogenate was sonicated on ice for four 15-s intervals, and debris was pelleted by centrifugation at 10,000 × g for 5 min at 4°C. The pellet was re-extracted in 0.5 ml of extraction buffer and the supernatants were combined. Multiple small aliquots (25–50 μl) were flash-frozen in liquid nitrogen and stored at ~80°C; aliquots were thawed only once.

**Ap endo Assay.** Ap endo activity in high-speed supernatants of tissue sonicates was quantitated by using a standard, highly sensitive assay that measures the conversion of plasmid DNA from supercoiled to relaxed form caused by incision at an abasic site (3). Activity (fmol abasic sites incised/cell/min, abbreviated to fmol/cell/min) is the mean established in at least three separate determinations that differed, in general, by less...
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The activities were expressed with reference to cell number, determined by counting cells in a tissue suspension as described previously (26). Activities are expressed with reference to cell number, determined by counting cells in a tissue suspension as described previously (26).

DNA substrate was prepared by a modification of a previously described protocol (3). Supercoiled pKT100 DNA (0.17 \mu{g}/ml) was incubated for 20 min at 65°C in 0.5 mM NaCl, 0.1 mM sodium citrate (pH 3.5). The resulting depurinated DNA, containing, on average, 1.5 abasic sites/molecule, was precipitated with isopropanol and resuspended at 0.1 mM MgCl2, 0.5 mM CoCl2, 100 \mu{g}/ml BSA, and extract equivalent to 10 to 10^4 cells. After incubation for 15 min at 37°C, reaction products were resolved on a 0.8% agarose gel in 40 mM Tris-acetate, 2 mM EDTA. The gel was stained with ethidium bromide to visualize supercoiled and nicked, relaxed plasmid DNA,

Table 1: Glioma and patient characteristics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>All</th>
<th>Newa</th>
<th>Surgeryb</th>
<th>RTc</th>
<th>RT + AA^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumors</td>
<td>84</td>
<td>41</td>
<td>19</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Patient age, yr</td>
<td>45 ± 14</td>
<td>48 ± 15</td>
<td>43 ± 13</td>
<td>41 ± 13</td>
<td>42 ± 11</td>
</tr>
<tr>
<td>Range</td>
<td>(18–78)</td>
<td>(21–78)</td>
<td>(23–70)</td>
<td>(18–70)</td>
<td>(22–59)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>10 (12%)</td>
<td>6 (15%)</td>
<td>3 (16%)</td>
<td>0</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>10 (12%)</td>
<td>5 (12%)</td>
<td>0</td>
<td>3 (19%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>39 (46%)</td>
<td>18 (44%)</td>
<td>9 (47%)</td>
<td>7 (44%)</td>
<td>5 (62%)</td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>15 (18%)</td>
<td>8 (19%)</td>
<td>2 (11%)</td>
<td>5 (31%)</td>
<td>0</td>
</tr>
<tr>
<td>Oligo-astrocytoma</td>
<td>10 (12%)</td>
<td>4 (10%)</td>
<td>5 (26%)</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Newly operated tumors.
b Tumors recurrent after surgery only.
c Tumors recurrent after surgery and radiotherapy (RT).
d Tumors recurrent after surgery, radiotherapy and alkylating agent (AA)-based chemotherapy.

RESULTS

Tumor and Patient Characteristics. Eighty-four glial tumors were obtained from 80 patients (Table 1), ranging in age from 18 to 78 years (mean ± SD, 45 ± 14 years). The male:female ratio was 1.9, in accord with the gender bias for gliomas (29). Fifty-nine tumors (70%) were astrocytic gliomas, the most frequent human brain tumor diagnosis (29). This group included 10 astrocytomas, 10 anaplastic astrocytomas, and 39 glioblastomas. Other tumors were 15 oligodendrogliomas and 10 mixed oligo-astrocytomas; 6 oligodendrogliomas and 6 oligo-astrocytomas were high grade with anaplastic features. As indicated in Table 1, tumors were divided among four treatment groups. Forty-one tumors were newly operated, 16 were recurrent after surgery alone, 16 were recurrent after surgery and radiotherapy and 8 were recurrent after surgery, radiotherapy and alkylating agent-based chemotherapy. Histologically normal brain adjacent to tumor was obtained from 58 patients. These individuals were similar to the entire patient population in age (45 ± 14 years), sex ratio (1.8, male:female) and distribution of diagnoses (Table 4).

Ap endo Activity Varies Widely in Adult Gliomas. Ap endo activity was quantitated by using a classic assay in which cleavage at an abasic site converts supercoiled plasmid DNA to a relaxed form (3). This assay has been used to measure Ap endo activity in extracts of human tissues, e.g., lymphocytes (30), brain (31, 32), and breast cancer and adjacent normal tissue (27). In accord with others (e.g., Ref. 27), we observed that native,
supercoiled plasmid DNA was not incised, that no activity was detectable in the absence of Mg²⁺, and that activity was linear with added extract. We also observed that activity was additive for mixtures of extracts containing high and low Ap endo activity, indicating that low activity was not attributable to the presence of an inhibitor (data not shown). The large quantity of Ape1 in cells permits monitoring of activity in fewer than 100 cells and obviates interference by other enzymes. Assay data for a representative tumor/normal tissue pair are illustrated in Fig. 1, in which both linearity with added extract, and the sensitivity of the assay are apparent. Ape1 probably accounts for most, if not essentially all, of the activity measured, in accord with its abundance and robust cleavage activity (1, 4) and with our finding that an increase in Ape1 protein accompanied the elevated Ap endo activity found in tumors (see Fig. 4 below). Ape2 also cleaves abasic sites, but its weak incision activity and apparent paucity in cells suggests that its contribution may be insignificant (14). The lyase activity of certain DNA glycosylases is also a possible contributor, but this source may be negligible as well; e.g., human 8-oxoguanine-DNA glycosylase can cleave abasic sites in vitro, but the catalytic efficiency (kcat/Km) is 400-fold lower than that of Ape1 (33).

Ap endo activity in 84 gliomas ranged 545-fold from 0.00077 to 0.42 fmol/cell/min (Fig. 2), the mean being 0.072 ± 0.095 fmol/cell/min. The large inter-individual variability is in accord with our previous observations for the DNA repair protein O6-methylguanine-DNA methyltransferase that differed 300-fold among 152 adult gliomas (26). No relationship was apparent between activity and either age or sex in newly operated tumors. No significant difference was observed between newly operated tumors and tumors recurring after surgery alone; after surgery and radiation; or after surgery, radiation and alkylating agent chemotherapy (Table 2), suggesting that treatment with radiation and alkylating agents was not associated with long-term changes in Ap endo activity.

**Ap endo Activity Is Greater in High-Grade Than in Low-Grade Gliomas.** Mean Ap endo activity differed 2.4-fold among glioma diagnoses, varying from 0.036 fmol/cell/min

<table>
<thead>
<tr>
<th>Tumors</th>
<th>n</th>
<th>Activity (fmol abasic sites nicked/cell/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>84</td>
<td>0.072 ± 0.095</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>10</td>
<td>0.036 ± 0.039</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>10</td>
<td>0.087 ± 0.12</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>39</td>
<td>0.086 ± 0.11</td>
</tr>
<tr>
<td>Oligodendroglia</td>
<td>15</td>
<td>0.043 ± 0.058</td>
</tr>
<tr>
<td>Oligo-astrocytoma</td>
<td>10</td>
<td>0.087 ± 0.089</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly operated</td>
<td>41</td>
<td>0.058 ± 0.082</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>19</td>
<td>0.10 ± 0.098</td>
</tr>
<tr>
<td>Surgery + radiotherapy</td>
<td>16</td>
<td>0.073 ± 0.10</td>
</tr>
<tr>
<td>Surgery + radiotherapy + alkylating agents</td>
<td>8</td>
<td>0.066 ± 0.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Glioma Ap endo activity by diagnosis and prior treatment</th>
</tr>
</thead>
</table>

*Mean ± SD (fmol abasic sites nicked/cell/min).
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The S-phase fraction (%S) of 61 unselected gliomas for which flow cytometric data were available ranged 1000-fold from 0.012% to 12%, the average being 3.7%. The mean Ap endo activity of histologically normal brain adjacent to 58 gliomas was 0.0096 ± 0.012 fmol/cell/min. Activity ranged approximately 413-fold from 0.00015 to 0.062 fmol/cell/min (Fig. 2). Mean activity in normal brain did not differ significantly between tissue accompanying newly operated tumors and tumors recurring after surgery, or surgery and radiotherapy, or surgery, radiotherapy and alkylating agent therapy (Table 4), suggesting that therapy was not associated with long-term changes in Ap endo activity. Likewise, mean activity did not differ significantly for normal brain adjacent to newly operated tumors differing by diagnosis or grade.

**Table 3** Ap endo activity by tumor grade

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>High grade</th>
<th>Low grade</th>
<th>High/Low</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Activity*</td>
<td>n</td>
<td>Activity*</td>
</tr>
<tr>
<td>All gliomas</td>
<td>61</td>
<td>0.090 ± 0.11</td>
<td>23</td>
<td>0.026 ± 0.028</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>49</td>
<td>0.086 ± 0.11</td>
<td>10</td>
<td>0.036 ± 0.039</td>
</tr>
<tr>
<td>Oligodendroglialoma</td>
<td>6</td>
<td>0.081 ± 0.078</td>
<td>9</td>
<td>0.017 ± 0.014</td>
</tr>
<tr>
<td>Oligo-astrocytoma</td>
<td>6</td>
<td>0.13 ± 0.091</td>
<td>4</td>
<td>0.020 ± 0.006</td>
</tr>
</tbody>
</table>

*Mean ± SD (fmol abasic sites incised/cell/min).

**Fig. 3** Ap endo activity as a function of S-phase fraction in diploid gliomas and aneuploid cell fraction in aneuploid gliomas. Activity is shown for 29 gliomas comprised solely of diploid cells (A) and 34 gliomas containing cells with an aneuploid DNA content (B). The regression lines are indicated.

for astrocytoma to 0.086–0.087 fmol/cell/min for glioblastoma, anaplastic astrocytoma and oligo-astrocytoma (Table 2; Fig. 2). Importantly, activity was 3.5-fold greater in high-grade (malignant) than in low-grade tumors (0.090 ± 0.11 versus 0.026 ± 0.028 fmol/cell/min), a difference that was highly significant (t = 4.39; P = 4.0 × 10⁻⁵). As shown in Table 3, activity was greater in malignant tumors of all diagnostic types, the difference between high and low grade ranging from 2.4-fold for astrocytoma (P = 0.02) to 6.5-fold for oligo-astrocytoma (P ≤ 0.03).

**Ap endo Activity Is Correlated with S-phase Fraction.** The S-phase fraction (%S) of 61 unselected gliomas for which flow cytometric data were available ranged 1000-fold from 0.012% to 12%, the average being 3.7% ± 2.8%. The mean S-phase fraction of 43 high-grade tumors was 2.7-fold greater that of 18 low-grade tumors (4.8 ± 3.0% versus 1.8 ± 1.2%; t = -5.55; P = 7.5 × 10⁻⁷), in accord with the increased proliferation characteristic of high-grade gliomas (34, 35). No difference in mean %S was observed between 29 diploid and 32 aneuploid gliomas (3.3 ± 2.2% versus 4.0 ± 3.2%).

Analysis of the 29 diploid gliomas revealed a significant relationship between Ap endo activity and %S (r = 0.443; t = 2.57; P = 0.02; Fig. 3A). In contrast, there was no relationship between activity and S-phase fraction in 32 aneuploid gliomas (r = 0.021; t = 0.115; P > 0.90). However, the aneuploid tumors could be divided into two groups: (a) A majority sub-sample of 25 tumors with %S < 6.5%, in which the regression of Ap endo activity on S-phase fraction (r = 0.458; t = 2.47; P ≤ 0.025) was essentially identical to that observed for diploid tumors; and (b) a minority sub-sample of seven “outlier” tumors with %S > 6.5% that had a significantly lower mean Ap endo than both the majority sub-sample of aneuploid gliomas (0.032 ± 0.014 versus 0.071 ± 0.081 fmol/cell/min; P ≤ 0.03) and the diploid gliomas (0.032 ± 0.014 versus 0.082 ± 0.11 fmol/cell/min; P ≤ 0.025).

**Ap endo Activity in Aneuploid Tumors Is Correlated with the Fraction of Aneuploid Cells.** Mean Ap endo activity did not differ significantly between 29 diploid and 34 aneuploid tumors whose DNA content was determined by flow cytometry (0.082 ± 0.11 versus 0.063 ± 0.073 fmol/cell/min; P > 0.43). However, linear regression analysis revealed a strong relationship between Ap endo activity and the fraction of aneuploid cells (r = 0.577; t = 3.99; P ≤ 4.0 × 10⁻⁴; Fig. 3B). The aneuploid tumors contained 1.1% to 100% of cells with a DNA content ranging from 1.1 to 2.0-fold greater than diploid (mean ± SD = 1.8 ± 0.2); Ap endo was not correlated with DNA content (r = 0.098; t = 0.500; P > 0.60).

**Ap endo Activity in Histologically Normal Brain Adjacent to Gliomas.** The mean Ap endo activity of histologically normal brain adjacent to 58 gliomas was 0.0096 ± 0.012 fmol/cell/min. Activity ranged approximately 413-fold from 0.00015 to 0.062 fmol/cell/min (Fig. 2). Mean activity in normal brain did not differ significantly between tissue accompanying newly operated tumors and tumors recurring after surgery, or surgery and radiotherapy, or surgery, radiotherapy and alkylating agent therapy (Table 4), suggesting that therapy was not associated with long-term changes in Ap endo activity. Likewise, mean activity did not differ significantly for normal brain adjacent to newly operated tumors differing by diagnosis or grade.

**Glioma Ap endo Activity Is Elevated Relative to Adjacent Normal Brain.** Mean Ap endo activity in 58 tumors was 7.3-fold higher than in adjacent, histologically normal brain.
Recent technical advances in radiotherapy (36, 37) and development of new alkylating agent–based chemotherapy protocols (38–40) have prolonged survival in some patients, yet the overall prognosis remains dismal, with an overall 2 year survival rate less than 20%. Clinical progress has been impeded, in part, by an incomplete understanding of DNA repair-mediated resistance mechanisms that may limit the efficacy of tumoricidal DNA damaging agents. Ape1 is a key enzyme in base excision repair of DNA damage (1, 2, 4, 5) and protects mammalian cells (15–17, 23) against the lethality of ionizing radiation and alkylating agents. The function of Ape1 in limiting the cytotoxicity of these DNA damaging agents suggests a role in glioma resistance to adjuvant ionizing radiation and alkylating agent therapy. Here, we provide information, currently lacking, concerning Ap endo activity in human gliomas and normal brain, and examine the relationship of glioma Ap endo activity to clinically relevant tumor characteristics.

**DISCUSSION**

Radiotherapy and alkylating agent–based chemotherapy are mainstays in the adjuvant therapy of malignant gliomas (21, 22). Recent technical advances in radiotherapy (36, 37) and development of new alkylating agent–based chemotherapy protocols (38–40) have prolonged survival in some patients, yet the overall prognosis remains dismal, with an overall 2 year survival rate less than 20%. Clinical progress has been impeded, in part, by an incomplete understanding of DNA repair-mediated resistance mechanisms that may limit the efficacy of tumoricidal DNA damaging agents. Ape1 is a key enzyme in base excision repair of DNA damage (1, 2, 4, 5) and protects mammalian cells (15–17, 23) against the lethality of ionizing radiation and alkylating agents. The function of Ape1 in limiting the cytotoxicity of these DNA damaging agents suggests a role in glioma resistance to adjuvant ionizing radiation and alkylating agent therapy. Here, we provide information, currently lacking, concerning Ap endo activity in human gliomas and normal brain, and examine the relationship of glioma Ap endo activity to clinically relevant tumor characteristics.

**Glioma Ap endo Activity Is Related to Tumor Characteristics.** We used a classic, highly sensitive, plasmid nicking assay to quantitate Ap endo activity in 84 gliomas of different diagnoses and grades. As mentioned earlier, it is likely that the activity we measured is attributable predominantly, or essentially entirely, to Ape1. Activity varied >500-fold (Fig. 2), and was greater in high-grade than in low-grade tumors of each diagnostic type (P ≤ 0.40 × 10⁻⁵ for all tumors; Table 3). The latter finding suggests a relationship between Ap endo activity and a feature(s) of malignant gliomas, such as high proliferative potential (34, 35). In fact, activity was significantly associated with S-phase fraction in diploid gliomas (P ≤ 0.02; Fig. 3A) and in a majority sub-sample of aneuploid tumors (P ≤ 0.03).

**Table 4** Ap endo activity in 58 pairs of gliomas and adjacent normal brain by diagnosis, grade and treatment

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Activitya</th>
<th>Fold elevationb</th>
<th>P≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>All gliomas</td>
<td>0.070 ± 0.10</td>
<td>0.0096 ± 0.012</td>
<td>7.3</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>6</td>
<td>0.046 ± 0.044</td>
<td>4.9</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>8</td>
<td>0.078 ± 0.13</td>
<td>8.2</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>26</td>
<td>0.086 ± 0.12</td>
<td>7.2</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>11</td>
<td>0.041 ± 0.055</td>
<td>5.9</td>
</tr>
<tr>
<td>Oligo-astrocytoma</td>
<td>7</td>
<td>0.069 ± 0.089</td>
<td>11</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>13</td>
<td>0.030 ± 0.031</td>
<td>4.2</td>
</tr>
<tr>
<td>High grade</td>
<td>43</td>
<td>0.084 ± 0.11</td>
<td>7.6</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly operated</td>
<td>25</td>
<td>0.056 ± 0.083</td>
<td>8.2</td>
</tr>
<tr>
<td>Surgery</td>
<td>15</td>
<td>0.097 ± 0.10</td>
<td>10</td>
</tr>
<tr>
<td>Surgery + RTa</td>
<td>10</td>
<td>0.069 ± 0.13</td>
<td>5.8</td>
</tr>
<tr>
<td>Surgery + RT + AAa</td>
<td>8</td>
<td>0.066 ± 0.13</td>
<td>4.7</td>
</tr>
</tbody>
</table>

a Mean ± SD (fmol abasic sites incised/cell/min).
b Mean tumor activity/mean adjacent normal activity.
c RT, radiotherapy.
d Alkylating agent (AA)-based chemotherapy.

(0.070 ± 0.10 versus 0.0096 ± 0.012 fmol/cell/min; t = −4.55, P ≤ 3.0 × 10⁻⁵; t test for paired samples). Elevated means were observed for all diagnostic and treatment groups (Table 4). As indicated in Fig. 4A, tumor activity was increased in 93% (54/58) of tumor-normal pairs, the increase being greater than 10-fold in 43% (25/58) of pairs. The ratio of tumor to normal Ap endo activity, averaged overall gliomas, was 13, and did not differ significantly among diagnoses, ranging from 9 for mixed oligo-astrocytoma to 17 for astrocytoma. These data indicate that elevation of Ap endo activity is characteristic of neocarcinogenesis. In the 8 cases examined, an increase in Ape1 protein, detected by Western blotting, accompanied the elevation of Ap endo activity, as illustrated in Fig. 4B for 6 tissue pairs.

Regression analysis showed a strong relationship between Ap endo activity in normal brain and adjacent tumor (r = 0.685; t = 7.03; P ≤ 3.1 × 10⁻⁵). Unexpectedly, we observed an inverse relationship between activity in normal brain and the magnitude of elevation of tumor activity (r = 0.318, t = −2.51; P ≤ 0.015). In contrast, there was no relationship between the magnitude of elevation and glioma activity (r = 0.0115; t = 0.868; P > 0.38). Likewise, the elevation of tumor Ap endo activity was unrelated to tumor grade (r = −0.567; P > 0.56), ploidy (r = 1.08; P > 0.28), fraction of aneuploid cells (r = 0.122; t = 0.564; P > 0.57) or fraction of S-phase cells (r = 0.017; t = −0.107; P > 0.91).
Apurinic Endonuclease in Adult Gliomas

Regression analysis of the aneuploid gliomas showed that Ap endo activity is significantly correlated with aneuploid cell fraction ($P \leq 4.0 \times 10^{-4}$; Fig. 3B). Interestingly, we have also found a significant association of aneuploid cell fraction and levels of the DNA repair protein $O^\bullet$-methylguanine-DNA methyltransferase in a sample of 94 adult gliomas (26). As mentioned, Ap endo can be causally linked with proliferation via oxidative metabolism; however, we are unaware of biological evidence linking aneuploidy, per se, with Ap endo. While an association of aneuploidy with Ap endo activity is intriguing, our data, of course, provide no evidence of causal relationships.

To further assess the relationships of Ap endo activity with both S-phase and aneuploid cell fraction, we carried out multiple regression analysis with percent aneuploid cells ($%A$) and $%S$ as covariates. In all 61 diploid and aneuploid tumors, there is a stronger relationship of Ap endo with $%S$ ($P \leq 0.20$) than with $%A$ ($P < 0.50$). This relationship is more apparent for the 54 tumors exclusive of the outliers described in “Results” ($P \leq 6.0 \times 10^{-4}$ for $%S$ versus $P \leq 0.17$ for $%A$). However, when examining only the 32 aneuploid tumors, we found a strong relationship between $%A$ and activity ($P \leq 5.6 \times 10^{-4}$) that is independent of $%S$ ($P \leq 0.38$), a relationship that was strengthened when analysis was limited to the 25 aneuploid tumors with $%S < 6.5$% ($P \leq 0.013$ for $%A$ versus $P < 0.19$ for $%S$). These analyses suggest that, in aneuploid tumors, there is a complex relationship between Ap endo activity, and aneuploid cell fraction and S-phase fraction, in which $%A$ appears to be a dominant determinate. We mention these findings for the sake of completeness; a larger sample will be required to assess their potential significance.

**Elevation of Glioma Ap endo Activity Accompanies Neurocarcinogenesis and Is Potentially Significant for Radiotherapy and Alkylating Agent Chemotherapy.** Our results indicate that elevation of Ap endo activity is a hallmark of glial tumorigenesis. Fully 93% (54 of 58) of tumor-normal pairs in our sample exhibited an increase, the average tumor: normal activity ratio being 13 (Fig. 4A). Not unexpectedly, the elevation of activity was accompanied by increased tumor Ape1 protein in the tissue pairs that we examined (e.g., Fig. 4B); we should note that the activity we observed could be greatly affected by interaction of Ape1 with other proteins (e.g., heat shock protein 70; Ref. 44), such that the ratio of Ape1 protein and Ape1-catalyzed abasic site cleavage activity may vary among tissue samples. Elevated levels of tumor Ape1 protein have been reported for adult cervical, prostate and epithelial ovarian carcinomas as well as for rhabdomyosarcomas and germ cell tumors in children (Ref. 1 and references therein; Refs. 25, 45). Our results do not disclose whether activity of the newly discovered Ape2 is elevated in gliomas. Nevertheless, available evidence indicates that the elevation of Ap endo activity that we have observed is attributable primarily or perhaps exclusively to Ape1.

We observed that Ap endo activity in gliomas is closely correlated with activity in normal brain ($P \leq 3.1 \times 10^{-9}$), suggesting that the increase during tumorigenesis is constrained by one or more characteristics of progenitor tissue. One such characteristic may be the levels of other enzymes in base excision repair pathways. Maintenance of a balance among these activities may confer a selective advantage, because recent studies with yeast and mammalian cells (5) suggest that unbalanced expression of base excision repair activities may promote increased DNA damage-induced genomic instability and cytotoxicity. We also observed that the fold-elevation of tumor Ap endo activity (tumor: normal ratio) was inversely correlated with the level in normal brain ($P \leq 0.015$). This finding suggests that there may be a maximum level of Ape1 that is compatible with survival. Factors contributing to a possible upper limit might include avoidance of a concentration of DNA strand breaks that exceed the cell’s repair capacity. Overall, the level of Ape1 activity in tumor tissue may reflect the roles of Ape1, not only in DNA repair, but in other cellular processes that govern survival, and may represent the complex interplay of a multitude of selective pressures.
A clinically significant consequence of the elevated Ap endo activity accompanying gliomagenesis may be enhanced resistance to radiotherapy and alkylating agent-based chemotherapy. This possibility is underscored by the magnitude of the elevation, which was greater than 5-fold in 59% of cases and greater than 10-fold in 43% of cases. It is important in this regard that even a 2-fold increase in Ap endo activity in cultured human tumor cells, mediated by over-expression of Ape1, enhanced resistance to bleomycin and ionizing radiation (25). In accord, the radiosensitivity of primary cultures of human cervical carcinoma cells is correlated with Ape1 protein level (24). Determination of the significance of Ap endo activity for glioma response to radiation and alkylating agents will rest on examination of the relationship between activity and clinical outcome. The wide range of activity we observed in gliomas should facilitate this analysis.

ACKNOWLEDGMENTS

We thank Lawrence Loeb for his interest and support and Douglas Kolstoie for technical assistance.

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Apurinic/Apyrimidinic Endonuclease Activity Is Elevated in Human Adult Gliomas

Michael S. Bobola, A. Blank, Mitchel S. Berger, et al.


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