O^-Methylguanine-DNA Methyltransferase in Pediatric Primary Brain Tumors: Relation to Patient and Tumor Characteristics


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ABSTRACT

The DNA repair protein O^-methylguanine-DNA methyltransferase (MGMT) confers resistance to methylating and chloroethylating agents in pediatric medulloblastoma- and glioma-derived cell lines and xenografts. Here, we assayed MGMT activity in 110 pediatric brain tumors to establish correlates with patient and tumor characteristics. We also assayed MGMT in histologically normal brain adjacent to 22 tumors to characterize changes in activity accompanying neurocarcinogenesis. MGMT activity was detected in 94% of tumors, ranging ca. 1,500-fold from 0.34 to 498 fmol/10^6 cells (~205-300,000 molecules/cell). Mean activity was 25 ± 66 fmol/10^6 cells, including six specimens with undetectable activity (Mer^- phenotype; <0.25 fmol/10^6 cells or 151 molecules/cell). MGMT content varied 10-fold among diagnostic groups and was associated with degree of malignancy, as evidenced by a 4-fold difference in activity between high- and low-grade tumors (P = 0.03). Tumor MGMT content was age dependent, being 5-fold higher in children 3–12 years old than in infants (P = 0.015) and adolescents (P = 0.015). Mean activity in tumors was 9-fold higher than in adjacent histologically normal brain (21 ± 44 versus 2.4 ± 4.0 fmol/10^6 cells; P = 0.05). By comparing tumor and adjacent normal tissue from the same patient, we found that 68% of cases exhibited an elevation of tumor activity that ranged from 2- to >590-fold. Moreover, 67% of Mer^- normal tissue was accompanied by Mer^+ tumor. These observations indicate that MGMT activity is frequently elevated during pediatric neurocarcinogenesis. Significantly, enhanced MGMT activity may heighten resistance to alkylating agents, suggesting a potential role for MGMT inhibitors in therapy.

INTRODUCTION

Primary CNS^3 tumors are the most common solid malignancy of childhood, occurring in ~2200 children annually (1). Whereas the majority (60–70%) are gliomas (astrocytomas, oligodendrogliomas, and ependymomas) histologically similar to those found in adults (2, 3), a sizable minority consists of diagnostic types uncommon in adults, including PNETs and mixed neuronal-glia tumors (2, 3). Moreover, unlike adult gliomas, which occur predominantly in the cerebral hemispheres, ~50% occur in the cerebellum and brain stem (3), suggesting that a subset of childhood tumors may have a unique pathogenesis. Although survival rates for some tumor types have improved in the last three decades, the prognosis for malignant pediatric CNS tumors remains grim, with a 5-year survival rate of 50%. As a consequence, brain tumors account for 26% of all pediatric cancer deaths (4). In light of evidence that the incidence of childhood brain tumors has been increasing at an annual rate of 2% (5, 6), development of more effective therapies remains an urgent priority.

Chloroethylating and methylating agents, when used in single agent or combination chemotherapy, are among the most effective antitumor drugs for treatment of malignant pediatric and adult brain tumors (7–9). However, intrinsic and acquired resistance to alkylating agents limits their usefulness. A large body of work with pediatric brain tumor-derived cell lines and xenografts has demonstrated that the DNA repair protein MGMT can contribute to alkylating agent resistance (10–14). MGMT exerts its protective effect by removing cytotoxic chloroethyl and methyl adducts from the O^- position of guanine to an internal cysteine, yielding guanine and S-alklycysteine (15). Because the alkyl receptor site is not regenerated, the number of O^-alkylguanine adducts that can be removed from DNA in vivo is limited by the number of MGMT molecules and the rate of synthesis of the protein. Recent evidence that MGMT limits the cytotoxicity of cyclophosphamide (16), an agent frequently used to treat newly diagnosed and progressive childhood gliomas and medulloblastomas (17, 18), suggests an even wider role for MGMT in pediatric CNS tumor resistance.

Most neoplastic human tissue specimens, including those

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from brain, express MGMT activity (19–22), raising the possibility that MGMT contributes to the drug resistance of tumors in vivo. In a survey of 152 adult gliomas, we found a 300-fold range of detectable MGMT activity (21). Importantly, 24% of specimens had no detectable activity [i.e., were Mer− (methyl repair deficient)], suggesting that this appreciable fraction of brain tumors may be more sensitive to alkylating agents. In accord, data from our laboratory suggest that Mer− adult glioma cells are preferentially killed by alkylators in vivo (22). To expand our analysis to childhood tumors, we have now assayed MGMT activity in 110 pediatric primary brain tumors and in histologically normal brain adjacent to 22 tumors. Our analysis of the largest collection of such tissue samples examined to date revealed that pediatric brain tumors have a wide range of MGMT activity and a low frequency of Mer. We also demonstrate that tumorigenesis in pediatric brain is most often accompanied by an increase in MGMT activity. Our findings may be significant for understanding clinical response to alkylating agent therapy, and they suggest a therapeutic role for MGMT inhibitors.

MATERIALS AND METHODS

Tissue. Tumors were resected at Children’s Hospital and Medical Research Center and the University of Washington Medical Center from 1991 to 1999. Ninety-five tumors were newly diagnosed, and 15 were recurrent after prior therapy (Table 1). None of the recurrent tumors was from patients from whom a newly diagnosed specimen was obtained. Care was taken to ensure that tumor was distant from the interface with normal tissue. All tissues were reviewed by a panel of neuropathologists affiliated with the University of Washington. Diagnosis, along with an S-phase fraction determined by flow cytometry of some unselected tumors, was obtained from the final neuropathology report. The S-phase cell fraction of some, unselected tumors was determined by flow cytometry. Normal tissue was microscopically free of hypercellularity, infiltrating tumor, endothelial proliferation, edema, and gliosis. Demographic information together with course and response to therapy was obtained from medical records. The precautions taken to preserve tissue viability and enzymatic activities during transport of specimens, and the procedure for determining cell number are described elsewhere (21, 22). The MGMT activity of 27 tumors and 13 normal brain samples was reported previously (19, 24).

MGMT Assay. The MGMT content of extracts (i.e., high-speed supernatants of whole tissue sonicates) was assayed by quantitating transfer of radioactivity from a DNA substrate containing \(^{3}H\)methylguanine to protein, as previously described in detail (21, 22). MGMT content is the mean of at least five determinations that generally differed by no more than 20%. Validation of this assay for extracts prepared from tumor and normal brain and controls indicating that the wide range of MGMT activity observed is unlikely to be due to degradation of MGMT and/or its \(^{3}H\)DNA substrate during extraction and assay, or to a diffusible inhibitor in extracts, have been described elsewhere (21, 22).

We define Mer− phenotype here as MGMT activity <0.25 fmol/10^6 cells or 151 molecules/cell. Mer− phenotype is a functional term that refers to the limit of detection in the particular assay used. In our biochemical assay, the definition of Mer− phenotype was established by: (a) the specific activity of the O^6-[methyl-\(^{3}H\)]methylguanine moiety in the DNA substrate (20 Ci/mmol); (b) counting efficiency equal to 14%; (c) the requirement that extract from at least 7.7 \times 10^6 cells be included in the assay; and (d) the requirement that extract from 7.7 \times 10^6 cells yield <12 cpm (86 dpm) above an unincubated control that displays 25–40 cpm when counted for 10 min. On average, the Mer− tumor and normal brain samples reported here yielded 2.2 ± 4.0 cpm (mean ± SD) above the unincubated control; these counts were sporadic.

Statistical Analysis. Standard statistical procedures (25) were applied using Microsoft Excel (Microsoft, Redmond, WA). Comparison of means was by Student’s t test assuming unequal variances. Relationships and trends between continuous

### Table 1  Tumor and patient characteristics

<table>
<thead>
<tr>
<th>Diagnosisa</th>
<th>All tumors</th>
<th>Newly diagnosed</th>
<th>Recurrentb</th>
</tr>
</thead>
<tbody>
<tr>
<td>All gliomas</td>
<td>66 (60%)</td>
<td>53 (56%)</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>35 (32%)</td>
<td>31 (33%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Anaplastic astrocytoma + glioblastoma</td>
<td>11 (10%)</td>
<td>6 (6%)</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>17 (15%)</td>
<td>14 (15%)</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>All nonglial tumors</td>
<td>44 (40%)</td>
<td>42 (44%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Medulloblastoma/PNET</td>
<td>29 (26%)</td>
<td>27 (28%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Ganglioglioma/DNT</td>
<td>12 (11%)</td>
<td>12 (13%)</td>
<td>0</td>
</tr>
<tr>
<td>Neuroepithelial tumors</td>
<td>3 (3%)</td>
<td>3 (3%)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Six after surgery alone, four after surgery and radiation therapy, three after surgery and chemotherapy, and two after surgery, radiotherapy, and chemotherapy.

b World Health Organization classification (23).
c Three anaplastic astrocytomas and eight glioblastoma multiformes.
d Neuroepithelial tumors include ganglioglioma, gliomatosis cerebri, and spongioblastoma.
e Includes pilocytic astrocytoma, low-grade astrocytoma, astroblastoma, gliomatosis cerebri, and spongioblastoma.

\( ^{3}H\)
variables were assessed by regression analysis. Results are reported as two tailed \( P \)s. Statistically significant relationships were determined at the 95\% confidence level. Because Mer\(^-\) samples may have an activity between 0 and 0.25 fmol/10\(^6\) cells, an activity of 0.125 fmol/10\(^6\) cells, i.e., one-half the lower limit of detection, was assigned to these specimens for purposes of calculation. An exception was the estimation of elevation or depression in tumor/normal pairs containing a Mer\(^-\) specimen; in these cases, the limit of detection, i.e., 0.25 fmol/10\(^6\) cells, was used to yield the most conservative estimate of the fold change.

**RESULTS**

**Patient and Tumor Characteristics.** Primary brain tumors were obtained from 60 male and 50 female patients, ranging from 0.5 to 17 years of age (mean ± SD, 7.5 ± 4.5 years). Tumors were classified by histology (Table 1) using the revised World Health Organization criteria (23). Sixty-six (60\%) were gliomas, the most frequent CNS tumor in children (2, 3). This group included 35 astrocytomas, 3 anaplastic astrocytomas, 8 glioblastomas, 17 ependymomas (including 3 with high-grade, anaplastic features), and 3 oligodendrogliomas. The 44 remaining tumors were composed of 29 embryonal tumors (27 medulloblastomas and 2 PNETs), 12 mixed neuronal-glial tumors (8 gangliogliomas and 4 DNTs), and 3 neuroepithelial tumors of uncertain origin. The number of supratentorial (i.e., cerebral) and infratentorial (i.e., cerebellar and brain stem) tumors did not differ appreciably (53 versus 57). High-grade diagnoses (i.e., anaplastic astrocytoma, glioblastoma, medulloblastoma/PNET, anaplastic ependymoma, and 2 neuroepithelial tumors) constituted a minority of tumors (45 of 110, i.e., 41\%). The distribution of our tumor sample by diagnosis, location, and grade was comparable with that reported previously (2, 3). As indicated in Table 1, 95 tumors were newly diagnosed and 15 were recurrent after previous therapy. Age, male:female ratio, and distribution of diagnoses were similar between newly operated and recurrent tumors. Histologically normal brain adjacent to tumor was obtained from 22 patients. These individuals were similar to the entire patient population in age (8.4 ± 5.3 years; range, 0.7 to 17 years) and distribution of diagnoses (Table 4).

**Variability of Tumor MGMT Activity.** As shown in Fig. 1, measurable MGMT activity ranged ~1500-fold from 0.34 to 498 fmol/10\(^6\) cells (i.e., 205–300,000 molecules/cell). Variability >100-fold was observed for medulloblastoma/PNET (~1200-fold), anaplastic astrocytoma/glioblastoma (~530-fold), and ependymoma (~140-fold). These findings are in accord with the 300-fold range in MGMT content observed for adult gliomas (21, 22). Only 6 tumors (5.5\%) lacked detectable MGMT activity, i.e., exhibited Mer\(^-\) phenotype (<0.25 fmol/10\(^6\) cells or 151 molecules/cell). Mer\(^-\) was observed only for astrocytic gliomas and ganglioglioma/DNT (Table 2). There was no correlation between MGMT and apurinic/apyrimidinic endonuclease activity in 38 tumors (\( r = 0.01; t = -0.05; P > 0.95 \)), indicating that the wide range of MGMT levels was not due to degradation during extraction. In addition, additive amounts of activity were found for mixed extracts of high- and low-MGMT specimens, demonstrating that the heterogeneity of MGMT activity was not due to degradation of MGMT during assay.

The mean MGMT activity for all tumors was 25 ± 66 fmol/10\(^6\) cells and did not differ significantly between male and female patients (30 ± 77 versus 18 ± 50 fmol/10\(^6\) cells; \( P > 0.30 \)). Likewise, the fraction of Mer\(^-\) tumors did not differ by sex (5\% versus 6\%). Newly diagnosed and recurrent tumors did not differ in MGMT content (26 ± 69 versus 16 ± 45 fmol/10\(^6\) cells; \( P > 0.45 \)) or frequency of Mer\(^-\) specimens (5\% versus 7\%). MGMT activity also did not differ significantly between supratentorial and infratentorial tumors (18 ± 44 versus 31 ± 82 fmol/10\(^6\) cells; \( P > 0.25 \)).

**MGMT Activity in High- versus Low-Grade Tumors.** Mean activity varied 10-fold among diagnoses from 53 ± 80 fmol/10\(^6\) cells for anaplastic astrocytoma/glioblastoma to 4.6 ± 6.1 fmol/10\(^6\) cells for astrocytoma (Fig. 1; Table 2). The low MGMT activity of astrocytoma and ganglioglioma/DNT was due, in part, to the relatively high frequency of Mer\(^-\) specimens in these groups (Table 2).

MGMT content was associated with degree of malignancy
MGMT in Pediatric Brain Tumors

Table 2  MGMT activity and frequency of Mer<sup>−</sup> phenotype

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean MGMT&lt;sup&gt;c&lt;/sup&gt; (fmol/10&lt;sup&gt;6&lt;/sup&gt; cells)</th>
<th>Mer&lt;sup&gt;−&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors</td>
<td>110</td>
<td>25 ± 66&lt;sup&gt;a&lt;/sup&gt; (0.34–498)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5 (6/110)</td>
</tr>
<tr>
<td>By diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All gliomas</td>
<td>66</td>
<td>19 ± 45 (0.38–199)</td>
<td>6.1 (4/66)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>35</td>
<td>4.6 ± 6.1 (0.34–26)</td>
<td>8.6 (3/35)</td>
</tr>
<tr>
<td>Anaplastic astrocytoma + glioblastoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>53 ± 80 (0.38–199)</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>17</td>
<td>28 ± 54 (1.3–181)</td>
<td>0</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>3</td>
<td>11 ± 5.3 (4.9–15)</td>
<td>0</td>
</tr>
<tr>
<td>All nonglial tumors</td>
<td>44</td>
<td>33 ± 89 (0.41–498)</td>
<td>4.5 (2/44)</td>
</tr>
<tr>
<td>Medulloblastoma/PNET</td>
<td>29</td>
<td>45 ± 108 (0.41–498)</td>
<td>0</td>
</tr>
<tr>
<td>Gangglioglioma/DNT</td>
<td>12</td>
<td>8.2 ± 17 (0.78–56)</td>
<td>17 (2/12)</td>
</tr>
<tr>
<td>Neuroepithelial tumors&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3</td>
<td>24 ± 22 (6.8–48)</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD.
<sup>b</sup> Range of detectable activity.
<sup>c</sup> Three anaplastic astrocytomas and eight glioblastoma multiformes.
<sup>d</sup> Astroblastoma, gliomatosis cerebri, and spongioblastoma.

as evidenced by a 4-fold difference in activity between high- and low-grade tumors (44 ± 95 versus 11 ± 30 fmol/10<sup>6</sup> cells; t = -2.24, P = 0.03). This trend was observed for both glial (44 ± 73 versus 13 ± 32 fmol/10<sup>6</sup> cells; P = 0.13) and nonglial tumors (44 ± 104 versus 8.1 ± 16 fmol/10<sup>6</sup> cells; P = 0.07). In contrast, we observed no difference of MGMT activity by grade among 152 adult gliomas (21). The greater activity of high-grade tumors reflected a 3-fold lower frequency (2.2% versus 7.7%) of Mer<sup>−</sup> phenotype and a 5-fold greater fraction (20% versus 4.6%) of specimens with activity >50 fmol/10<sup>6</sup> cells (i.e., >30,100 molecules/cell).

Although MGMT activity differs significantly by grade, there is considerable overlap between high- and low-grade diagnoses (Fig. 1). This is most noticeable for anaplastic astrocytoma/glioblastoma and medulloblastoma/PNET, of which 32% (13 of 41) have an activity that is at least an order of magnitude lower, i.e., = 1.1 fmol/10<sup>6</sup> cells or 660 molecules/cell, than the mean for low-grade tumors. Conceivably, these malignant tumors may be more responsive to alkylating agent-based chemotherapies.

Among 69 unselected tumors, the fraction of S-phase cells, determined by flow cytometry, ranged from 0 to 16%. In accord with previous observations (26), the mean S-phase fraction of 34 high-grade tumors was 2.3-fold greater than that of 35 low-grade tumors (4.8 ± 3.8% versus 2.1 ± 2.5%; t = 3.46; P = 0.01). However, regression analysis revealed no correlation between MGMT content and S-phase fraction (r = 0.069, P > 0.55), indicating that proliferation is not a major determinant of activity.

**Age Dependence of MGMT.** Tumors from children 3–12 years old have 5-fold greater MGMT activity than infants 0.5–3 years old (middle) is 5-fold higher than in infants 0.5–3 years old (left) and adolescents 12–17 years old (right).

**MGMT in Adjacent Histologically Normal Brain.** The mean MGMT activity of 22 specimens of histologically normal, subcortical cerebrum adjacent to tumor was 2.4 ± 4.0 fmol/10<sup>6</sup> cells (i.e., 1450 ± 2410 molecules/cell). Detectable activity ranged 72-fold from 0.25 to 18 fmol/10<sup>6</sup> cells (i.e., ~150–

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*Fig. 2 MGMT activity as a function of age. Mean MGMT activity (large crosses) in tumors from children 3–12 years old (middle) is 5-fold higher than in infants 0.5–3 years old (left) and adolescents 12–17 years old (right).*
10,900 molecules/cell); 6 specimens (27%) were Mer−. Mean and range of activity and Mer− frequency are in accord with our earlier analysis of 19 pediatric patients, which included 13 of the specimens reported here (24). Diagnosis of adjacent tumor and prior therapy had no significant effect on activity or on Mer− frequency (Table 4). In addition, activity did not differ between infants, children and adolescents (Table 3).

**Comparison of MGMT in Tumor and Adjacent Normal Brain.** The mean activity of 22 tumors adjacent to histologically normal brain (see above) was 8.8-fold higher than in the normal tissue (21 ± 44 versus 2.4 ± 4.0 fmol/10^6 cells; t = −2.08; P = 0.05), a trend observed for glial and nonglial tumors, and for newly operated and previously treated tumors (Table 4). The difference in MGMT activity between tumor and adjacent normal brain reflected, in part, a 2-fold higher frequency of Mer− normal tissue. The effects of tumorigenesis on MGMT activity were further characterized by pairwise analysis. In a majority of pairs (15 of 22, 68%), tumor activity was higher than in adjacent normal brain (31 ± 50 versus 2.4 ± 2.7 fmol/10^6 cells; t = 2.18; P = 0.05). As shown in Fig. 3, the elevation varied widely, ranging from 2- to nearly 600-fold, the average being 35-fold; notably, four normal brain specimens were Mer−, suggesting that acquisition of Mer+ phenotype occurred during tumorigenesis. The elevation of tumor activity occurred in a majority of both glial and nonglial tumors (Fig. 3). In another four pairs (18%), tumor activity was 1.7- to at least 17-fold lower than in normal brain, including one pair in which Mer+ normal brain accompanied Mer− tumor. For the remaining three pairs, tumor and normal activity did not differ; this group included two pairs of Mer− tumor and normal tissue, indicating that the Mer− phenotype was conserved during transformation. The analysis of paired specimens suggests that the progenitor tissue of pediatric primary brain tumors is predominantly Mer−, as opposed to the predominantly Mer+ progenitor tissue of adult gliomas (20–22, 24), and that neurocarcinogenesis in the pediatric population is frequently accompanied by elevation of MGMT activity.

**DISCUSSION**

Alkylating agent-based chemotherapy after surgical resection alone or resection and radiotherapy produces clinically documented increases in survival for pediatric gliomas and medulloblastoma (7–9). Chemotherapy is of particular impor-
tance in the adjuvant therapy of infants and young children who are normally spared radiotherapy because of long-term deleterious effects of radiation on physical and mental development (27). The efficacy of chemotherapy, however, is limited, in part by tumor resistance to DNA damaging agents. The poor prognosis for malignant pediatric CNS tumors emphasizes the necessity to characterize DNA repair-mediated resistance mechanisms to develop strategies to overcome resistance to alkylators of proven clinical benefit. MGMT protects human tumor cells (reviewed in Ref. 15), including those derived from pediatric brain tumors (10–14), against the lethality of clinically used alkylating agents. The function of MGMT in limiting alkylation cytotoxicity suggests a role in the resistance of pediatric brain tumors to alkylating agent therapy. We initiated this study to provide information, currently lacking, concerning MGMT activity in pediatric CNS tumors and normal brain, and to examine the relationship of MGMT activity to clinically relevant tumor characteristics.

By using a sensitive biochemical assay, we measured MGMT activity in 110 pediatric brain tumors of different diagnoses and grades. Activity varied ca. 1500-fold (Fig. 1) and was significantly greater in high-grade than in low-grade tumors of all diagnostic types. Hongeng et al. (20) also observed greater MGMT activity in high-grade tumors in a survey of 60 pediatric CNS neoplasms. The association of MGMT with tumor grade suggests a relationship between activity and some characteristic(s) of malignant brain tumors, such as greater growth rate. However, we observed no correlation between MGMT level and proliferative rate, measured as S-phase fraction, indicating that proliferation is not a major determinant of MGMT activity. Alternatively, MGMT content could reflect generation of endogenous DNA damage. The elevated rates of oxidative glycolysis (28) and of nitric oxide synthase activity characteristic of higher grade tumors (29) may increase levels of putative endogenous alkylators such as lipid peroxidation products (30) and nitrosated peptides and amino acids (31, 32), thus creating a selective pressure for increased MGMT activity. Enhanced endogenous alkylation and the consequently greater selection pressure could also be responsible for the relatively low frequency of Mer− tumors in pediatric compared with adult primary brain tumors [6% versus 24% (22)].

Our analysis revealed that MGMT activity is age dependent in pediatric brain tumors. The mean activity in tumors from children between 3 and 12 years old was significantly higher than in infants and adolescents (Table 3). Notably, this trend was observed regardless of tumor characteristics (e.g., diagnostic type and grade) and for both sexes (Table 3), suggesting that the difference in MGMT activity between the age groups is not a reflection of these tumor or patient characteristics. Conceivably, the age dependence of MGMT reflects processes associated with the physical and functional maturation of the CNS during childhood.

Comparison of MGMT activity in paired tumor and adjacent normal brain emphasizes that elevation of MGMT activity is a hallmark of pediatric neurocarcinogenesis. Elevation was observed in two-thirds of cases and frequently encompassed conversion from Mer− to Mer+ phenotype. These observations are consistent with the epigenetic regulation of MGMT observed in human tumors (33) and cultured cells (34–36). We also observed an elevation of MGMT activity in a majority of adult glioma/normal brain pairs (21, 22), suggesting that selective pressure for increased MGMT activity may prevail during human neurocarcinogenesis, perhaps driven by endogenous alkylation (31, 32). The elevated MGMT activity in human primary brain tumors likely reflects the need in proliferating cells for greater capacity to repair O6-alkylguanine adducts before replication.

A clinically significant consequence of the elevated MGMT activity accompanying pediatric CNS tumorigenesis may be enhanced resistance to alkylating agent-based chemotherapy. This possibility is underscored by the magnitude of the elevation, which was >5-fold in 45% of cases and >10-fold in 27% of cases. It may be significant in this regard that some (37, 38) but not all (22) studies have suggested that MGMT content may be predictive of the response of adult high-grade gliomas to chloroethlylating agent-based chemotherapy. Therefore, our findings suggest that substrate analogue inhibitors that ablate MGMT activity such as O6-benzylguanine (39) may improve response of pediatric brain tumors to clinically relevant alkylators by rendering tumor cells functionally Mer−. As noted earlier, our analysis of MGMT activity in adult gliomas indicates that Mer− cells are preferentially killed by alkylating agents (22). Ultimately, determination of the significance of MGMT for the response of malignant pediatric brain tumors to alkylating agents will rest on examination of the relationship between activity and clinical outcome. The wide range of activity observed in malignant pediatric CNS tumors, including a substantial minority with relatively low MGMT content, should facilitate this analysis.

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REFERENCES


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