The Proliferative Potential of Human Ependymomas Measured by In Situ Bromodeoxyuridine Labeling

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Twelve patients with ependymomas received a 30- to 60-minute intravenous infusion of bromodeoxyuridine (BrdU), 150 to 200 mg/m² at surgery, to label tumor cells in the DNA synthesis phase. Labeled cells were detected in excised tumor specimens by indirect immunoperoxidase staining using anti-BrdU monoclonal antibody as the first antibody. The BrdU labeling index (LI, defined as the percentage of labeled cells in relation to the total number of cells scored) was calculated for each specimen. All four spinal cord ependymomas had a BrdU LI of less than 1%, which is consistent with our clinical experience that most such tumors grow slowly and have an excellent prognosis. Five of the eight intracranial ependymomas also had a low BrdU LI of approximately 1% or less, and three had a BrdU LI of 3.2%, 3.4%, and 4.8%. The latter three tumors, only one of which was diagnosed as a malignant ependymoma at the time of study, were either recurrent or recurred within 2 years after gross or subtotal removal. Cytologic analysis of cerebrospinal fluid (CSF) was performed in five cases; CSF seeding of tumor cells was found in only one patient, who had a malignant ependymoma. A high BrdU LI did not always correlate with CSF seeding. Measurement of the LI using BrdU and anti-BrdU monoclonal antibodies can provide more accurate information on the proliferative potential of individual tumors and may lead to a more rational grading system of ependymomas. The results of such studies do not always predict the potential for CSF seeding.


Ependymomas grow slowly, are well-demarcated from surrounding tissue, and are generally considered to have a low malignant potential. Nevertheless, the prognosis of patients with these tumors, especially intracranial ependymomas, has been relatively poor. Although adjuvant radiation therapy has considerably increased the survival time over the past two decades, ependymomas are still one of the life-threatening tumors of the central nervous system (CNS).1-12

The significance of histopathologic grading in predicting the prognosis of patients with ependymomas has long been controversial. Some authors have reported a good correlation between the histologic grade and survival,1,3,6,8,14,15 whereas others have found no correlation.2,4,7,9,12,13 Another unresolved question is whether prophylactic irradiation of the entire craniospinal axis is necessary in patients with ependymomas; the actual incidence and clinical significance of spread of tumor cells in the cerebrospinal fluid (CSF) is not clear.10,11,14,16

Since 1984, we have been investigating the proliferative potential of various CNS tumors in situ using bromodeoxyuridine (BrdU), a thymidine analogue, and anti-BrdU monoclonal antibody. To better understand the biologic behavior of ependymomas, to elucidate some of their clinical ambiguities, and to evaluate whether such study will help to predict malignant growth or possible recurrence, we analyzed the results of cell kinetic studies in 12 patients with ependymomas.

Materials and Methods

Permission to administer BrdU was received from the Human Experimentation Committee at the University of California, San Francisco, and from the National Cancer Institute. Informed consent was obtained from each patient or a responsible relative.
TABLE I. Clinical Features and BrdU Labeling Indices in 12 Patients With Ependymomas

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Tumor location</th>
<th>Extent of resection</th>
<th>Primary or recurrent</th>
<th>Duration of signs and symptoms</th>
<th>Histologic condition</th>
<th>CSF* seeding</th>
<th>BrdU LI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>M</td>
<td>C6-T2</td>
<td>Gross total</td>
<td>P</td>
<td>4 yr</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>M</td>
<td>C6-T2</td>
<td>Partial</td>
<td>P</td>
<td>8 yr</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>M</td>
<td>C2-C7</td>
<td>Gross total</td>
<td>R</td>
<td>3 yr</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>M</td>
<td>C1-C4</td>
<td>Partial</td>
<td>P</td>
<td>6 yr</td>
<td>Subependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>F</td>
<td>Lateral ventricle</td>
<td>Subtotal</td>
<td>P</td>
<td>None</td>
<td>Subependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>F</td>
<td>IIIrd ventricle</td>
<td>Partial</td>
<td>P</td>
<td>3 wk</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>M</td>
<td>IVth ventricle</td>
<td>Subtotal</td>
<td>P</td>
<td>2 mo</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>F</td>
<td>IVth ventricle</td>
<td>Subtotal</td>
<td>P</td>
<td>1 mo</td>
<td>Ependymoma</td>
<td></td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>F</td>
<td>IVth ventricle</td>
<td>Gross total</td>
<td>R</td>
<td>2 mo</td>
<td>Ependymoma</td>
<td>1.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>M</td>
<td>IVth ventricle</td>
<td>Subtotal</td>
<td>R</td>
<td>8 mo</td>
<td>Ependymoma</td>
<td>3.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>F</td>
<td>IVth ventricle</td>
<td>Gross total</td>
<td>P†</td>
<td>3 wk</td>
<td>Malignant ependymoma</td>
<td>*</td>
<td>3.4 ± 1.6</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>M</td>
<td>IVth ventricle</td>
<td>Gross total</td>
<td>P‡</td>
<td>1 mo</td>
<td>Ependymoma</td>
<td></td>
<td>4.8 ± 1.4</td>
</tr>
</tbody>
</table>

* Confirmed by CSF cytology with or without myelography.
† Local recurrence 16 months after surgery.
‡ Local recurrence 3 months after surgery.

The histologic diagnosis was confirmed by examination of the tumor section adjacent to the section studied immunohistochemically. The tumors were categorized morphologically as ependymomas or malignant ependymomas. Subependymomas were included as a variant of ependymomas because of the close histologic relationship between these two types of tumors.

Nine ependymomas, one malignant ependymoma, and two subependymomas were analyzed. All patients received a 30- to 60-minute intravenous infusion of BrdU, 200 mg/m² in 100 ml of saline solution, shortly after induction of anesthesia but before biopsy of the tumor. Excised tumor specimens were fixed in 70% ethanol, embedded in paraffin, and cut into sections 6 μm thick. The slides were deparaffinized, immersed for 30 minutes in methanol containing 0.3% H₂O₂ to block endogenous peroxidase activity, and rinsed in distilled water.

Immunohistochemistry

The immunohistochemical staining technique used to detect BrdU-labeled cells has been described in detail elsewhere. Briefly, the sections were denatured with 2N HCl, immersed in purified anti-BrdU monoclonal antibodies (Becton Dickinson, Mountain View, CA), and then reacted with peroxidase-conjugated anti-mouse rabbit IgG antibodies (Zymed, South San Francisco, CA). The slides were developed for 10 to 15 minutes in diaminobenzidine tetrahydrochloride and H₂O₂ in Tris buffer and lightly counterstained with Gill No. 1 hematoxylin. Nuclei that incorporated BrdU were identified by the presence of a brownish reaction product.

Measurement of BrdU Labeling Index

The BrdU labeling index (LI) was calculated from each slide as the percentage of BrdU-labeled nuclei among total nuclei scored, excluding vascular components and hematogenous cells. Several viable areas that had a fairly even distribution of labeled cells were selected for examination; necrotic areas were avoided so as to minimize the effect of restricted delivery of BrdU. Two hundred to 600 tumor cells from each area and over 1000 cells in each specimen were evaluated.

Results

The clinical data on the 12 patients with CNS ependymomas and the BrdU LI of their tumors are summarized in Table 1. Four patients had spinal cord tumors; the mean age was 55 years. Histologically, three of the tumors were ependymomas and one was a subependymoma. All four spinal cord tumors had a BrdU LI of less than 1%.

Eight patients had intracranial ependymomas; the mean age was 9.6 years (P < 0.01 versus patients with spinal tumors). Six of the tumors were located in the fourth ventricle. Histologically, only one of the eight tumors was diagnosed as a malignant ependymoma. The malignant ependymoma had a fairly high BrdU LI of 3.4% (Fig. 1). Two of the seven ependymomas that showed no signs of anaplasia or malignant characteristics (e.g., nuclear pleomorphism, frequent mitosis, or necrotic foci) had high BrdU LI of 3.2% (Patient 9) and 4.8% (Patient 12) (Fig. 2).

During a follow-up period of 24 months, two patients had a tumor recurrence. Patient 11, whose tumor had a
BrdU LI of 3.4% (Fig. 1), had a recurrence 16 months after gross total removal. Patient 12, whose tumor had a BrdU LI of 4.8%, despite the lack of anaplastic features, had a recurrence 3 months after gross total removal. Patients 3 and 10 had recurrent ependymomas at the time of BrdU study. The intervals between the initial gross total resection and tumor recurrence were 3 years and 8 months, respectively.

Cytologic examination of cerebrospinal fluid (CSF) was performed in five cases. The results were positive in only one patient (Patient 11), who had a malignant ependymoma. This patient had no signs of disseminated lesions at the time of the study but later developed tumors in the spinal canal and in the supratentorial compartment. Negative results were obtained in Patient 12, whose tumor had the highest BrdU LI (4.8% ± 1.4%).
FIGS. 2A AND 2B. Tumor sections from a patient with ependymoma (Patient 12). (A) Section stained with hematoxylin and eosin shows characteristic pseudorosettes and fairly uniform nuclei without mitotic figures (×380). (B) Section stained using the immunoperoxidase method for BrdU shows more BrdU-positive nuclei than are found in other ependymomas with typical histologic features (×380).

in this series. A high BrdU LI did not always correlate with the presence of tumor cells in the CSF.

Discussion

Three of eight intracranial ependymomas in our study had a fairly high BrdU LI. Only one of the three tumors (BrdU LI, 3.4%) had histopathologically malignant features; it recurred 16 months after gross total resection. One tumor with a BrdU LI of 4.8% recurred 3 months after gross total resection despite the absence of histopathologically malignant features. The third tumor (BrdU LI, 3.2%) was a recurrent tumor that had taken 8 months to recur after the initial gross total removal. Although it is difficult to estimate the actual growth rate of a tumor from the LI alone, our results indicate that
tumors with high LI, irrespective of their histologic features, grow faster and are clinically more aggressive than tumors with low LI and have a strong tendency to recur.

The spinal cord ependymomas had a very low BrdU LI (<1%), which coincides with the observations of most authors that these tumors show minimal aggressive behavior and are usually associated with an excellent prognosis.\(^{3,5,9,11}\) In our previous studies, all slow-growing tumors, including pituitary adenomas, meningiomas, and neurinomas, and the majority of low-grade gliomas had a BrdU LI of less than 1%.\(^{19-21}\)

Subependymomas are histologically closely related to ependymomas and generally regarded as a variant.\(^{22}\) The two subependymomas in our study had a low LI of less than 1%, which is in agreement with the hypothesis that subependymomas are among the most benign glial tumors. Most subependymomas are found incidentally at autopsy.\(^{22}\)

Although ependymomas are grossly demarcated from the surrounding normal tissue, complete surgical removal of these tumors, especially those in the fourth ventricle, is difficult because of their anatomic relationship to vital important areas. To predict the prognosis and to design the appropriate treatment, it is essential to better understand the biologic activity and to estimate the regrowth rate of residual tumors. Our results indicate that cell kinetics studies with BrdU and anti-BrdU monoclonal antibodies can be used to predict the growth potential of ependymomas and their potential for recurrence more accurately than is possible from the results of histopathologic examination.

Ependymomas, like medulloblastomas, tend to spread in the subarachnoid space. The reported incidence of CSF seeding of ependymomas has varied from 0%\(^{23,24}\) to 64%,\(^{11}\) possibly because of the variety of diagnostic procedures used, including clinical or radiological assessment, CSF cytology, and autopsy. Therefore, in patients with ependymomas, the indication for prophylactic craniospinal irradiation after surgery remains open to discussion.\(^{10,11,14,16}\) In our study, only one of five patients with intracranial ependymomas in whom both cytologic studies of the CSF and myelography were performed were found to have tumor cells in the CSF; no other tumor was found. This patient had a histopathologically malignant tumor with a high BrdU LI (3.4%) and later developed metastatic tumors in the spinal canal and supratentorial compartment. The four other tumors had no malignant features, although a few had a high BrdU LI. These results suggest that the potential for CSF seeding of ependymomas does not necessarily correlate with the proliferative potential measured by the BrdU LI; a positive CSF cytology appears to be more predictive for the development of disseminated tumors.

BrdU labeling studies have provided a considerable amount of information on cell kinetics and biology of various CNS tumors.\(^{19-21,25,26}\) These data supplement the histopathologic diagnosis, thereby allowing more accurate prediction of prognosis and aiding in the design of treatment for individual patients. BrdU has been used as a radiosensitizing agent for more than 20 years without causing serious side effects.\(^{27-29}\) Although teratogenic and mutagenic effects could be induced with high doses or prolonged administration of BrdU,\(^{30}\) the doses required for in vivo labeling studies (150 to 200 mg/m\(^2\)) are small compared with the doses used therapeutically as a radiosensitizing agent.\(^{31,32}\) This has resulted in a very low serum concentration that experimentally has shown minimal induction of mutation and no increase in the sister chromatid exchange rate, which may relate to mutagenicity.\(^{17,33-36}\) Thus, BrdU labeling studies can be applied with fewer restrictions to elucidate the complex proliferation kinetics of human tumors in situ and to better understand their malignant potential.

REFERENCES


