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Predicting the recurrence of ependymomas from the bromodeoxyuridine labeling index

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Abstract. The usefulness of histopathological grading in predicting the prognosis of patients with ependymomas is controversial. To clarify the discrepancy between the histological malignancy and the prognosis of these tumors, we estimated the proliferative potential of 32 intracranial and intraspinal ependymomas and correlated the findings with the clinical behavior. Each patient received an intraoperative infusion of bromodeoxyuridine (BUdR, 200 mg/m^2 i.v.) before tumor removal; the BUdR labeling index (LI), or percentage of BUdR-labeled cells, was determined immunohistochemically in excised specimens. The mean BUdR LI ($\pm$ SD) of intracranial malignant ependymomas was 4.1 $\pm$ 2.8%. Nonmalignant intracranial and intraspinal ependymomas and subependymomas had mean LIs of 1.5 $\pm$ 0.9%, 1.1 $\pm$ 0.3%, and <1%, respectively. Overall, 44% of the tumors recurred. There were no statistically significant differences in the recurrence rates of intracranial and intraspinal ependymomas, including subependymomas (43% and 44%, respectively), or of intracranial ependymomas with LIs greater than 1.0% and less than 1.0% (67% and 44%, respectively). However, the early recurrence rate (within 24 months after treatment) of tumors with LIs greater than 1.0% was higher than that of tumors with LIs of less than 1.0% (100% vs. 25%, $P < 0.05$). The BUdR LI also showed a statistically significant inverse correlation with the time to recurrence. These findings indicate that BUdR LI reflects the proliferative potential of individual ependymomas and can be used to help predict the recurrence and estimate the prognosis of these tumors.

Key words: Brain tumor – Ependymoma – Bromodeoxyuridine – Recurrence – Proliferative potential – Labeling index

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The usefulness of histopathological grading in estimating the prognosis of patients with ependymomas is controversial [1, 5, 21]. Morphological characteristics, such as cellularity, mitotic activity, degree of anaplasia, focal necrosis, and vascular endothelial proliferation, have been used as histological criteria for diagnosing malignant ependymomas. The prognosis of malignant ependymomas is generally poorer than that of nonmalignant ependymomas [1, 5] but, as first pointed out by Kricheff et al. [12], there are exceptions. The prognosis of malignant ependymomas is highly variable [21]. To clarify the discrepancy between the histological malignancy and the prognosis of these tumors, the proliferative potential of 32 ependymomas was estimated from the bromodeoxyuridine (BUdR) labeling index (LI) [9] and correlated with the clinical behavior.

Material and methods

Patients

Thirty-two consecutive patients with intracranial or intraspinal ependymomas treated between 1984 and 1990 at the University of California, San Francisco (UCSF), were entered into the study. Permission to administer BUdR was received from the Human Experimentation Committee at UCSF and from the National Cancer Institute. Informed consent was obtained from each patient or a responsible relative.

There were 18 males and 14 females, aged 9 months to 75 years. At the time of diagnosis, 12 patients (38%) were less than 16 years old and eight (25%) were less than 2 years old. Most of the tumors were located in the IV ventricle or in the spinal cord.

Histology and immunohistochemistry

Each patient received a 30-min intraoperative infusion of BUdR (200 mg/m^2 i.v.) before tumor removal. Excised tumor specimens were immediately placed in chilled 70% ethanol and fixed for at least 12 h.

The fixed specimens were embedded in paraffin and cut into 5-μm sections. Serial sections were stained with hematoxylin-eosin and with immunohistochemical stains for BUdR. Tissue specimens

were reviewed by one of us (RLD) to confirm the diagnosis of nonmalignant or malignant ependymoma (anaplastic ependymoma) as defined by the World Health Organization [20].

The immunohistochemical staining procedure to detect BUdR-labeled cells has been described in detail [16, 17]. Briefly, the sections were deparaffinized, immersed for 30 min in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidase activity, and denatured with 4*N* hydrochloric acid. The tissue sections were incubated with anti-BUdR monoclonal antibody (Caltag, South San Francisco, Calif., USA) and reacted with peroxidase-conjugated anti-mouse rabbit immunoglobulin antibody (DAKO Corporation, Santa Barbara, Calif., USA). The slides were developed for 5 min in diaminobenzidine tetrahydrochloride and hydrogen peroxide in TRIS buffer and lightly counterstained with Gill No. 1 hematoxylin.

The BUdR LI was calculated as the percentage of BUdR-labeled cells in several viable areas that had an even distribution of labeled cells. Vascular components and hematogenous cells were excluded. Over 1000 cells in each specimen were evaluated.

Treatment

All patients were treated surgically. The extent of resection was total in seven (22%) of 32 cases, subtotal in 17 (53%), and partial in four (12.5%); four patients (12.5%) had a biopsy only. A cerebrospinal fluid (CSF) diversionary shunt was performed in 13 patients (41%).

Twenty-four patients (75%) received postoperative radiation therapy, which was delivered regionally in 20 (83%) cases, to the whole brain in two (8%), and to the entire neuraxis in two (8%). The total dose ranged from 45 Gy to 72 Gy. Seven patients with primary tumors and five with recurrent tumors received chemotherapy.

Statistical analysis

Statistical analysis was performed using StatView 512 + (Brain Power Inc., Calabasas, Calif., USA). Logarithmic interpolation was applied to the correlation between BUdR LI and time to recurrence.

Results

The clinical, surgical, and histopathological findings and the results of BUdR labeling studies are summarized in Tables 1–4.

Histology and labeling index

Among the 23 intracranial tumors, seven (78%) of nine malignant ependymomas had BUdR LIs of more than 1% (mean 5.0 ± 2.5%), whereas only two (22%) of nine nonmalignant ependymomas had LIs above 1% (mean 3.1% ± 0.1%). All five intracranial subependymomas

Table 1. BUdR labeling index (LI), tumor location, and outcome in 23 patients with intracranial ependymomas

Histological type	No. of cases	Outcome		LI (%)			Location (ventricle)		
		Alive	Dead	Mean ± SD	>1.0	<1.0	Lateral	III	IV
Malignant	9 (39%)	7	2	4.1 ± 2.8	7 (5.0 ± 2.5)	2	1	0	8
Nonmalignant	9 (39%)	8	1	1.5 ± 0.9	2 (3.1 ± 0.1)	7	2	2	5
Subependymoma	5 (22%)	4	1	<1.0	0	5	3	0	2
Total	23	19	4	2.4 ± 2.3	9 (4.5 ± 2.4)	14	6 (26%)	2 (9%)	15 (65%)

Table 2. Surgical procedures and recurrence in 23 patients with intracranial ependymomas

Histological type	Extent of resection				Overall recurrence						Early recurrence	
	Total	Subtotal	Partial	Biopsy	At study	After study	Total	Mean LI ± SD (%)	LI >1.0	LI <1.0	LI >1.0	LI <1.0
Malignant	0	8	1	0	4	2	6	5.2 ± 2.7	5	1	5	0
Nonmalignant	2	6	0	1	3	1	4	1.6 ± 1.0	1	3	1	1
Subependymoma	3	1	1	0	0	0	0	N/A	0	0	0	0
Total	5 (22%)	15 (65%)	2 (9%)	1 (4%)	7	3	10 (43%)	3.7 ± 2.9	6	4	6	1

Table 3. BUdR labeling index (LI) and surgical procedures in nine patients with intraspinal ependymomas. NA, Not applicable

Histology	No. of cases	LI (%)			Extent of resection			
		Mean ± SD	>1.0	<1.0	Total	Subtotal	Partial	Biopsy
Malignant	0	NA	0	0	0	0	0	0
Nonmalignant	8 (89%)	1.1 ± 0.3	1 (2.0%)	7	2	2	2	2
Subependymoma	1 (11%)	<1.0	0	1	0	0	0	1
Total	9	1.1 ± 0.3	1	8	2 (22%)	2 (22%)	2 (22%)	3 (34%)

Table 4. Recurrence in intraspinal ependymomas

Histology	Recurrence			
	At study	After study	Total	LI (%) (Mean ± SD)
Malignant	0	0	0	NA
Nonmalignant	4	0	4	<1.0
Subependymoma	0	0	0	NA
Total	4	0	4 (44%)	<1.0

had LIs below 1%. Among the nine intraspinal tumors, there were eight nonmalignant ependymomas, only one of which had an LI below 1% (mean 1.1 ± 0.3%), and one subependymoma with an LI below 1%.

Outcome and labeling index

The median follow-up period was 10 months (range 1–192 months). Twenty-eight patients (88%) are still alive. Three patients died of tumor recurrence. Two of these had BUdR LIs of 4.8% and 8.0%. The third, an 18-month-old child, presented in an acute coma and died after partial removal of a nonmalignant ependymoma with an LI of less than 1% that extensively involved the brainstem. One patient with a subependymoma died of acquired immunodeficiency syndrome.

Recurrence and LI

Fourteen (44%) of the 32 tumors recurred. The recurrence rates for intracranial and intraspinal ependymomas were 56% and 50%, respectively; none of the subependymomas recurred. The mean LI was higher in recurrent than in nonrecurrent tumors (3.0 ± 2.7% vs. 1.3 ± 0.6%, $P < 0.05$). The LIs of seven intracranial ependymomas that recurred locally were not significantly different from those of three that recurred locally and disseminated into CSF (4.0 ± 3.1% vs. 3.1 ± 1.6%). Among intracranial ependymomas, there was no difference in the LIs of nonmalignant tumors that recurred and those which did not, but among malignant tumors, the mean LI was higher in recurrent than in nonrecurrent tumors (5.2 ± 2.7% vs. 1.9 ± 0.7%; $P < 0.05$). There was no significant difference in the recurrence rates of malignant and nonmalignant ependymomas. Among intracranial ependymomas, six (67%) of nine tumors with LIs above 1% recurred, compared with only four (44%) of nine tumors with LIs below 1% (excluding subependymomas); the difference in recurrence rates was not statistically significant. Thus, the overall recurrence rate was reflected no more accurately by the BUdR LI than by the histological grade.

The BUdR LI did, however, predict early recurrence. All six recurrent intracranial ependymomas with LIs above 1% recurred within 24 months after treatment, compared with only one of four tumors with LIs below 1%. These two values for early recurrence rates were statistically different. Among intraspinal tumors, recurrences were seen 4–14 years after treatment. For both intracranial and intraspinal tumors, there was a good inverse correlation between the time to recurrence and the BUdR LI (Fig. 1).

Two cases in which the biological behavior correlated with the BUdR LI rather than with the histological diagnosis are presented below.

Case 1. This 9-year-old boy presented with nystagmus of 4 months' duration at the age of 18 months (1982). A computerized tomography (CT) scan showed an enhancing 2-cm mass adjacent to the IV ventricle. A craniotomy was performed, and about 70% of the tumor was removed. The histological diagnosis was malignant ependymoma. Radiation therapy was administered postoperatively (40 Gy to the whole brain, 48 Gy to the posterior fossa, and 25 Gy to the spine). The patient did well for almost 7 years, when he presented with headaches (1989). CT scans and magnetic resonance (MR) images showed a small, locally recurrent tumor. At surgery, BUdR was administered and the recurrent tumor was totally removed. Light microscopic examination of the resected tumor specimen showed moderate cellularity, moderate mitotic activity, moderate cellular and nuclear pleomorphism, and focal necrosis compatible with a di-

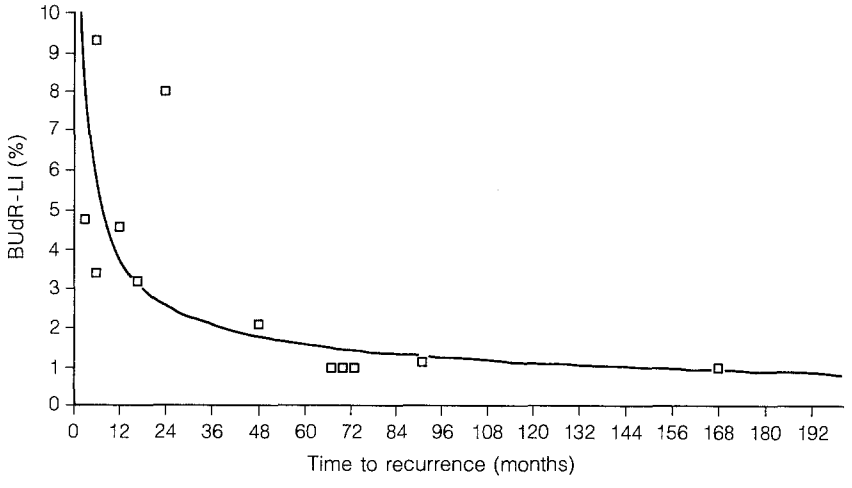
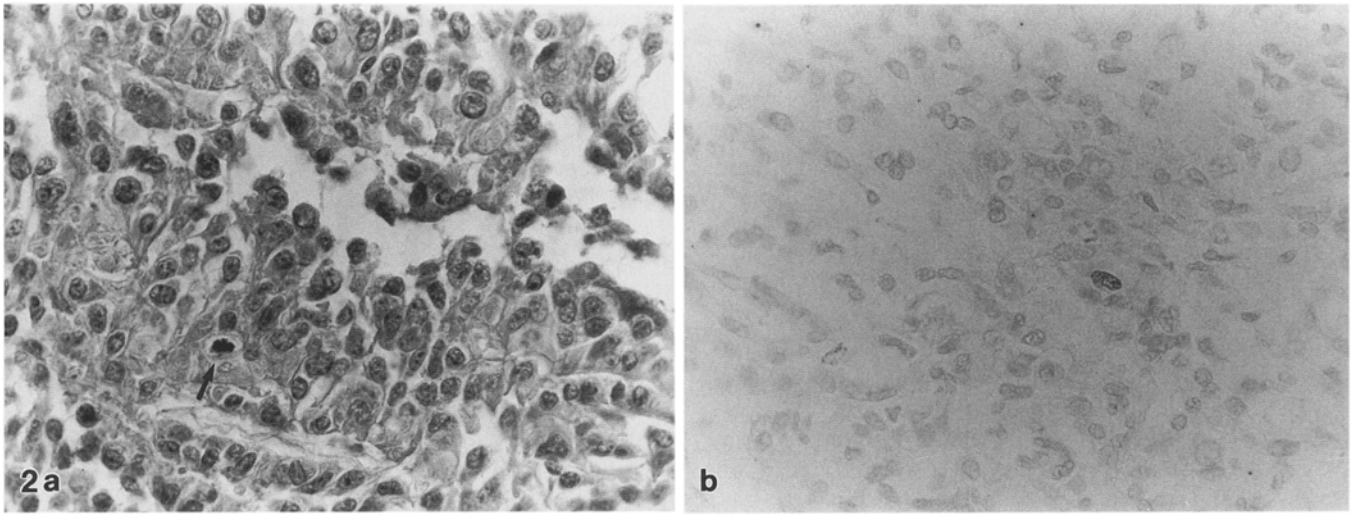


Fig. 1. The bromodeoxyuridine labeling index (BUdR LI) correlated inversely with the time to recurrence in both intracranial and intraspinal tumors ($y = 14.911x^{-0.55283}$; $R^2 = 0.685$)



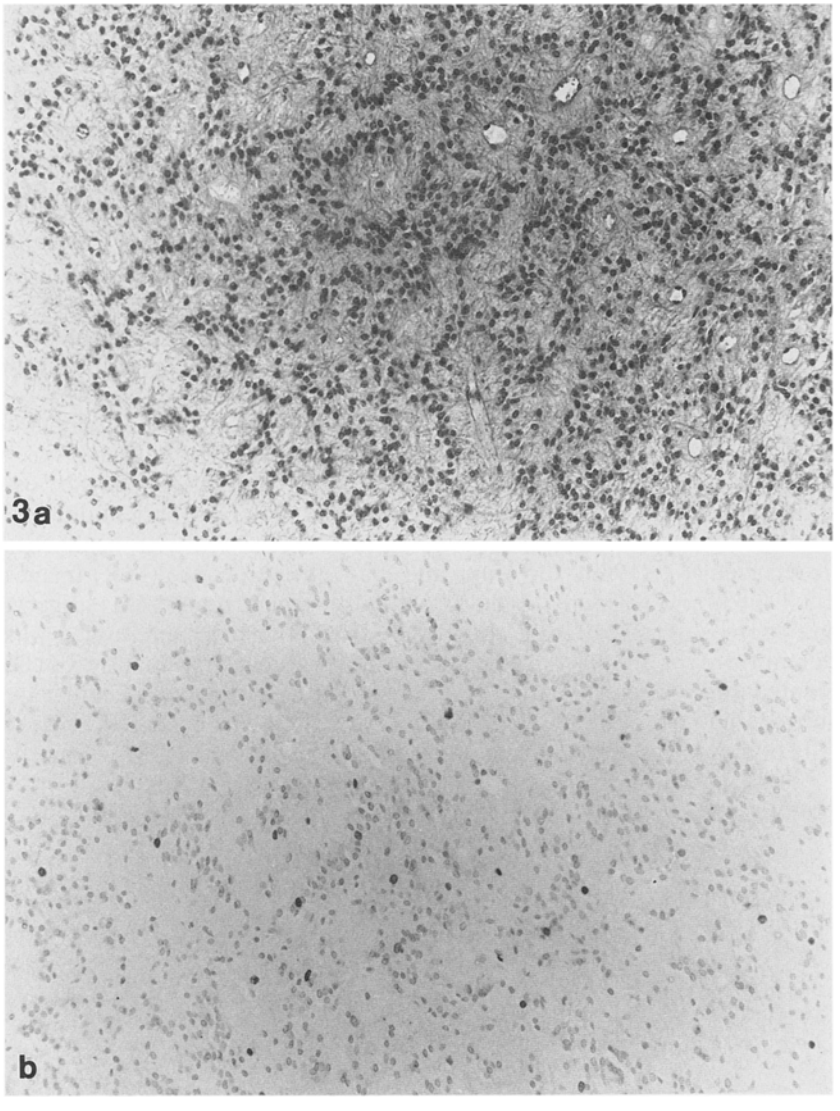


Fig. 2. **a** Photomicrograph of malignant ependymoma shows moderate cellular and nuclear pleomorphism, moderate cellularity, and moderate mitotic activity (*arrow*) compatible with malignant ependymoma. Hematoxylin-eosin; original magnification ×200. **b** Photomicrograph of an adjacent section showing few cells labeled with BUdR (LI <1%). Indirect immunoperoxidase stain; original magnification ×200

Fig. 3. **a** Photomicrograph of nonmalignant ependymoma showing less pleomorphic nuclei and cells, well-preserved perivascular pseudorosettes, and no mitotic activity. Hematoxylin-eosin; original magnification ×200. **b** Photomicrograph of an adjacent section showing many cells labeled with BUdR (LI 3.2%). Indirect immunoperoxidase stain; original magnification ×200

agnosis of malignant ependymoma (Fig. 2a), but the BUdR LI was < 1.0% (Fig. 2b).

Case 2. This 31-month-old girl presented with vomiting. Seventeen months earlier, a nonmalignant ependymoma had been totally resected at another hospital. CT scans and MR images showed a large IV ventricular mass, indicating local recurrence. A suboccipital craniectomy was performed, and 90% of the tumor was removed. The diagnosis of nonmalignant ependymoma was confirmed

(Fig. 3a), but the BUdR LI was 3.2% (Fig. 3b). The patient's parents refused to allow radiation therapy, and she died of tumor recurrence 9 months later.

Discussion

Primary spinal cord ependymomas have been reported to have an excellent prognosis, with 5-year survival rates of 70–100% [3, 4, 7, 8, 11]. In our series, all spinal cord tumors had LIs below 1%, with one exception (LI 2%), and appeared to have low proliferative potential. This result is compatible with the slow-growing nature of these tumors proved by their low early recurrence rate (0%).

Subependymomas are rare intracranial tumors. Matsumura et al. [14] reported that asymptomatic subependymomas constituted 0.4% of 1000 serial routine necropsy findings and symptomatic subependymomas constituted 0.7% of 1000 serial intracranial neoplasms. Although subependymoma is regarded as a variant of ependymoma, it is believed to be an extremely benign tumor [22]. None of the six subependymomas in our series recurred, and all had LIs below 1%, which coincides with their actual biological behavior.

The clinical course of intracranial ependymomas is highly variable; the survival rates range from 28% to 70% [5, 6, 13, 15, 18, 23, 25]. Longer-term survival has been demonstrated in patients who received more than 45 Gy of radiation therapy [10, 19, 24]. Wallner et al. [25] recommended partial brain irradiation for most ependymomas and whole brain plus spinal irradiation for anaplastic ependymomas. Among patients with incompletely resected ependymomas, postoperative radiation therapy is now standard therapy [2, 5, 19]. Although it may not be curative, radiation therapy slows the growth rate of ependymomas and thus affects the correlation between the proliferative potential reflected by the BUdR LI and the time to recurrence.

Recently, Nazar et al. [18] examined several prognostic factors influencing survival in children with infratentorial ependymomas. The 5-year survival rate was higher after total resection (86.7%) than after subtotal resection (29.5%). In our series, five tumors were totally resected and only one, with a BUdR LI of 3.0%, recurred; however, the follow-up period was short (8–40 months; mean 27.6 ± 12 months). Eight of 14 ependymomas that recurred had an LI of less than 1.0%. All of them recurred several years after partial or subtotal removal. Thus, even a tumor with a low proliferative potential will eventually recur after incomplete resection.

The correlation between the histopathological findings and postoperative survival has been controversial. Ross and Rubinstein [21] reported that features such as high cellularity, increased mitotic activity, cellular or nuclear pleomorphism, focal necrosis, and vascular endothelial proliferation did not predict recurrence or other subsequent clinical behavior. Nazar et al. [18], however, found that histologic features such as mitotic index, cellular density, and necrosis did influence survival. Our study showed some correlation between the BUdR LI and the clinical behavior of ependymomas. With only five exceptions, a BUdR LI above 1% correlated with histological malignancy: two malignant ependymomas had LIs below 1%, and three nonmalignant ependymomas had LIs above 1%. One of the two patients with malignant ependymomas has been recurrence-free for 2 years after subtotal resection; in the other patient (case 1) the tumor recurred 7 years after incomplete resection. Of the three nonmalignant ependymomas, one with an LI of 3.2% recurred 17 months after treatment. The other two tumors, with LIs of 2.9% and 2.0%, have not recurred, but the follow-up times are only 4 months and 9 months, respectively.

Although these data seem to show that the BUdR LI of ependymomas correlates well with their clinical behavior, it is difficult to correlate the BUdR LI with the overall recurrence rate. Excluding subependymomas, only five (28%) of 18 intracranial tumors were totally resected, and residual tumors will eventually recur regardless of their rate of growth. However, the early recurrence rate was very high (100%) in tumors with LIs above 1% and low (25%) in those with LIs below 1%, and the time to recurrence correlated inversely with the BUdR LI. Thus, we can conclude that the BUdR LI appropriately reflects the proliferative potential of ependymomas.

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Note added in proof. After we submitted this paper, a large series of ependymomas was reported by Schiffer et al. [1, 2]. Their statistical analysis of 298 ependymomas showed that only the number of mitoses was an important prognostic factor. This finding is compatible with our data indicating that the BUdR LI is a trustworthy prognostic factor in patients with ependymomas. Both the number of mitoses and the BUdR LI directly indicate the proliferative potential of the tumor. As Schiffer et al. pointed out, the current histological criteria for malignant ependymoma seem less useful and therefore more appropriate criteria should be established.

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