

Postoperative Residual Tumor Growth of Meningioma Can Be Predicted by MIB-1 Immunohistochemistry

Hiroshi Nakaguchi, M.D.
 Takamitsu Fujimaki, M.D., Ph.D.
 Akira Matsuno, M.D., Ph.D.
 Reiko Matsuura, D.V.M.
 Akio Asai, M.D., Ph.D.
 Ichiro Suzuki, M.D., Ph.D.
 Tomio Sasaki, M.D., Ph.D.
 Takaaki Kirino, M.D., Ph.D.

Department of Neurosurgery, The University of Tokyo Hospital, Tokyo, Japan.

BACKGROUND. Meningiomas are benign tumors that can be cured by surgical removal. However, tumors located deeply within or close to vital structures cannot be removed completely and require repeated surgery. This study was designed to clarify whether immunohistochemical study using MIB-1 monoclonal antibody is useful for determining the rate of regrowth for this tumor.

METHODS. Tumor volume doubling time (Td) was measured by using computed tomography or magnetic resonance imaging neuroimages during 29 different follow-up periods after surgery. MIB-1 monoclonal antibody was used to stain Ki-67 proliferating cell antigen in surgical specimens, and the MIB-1 staining index (SI) was determined independently of neuroimaging analysis. These two values and other clinical parameters were analyzed statistically.

RESULTS. The Td values varied from 19–6830 days (median, 350 days); the Td values were <365 days in 15 cases, 365–730 days in 8 cases, and >730 days in 6 cases. There was no significant correlation between age and Td value, but all 6 patients whose Td values were >2 years were age >50. There was a strong inverse correlation between log(Td) and MIB-1 SI ($P < 0.001$). In three cases, more than three surgical procedures were performed for tumor recurrence. The MIB-1 values did not increase at the time of the first recurrence but increased at later recurrences. The calculated Td values also were not shortened until the second recurrence.

CONCLUSIONS. Using the MIB-1 SI makes it possible to predict the regrowth potential of a tumor after initial surgery. *Cancer* 1999;85:2249–54.

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KEYWORDS: tumor volume doubling time, Ki-67 antigen, MIB-1 antibody, meningioma, recurrence.

Meningiomas are benign tumors that can be cured by surgical removal. However, 20–50% of the patients require repeated surgery because of tumor recurrence.^{1–3} Because repeated surgery is more difficult and is associated with an increased complication rate, the prognosis for such patients is unfavorable. The growth potential of meningioma is variable, but microscopic morphologic classifications, such as that of the World Health Organization (WHO), cannot predict its clinical characteristics. Recently, the MIB-1 monoclonal antibody has been used frequently to stain Ki-67 antigen, which is present in all proliferative cells, in order to investigate the growth potential of various systemic and intracranial neoplasms.^{4,5} We recently demonstrated that a longer recurrence free period in meningioma patients is well correlated with a lower MIB-1 staining index (MIB-1 SI; positive cell ratio) of surgical specimens.⁶ The aim of this study was to investigate whether the actual growth of meningioma is correlated with the MIB-1 SI in a series of clinical cases and to

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Hiroshi Nakaguchi and Takamitsu Fujimaki contributed equally to this work.

Address for reprints: Takamitsu Fujimaki, M.D., Ph.D., Department of Neurosurgery, The University of Tokyo Hospital, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.

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TABLE 1
Number of Cases and Instances, Range of MIB-1 Staining Index, and Tumor Volume Doubling Time of Each Histologic Subgroup

Histologic subtype	No. of cases	No. of observed instances	MIB-1 index (%) range (median)	Tumor volume doubling time (days) range (median)
Meningotheliomatous	15	16	0.1-7.0 (1.5)	163-6830 (463)
Fibroblastic	4	7	1.5-9.9 (3.0)	19-444 (339)
Transitional	1	1	7.7	127
Atypical	2	2	2.3, 3.4	350, 488

TABLE 2
Localization and Histologic Subclassification of Meningiomas

Localization	No. of cases	Histologic subclassification			
		Meningotheliomatous	Fibroblastic	Transitional	Atypical
Clivus/cerebellopontine angle	6	5			1
Parasagittal/falx	5	3	1		1
Sphenoid ridge/frontal base	4	2	2		
Tentorial	4	3		1	
Sylvian/ventricular	2	1	1		
Convexity	1	1			
Total	22 cases	15	4	1	2

examine whether the MIB-1 SI can be used to predict tumor recurrence. We measured the tumor volume doubling times (Td) of meningiomas in neuroimages and analyzed their correlation with MIB-1 SI in individual cases.

MATERIALS AND METHODS

Case Materials

Between January 1980 and August 1993, we treated 130 patients with intracranial meningioma at the University of Tokyo Hospital. Twenty-two patients (14 females and 8 males; mean age, 51.1 years) showed meningioma regrowth, and 29 tumor resections were performed for these patients (three times in two patients and four times in 1 patient). In these cases of recurrence, postoperative tumor growth was monitored by neuroimaging (computed tomography [CT] or magnetic resonance imaging [MRI] scan) to determine the Td value (described in detail below) during 26 follow-up intervals. In two other cases, preoperative tumor growth was observed on neuroimages, and the data were compared with those obtained during postoperative follow-up (until recurrence). The surgical samples were fixed with formalin, and paraffin embedded tissues were stained with hematoxylin and eosin (H&E) and with anti-Ki67 monoclonal antibody (MIB-1). There were 15 meningotheliomatous meningiomas, 4 fibroblastic meningiomas, 1 transitional meningioma, and 2 atypical meningiomas (Table 1). Malignant meningiomas were excluded from this study

because they often invade into the brain parenchyma, which sometimes makes the measurement of the tumor volume on the neuroimages difficult. The locations and histological subclassification of the tumors are shown on Table 2. In only 7 instances was the extent of resection considered to be total by the surgeon (Simpson Grade 1),⁷ in other instances, it was incomplete or partial resection.

MIB-1 Immunohistochemistry

The MIB-1 immunostaining method used was essentially the same as that described previously.^{6,4} Briefly, sections (4 μ m thick) of routinely processed paraffin blocks were mounted on 3-aminopropylmethoxysilane-coated slides, deparaffinized in xylene, and rehydrated through a graded ethanol series to water. After blocking of endogenous peroxidase activity with 0.3% H₂O₂ in methanol for 30 minutes, the slides were dipped in 0.01 M citrate, pH 6.0, and the Ki-67 antigen was enhanced by microwave irradiation for 30 minutes (10 \times 3 minutes). The slides were then immunostained with a commercially available preparation of MIB-1 (mouse; diluted 1:100 in bovine serum albumin; phosphate-buffered saline, pH 7.4; Immunotech, S.A., Marseille, France) at room temperature for 1 hour. Biotinylated antibody against mouse immunoglobulin G (goat; Vectastain; Vector Laboratories, Burlingame, CA) was applied as a secondary antibody for 30 minutes. Immunoreactions were visualized by using streptavidin-biotin complex (ABC) labeled with

horseradish peroxidase and a Vectastain ABC kit (Vector Laboratories) for 30 minutes and developed with freshly prepared 3,3'-diaminobenzidine tetrahydrochloride dissolved in 0.05 M Tris-HCl, pH 7.6, and 0.017% H₂O₂ for 7 minutes. Nuclear staining was carried out with hematoxylin for 5 seconds. Tissue from a brain metastasis of colon adenocarcinoma was used as a positive control, and normal murine serum was substituted for the primary antibody as a negative control. All tissue sections were examined at high power magnification ($\times 400$). The number of cells stained positively with MIB-1 and the total number of tumor cells were counted in several representative fields containing more than 1000 cells. The ratio was expressed as the MIB-1 SI (%). In areas in which MIB-1-immunopositive cells were distributed heterogeneously, the area containing the largest number of MIB-1-immunostained cells was considered to represent the proliferative activity of the tumor.

Measurement of Tumor Volume and Calculation of Td

By using enhanced CT or MRI images, the tumor volume was measured. The area of each scan was measured by using a digital planimeter (Uchida-Yoko, Tokyo, Japan), and the volume of each tumor was determined by summing the areas of all the sections and multiplying by the thickness of each scan.

From serial CT or MRI images, Td values were calculated by using the formula

$$Td = t \times \log 2 / (\log V_b - \log V_a)$$

where V_a is the tumor volume at initial imaging, and V_b is the tumor volume at t days after V_a . When no tumor was found on postoperative images, the theoretical residual volume of 0.1 cm³ according to the assumption of Cho et al.⁸ was applied.

Statistical Analysis

The correlation between MIB-1 SI and Td was determined by logarithmic regression analysis. Student's t test was used to analyze the mean value of MIB-1 SI. Data were analyzed by using StatView statistical software (Abacus Concepts, Berkeley, CA). Differences were considered significant at $P < 0.05$. Measurements of MIB-1 SI and Td were determined independently by different investigators (Td, H.N.; MIB-1 SI, A.M. and T.F.), and the correlations were analyzed after all values had been determined.

RESULTS

MIB-1 SI of the Examined Cases

The MIB-1 SI for all 99 patients who did not have tumor recurrence and were not analyzed for Td

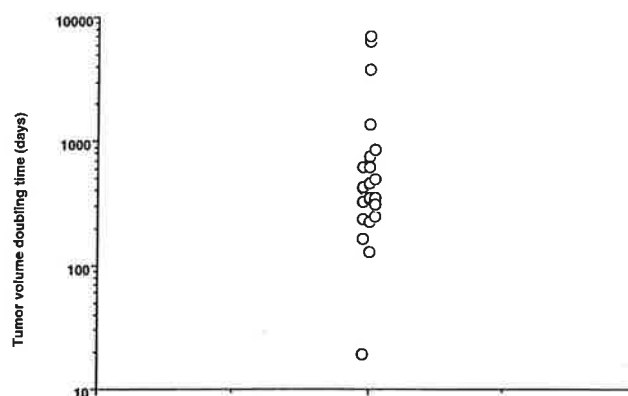


FIGURE 1. Distribution of tumor volume doubling time (Td) in follow-up on 29 patients (22 cases). The postoperative Td values ranged from 19 days to 6830 days. The Td values were <1 year in 15 cases, between 1 year and 2 years in 8 cases, and >3 years in 6 cases. The Td value was >10 years in 2 cases.

ranged from 0.1% to 10.4%, with a mean of 2.6%. The mean MIB-1 SI of the 29 tumor specimens from 22 patients that were analyzed for Td ranged from 0.1% to 9.9% (mean, 3.1%). These values from the two groups were not significantly different. These data indicate that the proliferative potential of the tumors that were analyzed for Td was identical to that of the "common" meningioma group of patients who were treated at our institute.

Td

Postoperative Td values were measured at follow-up in 29 cases (22 patients). In 2 other patients for whom preoperative tumor enlargement was followed on MRI images, the Td values also were determined. The postoperative Td values for meningiomas ranged from 19 days to 6830 days (median, 350 days). The Td values were <365 days (1 year) in 15 cases, 366–730 days in 8 cases, and >730 days in 6 cases. In 2 cases, the Td values exceeded 3650 days (Fig. 1). There was no statistically significant correlation between age and Td value; however, all 6 of the patients with Td values that exceeded 730 days were >50 years of age (Fig. 2). In 2 patients whose preoperative Td values were measured, the Td values were 147 days (74 years of age at surgery) and 647 days (56 years of age), respectively.

Td values and MIB-1 indices for each subgroup are shown in Table 1. In two patients with atypical meningioma, these values did not differ significantly from those of the other subgroup, probably due to the small numbers of patients in this group.

Correlation between MIB-1 SI and Td

The calculated postoperative Td values were plotted against the MIB-1 SI values (Fig. 3). An increased

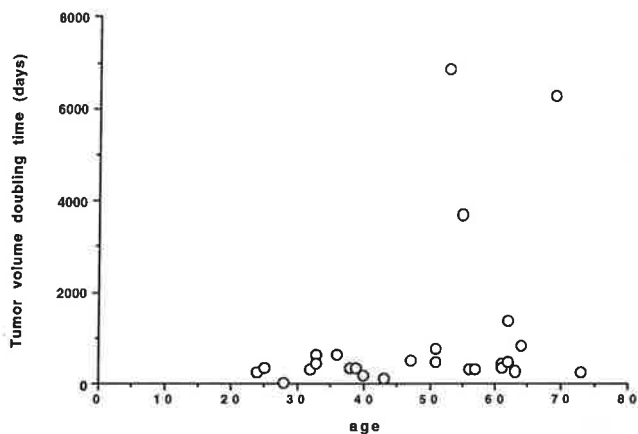


FIGURE 2. Correlation between age and tumor volume doubling time (Td) value. There was no statistically significant correlation between these parameters; however, all of the 6 patients with Td values that exceeded 2 years were >50 years of age.

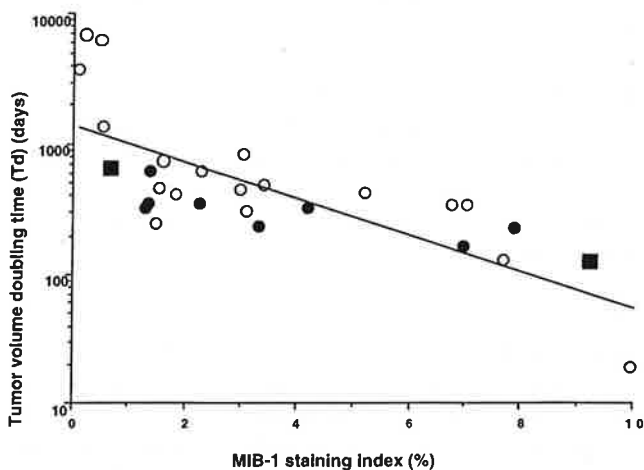


FIGURE 3. Correlation between tumor volume doubling time (Td) and MIB-1 staining index (SI). Open circles and solid circles indicate patients in whom postoperative tumor doubling (prospective growth) was measured. Solid circles indicate patients with tumors that were so small that they were not visualized in postoperative neuroimages. Solid squares indicate patients for whom preoperative tumor growth was observed in neuroimages. Calculated Td values were plotted against MIB-1 SI at surgery for comparison with "prospective" cases.

MIB-1 SI was correlated with a shorter Td value. In all of the cases that showed an MIB-1 value of >3%, the Td value was <730 days (2 years). The Td value can be calculated from the MIB-1 SI at surgery by using the formula

$$\log Td = 31.4 - 0.14 \times \text{MIB SI}$$

$$R^2 = 0.556 (P < 0.0001).$$

The calculated Td value and MIB-1 SI for two patients

in whom tumor increase was observed before surgery were plotted on the same graph (Fig. 3). These two cases also are close to the correlation curve that was drawn according to the above formula.

Changes in MIB-1 SI and Td Values during the Clinical Course

In three patients, repeated surgery was performed for recurrent tumors. The calculated Td and MIB SI values for the observation period (or interval after surgery) are presented in Table 3. MIB-1 values did not increase at the first recurrence but did increase at the second recurrence in case 2. The calculated Td value also was not shortened until the third surgery but became shorter afterward in one case. There were no clear trends in the changes in Td or MIB-1 values in cases of repeated recurrences.

DISCUSSION

Meningioma, as a single entity, is the most common of all intracranial neoplasms. It usually is benign in nature and arises from the arachnoid cells or the arachnoid membrane that covers the brain. The WHO has classified meningioma into three categories: benign (Grade 1), atypical (Grade 2), and malignant (Grade 3). Grade 1 meningioma includes 12 subtypes. Atypical or malignant meningiomas tend to grow faster, but even Grade 1 meningioma recurs frequently.¹⁻³ However, the subtypes of Grade 1 meningioma reflect the histologic characteristics, and not the growth rate, of individual tumors. Sometimes, a Grade 1 meningioma may recur within 1 year after surgery and may behave clinically as a higher grade tumor. Pathological specimens of these cases obtained at initial surgery do not always have atypical features, such as a high mitotic rate, increased cellularity, focal necrosis, cellular pleomorphism, and invasion of the adjacent brain parenchyma. It is not possible to determine the proliferative potential of these tumors based on classical histologic classification using H&E sections.⁶

To evaluate the proliferative potential or cell kinetics of brain tumors, various techniques have been developed, including measurement of the S-phase fraction by [³H]-thymidine autoradiography⁹ or immunohistochemical staining after intravenous infusion of bromodeoxyuridine (BUdR).^{10,11} Recently, immunohistochemical methods using antiproliferating cell nuclear antigen (anti-PCNA) or the silver-stained nucleolar organizer region protein (AgNOR) count also have been developed to assess the proliferative potential of tissues.^{12,13} The BUdR method, for example, is accurate for detecting S-phase cells; Cho et al. showed that the tumor doubling time of meningioma is well correlated with the BUdR labeling index. How-

TABLE 3
Change in Tumor Volume Doubling Times and MIB-1 Staining Indices in Cases Showing Multiple Tumor Recurrence

Case	Age (yrs)	Gender	Histologic subclassification	MIB-1 value at first operation (%)	Td after first operation in days (observation period)	MIB-1 value at second operation (%)	Td after second operation in days (observation period)	MIB-1 value at third operation (%)	Td after third operation in days (observation period)
1	61	M	Fibroblastic	1.8	417 (133)	3.0	444 (637)	1.5	247 (56)
2	32	F	Meningotheliomatous	4.2	322 (2238)	5.2	427 (2678)	14.9	n.d.
3	34	M	Fibroblastic	7.8	222 (2097)	6.7	342 (623)	3.0	n.d.

Td: tumor volume doubling time; M: male; F: female; n.d.: not determined.

ever, the BUdR method requires intravenous administration of BUdR prior to biopsy or incubation of the tumor tissue in a BUdR-containing solution immediately after tumor resection; therefore, it cannot be applied to historic archive specimens. The value of the anti-PCNA antibody method and that of the AgNOR count method also have been questioned in the field of neurooncology.¹³ MIB-1 monoclonal antibody staining with the Ki-67 antigen is applicable to historic archive specimens, allowing retrospective analysis, and is now used for a variety of tumors.^{4-6,12,14} The usefulness of MIB-1 immunohistochemistry for analysis of meningioma growth also has been reported in a smaller number of patients.¹⁵

We have shown previously that meningioma with a higher MIB-1 SI recurs earlier.⁶ Symptomatic recurrence of meningioma is affected by various factors. For some meningiomas, total resection with wide excision of the attachment area is difficult because of the location or involvement of vital structures, such as cranial nerves or important blood vessels. In the present series, surgeons were able to achieve complete resection in only seven patients, probably due to the localizations of the tumors, which were different from common sites.¹⁶ Even for the seven tumors in which total resection was achieved, six were located in the parasagittal area or the cerebellopontine angle, where complete resection is difficult. The fact that these tumors recurred later proved that the resection had not been truly complete. Thus, the extent or mode of meningioma resection is not uniform. The residual tumor mass varies among cases, and the time to tumor recurrence is affected by the residual tumor volume. The period taken for recurrence also varies among patients; if the tumor is located in an important area, such as the motor cortex or Broca's speech center, then recurrence is detected early because of apparent symptoms. However, if the tumor is located in a silent area, then the patient will not develop symptoms until the tumor has grown to a certain size. Thus, it would be more accurate to compare actual

tumor growth with the MIB-1 value than with the interval until the second surgery. Moreover, it would be very helpful if tumor growth rate could be predicted in each case, especially in cases where total removal could not be achieved, because the interval to the next follow-up imaging study could be determined, thus avoiding unnecessary examinations and saving medical costs.

Generally, meningiomas are well demarcated on CT or MRI images and, thus, are suitable for volume measurement. However, it still is not easy to collect a sufficient number of patients for whom serial images are available for analysis. In seven patients (one with atypical meningioma and six with meningotheliomatous meningioma), the residual tumors were very small and could not be identified on neuroimages; therefore, we used Cho et al.'s assumption that the residual tumor volume after surgical resection is 0.1 cm³.⁸ In the latter study, although serial CT or MRI images were not available, the result was well correlated with clinical behavior. In the current study, the correlation between Td and MIB-1 SI was consistent among those patients for whom serial images were available and among those for whom the assumption of Cho et al. was applied. Accordingly, we conclude that the assumption of Cho et al. is appropriate.

In the analysis of tumor growth kinetics, there is always argument about whether the value obtained from a surgical specimen really represents the kinetics of the whole tumor. We used the highest value for each surgical specimen, and the result was found to correlate well with the actual tumor volume growth rate. Thus, our findings confirm that the highest value obtained by cell kinetics analysis using surgical specimens can be used to predict the growth rate, as shown previously with the BUdR method.

This is the first study to analyze the true correlation between MIB-1 SI at surgery and Td after surgery in a substantial clinical series of patients. Our results suggest that this value can be used to predict tumor

recurrence or to determine the appropriate observation interval for patients with meningioma.

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