Measurement of Cell Kinetic Parameters in Meningiomas

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Abstract

Cell kinetic parameters were measured in four cases of meningioma showing regrowth. The cell cycle time (T_c) and tumor doubling time (T_d) were established by histological evaluation of the biopsy specimens and calculation of the growth curve based on computed tomography (CT)/magnetic resonance (MR) images. MIB-1 mouse monoclonal antibody, which recognizes an epitope of the Ki-67 antigen, was used to establish the MIB-1 staining index (SI) (MIB-1-positive nuclei/5000 tumor cells) for each tumor. The changes in tumor volume were calculated from CT/MR images, and the T_d was estimated from the growth curve. The T_c was calculated using Steel’s formula from the MIB-1 SI and tumor volume. MIB-1 SI varied from 0.0055 to 0.0468, T_d from 270 to 1429 days, and T_c from 10.4 to 15.9 days. Furthermore, the potential doubling time (T_p) was calculated using the corresponding T_c and MIB-1 SI (growth fraction) for the four cases; these values varied from 197 to 1645 days and were very close to the T_d values. This method of evaluation of tumor T_c and calculation of T_p may be useful for predicting the postoperative course in meningiomas.

Key words: cell cycle, cell kinetics, meningioma, MIB-1

Introduction

Tumor biological behavior and progression are closely related to the cell kinetic parameters of the neoplastic cells. Data for such parameters in meningiomas refer mainly to tumor doubling time (T_d)\(^{3,11,12}\) and to potential doubling time (T_p).\(^{9,17}\) Since tumor regrowth is not uncommon after incomplete resection of meningiomas, accurate evaluation of cell kinetic parameters employing biopsy specimens may establish the natural course of the tumor more clearly and help to determine the timing of reoperation on the residual tumor.

Ki-67 monoclonal antibodies (mAbs) react with a human nuclear antigen expressed in all proliferating and dividing cells during all active parts of the cell cycle.\(^{6}\) Therefore, Ki-67 mAbs have been used to estimate the growth fraction (GF) of various types of brain tumors.\(^{1,4,7,14-16,21,22}\)

The present study evaluated the GF in four meningiomas using MIB-1 mAb, a recently developed Ki-67 mAb,\(^{2}\) calculated the tumor size and growth rates based on analysis of neuroimaging, and established the cell cycle time (T_c) and T_d.

Materials and Methods

I. Patients

During the past 10 years, 136 patients with meningioma have been treated at the Department of Neurosurgery in Kyoto Prefectural University of Medicine and Matsushita Memorial Hospital. Among these 136 patients, 36 underwent incomplete resection of the tumor (exceeding Simpson’s surgical grade 3).\(^{18}\) Six residual tumors showed regrowth on computed tomography (CT) or magnetic resonance (MR) imaging. Four of these six meningiomas were examined in the present study.

II. MIB-1 staining index (SI)

Routine histological examination and immunohistochemistry were performed on biopsy specimens from the four patients. Immunohistochemical staining used the streptavidin/peroxidase method (Dako StreptABComplex/HRP Duet mouse/rabbit kit; Dako Co., Santa Barbara, Cal., U.S.A.), and MIB-
1 mouse mAb (lot 006; Dianova, Hamburg, Germany), which recognizes an epitope of the Ki-67 antigen.13

Five thousand tumor cell nuclei in 8 to 12 randomly selected high-power fields (×400) were counted for each specimen. Endothelial cells were excluded from the counting. The MIB-1 SI (MIB-1-positive nuclei/5000 tumor cells) was calculated for each tumor.

III. Growth curve from CT/MR images

Tumor volume was calculated from CT/MR images using a slide scanner and Image 1.44 (National Institutes of Health, Bethesda, Md., U.S.A).5 The Image 1.44 program analyzes scanned images of CT/MR images and measures the size of the regions of interest (in cm²). The areas of contrast enhancement were measured in each segmented image. The volume of the tumor (in cm³) was calculated by multiplying by the slice thickness. The tumor volume was calculated at several time points after the operation. The growth curve of the tumor was drawn using simple linear regression analysis.

IV. Calculation of $T_c$ and $T_d$

$T_c$ was calculated by Steel’s formula10 based on the following assumptions.

In general, when all cells of the tumor are actively dividing and cell loss is negligible, the cell population of the tumor at time point $t$ ($N_t$) is given by the following equation:

$$N_t = N_0 \times 2^{t/T_c} \quad (1)$$

where $N_0$ is initial tumor cell population. Since part of the tumor cell population is not actively dividing, the resting cell population should also be considered in the equation. Using the GF of the tumor, the equation can be modified as follows:

$$N_t = N_0(1 + GF)^{t/T_c} \quad (2)$$

In this equation, if the cell density of the tumor is constant, the tumor cell population may be replaced with the tumor volume:

$$V_t = V_0(1 + GF)^{t/T_c} \quad (3)$$

where $V_t$ is the tumor volume at time $t$, and $V_0$ is the initial tumor volume. In the present study, GF was given by MIB-1 SI, and $V_0$, $V_t$, and $t$ were also given by the clinical data. Therefore, the $T_c$ for each tumor could be calculated from the above equation.

As $T_d$ is the actual doubling time of the proliferating cell population, $T_d$ was estimated directly from the growth curve drawn using simple linear regression analysis for each case. Therefore, $T_c$ and $T_d$ were calculated independently.

V. Estimation of $T_p$

$T_p$ is the theoretical population doubling time during exponential growth assuming that cell loss is nil. According to Steel,20 the population of the tumor at $N_i$, when the population follows exponential growth, is given by the following equation:

$$N_i = N_0 \exp(bt) \quad (4)$$

where $b$ is the growth constant. This equation is analogous to Equation (1). In the interval of one cell cycle time ($t = T_c$), the equation can be rewritten as follows using the GF of the tumor:

$$(1 + GF)N_0 = N_0 \exp(bT_c) \quad (5)$$

and therefore

$$b = \log_e(1 + GF)/T_c \quad (6)$$

When $t$ is $T_p$, $N_i$ will be double the initial population (from the definition of $T_p$):

$$2N_0 = N_0 \exp(bT_p) \quad (7)$$

and

$$b = \log_e 2/T_p \quad (8)$$

From Equations (6) and (8), $T_p$ can be calculated by the following equation:

$$T_p = T_c \log_2/\log(1 + GF) \quad (9)$$

In the present study, GF was given by MIB-1 SI. Although the actual $T_c$ of meningioma in situ is unknown, the calculated $T_c$ in each case was employed in the present study. Using the range of $T_c$ for the four cases in this study and the MIB-1 SI in each case, the $T_p$ was estimated for each case.

Results

Table 1 summarizes the cell kinetic parameters and relevant clinical data.

Case 1: A 31-year-old female was admitted to our hospital because of headaches. Partial removal of a large falcotentorial meningioma was performed on June 21, 1990. After surgery, the calculated tumor volume was 14.4 cm³ ($V_0$), and 1233 days (t) later, had reached 27.6 cm³ ($V_t$). The MIB-1 SI of the surgical specimen was 0.0055 (GF). The calculated $T_c$ derived from these values was 10.4 days.

Figure 1 shows the growth curve and representative MR images at time points A and B. The relationship between tumor volume ($Y$) and time ($X$) is given by $Y = 14.74 + 0.0105X$ ($r = 0.970$, $p < 0.01$). The $T_d$ directly measured by this relationship was 1429 days.

Case 2: A 66-year-old female underwent operation

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Table 1  Clinical and cell kinetic parameters of meningiomas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/</th>
<th>Subtype</th>
<th>$V_0$ (cm$^3$)</th>
<th>$t$ (days)</th>
<th>$V_t$ (cm$^3$)</th>
<th>SI (=GF)</th>
<th>$T_c$ (days)</th>
<th>$T_d$ (days)</th>
<th>$T_o$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31/F</td>
<td>fibroblastic</td>
<td>14.4</td>
<td>1233</td>
<td>27.6</td>
<td>0.0055</td>
<td>10.4</td>
<td>1429</td>
<td>1645 ± 380</td>
</tr>
<tr>
<td>2</td>
<td>66/F</td>
<td>transitional</td>
<td>11.6</td>
<td>672</td>
<td>24.6</td>
<td>0.018</td>
<td>15.9</td>
<td>619</td>
<td>505 ± 116</td>
</tr>
<tr>
<td>3</td>
<td>26/M</td>
<td>meningothelial</td>
<td>2.0</td>
<td>407</td>
<td>6.5</td>
<td>0.0468</td>
<td>15.7</td>
<td>270</td>
<td>197 ± 45</td>
</tr>
<tr>
<td>4</td>
<td>70/M</td>
<td>meningothelial</td>
<td>6.0</td>
<td>1907</td>
<td>62.6</td>
<td>0.0154</td>
<td>12.4</td>
<td>340</td>
<td>589 ± 136</td>
</tr>
</tbody>
</table>

on August 7, 1986, for a falx meningioma. The MIB-1 SI of the tumor was 0.018 (GF). After surgery, continuous growth of the tumor was seen. The initial tumor volume was 11.6 cm$^3$ ($V_0$), and had become 24.6 cm$^3$ ($V_t$) 672 days ($t$) later. The $T_c$ calculated from these values was 15.9 days.

Figure 2 shows the CT/MR images of the tumor and its growth curve. The relationship between tumor volume ($Y$) and time ($X$) is given by $Y = 11.6 + 0.0194X$. The $T_d$ directly measured by this relationship was 619 days.

Figure 3 shows the MR images of the tumor and its growth curve. The relationship between tumor volume ($Y$) and time ($X$) is given by $Y = 14.74 + 0.0105X$ ($r = 0.970$, $p < 0.01$).

**Case 3:** A 26-year-old male was admitted because of a focal seizure of the left leg. Subtotal removal of a right falx meningioma was performed on May 10, 1990. The tumor volume after removal was 2.0 cm$^3$ ($V_0$), and had increased to 6.5 cm$^3$ ($V_t$) 407 days ($t$) later. The MIB-1 SI of the tumor was 0.0468 (GF). From these values, the $T_c$ was calculated as 15.7 days.

**Case 4:** A 70-year-old male was admitted because of dizziness. Partial removal of a right tentorial meningioma was performed on January 16, 1986. The initial tumor volume after operation was 6.0 cm$^3$ ($V_0$), and had become 62.6 cm$^3$ ($V_t$) 1907 days ($t$) later. The MIB-1 SI of the tumor was 0.0154 (GF). The $T_c$ calculated from these values was 12.4 days.

Figure 3 shows the MR images of the tumor and its growth curve. The relationship between tumor volume ($Y$) and time ($X$) is given by $Y = 1.62 + 0.0115X$ ($r = 0.980$, $p < 0.001$). The $T_d$ directly measured by this relationship was 270 days.

**Case 4:** A 70-year-old male was admitted because of dizziness. Partial removal of a right tentorial meningioma was performed on January 16, 1986. The initial tumor volume after operation was 6.0 cm$^3$ ($V_0$), and had become 62.6 cm$^3$ ($V_t$) 1907 days ($t$) later. The MIB-1 SI of the tumor was 0.0154 (GF). The $T_c$ calculated from these values was 12.4 days.

The CT images of the tumor and its growth curve are shown in Fig. 4. The relationship between tumor volume ($Y$) and time ($X$) is given by $Y = 3.20 + 0.0294X$ ($r = 0.993$, $p < 0.001$). The $T_d$ directly measured by this relationship was 340 days.
Fig. 3 Case 3. Representative MR images of a right falx meningioma at time points A and B. Growth curve showing the relationship between tumor volume (Y) and time (X) is given by $Y = 1.62 + 0.0115X$ ($r = 0.980$, $p < 0.001$).

Fig. 4 Case 4. Representative CT images at time points A and B. Growth curve showing the relationship between tumor volume (Y) and time (X) is given by $Y = 3.20 + 0.0294X$ ($r = 0.993$, $p < 0.001$).

The calculated $T_d$ in Cases 1 to 4 ranged between 10 and 16 days. Therefore, the $T_p$ was estimated to be $1645 \pm 380$ days in Case 1, $505 \pm 116$ days in Case 2, $197 \pm 45$ days in Case 3, and $589 \pm 136$ days in Case 4.

**Discussion**

Previously, measurement and evaluation of cell kinetic parameters in meningiomas has used two approaches: retrospective measurement of $T_d$ from the growth curve based on CT/MR images$^{3,11,12}$; and calculation of the tumor $T_p$ using a double labeling method (bromodeoxyuridine and iododeoxyuridine) based on a mathematical model of cell proliferation.$^{8,17}$ The importance of evaluating the $T_d$ in meningiomas was stressed, because the $T_d$ has been considered as useful for predicting the natural course in individual patients.

The $T_d$ calculated for cases of benign meningioma has been reported as ranging from 138 to 1045 days (mean 415 days),$^{12}$ 159 to 440 days,$^3$ and 197 to 7943 days.$^{11}$ The range of $T_d$ in our study varied from 270 to 1429 days. The $T_d$ is the actual tumor doubling time calculated from retrospective analysis of CT/MR images, so correlates with the natural course of the tumor. However, $T_d$ cannot be used to predict the natural course of the residual tumor just after the operation.

Study of a series of human malignant glial tumors using $[^3H]$thymidine suggested that the biological behavior and prognosis were closely related to the GF of each tumor and not to the $T_c$.$^{10}$ Recently, $T_c$ has been found to differ according to the histological type of the neoplastic cells. For example, the $T_c$ in glioblastomas is shorter than that in malignant or in differentiated astrocytomas.$^{16}$ Therefore, the proliferation activity of glioma may depend on $T_c$ as well as on GF, and knowledge of the exact value of $T_c$ may be important for the planning of adjuvant postoperative therapy.

The present study evaluated the $T_c$ based on the tumor volume calculated from the CT/MR images (clinical data) and from the MIB-1 SI (pathological data). We assumed that the cell loss in the meningioma is negligible when using Steel's formula for calculating $T_c$.$^{19}$ Unlike malignant gliomas, necrosis is not commonly seen in meningioma. Therefore, cell loss in meningioma is lower than in malignant gliomas. The range of calculated $T_c$ in this study varied from 10 to 16 days. The objective of calculating the range of $T_c$ was to estimate $T_p$ and therefore to predict the behavior of the residual tumor just after the operation. Using the range of $T_c$, $T_p$ can be calculated from the MIB-1 SI of surgical specimens. In all
our cases, the value of $T_p$ (the theoretical doubling time) was found to be quite close to that of $T_d$ (the actual doubling time). Therefore, such an estimation of $T_p$ in meningiomas is fairly reliable. Accurate evaluation of the range of $T_c$ in situ based on data from many cases is necessary. Measurement of MIB-1 SI in individual patients will become of major importance for the planning of postoperative therapy.

$T_p$ in benign meningioma was found to vary from 6 to 39 days. Calculation of $T_d$ from this $T_p$, assuming a hypothetical tumor cell loss factor of 0.8 to 0.9, showed that the $T_d$ ranged from 30 to 390 days. The value for $T_d$ agreed with other reported values, including ours, but the $T_p$ value is quite different from ours. This difference may be related to the calculation method used. Use of a hypothetical tumor cell loss factor of 0.8 to 0.9 was based on a previous study of glioblastoma. In the present study cell loss in meningioma was assumed to be negligible. Also, $T_p$ was calculated from only histological data using a mathematical model, but the present study evaluated $T_p$ from clinical and pathological data. Since the histological findings and biological behavior of meningioma are apparently different from those of glioblastoma, the assumption of a tumor cell loss factor of 0.8 to 0.9 in meningiomas does not appear reasonable. In fact, our preliminary results suggest that the cell loss factor in meningiomas could be lower than 0.44.

Since the measurement of $T_d$ requires a retrospective analysis of tumor growth, the evaluation of $T_p$ is essential for the prediction of the natural course of meningiomas. The present study included very few cases, but the method described for the evaluation of $T_c$ and $T_p$ is very simple and potentially very useful for establishing the clinical prognosis and planning adjuvant treatment for patients with residual meningioma.

References


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Commentary

Nakagawa et al. present four cases (2 female, 2 male) of recurrent meningioma after incomplete resection exceeding Simpson’s grade 3. Cell kinetic parameters were measured using MIB-1 staining index and growth curves from CT-MR images. They state that their method of determining cell-cycle time and tumor doubling time may be useful for predicting the postoperative course in meningiomas. This statement, based on only four cases, is not new. In 1993 Black1) highlighted the present status of the biology of meningiomas. The postoperative course can be predicted using the WHO classification and the Simpson classification which shows recurrence rates for grades III and IV of 29% and 40%, respectively. As another useful predictor of recurrence, flow-cytometry has been suggested.2) The management of meningiomas is still a surgical challenge since the days of Harvey Chshing, even with the knowledge of the molecular biology of these tumors gained during the last years.

References


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If we could easily and inexpensively predict the natural course of residual meningioma, we could better choose between conservative therapy or adjuvant therapy, including radiation therapy. In this article by Nakagawa et al., the authors present a clever method for predicting the natural course of residual meningioma by calculating the potential doubling time ($T_p$) from MIB-1 SI measured in each meningioma. However, as they mention in the article, the four meningiomas are too few to accurately determine the cell-cycle time ($T_c$) of meningiomas. I hope that the authors will continue their efforts to establish an accurate $T_c$ by studying more meningiomas. It is reasonable to assume that $T_c$ may be different in malignant meningiomas and radiation-treated meningiomas. In this article, the meningothelial-type meningiomas tend to show shorter $T_p$ than fibroblastic-type meningiomas. If there is a correlation between $T_p$ and histological type, we may be able to estimate the prognosis of the residual meningioma without MIB-1 staining. Furthermore, we sometimes encounter calcified meningiomas that remain the same size for a long time. Using the method in this article, we can confirm that calcified meningiomas show a long $T_p$.

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