Correlation of Histopathologic Classification with Proliferative Activity and DNA Ploidy in 120 Intracranial Meningiomas, with Special Reference to Atypical Meningioma

Histologic classification of 120 meningiomas was correlated with their proliferative fraction and DNA ploidy using immunohistochemistry and flow cytometry to differentiate histologically atypical meningiomas from benign ones. Histologically, the 120 meningiomas included 101 benign (43 meningotheliomatous, 40 transitional, 11 fibroblastic, 2 secretory, 2 microcystic, 2 angiomatous, and 1 psammomatous), 15 atypical, and 4 malignant meningiomas. As a histologic spectrum between the benign and malignant meningiomas, atypical meningiomas were defined by the presence of two of the following criteria; high cellularity, focal necrosis, uninterrupted growth pattern, and certain cytologic findings i.e., high nuclear/cytoplasmic ratio, coarse chromatin, and prominent nucleoli. In 56 cases, immunostaining for proliferating cell nuclear antigen showed higher proliferating cell fraction in atypical and malignant meningiomas than that in benign meningiomas($p<0.05$). In the flow cytometric analysis, aneuploidy was more often seen in atypical meningiomas compared to benign meningiomas($p<0.05$). We found that benign, atypical, and malignant meningiomas could be histologically classified and correlated with proliferative activity and DNA ploidy pattern. Therefore, atypical meningiomas should be distinguished from benign meningiomas by histopathologic examination and confirmed by studies on their proliferation fractions and DNA ploidies.

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Key Words : Correlation, Histopathologic classification, Proliferating cell nuclear antigen(PCNA), DNA ploidy, Atypical meningioma

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INTRODUCTION

Meningiomas are common intracranial nonglial tumors, with the reported incidence being 13~19% of all primary intracranial tumors (1, 2). The definition of malignancy in meningiomas is beset by frequent discordance between histologic and biologic features. In spite of uncertainties and imperfect relationship between tumor grade and clinical outcome, it is important to categorize meningiomas according to their level of differentiation and degree of aggressiveness as estimated by the relationship of the tumor to the underlying brain. Meningiomas are recommended to be largely grouped into 1) numerically predominant typical or benign meningioma ; 2) a less common atypical tumor that is prone to recur ; and 3) rare lesions that are overtly malignant or anaplastic (3). Atypical meningiomas were recently included in the new World Health Organization (WHO) classification of central nervous system tumors (4), where they are defined as "meningiomas in which often several of the following features are evident; frequent mitosis,

high cellularity, small cells with high nuclear cytoplasmic ratio and/or prominent nucleoli, uninterrupted patternless or sheet-like growth, and foci of necrosis (1). Although this is an excellent description of ominous histopathological features, terms such as "often" and "several" might be considered somewhat vague for a detailed correlative study. There has also been difficulty in specifying the rate of recurrence for atypical meningiomas due in part to the inconsistent definition of "atypical meningioma". For this matter, the use of ploidy analysis and proliferation markers appear ideally suited to distinguish benign from atypical meningiomas and also to predict the recurrence (3,5,6). Ki-67, bromodeoxyuridine(BrdU), ^{3}H-thymidine, AgNOR counts, and proliferating cell nuclear antigen (PCNA) have been used as the proliferative markers of central nervous system tumor (3,8,9). However, it is still controversial as to whether DNA ploidy level and the proliferating indices of meningiomas are definitely correlated with their histologic grades (10,11).

It is the purpose of our study to correlate histological grades with the proliferative activity and DNA ploidy

using 120 meningioma cases, with a special emphasis on the entity of atypical meningioma.

MATERIALS AND METHODS

We collected 125 intracranial meningiomas that were diagnosed at Seoul National University Hospital during a period of 6 years from January 1988 to December 1993. The histologic review of the histology slides was done, based on the new WHO classification (1992) scheme. Therefore, four hemangiopericytomas and one hemangioblastoma were excluded.

Histopathologic evaluation

The meningiomas were first divided into three categories; benign, atypical, and malignant meningiomas. The benign meningiomas were histologically characterized by bland-looking syncytial or fibroblastic meningothelial cells, and divided into three large histologic spectra, including meningotheliomatous, transitional, and fibroblastic meningiomas, depending on the major proportion of each component. The atypical meningiomas were grouped by the presence of more than two of seven criteria, i.e., increased cellularity, pleomorphism, prominent nucleoli, increased mitotic activity, uninterrupted sheet-like growth, focal necrosis, small cells with high nuclear cytoplasmic ratio. The malignant meningiomas were diagnosed by considerable cytologic atypia, frequent mitoses, parenchymal invasion, and necrosis.

Immunohistochemical and flow cytometric studies

PCNA immunostaining

Paraffin sections of 56 cases (43 benign, 10 atypical, 3 malignant meningiomas) were chosen and cut with 4 μm in thickness, deparaffinized and immersed for 10 minutes in phosphate buffer solution in methanol with 3% hydrogen peroxide. After blocking with normal serum or non-immune goat serum for 25 min, the specimens were incubated with monoclonal antibodies against PCNA (DAKO, Code No. M-879, PC10 clone, IgG 2a kappa), with the dilution of 1:50. Avidin-biotin-peroxidase complex was added, and the reaction product was visualized by developing 0.05% diaminobenzidine in Tris Buffer (pH 7.6) and 0.01% H_2O_2. After washing and incubation in 0.1 M sodium acetate buffer (pH 4.0), the nuclei in the background were counterstained with ethyl green for 30 min.

PCNA labeling index measurement by cell image analyzer

The PCNA-positive nuclei were counted in 10 high-power fields of the light microscopy in an immunostained slide, using cell image analyzer system (CAS 200, Becton-Dickinson Co.). The image analyzer showed green and brown colors in negative and positive nuclear areas, respectively. Although the positive nuclei showed some variation in the intensity of immunostaining, they were all considered positive cells. PCNA labeling index was obtained from the percentage of the labelled cells among all positive and negative tumor cells. This process was performed repeatedly in 10 different fields per case, and the merged value was the average proliferation index (PI) of the case.

Flow cytometric study for DNA ploidy

Fifty μm-thick paraffin sections of 48 cases including 38 benign, 7 atypical, and 3 malignant meningiomas were made. In every case, four or five sections were placed in glass centrifuge tubes, dewaxed in 10 ml of xylene for 30 minutes and then immersed in another xylene solutions. After removal of xylene, they were rehydrated first in 10 ml of absolute alcohol for 50 minutes, followed by 95%, 75%, and 50% alcohol solutions, and finally in distilled water for 24 hours. The tissues were digested in 0.5% pepsin in phosphate-buffered solution (PBS, pH 1.5) for 30 minutes in 37°C water bath with the use of an additional sonication stage for 30 seconds at 10-minute intervals to release nuclei. The cell suspension was filtered through a 50 μm nylon mesh into a centrifuge tube filled with cold PBS. It was spun for 4~5 minutes at 1500 rpm, the pellet was resuspended with 1 ml of cold PBS, and whole-cell DNA staining was performed using 2 ml of propidium iodide (0.1 mg/ml in PBS at a pH of 7.4). With addition of 100 μl of citrate buffer, it was again filtered through the nylon mesh. Flow cytometry was performed on the FACScan[TM] with Doublet Discrimination Module (DDM) and cellFIT[TM] software at an excitation wavelength of 488 nm. The emission spectra of propidium iodide was measured through a 620 nm-long pass filter and 1% neutral density filter. The fluorescent intensity of 10,000 cells from each specimen was measured and considered to be proportional to the total DNA content of the cell. The coefficient of variation (CV) of the G0/G1 peak was used to assess the quality of the sample. With the reliable CV values between 3~5, the graphs of DNA content were evaluated for the aneuploidy lines.

Correlation of the morphology with proliferation indices and DNA ploidy

The individual proliferation index (PI) values of 56 meningiomas were plotted on the graph, according to the

histological groups of benign, atypical and malignant meningiomas. The mean PIs of benign and atypical meningioma groups were separately measured to determine if there was a significant difference. In DNA ploidy analysis of 48 cases, the proportions of the aneuploidy cases were calculated according to the histologic types. Using 45 cases in whom both PCNA immunostaining and DNA ploidy analysis were performed, PI values of each aneuploidy and diploidy group were separately dotted, with the means and standard deviations. In comparison of the mean PI values between benign and atypical meningiomas and between diploidy and aneuploidy groups, the differences were statistically analyzed by student t-test.

RESULTS

One hundred and twenty cases of meningiomas in our series could be divided into 101 benign (84.2%), 15 atypical (12.5%), and 4 malignant meningiomas (3.3%), respectively (Table 1). Most of 101 benign meningiomas were made of classic syncytial or spindle-fibroblastic meningeal tumor cells, and included 43 meningothelial, 40 transitional, and 11 fibroblastic types, with remaining minor variants of psammomatous (n=1), microcystic (n=3), secretory (n=2), and angiomatous (n=1) types. Fifteen cases were classified as atypical meningiomas by applying the following diagnostic criteria, i.e. high cel-

Table 1. Distribution of histologic types of 120 meningiomas in this series

Histologic types	No. of cases(%)
Benign	101 (84.2%)
meningotheliomatous	43 (35.8%)
transitional	40 (33.3%)
fibroblastic	11 (9.1%)
angiomatous	2 (1.6%)
microcystic	2 (1.6%)
secretory	2 (1.6%)
psammomatous	1 (0.8%)
Atypical	15 (12.5%)
Malignant	4 (3.3%)
Total	120 (100%)

lularity, pleomorphism, mitosis, focal necrosis, prominent nucleoli, uninterrupted sheet-like growth, and small cells with high N/C ratio. Most of our atypical cases revealed high cellularity, pleomorphism, uninterrupted growth pattern, and small cells with high N/C ratio (Fig. 1 and 2), whereas mitotic figures, prominent nucleoli, and focal necrosis were relatively rarely found. All four malignant meningiomas showed a marked cellular pleomorphism with anaplasia, widespread invasion into underlying brain tissue, large areas of necrosis, and frequent mitoses.
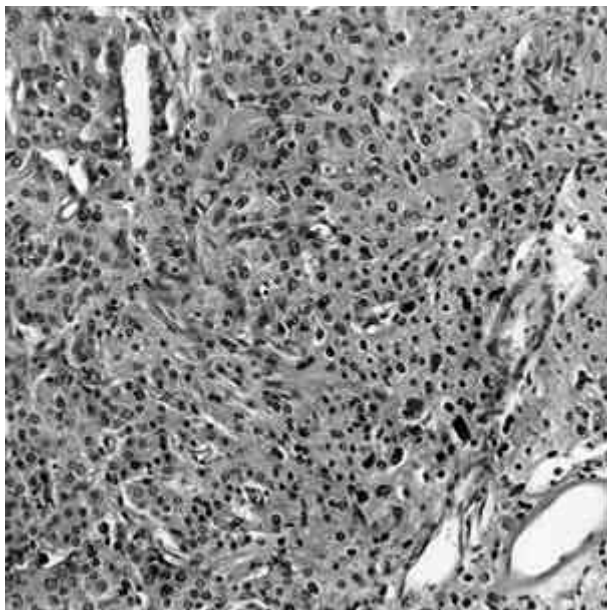

Fig. 1. A case of atypical meningioma showing high cellularity and marked pleomorphism, and uninterrupted growth pattern (H&E).
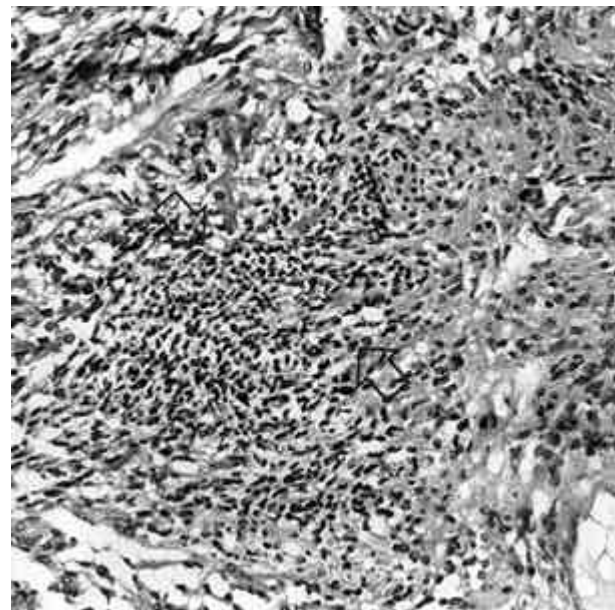

Fig. 2. Another case of atypical meningioma showing small cells with high N/C ratio (arrows) in addition to high cellularity and pleomorphism (H&E).

Table 2. PCNA labeling indices of 56 meningiomas of different histologic types

Histologic types (No. of cases)	Mean proliferation index
Benign (43)	20.1%
meningotheliomatous (16)	18.3%
transitional (19)	21.0%
fibroblastic (4)	17.6%
secretory (2)	25.0%
microcystic (1)	41.2%
angiomatous (1)	20.1%
Atypical (10)	28.8%
Malignant (3)	29.8%

Proliferating cell nuclear antigen (PCNA)-immuno-histochemical staining of 56 cases revealed a marked variability in number, intensity, and distribution pattern. This was partly true even within a same case. Proliferation index (PI) values of the benign meningiomas ranged widely and there was no significant difference among histological variants (Table 2 and Fig. 3). However, the difference between average PCNA-labelling indices of benign, atypical, and malignant group revealed a statistical significance between the benign (20.1±9.0

%) and atypical(28..8±10.8%) or malignant meningiomas (p<0.05) (Fig. 4).

In the flow cytometric analysis, DNA curves of 48 cases were divided into aneuploidy and diploidy patterns. Aneuploidy pattern was shown in 12 cases (6 benign, 4 atypical, and 2 malignant meningiomas) and diploidy in 36 cases (32 benign, 3 atypical, and 1 malignant meningioma). The proportion of aneuploidy was higher in atypical or malignant meningiomas than in benign meningiomas (Table 3). The aneuploidy cases showed higher mean PI values (31.1±10.8%) than those(19.9± 9.2%) of diploidy cases (p<0.05) (Fig. 5).

DISCUSSION

Because of its operability, the meningioma is the most common brain tumor that is accessioned in Pathology Departments of most institutes including our hospital (12). Complete removal and remarkable postoperative improvement are the usual story of meningioma with exceptional cases of malignant meningiomas. It is well-known that a great majority of meningiomas shows an excellent clinicopathologic correlation. However, there are cases that show apparent discrepancy between clinical course and histological appearance.
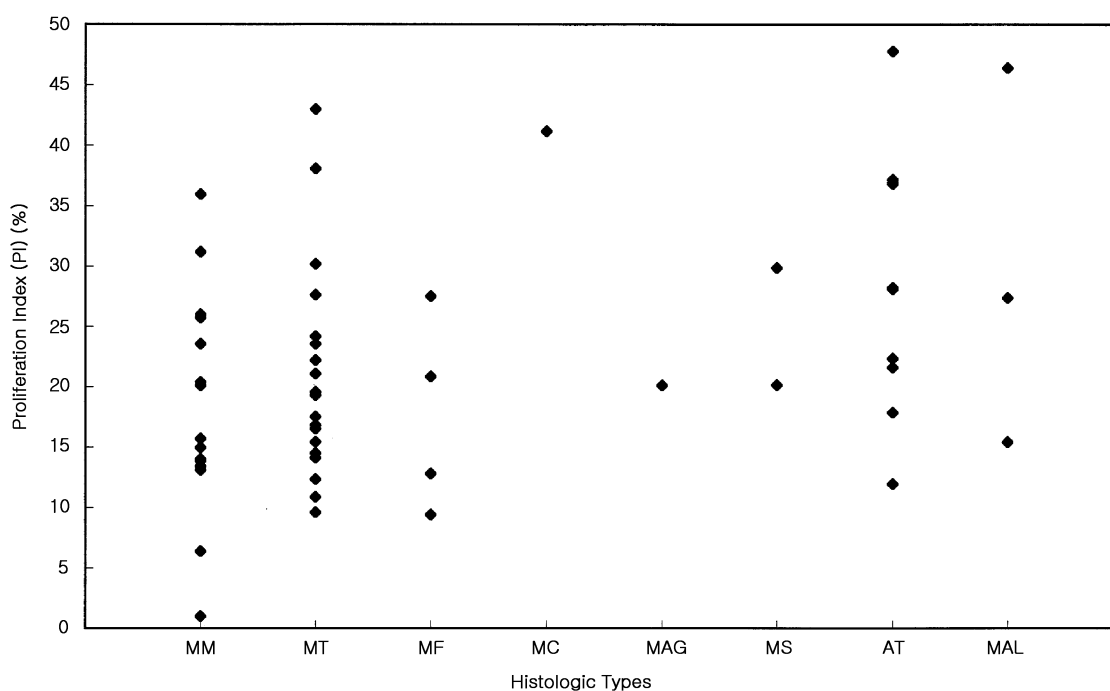

Fig. 3. Distribution of PI values of 56 meningiomas according to the histologic types. The solid dots represent PI values. (MM: Meningotheliomatous, MT: Transitional, MF: Fibroblastic, MC: Microcystic, MAG: Angiomatous, MS: Secretory, AT: Atypical, MAL: Malignant)

Table 3. Proportion of aneuploidy and diploidy of 48 meningiomas of different histologic types

Histologic Types (No. of cases)	No. of aneuploidy	No. of diploidy	% of aneuploidy
Benign (38)	6	32	15.7%
meningotheliomatous (12)	2	10	
transitional (18)	1	17	
fibroblastic (4)	0	4	
secretory (2)	2	0	
microcystic (1)	0	1	
angiomatous (1)	1	0	
Atypical (7)	4	3	57.1%
Malignant (3)	2	1	66%

It has been conventional to devide the meningiomas into benign and malignant types. And various histological variants were described in the literature, many of which were included in the new WHO classification of brain tumors (4). Our series in this study showed comparable incidence of various types of benign meningiomas, with the dominance of meningothelial and transitional type. However, by this study, we reassessed ourselves that major histological variants, especially meningothelial, transitional, and fibroblastic types are a more likely continuum rather than a set of distinct

entities as described by Burger and Scheithauer (3). In this study, approximately 80 percent of 120 meningiomas were represented by meningothelial, transitional, and fibroblastic types of benign meningiomas, that were frequently mixed in the same tumor. We have excluded hemangiopericytoma and hemangioblastoma based on the new WHO classification with which we concur.

When we encounter a meningioma that shows histological findings lying between classical meningothelial meningioma and malignant meningioma, we often think of atypical meningioma and try to correlate with
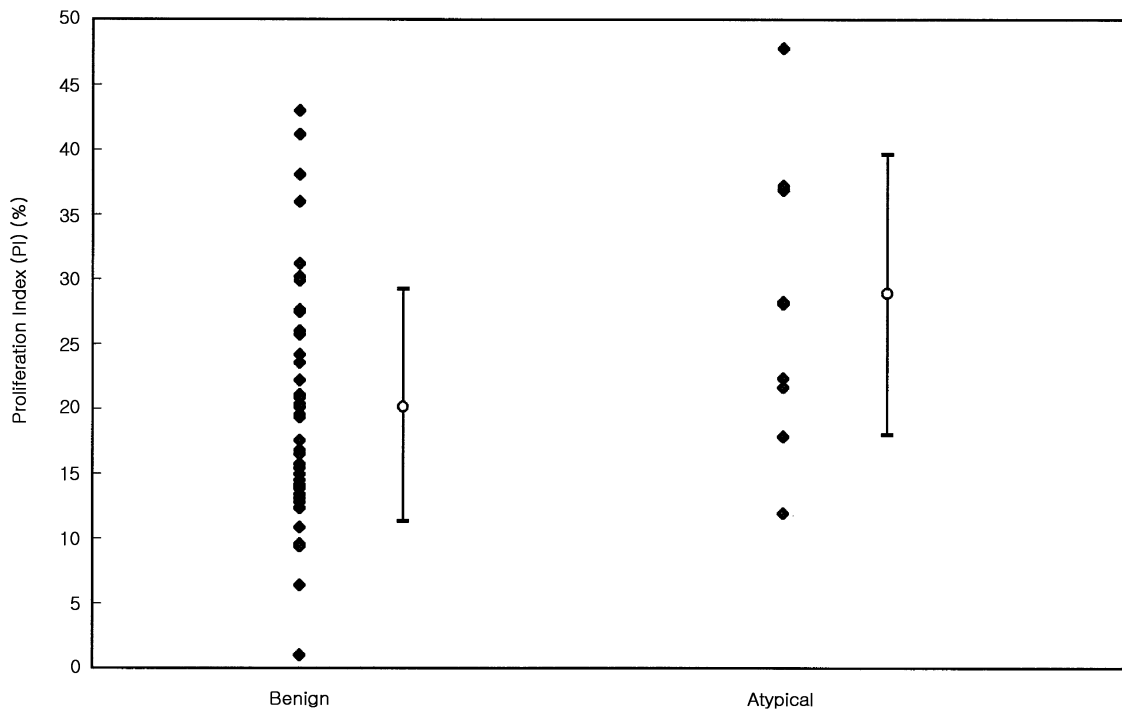

Fig. 4. Compatison of PI values between benign (n=43) and atypical (n=10) meningiomas. The black dots represent the individual PI values. The open dots with bars represent the mean PI±SD.
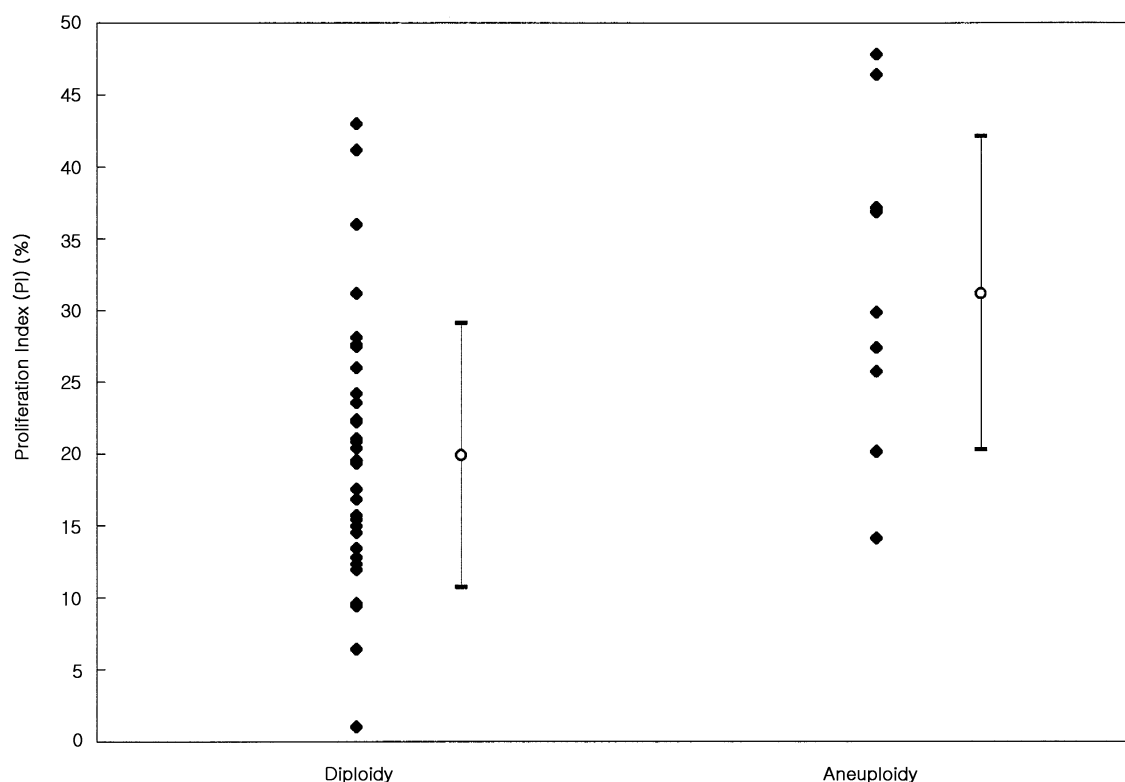
Fig. 5. Comparison of PI values between diploidy (n=34) and aneuploidy (n=11) groups in 45 cases of meningiomas.

the clinical features. Therefore, the term atypical meningioma has been diagnosed by a different set of criteria. Maier et al. (1) described an intermediate group of meningiomas exhibiting signs of rapid growth (focally increased cellularity and increased mitotic rate : at least five mitotic figures per 10 high-power fields in the most active area). Jaaskelainen et al. (13) described atypical meningioma as grade II out of IV by combining six histological criteria (loss of architecture, increased cellularity, nuclear pleomorphism, mitotic figures, focal necroses, and brain infiltration) and reported its increased growth rate. The new WHO classification of CNS tumors (4) defines atypical meningiomas as "meningiomas in which often several of the following features are evident ; frequent mitoses, high cellularity, small cells with high nuclear cytoplasmic ratios and/or prominent nucleoli, uninterrupted patternless or sheet-like growth, and foci of necrosis". Therefore, an establishment of strict criteria for the entity of atypical meningioma is critical, particularly considering the recurrence rate of 35~38% in atypical meningioma, in contrast to 3~7% in classical meningiomas and 73~78% in malignant meningiomas (1,14). We have to establish more consistent and reproducible parameters to diagnose the atypical meningioma. For that matter, combination of histological criteria and

proliferative activity study could be the ideal way of diagnosing the atypical meningioma. Our study revealed statistically significant higher PI values in the atypical meningiomas compared to conventional benign meningiomas, and the PI values of malignant meningiomas were slightly higher than atypical meningiomas (Fig. 4 and Table 2). We also found the aneuploidy tumors showed higher proliferation rates than the diploidy ones (Fig. 5). And the atypical meningiomas showed the significantly higher proportion of aneuploidy compared to the benign meningiomas (Table 3). We understand there is a controversy in correlating the quantitative parameters and histologic grading (15~17). A previous study (11) using PCNA immunostainings on atypical meningiomas failed to demonstrate the correlation between the histologic grading and proliferation indices, although Salmon et al. (18) reported that PCNA might be helpful for determining malignant or aggressive meningiomas, with no significant difference between recurrent and nonrecurrent meningiomas. Reports on DNA ploidy in relation to the histologic grades of the meningioma are rare, but Salmon et al. (19) found no significant difference in the proportion of aneuploidy or diploidy between classic and malignant meningiomas. Furthermore, they could not confirm the correlation between the proliferative activity

and ploidy pattern in the meningiomas (20).

In conclusion, our study shows that histologically atypical meningiomas show higher proliferating fractions and more frequent aneuploidy patterns than the classical benign meningiomas. And a positive correlation between the proliferating activities and DNA ploidy pattern was found in meningiomas. Therefore, combination of histological criteria and proliferative activities of the tumor is important to distinguish benign from malignant meningioma, particularly in diagnosing atypical meningioma.

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