

Comparison of Proliferative Activity in Each Histological Subtypes of Benign and Atypical Intracranial Meningiomas by PCNA and Ki-67 Immunolabeling*

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= Abstract =

양성 뇌수막종의 조직학적 아형 및 이형성 뇌수막종에서
PCNA와 Ki-67 표지지수의 비교

가

최승진 · 장은덕** · 권성오 · 계대근 · 박춘근 · 이상원 · 강준기

Objective : The clinical prognosis and biological behavior of atypical and especially malignant meningiomas are well known to be worse than benign meningioma, but the degree of biological aggressiveness in each classical subtypes of benign meningioma is controversy. This study was performed to see whether there is a difference in the proliferative activity between each different histological subtypes of benign meningioma as well as atypical meningioma.

Methods : Paraffin - embedded surgical specimens of 27 meningiomas, including two recurrent tumors, were studied to evaluate proliferative activity by immunohistochemical method with monoclonal antibodies to proliferating cell nuclear antigen(PCNA) and MIB - 1. The specimens consisted of 8 cases of meningothelial, 9 cases of transitional, 5 cases of fibroblastic subtypes and 5 cases of atypical meningiomas.

Results : Mean PCNA labeling indices of meningothelial, transitional and fibroblastic meningiomas were $4.82 \pm 5.10\%$, $9.01 \pm 4.25\%$ and $5.66 \pm 5.32\%$, but that of atypical meningiomas was $27.62 \pm 19.67\%$, noting a higher value compared to all three subtypes of benign meningiomas. Mean Ki - 67 labeling indices of the above 3 subtypes were $0.43 \pm 0.85\%$, $0.44 \pm 1.08\%$ and $0.24 \pm 0.18\%$, and that of atypical meningiomas was also revealed to be of higher value ($0.84 \pm 0.59\%$). PCNA and Ki - 67 labeling indices were not statistically different between histological subtypes of benign meningioma ($p > 0.05$), but the differences of both immunolabeling between benign and atypical meningiomas were statistically significant ($p < 0.05$).

Conclusion : Immunolabeling of PCNA and Ki - 67 in intracranial meningiomas reveals no prognostic difference between meningothelial, transitional and fibroblastic subtypes in classical benign meningiomas by measuring expression of PCNA and Ki - 67, but it seems to be helpful in differentiating benign and atypical meningioma, later showing more proliferative activity and biological aggressiveness.

KEY WORDS : Intracranial meningioma · Histological subtypes · Atypical meningioma · PCNA labeling index · Ki - 67 labeling index.

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Introduction

Intracranial meningiomas exhibit about 15 percent of primary intracranial tumors, and are frequently encountered as extra-axial brain tumors in neurosurgical field¹⁴⁾²⁶⁾. Histopathologically, meningiomas are classified as benign, atypical, papillary and malignant types according to its behavior¹⁾, 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 nucleoli²¹⁾. Clinically, the more anaplastic form has revealed a worse prognosis³⁰⁾. Most meningiomas are benign and the extent of surgical resection has been considered an important factor in predicting recurrence of tumor²¹⁾, but 2.3 - 30% of recurrence rates in benign meningioma were reported despite of complete surgical resection⁶⁾²³⁾. In the subtypes of benign meningioma, it is not well known whether there are any differences in biological and clinical aggressiveness between them. To predict the prognosis of neoplasm, the evaluation of cell proliferation rate can be used because it is known to be correlated with biological aggressiveness¹⁰⁾.

In this study, the authors made use of immunohistochemical stain with monoclonal antibodies of proliferating cell nuclear antigen(PCNA) and Ki-67 to investigate proliferating activity of meningeal tumor cells and compared it according to the subtypes of classic benign meningioma (meningothelial, fibroblastic, transitional) and atypical meningioma.

Materials and Methods

1. Materials

The authors studied 27 paraffin-embedded surgical specimens of intracranial meningiomas, including 2 recurred tumors, obtained during surgical operations at the Department of Neurosurgery. The patient population consisted of 17 women and 8 men ranging from 20 to 75 years in age(mean 54.6 years). Histologically, the specimens were divided into 22 classical benign meningiomas with typical histological patterns, without prominent nuclear mitotic activity or hypercellularity¹⁴⁾, and 5 atypical meningiomas with increased cellularity and at least five mitotic figures per 10 high-power(400 ×) fields²¹⁾. Histologic subtype of

classical benign meningiomas was evaluated as 8 specimens of meningothelial, 9 specimens of transitional and 5 fibroblastic specimens(Table 1). In two recurrent meningiomas, one case was fibroblastic meningioma in both first and second operative specimens, and another case was meningothelial subtype at first operation but showed atypical meningioma in second operative specimen.

2. Immunohistochemistry for PCNA

All surgical specimens for immunohistochemistry were fixed in 10% formalin and embedded in paraffin. After cutting into 5 μ m thickness, the specimens were deparaffinized with 100% acetone and stabilized with iodine solution(DBS, Fremont, CA. Before adding the primary antibody, Specimen slides were microwaved to a temperature of 121 for 20 minutes in a citrate buffer(pH 6.0 ; 0.01mol/L). After cooling in the same solution and rinsing in immunoassay buffer, slides were blocked with 3% hydrogen peroxide and water for 2 minutes at 45 . After blocking with non-immune goat serum for 30 minutes at room temperature, tissue sections were incubated with anti-PCNA monoclonal mouse antibody clone PC10(IgG2a kappa, DBS, Code No. PDM 014, Fremont, CA) for 60 minutes at room temperature. Following the addition of avidin-biotin-peroxidase complex, the antigenantibody reaction was visualized by adding 3,3'-diamino-benzidine/H₂O₂. The slides were counterstained with Mayer' hematoxylin and mounted.

3. Immunohistochemistry for Ki-67

Same methods and procedures for tissue preparation were performed as in PCNA immunostaining. Monoclonal mouse antibody to Ki-67 clone MIB-1(IgG1, kappa, DBA, Code No. PDM 026, Fremont, CA) was used as primary

Table 1. Tumor location and histological type of meningiomas

Location	Meningothelial	Transitional	Fibroblastic	Atypical	Total
Convexity			2	2	4
Parasagittal/ Falx	2	5	2	1	10
Sphenoid wing	3	2			5
Temporal base	1			2	3
Petroclival/ CPA	2		1		3
Tuberculum sellae/ Cavernous sinus		2			2

CPA : cerebellopontine angle

antibody. 3,3'-diaminobenzidine was also used as a chromogen, and counterstaining with Mayer's hematoxylin was done.

4. Measurement of PCNA and Ki-67 labeling indices by cell image analyzer

After identifying the highest density areas of brown-colored DAB-positive nuclei by light microscopy at 100 × magnification fields of each PCNA and Ki-67 immunostained slides, positive cells were counted in 10 high-power fields (200 ×) of the light microscopy, using cell image analyzer system (CAS 200, Becton-Dickinson Co.). Regardless of the degree of immunostaining, brown-colored nuclei were considered immune-reactive and determined positive (Fig. 1 and 2). PCNA and Ki-67 labeling indices were expressed as a percentage of positive brown-colored nuclei to all tumor nuclei.

5. Statistical analysis

Results were expressed as mean ± SEM. Data was analyzed by the use of SPSS/PC software. Statistical comparisons were performed by the use of an independent t-test. A value of $p < 0.05$ was considered significant.

Results

Positive nuclei could be confirmed and easily counted in immunostained specimens with not only anti-PCNA, but also with MIB-1 monoclonal antibody. In classical benign meningiomas, mean PCNA labeling indices according to subtypes were 4.82% in meningothelial, 9.01% in transitional and 5.66% in fibroblastic. And mean Ki-67 labeling indices were 0.43% in meningothelial, 0.44% in transitional and 0.24% in fibroblastic. The differences between these proliferative activities were not statistically signifi-

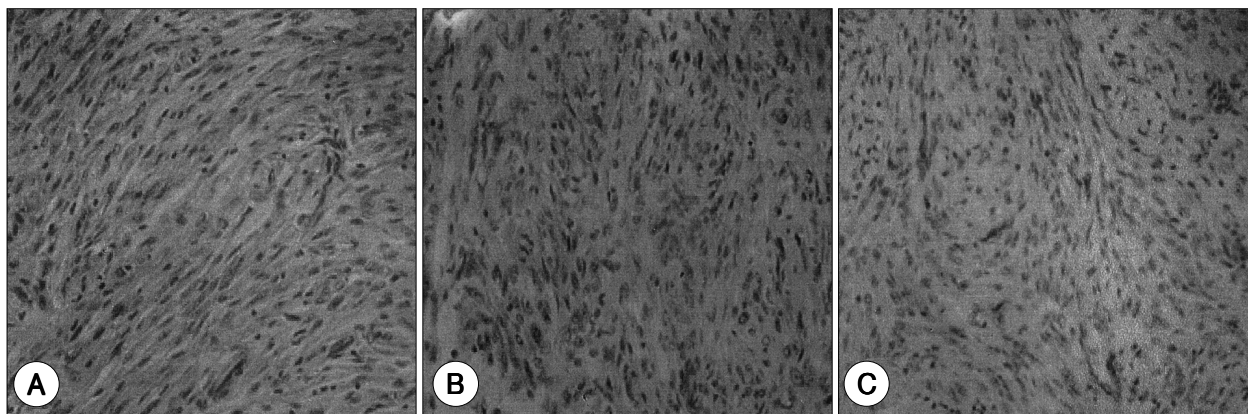


Fig. 1. Photomicrograph of fibroblastic meningioma. A : H&E staining (100 ×). B, C : Immunohistochemical staining for PCNA (B) showing fine nuclear positivity, in contrast with immunohistochemical staining for Ki-67 (C) which shows nearly no positive nuclear immune reaction (100 ×).

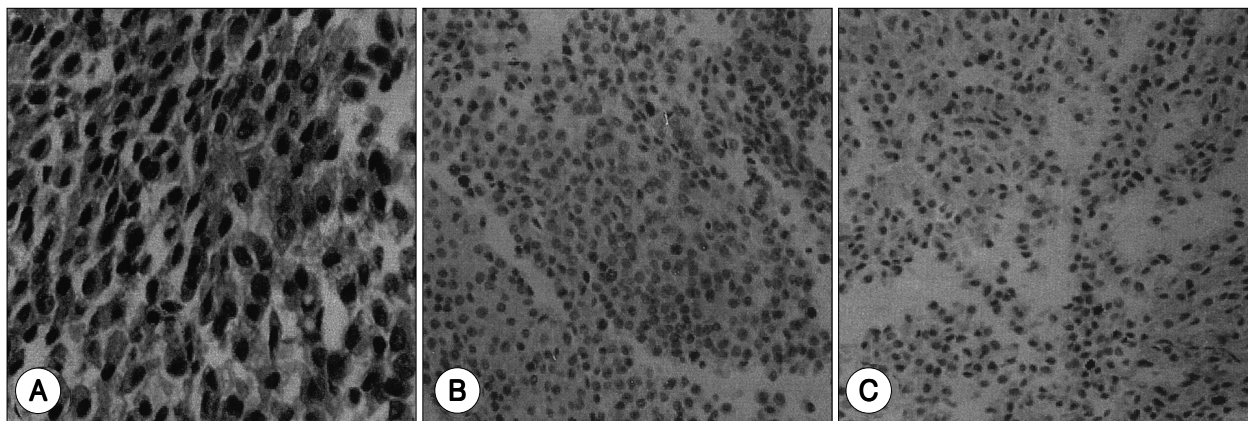


Fig. 2. Photomicrograph of atypical meningioma. A : H&E staining (200 ×). B, C : Immunohistochemical staining for PCNA (B) and for Ki-67 (C) demonstrating more immunopositive nuclei compared to fibroblastic meningioma (100 ×).

Table 2. Correlation of PCNA and Ki-67 labeling indices in various histologic types

Histology	PCNA LI (mean ± SEM)	Ki-67 LI (mean ± SEM)	No. of cases
Meningothelial	4.82 ± 5.10%	0.43 ± 0.85%	8
Transitional	9.01 ± 4.25%	0.44 ± 1.08%	9
Fibroblastic	5.66 ± 5.32%	0.24 ± 0.18%	5
Atypical*	27.62 ± 19.67%	0.84 ± 0.59%	5

LI : labeling index

* : P = 0.02, statistically significant

Table 3. Immunolabelings and mitotic figures of 5 atypical meningiomas

Case	PCNA LI	Ki-67 LI	Mitosis(No./10 HPFs)
1	15.0%	0%	6
2	17.3%	1.0%	8
3	61.5%	0.5%	11
4	28.3%	1.2%	9
5	16.0%	1.5%	16

LI : labeling index

HPFs : high power fields(400 ×)

cant($p > 0.05$). But in atypical meningiomas, mean PCNA and Ki-67 indices were 27.62% and 0.84%, showing higher values than in all subtypes of benign meningiomas (Table 2) and the differences between benign meningiomas and atypical meningiomas were statistically significant ($p < 0.05$), and then proliferative activity in atypical meningiomas was revealed higher than in benign meningiomas. In comparison between mitotic figures and immunolabeling indices of all five atypical meningiomas, although there is little statistical significance due to insufficient number of cases, Ki-67 labeling index used MIB-1 monoclonal antibody seems like to be correlated with mitotic figure in this study (Table 3).

Discussion

For predicting the prognosis of intracranial meningiomas, high mitotic index in histological feature has been thought to be a major indicator for tumor recurrence^{7,22} like other neoplasms though it is being debated by some other authors^{5,28}. But only counting mitoses cannot provide all information on biological aggressiveness and recurring tendency of tumor, because the mitotic compartment constitutes the smallest portion of cycling tumor cells²⁰. Though classical benign meningiomas show few or no mitoses in histological feature, there have been recurrences despite complete surgical removal^{6,22,23}. Therefore, the prediction of recurrence and prognosis of meningiomas cannot be

only confined within histological findings.

Proliferation rate has shown to be correlated with biological aggressiveness¹⁰. Methods for evaluating the proliferative activity of meningiomas include static and flow DNA cytometry, counts of argyrophilic nucleolar organizer regions (AgNORs), in vivo or in vitro uptake of bromodeoxyuridine (BrdU), and immunohistochemical studies with monoclonal antibodies of proliferating cell nuclear antigen (PCNA) and Ki-67^{12,19,29}.

Recently, of these methods, immunohistochemical studies against PCNA and Ki-67 are the most widely used. PCNA is a 36-kD nuclear auxiliary protein of polymerase and is highly expressed in the G1/S phase, declines through G2 phase, and reaches low levels at M phase and interphase during the cell cycle^{3,17}. PCNA labeling index (LI) is generally well correlated with tumor grade and clinical prognosis in brain tumors^{2,15,27}. Ki-67 is a protease sensitive, nonhistone protein (345 kD and 395 kD) which is known to be expressed in all phases of the cell cycle except G0 phase or quiescent cells⁸ and is the most widely used proliferation marker.

The sensitivity of immunolabelings of PCNA and Ki-67 for estimating proliferative activity of tumor cells is generally accepted as reliable methods. Ki-67 labeling index is considered more specific than PCNA labeling index of which a broad overlapping between different grades was observed in brain tumors^{15,16}. In meningiomas, one series of histologic and immunohistochemical studies showed that PCNA index is closely correlated with mitotic index, and it was noted that it may serve as a useful adjunct in evaluating the proliferative potential of meningiomas and predicting the clinical course¹². But several authors have stated that PCNA immunolabeling had a limited value due to possibility of modifications by preoperative steroid therapy⁹ and by tissue handling and fixation⁴. The prognostic usefulness of Ki-67 labeling index has also been observed by others^{11,24}. Langford et al¹⁸ showed a significant correlation between the MIB-1 and BrdU measurements and noted that MIB-1 proliferation index can be used instead of BrdU to determine the proliferative potential of meningiomas. They also revealed that it was not significantly affected by steroid pretreatment or age.

The extent of PCNA and Ki-67 expression in meningiomas is known to be mainly correlated with the grade or degree of a tumor's anaplastic nature. Hsu et al noted with immunoperoxidase staining for PCNA that the benign

group had low numbers of labeled nuclei, and a majority of atypical and malignant groups revealed high numbers of PCNA-positive nuclei¹²⁾. On the other hand, it was reported that PCNA indices of WHO-grade and / showed no statistical difference, but the differences of these groups for the mean and for the maximal Ki-67 index were statistically significant²⁹⁾. According to subtypes of benign meningioma, Roggendorf et al²⁵⁾ described a higher Ki-67 index in transitional meningiomas as compared with other subtypes, and Ide et al¹³⁾ noted that meningothelial meningiomas tended to have higher PCNA staining indices than fibrous subtype. But other studies showed no significant differences in proliferative indices between subtypes of benign meningioma¹²⁾²⁹⁾.

In this study, we could not reveal meaningful differences of proliferative indices between the 3 subtypes of benign meningioma, but we could find high PCNA and Ki-67 labeling indices in atypical meningiomas compared to benign meningiomas with statistically significant differences.

Conclusion

This result lead us to conclude that there seems to be no difference of proliferative activity between meningothelial, transitional and fibroblastic subtypes in classical benign meningiomas by measuring expression of PCNA and Ki-67. Like other methods to measure the proliferative potential of tumors, estimating proliferative activity using PCNA and Ki-67 labeling indices in meningiomas can be helpful to differentiate benign and atypical types and predict the biological behavior. Therefore, combination of histological criteria and proliferative activities of the tumor is important to distinguish benign from atypical meningioma.

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