

Ki-67, Proliferating Cell Nuclear Antigen, Flow Cytometry를 이용한 수막종의 증식력 분석

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= Abstract =

Analysis of Proliferative Potentials in Meningiomas by Ki-67, Proliferating Cell Nuclear Antigen, and Flow Cytometry

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Objective : In this study, we investigated the relationship between the histologic grading of meningiomas and proliferative potentials determined by the Ki - 67, proliferating cell nuclear antigen(PCNA) and flow cytometry (FCM) with the aim of determining whether these potentials can be used as a parameter to the proliferative activity, in particular of atypical and malignant meningiomas.

Methods : This study consisted of 47 meningiomas(6 malignant, 14 atypical, and random sampled 27 benign meningiomas). By immunohistochemical staining of Ki - 67 and PCNA on formalin - fixed, paraffin - embedded sections, the anti - human rabbit polyclonal antibody against Ki - 67 antigen and anti - PCNA monoclonal antibody(PC10) scores were counted. FCM was also performed on paraffin - embedded tissue using a selective staining technique for DNA. DNA ploidy, S - phase fraction, and proliferative index(PI) were determined.

Results : The results are summarized as follows ;

- 1) Proliferation rates as assessed by Ki - 67 and PCNA closely correlated with the degree of anaplastic histologic features.
- 2) Proliferative potentials determined by FCM(S - phase fraction and PI) were not able to distinguish between benign and atypical/malignant meningiomas.
- 3) DNA ploidy was not a useful indicator of histologic grade in these tumors.
- 4) Proliferative potentials such as Ki - 67 staining index(SI) and PCNA SI did not correlate with the ploidy pattern.
- 5) There was a linear correlation between Ki - 67 SI and PCNA SI, but we could not find a correlation between Ki - 67 SI and S - phase fraction or PI. Our results also did not show a statistically significant correlation between PCNA SI and S - phase fraction or PI.

Conclusions : We conclude that evaluation of the proliferative potentials with Ki - 67 and PCNA is important as an additional factor for the prediction of malignancy in meningiomas. A dual study of Ki - 67 and PCNA SIs on the same tissue might improve the accuracy with which the proliferative potential of a tumor can be predicted. We demonstrated that FCM in meningiomas is not valuable in predicting the behavior of these neoplasms, but we did observe a trend toward more malignancy with higher percent S - phase fraction and higher PI. Analysis of the S - phase fraction and PI might therefore be a useful tool to discriminate among histologic grades of meningiomas.

KEY WORDS : Meningiomas · Proliferative potentials · Ki - 67 · Proliferating cell nuclear antigen · Flow cytometry.

서론

15% 가 , (proliferative potential) (cell kinetics) [³H]thymidine uptake nucleolar organizer region (silver-stained argylophilic nucleolar organizer region-associated proteins (AgNORs)) , bromodeoxyuridine (BUDR) Ki-67 (proliferating cell nuclear antigen, PCNA) (flow cytometric analysis, FCM)

24)26)27) (grade) Ki-67, PCNA

대상 및 방법

1990 3 1997 2 47 가 29 (61.7%), 가 18 (38.3%) , 49.3 (: 19~75) 47 6 , 14 27 가

. 1) - 가 , 2) - () 가 (2 (nuclear pleomorphism), / (nuclear to cytoplasmic ratio), 가 , 3) (histologic group) 47 Ki-67, PCNA, 가

1. Ki-67

avidine-biotin complex , anti-human rabbit polyclonal antibody (A0049, DAKO) Ki-67 (staining index, SI) 1 : 500 , anti-human rabbit polyclonal antibody . Ki-67

2. PCNA index

avidine-biotin complex PCNA (anti-PCNA primary monoclonal antibody, PC10, DAKO) PCNA . 1 : 200

PCNA , PCNA PCNA

3. FCM

DNA DNA

50 μm, 5~6 xylene (rehydrated), 0.25% trypsin, propidium iodide (fluorescence), G₀G₁ peak, G₂M peak가 (aneuploid population), S G₂M, PI(%)=%S+%G₂M.

4. 통계적 분석
 Wilcoxon rank sum test (Ki-67, PCNA), Spearmen rank - order correlation, chi - square test (p<0.05).

결 과
 Ki-67, PCNA (Table 1)

1. Ki-67
 47 Ki-67, 0.1~25.0%, 4.68% ± 0.56%, 4.46%, 7.47 ± 9.23%, 5.45 ± 6.26%, Ki-67 2.5%

Table 1. Summary of the Ki-67 staining index, PCNA staining index, S-phase fraction, and proliferative index in 47 meningiomas*

| Histology | Ki-67 SI | PCNA SI | FCM | | |
|-----------|----------|---------|------------------|------|--------|
| | | | S-phase fraction | PI | Ploidy |
| B | 0.1 | 0.1 | 4.1 | 6.1 | d |
| B | 0.1 | 0.4 | 6.1 | 6.4 | d |
| B | 0.1 | 0.8 | 6.6 | 7.1 | d |
| B | 0.1 | 1.1 | 21.7 | 22.6 | d |
| B | 0.1 | 1.5 | 7.7 | 12.1 | d |
| B | 0.1 | 3.5 | 14.8 | 15.0 | d |
| B | 0.1 | 3.5 | 21.7 | 23.1 | a |
| B | 0.1 | 3.5 | 22.2 | 22.2 | d |
| B | 0.1 | 4.0 | 4.9 | 7.6 | d |
| B | 0.1 | 10.2 | 16.1 | 21.3 | a |
| B | 0.1 | 25.0 | 12.3 | 20.1 | d |
| B | 0.2 | 2.5 | 4.2 | 6.2 | d |
| B | 0.3 | 1.8 | 3.4 | 8.5 | d |
| B | 0.3 | 3.1 | 20.1 | 20.8 | d |
| B | 0.4 | 4.2 | 4.5 | 10.3 | d |
| B | 0.4 | 4.5 | 6.6 | 9.5 | d |
| B | 0.4 | 25.0 | 4.7 | 4.8 | d |
| B | 0.6 | 3.8 | 3.0 | 3.0 | d |
| B | 0.6 | 5.2 | 11.2 | 14.7 | d |
| B | 0.7 | 1.0 | 6.4 | 8.7 | d |
| B | 0.7 | 2.5 | 6.7 | 9.7 | d |
| B | 0.8 | 2.5 | 0.5 | 3.5 | d |
| B | 0.9 | 10.5 | 4.6 | 5.3 | d |
| B | 1.1 | 3.0 | 7.1 | 8.9 | d |
| B | 1.3 | 3.2 | 4.2 | 4.4 | d |
| B | 1.6 | 5.7 | 19.1 | 19.1 | d |
| B | 2.4 | 1.8 | 2.7 | 10.0 | d |
| A | 0.2 | 3.2 | 4.4 | 5.0 | d |
| A | 1.2 | 10.2 | 2.4 | 3.7 | d |
| A | 1.4 | 22.5 | 15.8 | 24.5 | a |
| A | 1.7 | 35.0 | 9.2 | 9.6 | d |
| A | 1.8 | 4.5 | 9.4 | 12.9 | d |
| A | 2.1 | 25.0 | 20.6 | 27.4 | a |
| A | 2.1 | 37.0 | 4.3 | 11.3 | d |
| A | 3.3 | 19.1 | 8.7 | 9.0 | d |
| A | 3.6 | 10.5 | 14.8 | 22.2 | d |
| A | 4.2 | 25.0 | 7.8 | 12.5 | d |
| A | 6.1 | 35.0 | 3.9 | 7.9 | d |
| A | 10.1 | 41.0 | 10.7 | 11.1 | d |
| A | 11.2 | 20.0 | 13.0 | 15.9 | d |
| A | 15.1 | 32.0 | 7.0 | 8.1 | d |
| M | 0.1 | 5.6 | 13.1 | 13.1 | d |
| M | 0.6 | 37.0 | 6.6 | 7.8 | d |
| M | 4.8 | 8.5 | 12.3 | 21.1 | a |
| M | 4.9 | 1.8 | 11.4 | 12.9 | d |
| M | 9.4 | 22.0 | 15.4 | 15.9 | d |
| M | 25.0 | 30.5 | 11.6 | 12.4 | d |

Mean ± SD 2.61 ± 4.68 11.9 ± 12.52 9.57 ± 5.91 12.24 ± 6.48
 * : B : benign A : atypical
 M : malignant FCM : flow cytometry
 PCNA : proliferating cell nuclear antigen
 SI : staining index PI : proliferative index(%S+G₂M)
 d : diploidy a : aneuploidy SD : standard deviation

Table 2. Ki-67 staining index, Proliferating cell nuclear antigen staining index, S-phase fraction, Proliferative index, according to histologic grade*

| | Histology | Mean ± SD |
|--|-----------|---------------|
| Ki-67 staining index ^a | B | 0.51 ± 0.56 |
| | A | 4.57 ± 4.46 |
| | M | 7.47 ± 9.23 |
| | A+M | 5.45 ± 6.26 |
| | Total | 2.61 ± 4.68 |
| Proliferating cell nuclear antigen staining index ^b | B | 4.96 ± 6.28 |
| | A | 22.86 ± 12.33 |
| | M | 17.57 ± 14.41 |
| | A+M | 21.27 ± 12.84 |
| | Total | 11.9 ± 12.52 |
| S-phase fraction ^c | B | 9.16 ± 6.74 |
| | A | 9.43 ± 5.18 |
| | M | 11.73 ± 2.90 |
| | A+M | 10.12 ± 4.67 |
| | Total | 9.57 ± 5.91 |
| Proliferative index ^d | B | 11.51 ± 6.60 |
| | A | 12.94 ± 7.17 |
| | M | 13.87 ± 4.40 |
| | A+M | 13.22 ± 6.36 |
| | Total | 12.24 ± 6.48 |

* : B : benign
M : malignant
a : p=0.0001
c : p=0.2585
A : atypical,
SD : standard deviation,
b : p=0.0001
d : p=0.2120(Wilcoxon rank sum test)

Ki - 67
(Wicoxon rank sum test, p=0.0001)
(Table 2). Ki - 67 가 (prolife-
rative activity)
가

2. PCNA

47 PCNA immunoperoxidase
0.1~41.0% (median,
4.8%), 11.9 ± 12.52%
PCNA 4.96 ± 6.28%
PCNA
22.86 ± 17.57%,
17.57 ± 14.41%
21.27 ± 12.84%
PCNA
가

PCNA
가 , PCNA
(Wilcoxon rank sum test, p=0.0001)(Table 2).

PCNA

3. FCM

0.5~22.2%
(median, 7.7%), 9.57 ± 5.91%
9.16 ± 6.74%
9.43 ± 5.18%, 11.73 ± 2.90% , 10.12 ± 4.67%
/ 가 (Wilcoxon rank
sum test, p=0.2585)(Table 2).

47 (PI) 3.0~
27.4%(median, 10.3) , 12.24 ± 6.48%
11.51 ± 6.60%
12.94 ± 7.17%, 13.87 ± 4.40%
13.22 ± 6.36%
(; 3.0~23.1%,
; 3.7~27.4%, ; 7.8~27.4%). Wilcoxon rank
sum test
(p=0.2120)(Table 2).

(parameter)가 /
(89.4%),
47 5 (10.6%)
27 2 (7.4%), 14
2 (14.3%), 6 1
(16.7%) 20
3 (15%)가
12.0% 21.0%

Table 3. Ploidy pattern according to histologic grade*

| Histology | Diploidy(%) | Aneuploidy(%) | total |
|-----------|-------------|---------------|-------|
| B | 25(92.6) | 2(7.4) | 27 |
| A | 12(85.7) | 2(14.3) | 14 |
| M | 5(83.3) | 1(16.7) | 6 |
| A+M | 17(85.0) | 3(15.0) | 20 |
| Total | 42(89.4) | 5(10.6) | 47 |

* : B ; benign, A : atypical, M : malignant
 * : The difference between B and A+M was not statistically significant(chi-square test ; p=0.40)

Table 4. Ki-67 staining index, Proliferating cell nuclear antigen staining index according to ploidy pattern

| | Histology | Mean ± SD |
|---|------------|---------------|
| Ki-67 staining index according to ploidy pattern ^a | Aneuploidy | 1.7 ± 1.93 |
| | Diploidy | 2.71 ± 4.91 |
| PCNA staining index according to ploidy pattern ^b | Aneuploidy | 13.94 ± 9.33 |
| | Diploidy | 11.65 ± 12.92 |

SD : standard deviation
 PCNA : Proliferating cell nuclear antigen
 a : p=0.6384, b : p=0.3084(Wilcoxon rank sum test)

(chi - square test, p=0.404)(Table 3).

DNA

(indicator)

Ki - 67 5 0.1
 ~4.8%(median, 1.4%), 1.7 ± 1.93% , 42
 Ki - 67
 (nuclear fraction) 0.1~25%(median, 0.7%), 2.71%
 Ki - 67
 (Wilcoxon rank sum test, p=0.6384)(Table 4). 5

PCNA 3.5~25.0%

(median, 10.2%), 13.94% , 42
 PCNA 0.1~41.0%(median, 4.35%), 11.65 ± 12.92%
 PCNA
 (Wilcoxon rank sum test, p=0.3084)(Table 4).

Ki - 67 PCNA
 가

4. Correlation

Ki - 67 PCNA
 (linear correlation)가 (Spearman rank - order correlation, p=0.0001), Ki - 67
 (Spearman rank - order correlation, p=0.9710). Scatterplot

with regression line PCNA

(Spearman rank - order correlation, p=0.1778, 0.1328).

고 찰

가 가 ,
 (tumor histology) [3H]thymidine uptake
 , Ki - 67 AgNOR , BUdR
 , PCNA
 logy) 가 가 (tumor bio-24)26)27).

1. Ki-67
 Gerdes 9) Ki - 67 (monoclonal antibody) 10 (marker)
 . Ki - 67 G₁, S, G₂, M (nuclear antigen) G₀
 (growth fraction)
 13)15)18)22)27)29)

Ki - 67 가 가
 3)15)20)23)27). Cremerius 3)
 Ki - 67 가 가(2%
) , Kolles 15) Ki - 67 가
 (Ki - 67 : 11%)
 (Ki - 67 : 0.7%)

가 Nakasu 23) 120
 Ki - 67 MIB - 1
 Ki - 67

Ki - 67 가
 ±0.56%, Ki - 67 0.51
 4.57 ± 4.46%, 7.47 ± 9.23%
 Ki - 67 5.45 ± 6.26%
 가 Ki - 67 2.5%
 가 가

가

2)¹²)³⁰ Karamitopoulou ¹²) 51 PCNA
 $3.80 \pm 7.35\%$

Ki-67
¹³)²²) Moller ²²)
 Ki-67 가
 (0.6%, 1.6%, 3.1%)
 가

PCNA
 PCNA (over-expression)
 PCNA (immunoreactivity)

가 Zimmer ³⁰)
 PCNA
 / PCNA
 20%

Ki-67
³)¹⁶)¹⁸)²⁰)²¹)²⁷)²⁹) Matsuno ²⁰)
 MIB-1
 가 , Shibuya ²⁷) Ki- 가
 BUdR 가 4 PCNA
 . Langford ¹⁸) BUdR MIB-1 가

67 Burns ²) 5
 PCNA 가

MIB-1
 가 PCNA
⁴)¹⁰)¹¹)²²)²⁸) Shih ²⁸)
 가 PCNA 가

Ki-67 PCNA
 Moller ²²) PCNA
 가 , Ki-67
 PCNA 가 PCNA (hemangiopericytoma)
 Meixensberger ²¹) PCNA 가 Moller ²²)
 Ki-67 가 (2.6%, 9.7%, 19.9%). Hsu ¹⁰)¹¹)
 , 5 Ki-67 가 0.1~ PCNA 가
 4.8% (median, 1.4%), 1.7 ± 1.93% 1.16 ± 0.29%, 14.14 ± 2.07%, 21.37 ± 5.47%
 , 42 Ki-67 (median, 0.21%, 2.45 ± 0.16%).
 1.7 ± 1.93% 0.1~25.0% (median, 0.56 ±
 0.7%), Ki-67 0.21%, 2.45 ± 0.16%).
 . Yasue ²⁹)
 MIB-1 4.96 ± 6.28% , PCNA
 가 , 22.86 ± 17.57%, 17.57 ± 14.41%
 Ki-67 PCNA
 가

2. PCNA
 PCNA 36-kD
 , G1 S
 DNA DNA polymerase -
 (cofactor) ²)⁴)⁷)¹⁰ - ¹²)¹⁷)²²)²⁶)²⁸)³⁰)
 PCNA PCNA
 가 PCNA
 가 PCNA

PCNA, Moller²²⁾ PCNA Ki-67
 가 가 , Kujas¹⁶⁾ Ki-67
 67 PCNA 가
 Ki-67 PCNA
 PCNA
 3.5~25.0%, 13.94 ± 9.33%, 42
 0.1~41.0%, 11.65 ± 12.92%

3. FCM

¹⁾⁷⁾²⁹⁾ Appley¹⁾ 19 2 (10.5%)
 , Yasue²⁹⁾ 29 4 (13.8%)
 DNA , Garcia⁷⁾
 87 30 (34.5%)
 47 5 (10.6%)

esens¹⁹⁾ DNA
 DNA , Garcia
⁷⁾ 87 (S-phase to cell cycle)
 DNA 34.5%
 Nishizaki²⁴⁾ 13
 3 (23%) Finn
⁶⁾ 22 (angioblastic)
 DNA (hemangiopericytoma)
 DNA
 27
 2 (7.4%)
 14 2 (14.3%), 6
 20 1 (16.7%)
 3 (15%)

DNA
 DNA
¹⁴⁾²¹⁾²⁵⁾ Meixensberger
²¹⁾ DNA 134
 DNA
 DNA
 가 가 가
 DNA
 가
 Nishizaki²⁵⁾ DI
 , DI 가
 DI
 Kasai¹⁴⁾ DNA
 , DI 가
 가 ⁵⁾⁶⁾ Finn⁶⁾
 22 (hemangioperi-
 cytoma)
 S-phase 9%
 11% , Detta⁵⁾
 2.5
 Gracia⁸⁾
 S-phase 20%
 S-phase 0.5%
 22.2% 9.57 ± 8.91%
 S-phase 9.16 ± 6.74%
 5.18%, 11.73 ± 2.90%
 10.12 ± 4.67%
 가
 47 3.0% 27.4%
 12.24 ± 6.48%
 11.81 ± 6.60% , 12.94 ± 7.17%,
 13.87 ± 4.40%
 13.22 ± 6.36%

(3.0~23.1%, 3.7~27.4%, 7.8~27.4%).

PCNA, Ki-67

()

DNA, Ki-67, PCNA

가 Ki-67 PCNA

가 Ki-67 PCNA

Scatter plot with regression line

Ki-67 PCNA

가

가

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References

- 1) Appley AJ, Fitzgibbons PL, Chandrasoma PT, Hinton DR, Apuzzo ML : *Multiparameter flow cytometric analysis of neoplasms of the central nervous system : correlation of nuclear antigen P105 and DNA content with clinical behavior. Neurosurgery 27 : 83-96, 1990*
- 2) Burns DK, Mickey BE, Bigio EH : *Proliferating cell nuclear antigen (PCNA) expression in human gliomas and meningiomas. J Neuropathol Exp Neurol 51 : 362-367, 1992*
- 3) Cremerius U, Striepecke E, Henn W, Weis J, Mull M, Lippitz B, et al : *18FDG-PET in intracranial meningiomas versus grading, proliferation index, cellular density and cytogenetic analysis. Nuklearmedizin 33 : 144-149, 1994*
- 4) Demirtas E, Yilmaz F, Ovul I, Oner K : *Recurrence of meningiomas versus proliferating cell nuclear antigen (PCNA) positivity and AgNOR counting. Acta Neurochir 138 : 1456-1463, 1996*
- 5) Detta A, Kenny BG, Smith C, Logan A, Hitchcock E : *Correlation of proto-oncogene expression and proliferation in meningiomas. Neurosurgery 33 : 1065-1074, 1993*
- 6) Finn WG, Goolsby CL, Rao MS : *DNA flow cytometric analysis of hemangiopericytoma. Am J Clin Pathol 101 : 181-185, 1994*
- 7) Garcia RL, Coltrera MD, Gown AM : *Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissues. Comparison with flow cytometric analysis. Am J Pathol 134 : 733-739, 1989*
- 8) Garcia R, Bueno A, Castanon S, Ruiz-Barnes P, Maria de Campos J, Kusak E, et al : *Study of the DNA content by flow cytometry and proliferation in 281 brain tumors. Oncology 54 : 112-117, 1997*
- 9) Gerdes J, Schwab U, Lemke H, Stein H : *Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31 : 13-20, 1983*
- 10) Hsu DW, Hakim F, Biller BM, de la Monte S, Zervas NT, Klibanski A, et al : *Significance of proliferating cell nuclear antigen index in predicting pituitary adenoma recurrence. J Neurosurg 78 : 753-761, 1993*
- 11) Hsu DW, Pardo FS, Efirid JT, Linggood RM, Hedley-Whyte ET : *Prognostic significance of proliferative indices in meningiomas. J Neuropathol & Exp Neurol 53 : 247-255, 1994*
- 12) Karamitopoulou E, Perentes E, Melachrinou M, Maraziotis T : *Proliferating cell nuclear antigen immunoreactivity in human central nervous system neoplasms. Acta Neuropathol (Berl) 85 : 316-322, 1993*
- 13) Karamitopoulou E, Perentes E, Diamantis I, Maraziotis T : *Ki-67 immunoreactivity in human central nervous system tumors : A study with MIB-1 monoclonal antibody on archival*

- materials. *Acta Neuropathol (Berl)* 87 : 47-54, 1994
- 14) Kasai H, Kawamoto K : Cytogenical analysis of brain tumors by FISH (fluorescence in situ hybridization) and FCM (flow cytometry). *Noshuyo Byori* 12 : 75-82, 1995
 - 15) Kolles H, Niedermayer I, Schmitt C, Henn W, Feld R, Steudel WI, et al : Triple approach for diagnosis and grading of meningiomas : histology, morphometry of Ki-67/Feulgen stainings, and cytogenetics. *Acta Neurochir (Wien)* 137 : 174-181, 1995
 - 16) Kujas M, Rateau JG, Bruch JF, Van Effenterre R : Flow cytometry in the study of meningiomas. Preliminary results and attempt at clinical correlation. *Archives d Anatomie et de Cytologie Pathologiques* 44 : 77-82, 1996
 - 17) Landberg G, Roos G : Antibodies to proliferating cell nuclear antigen as S-phase probes in flow cytometric analysis. *Cancer Res* 51 : 4570-4574, 1991
 - 18) Langford LA, Cooksley CS, DeMonte F : Comparison of MIB-1 (Ki-67) antigen and bromodeoxyuridine proliferation indices in meningiomas. *Human Pathol* 27 : 350-354, 1996
 - 19) Mathiesen T, von Holst H, Askensten U, Collins PV : DNA-determination in the clinical management of patients with meningioma or hemangioblastoma. *Br J Neurosurg* 3 : 575-580, 1989
 - 20) Matsuno A, Fujimaki T, Sasaki T, Nagashima T, Ide T, Asai A, et al : Clinical and histopathological analysis of proliferative potentials of recurrent and non-recurrent meningiomas. *Acta Neuropathol (Berl)* 91 : 504-510, 1996
 - 21) Meixensberger J, Janka M, Zellner A, Roggendorf W, Roosen K : Prognostic significance of nuclear DNA content in human meningiomas : A prospective study. *Acta Neurochirurgica-Supplementum* 65 : 70-72, 1996
 - 22) Moller ML, Brandstrup O : No prediction of recurrence of meningiomas by PCNA and Ki-67 immunohistochemistry. *J Neuro-Oncol* 34 : 241-246, 1997
 - 23) Nakasu S, Nakajima M, Matsumura K, Nakasu Y, Handa J : Meningioma : proliferating potential and clinicoradiological features. *Neurosurgery* 37 : 1049-1055, 1995
 - 24) Nishizaki T, Orita T, Furutani Y, Ikeyama Y, Aoki H, Sasaki K : Flow cytometric DNA analysis and immunohistochemical measurement of Ki-67 and BUdR labeling indices in human brain tumors. *J Neurosurg* 70 : 379-384, 1989
 - 25) Nishizaki T, Ohshita N, Nagatsugu Y, Orita T, Ito H, Sasaki K : Clinical evaluation of DNA index in human brain tumors. *J Neuro-Oncol* 17 : 9-13, 1993
 - 26) Sasaki A, Naganuma H, Kimura R, Isoe S, Nakano S, Nukui H, et al : Proliferating cell nuclear antigen (PCNA) immunostaining as an alternative to bromodeoxyuridine (BrdU) immunostaining for brain tumors in paraffin embedded sections. *Acta Neurochir (Wien)* 117 : 178-181, 1992
 - 27) Shibuya M, Ito S, Miwa T, Davis RL, Wilson CB, Hoshino T : Proliferative potential of brain tumors : analysis with Ki-67 and anti-DNA polymerase alpha monoclonal antibodies, bromodeoxyuridine labeling, and nucleolar organizer region counts. *Cancer* 71 : 199-206, 1993
 - 28) Shih YH, Kadota Y, Sato K, Sueyoshi N, Shirai T : Immunohistochemical expression of proliferating cell nuclear antigen (PCNA) in intracranial glioma and meningioma. *Chung Hua i Hsueh Tsa Chih - Chinese Medical Journal* 53 : 193-197, 1994
 - 29) Yasue M, Akasaki Y, Numoto TR, Abe S, Abe T, Takeuchi Y, et al : MIB-1 immunostaining and DNA flowcytometry in meningiomas. *Noshuyo Byori* 13 : 17-20, 1996
 - 30) Zimmer C, Gottschalk J, Cervos-Navarro J, Martin H, Beil M, Jautzke G : Proliferating cell nuclear antigen (PCNA) in atypical and malignant meningiomas. *Path Res Pract* 188 : 951, 958, 1992