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# Evaluation of the malignancy of glioma using $^{11}$C-methionine positron emission tomography and proliferating cell nuclear antigen staining

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**Abstract** $^{11}$C-methionine positron emission tomography (PET) and proliferating cell nuclear antigen (PCNA) staining were performed in 13 cases of glioma to investigate the relationship between the uptake of L-[methyl]-$^{11}$C-methionine and the degree of malignancy and proliferative potential. The $^{11}$C-methionine uptake was significantly greater in high-grade gliomas compared to low-grade gliomas ($P<0.05$). The PCNA indexes were also significantly higher in the high-grade cases ($P<0.05$). Moreover, a strong positive correlation was found between the $^{11}$C-methionine values and the PCNA indexes ($P<0.005$), demonstrating that higher $^{11}$C-methionine uptake was associated with greater proliferative potential and greater malignancy. $^{11}$C-methionine PET is a potentially useful preoperative method to discriminate the malignacy of glioma.

**Key words** $^{11}$C-methionine · PET · PCNA · Glioma

## Introduction

Positron emission tomography (PET) has been used to investigate the malignancy, extent of spread, effectiveness of therapy, and the prognosis of brain tumors using various tracers to measure blood flow, amino acid metabolism, and glucose metabolism [2, 8, 10]. Various studies have found a correlation between tumor grade and $^{11}$C-methionine PET findings [4, 7, 21, 24]. However, the correlation between PET findings and histological indications of malignancy remains unclear. Moreover, the involvement of $^{11}$C-methionine in intracellular amino acid metabolism, particularly as related to protein and nucleic acid synthesis, remains controversial [14, 21, 25, 27]. Consequently, $^{11}$C-methionine PET studies have been used primarily for determining the extent of tumor spread [2, 10, 11, 22–24, 27].

Immunohistological studies have clearly demonstrated the effectiveness of markers such as Ki-67 [12, 26], bromodeoxyuridine [6], and PCNA [1] for the evaluation of the malignancy and prognosis of brain tumors [1, 6, 9, 13, 17–19]. PCNA is thought to be particularly effective as an indicator of brain tumor malignancy, since it can easily be used to evaluate the spread of brain tumors in paraffin sections [1, 17].

We investigated the correlation between the proliferative capacity of brain tumors as determined by the PCNA index and amino acid metabolism measured by $^{11}$C-methionine PET to assess the usefulness of this method for the preoperative diagnosis of the malignancy of brain tumors.

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## Materials and methods

This study included 13 patients with glioma who underwent preoperative magnetic resonance imaging (MRI) and $^{11}$C-methionine PET studies in our department since 1992. All patients underwent either excision of the tumor or biopsy.The tumors were classified according to the World Health Organization (WHO) classification of neuroepithelial tumors as one pilocytic astrocytoma, two fibrillary astrocytomas, one gemistocytic astrocytoma, one oligodendroglioma, one oligoastrocytoma, one gliomatosis cerebri, one anaplastic ependymoma, one anaplastic astrocytoma, and four glioblastomas. To compare the proliferative capacity, tumors in WHO grades 1 and 2 (seven cases) were classified as low-grade gliomas, and tumors in grades 3 and 4 (six cases) as high-grade gliomas.

$^{11}$C-methionine PET was performed using a Headtome IV (Shimadzu, Kyoto, Japan) located at the Cyclotron Center of Iwate Medical University. 12.27 kBq/kg of $^{11}$C-methionine (produced in a baby cyclotron) was injected intravenously and PET performed after 30 min of stabilization. This PET scanner has a 4.5-mm (full-width half-maximum) spatial resolution and a slice thickness of 6.5 mm. The four-surface detector ring with seven rows of image units was moved in increments of 6.5 mm, allowing 14 cross-section images to be obtained simultaneously [16]. The local radioactivity count was measured in a region of interest of 20-mm diame-

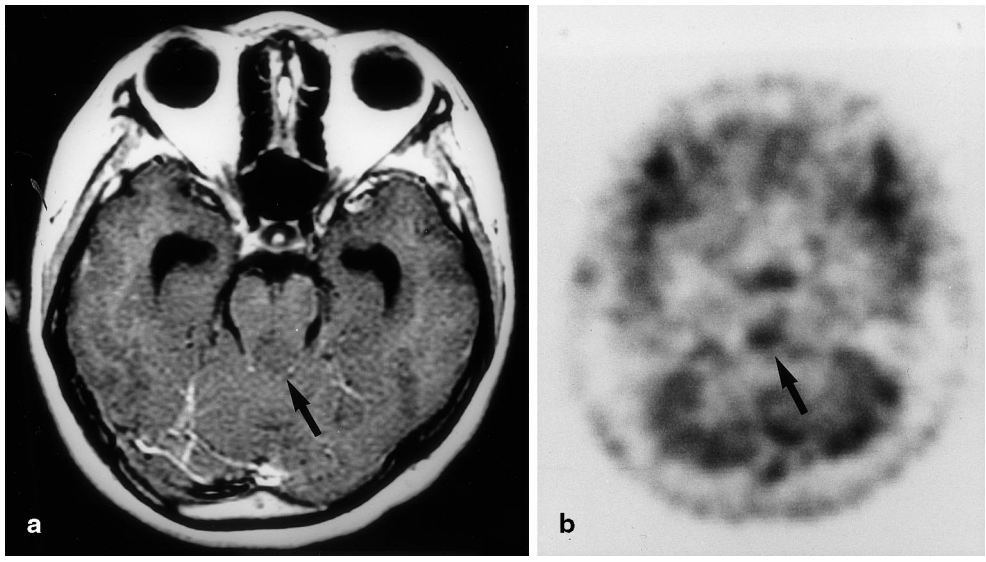


**Fig. 1a, b** A 19-year-old woman with pilocytic astrocytoma in the tectum. **a** MRI showing no enhancement in the lesion after infusion of gadolinium-DTPA. **b** $^{11}$C-methionine PET scan showing uptake in the lesion was at the same level as that of the surrounding normal tissue. The DUR of the tumor was 2.01, the DUR(T/N ratio) was 1.11, and the PCNA index was 0.1

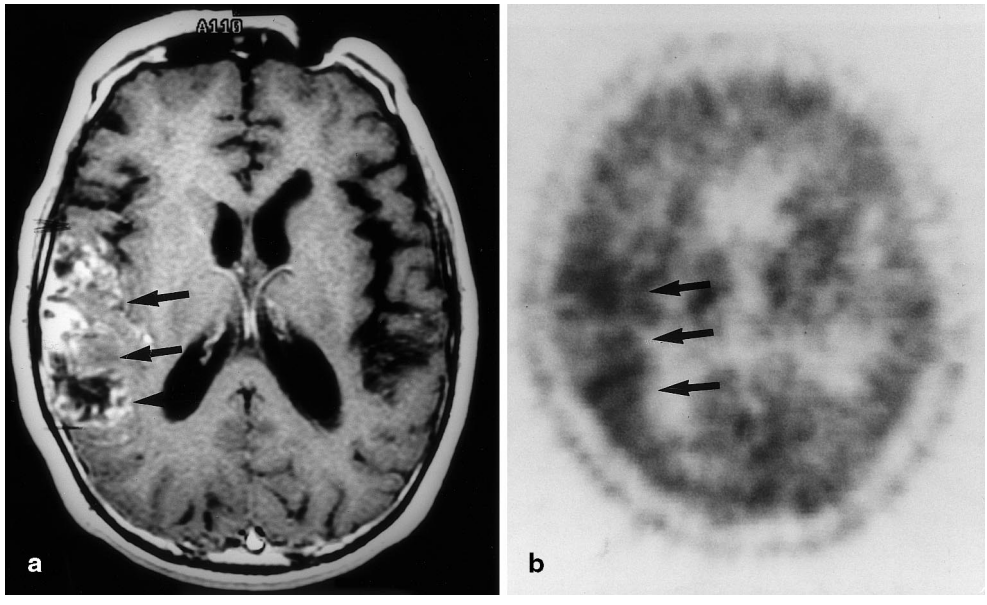


**Fig. 2a, b** A 76-year-old man with oligodendroglioma in the right temporal lobe. **a** MRI with gadolinium showing the mass lesion in the right temporal lobe is markedly enhanced. **b** $^{11}$C-methionine PET scan showing markedly increased uptake of $^{11}$C-methionine beyond the area of the abnormalities on the MRI and at the same level as that of the high-grade gliomas. The DUR of the tumor was 3.34, the DUR(T/N ratio) was 2.21, and the PCNA index was 3.7

ter, which included the region of maximal count in the PET image. The uptake of $^{11}$C-methionine was calculated as the differential uptake ratio (DUR) using the following equation: DUR=(pixel count/pixel volume)/(injected radioisotope activity/body weight) ± calibration factor.

Moreover, the ratio of the DUR on the side of the lesion to that in the normal, contralateral cortex (the T/N ratio) was also calculated. The PCNA index was measured using anti-PCNA monoclonal antibody immunostaining by the avidin-biotin-peroxidase complex method of Hsu [15]. Tumor tissue removed at surgery was placed in a 10% formalin solution for 24 h and 3-µm-thick paraffin sections were prepared. After deparaffinization of the sections, they were immersed in 0.3% $H_2O_2$ to remove inherent peroxidase. After washing in phosphate-buffered saline (PBS), sections were treated for 30 min at room temperature with tenfold diluted normal rabbit serum (Dakopatts) and reacted overnight at 4°C with 50-fold diluted PCNA mouse monoclonal antibody (PC 10, Novocastra Co.) as the primary antibody. After washing in PBS, sections were reacted for 30 minutes at room temperature with peroxidase standard streptoavizin (Nichirei) and then stained with 0.005% $H_2O_2$ diaminobenzidine solution (20 mg in 0.05 M Tris-HCl buffer, pH 7.6) and contrast-stained with hematoxylin. Approximately 2000 cells were examined at 400-fold magnification under a light microscope and the percentage of positive PCNA cells was determined [1]. Differences in values between the low- and high-grade gliomas were evaluated using Student's *t*-test, and correlations were evaluated with Spearman's correlation coefficient.

## Results

Study of the individual $^{11}$C-methionine PET findings showed that, in three of the seven cases of low-grade tumor, $^{11}$C-methionine uptake was seen at the site of the tumor, whereas in four cases the uptake was similar to that of the surrounding normal tissue (Fig. 1). The $^{11}$C-methionine uptake of 14.3% of low-grade cases was comparable to that seen in some of the high-grade cases (Fig. 2). In contrast, all six high-grade cases showed $^{11}$C-methionine uptake at the site of the tumor (Fig. 3). The

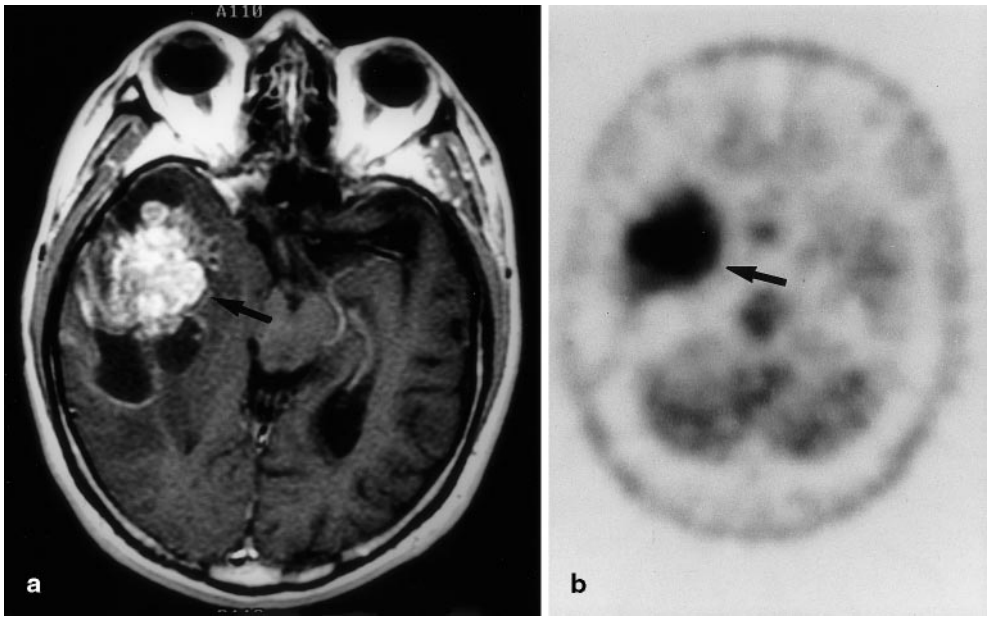


**Fig. 3a, b** A 56-year-old man with glioblastoma in the right temporal lobe. **a** MRI with gadolinium showing the mass lesion in the right temporal lobe is markedly enhanced. **b** $^{11}$C-methionine PET scan showing markedly increased uptake of $^{11}$C-methionine beyond the area of the abnormalities on the MRI. The DUR of the tumor was 3.76, the DUR(T/N ratio) was 2.58, and the PCNA index was 28.1

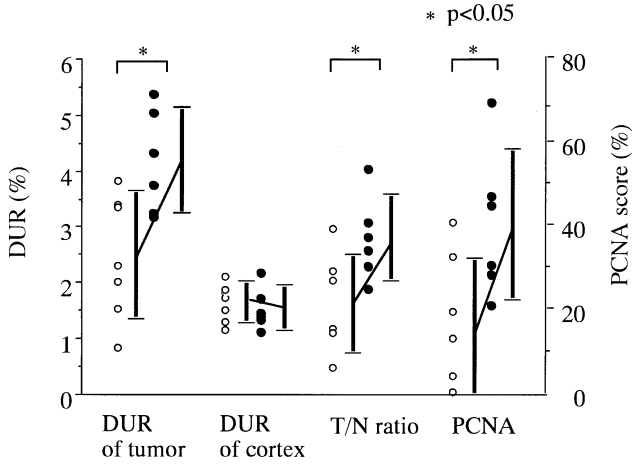


**Fig. 4** Comparisons of DUR (differential uptake ratio) and PCNA indexes in low-grade gliomas (*open circles*) and high-grade gliomas (*closed circles*). The mean DUR of the contralateral cortex were not significantly different between low-grade cases and high-grade cases. The mean DUR of the tumor, T/N ratio, and PCNA indexes were significantly higher in high-grade cases ($P$<0.05)

mean DUR was 2.46±1.1 in low-grade cases, and significantly higher ($P$<0.05) in high-grade cases (mean DUR=4.16±0.9). Moreover, the T/N ratio showed a significant difference ($P$<0.05) between 1.58±0.8 in low-grade cases and 2.79±0.7 in high-grade cases (Fig. 4).

The mean PCNA index of the low-grade cases was 15.5±16.0%, whereas that of the high-grade cases was 39.9±17.5% ($P$<0.05) (Fig. 4). The range of PCNA indexes was large in both groups. The low-grade cases had indexes ranging from 0.1 to 40.5%, whereas the high-grade cases had indexes between 20 and 70% (Fig. 4). There was a positive correlation between the DUR and the PCNA indexes (R∧2=0.397). A significant positive correlation was seen between the T/N ratios and the PCNA indexes (R∧2=0.677, $P$<0.005) (Fig. 5).

## Discussion

Previous $^{11}$C-methionine PET studies have found significantly more $^{11}$C-methionine uptake in high-grade glio-

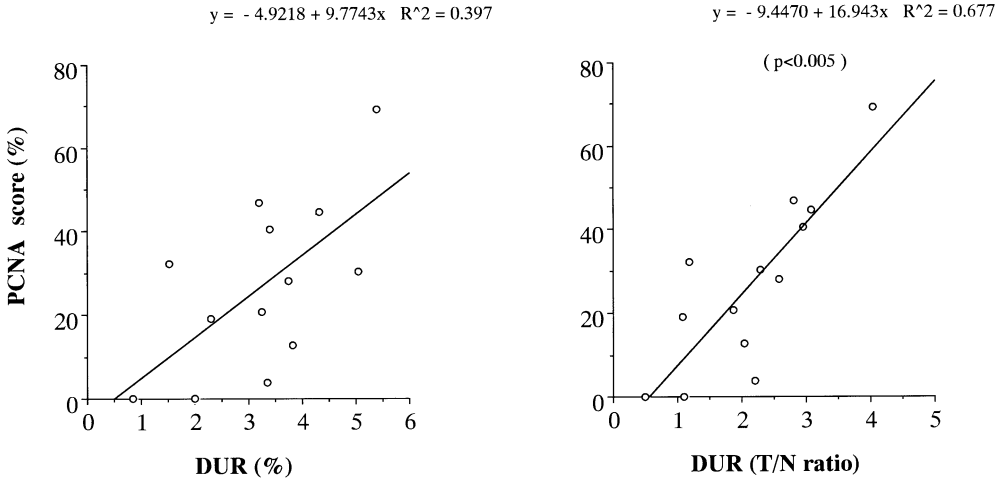


**Fig. 5** Correlations between DUR and PCNA indexes (*left*), and T/N ratio and PCNA indexes (*right*). A significant positive correlation is seen between the T/N ratios and the PCNA indexes (R=0.677, $P$<0.005)

mas than in low-grade ones [20, 7]. However, this difference$^{11}$C does not allow evaluation of malignancy in individual cases using only this criterion [24]. The present study also found significantly higher DUR and T/N ratios in high-grade cases compared to low-grade ones. However, study of individual cases showed 14.3% of low-grade glioma cases to have high DUR and T/N values. Evaluation of malignancy using morphological considerations commonly finds large differences in prognosis in cases classified into the same histological group. Low-grade gliomas often cannot be distinguished either clinically or histologically from high-grade gliomas, so high values for the DUR or T/N ratio may be indicative of high-grade glioma.

The correlation between PCNA index and histological malignancy and prognosis in patients with cerebral glioma is well-known [1, 3, 5, 6, 9, 13, 17–19, 28]. Significant differences in PCNA index between low- and high-grade glioma and astrocytic tumor have been reported [1]. The present study showed a clear positive correlation between PCNA index and both DUR and T/N ratios, indicating that $^{11}$C-methionine is taken up more rapidly and accumulates in highly proliferative tissues. These results also indicate that cases which were histologically classified as low-grade glioma but showed high levels of $^{11}$C-methionine uptake were in fact high-grade gliomas in terms of proliferative capacity.

Previous studies on the correlation between the uptake of $^{11}$C-methionine and malignancy of tumor tissue were based only on histological classification. Our study shows that both $^{11}$C-methionine PET and PCNA indexes give similar indications of the biological character of the tumor tissue. We conclude that $^{11}$C-methionine PET can provide valuable information concerning the proliferative capacity of the tumor for the preoperative diagnosis. The present preliminary study requires a follow-up study and investigation of the final prognoses in a larger number of patients. In addition, the present findings apply only to cerebral gliomas, so similar studies on other tumor types will be necessary.

## References


1. Beppu T, Arai H, Kanaya H, Sasaki K (1992) Measurement of PCNA labeling index in astrocytic tumors. No Shinkei Geka 20:1255–1259
2. Bergström M, Collins VP, Ehrin E, Ericson K, Eriksson L, Greitz T, Halldin C, von Holst H, Långström B, Lilja A, Lundqvist H, Nagren K (1983) Discrepancies in brain tumor extent as shown by computed tomography and positron emission tomography using 68Ga EDTA, $^{11}$C glucose, and $^{11}$C methionine. J Comput Assist Tomogr 7:1062–1066
3. Bravo R, Fey SJ (1981) Identification of a nuclear and a cytoplasmic polypeptide whose relative proportions are sensitive to changes in the rate of cell proliferation. Exp Cell Res 136:311–319
4. Bustany P, Henry JF, Sargent T, Zarifian E, Cabanis E, Collard P, Comar D (1983) Local brain protein metabolism in dementia and schizophrenia: in vivo studies with 11CL-methionine and positron emission tomography. In: Positron emission tomography of the brain (Heiss WD, Phelps ME, eds), Springer-Verlag, Berlin, pp 208–211
5. Celis JE, Celis A (1985) Cell-cycle-dependent variations in the distribution of the nuclear protein cyclin (proliferating cell nuclear antigen) in cultured cells: subdivision of S phase. Proc Natl Acad Sci U S A 82:3262–3266
6. Coltrera MD, Gown AM (1991) PCNA/cyclin expression and BrdU uptake define different subpopulations in different cell lines. J Histochem Cytochem 39:23–30
7. Derlon JM, Bourdet C, Bustany P, Chatel M, Theron J, Darcel F, Syrota A (1989) 11CL-methionine uptake in gliomas. Neurosurgery 25:720–728
8. Di Chiro G, DeLaPaz RL, Brooks RA, Sokoloff L, Kornblith PL, Smith BH, Patronas NJ, Kufta CV, Kessler RM, Johnston GS, Manning RG, Wolf AP (1982) Glucose utilization of cerebral gliomas measured by 18F-fluorodeoxyglucose and positron emission tomography. Neurology 32:1323–1329
9. Dierendonck JH, Wijsman JH (1991) Cell-cycle-related staining patterns of antiproliferating cell nuclear antigen monoclonal antibodies. Am J Pathol 138:1165–1172
10. Ericson K, Lilja A, Bergström M, Collins VP, Eriksson L, Ehrin E, von Holst H, Lundqvist H, Långström B, Mosskin M (1985) Positron emission tomography with ($^{11}$C methyl)-L-methionine, 11CD-glucose, and 68Ga EDTA in supratentorial tumors. J Comput Assist Tomogr 9:683–689
11. Ericson K, Blomqvist G, Bergström M, Eriksson L, Stone-Elander S (1987) Application of a kinetic model on the methionine accumulation in intracranial tumors studied with positron emission tomography. Acta Radiol 28:505–509
12. Gerdes J, Lemke H, Baisch H (1984) Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133:1710-1715
13. Hall PA, Levison DA (1990) Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 162:285–294
14. Hatazawa J, Ishiwata K, Itoh M, Kameyama M, Kubota K, Ido T, Matsuzawa T, Yoshimoto T, Watanuki S, Seo S (1989) Quantitative evaluation of L-methyl-C-11 methionine uptake in tumor using positron emission tomography. J Nucl Med 30:1809–1813
15. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedure. J Histochem Cytochem 29:577–580
16. Iida H, Miura S, Kanno I (1989) Design and evaluation of Headtome IV, a wholebody positron emission tomograph. IEEE Trans Nucl Sci 37:1006–1010
17. Kim DK, Hoyt J, Bacchi C, Keles GE, Mass M, Mayberg MR, Berger MS (1993) Detection of proliferating cell nuclear antigen in gliomas and adjacent resection margins. Neurosurgery 33:619–626
18. Kunishio K, Mishima N, Matsuhisa T (1990) Immunohistochemical demonstration of DNA polymerase a in human brain tumor cells. J Neurosurg 72:268–272
19. Landberg G, Roos G (1991) Antibodies to proliferating cell nuclear antigen as S-phase probes in flow cytometric cell cycle analysis. Cancer Res 51:4570–4574
20. Lilja A, Bergström K, Hartvig P, Spannare B, Halldin C, Lundqvist H, Långström B (1985) Dynamic study of supratentorial gliomas with L-methyl-$^{11}$C-methionine and positron emission tomography. AJNR Am J Neuroradiol 6:505–514
21. Lilja A, Lundqvist H, Olsson Y, Spannare B, Gullberg P, Långström B (1989) Positron emission tomography and computed tomography in differential diagnosis between recurrent or residual glioma and treatment-induced brain lesions. Acta Radiol 30:121–128
22. Mineura K, Sasajima T, Kowada M, Uesaka Y, Shishido F (1991) Innovative approach in the diagnosis of gliomatosis cerebri using carbon-11-L-methionine positron emission tomography. J Nucl Med 32:726–728

23. Mosskin M, von Holst H, Bergström M, Collins VP, Eriksson L, Johnström P, Noren G (1987) Positron emission tomography with $^{11}$C-methionine and computed tomography of intracranial tumors compared with histopathologic examination of multiple biopsies. Acta Radiol 28:673–681
24. Ogawa T, Shishido F, Kanno I, Inugami A, Fujita H, Murakami M, Shimosegawa E, Ito H, Hatazawa J, Okudera T, Uemura K, Yasui N, Mineura K (1993) Cerebral glioma: evaluation with methionine PET. Radiology 186:45–53
25. Phelps ME, Barrio JR, Huang SC (1985) Measurement of cerebral protein synthesis in man with positron computerized tomography: model assumptions and preliminary results. In: Metabolism of the human brain studied with positron emission tomography (Greitz T, ed), Raven Press, New York, pp 215–232
26. Shibata T, Burger PC (1987) The use of monoclonal antibody Ki-67 in determination of the growth fraction in pediatric brain tumors. Childs Nerv Syst 3:364–367
27. Shishido F, Uemura K, Inugami A, Tomura N, Higano S, Fujita H, Murakami M, Kanno I, Yasui N, Mineura K (1990) Value of $^{11}$C-methionine and PET in the diagnosis of low-grade gliomas. Kaku Igaku 27:293–302
28. Takasaki Y, Deng JS, Tan EM (1981) A nuclear antigen associated with cell proliferation and blast transformation. J Exp Med 154:1899–1909