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Proton magnetic resonance spectroscopy reflects cellular proliferative activity in astrocytomas

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Abstract We examined whether proton magnetic resonance spectroscopy (MRS) could provide accurate information on histological grade and cell proliferation in astrocytomas. We studied 23 patients with astrocytomas: five grade II, 10 grade III and eight with grade IV (glioblastoma multiforme). We performed proton MRS and determined the Ki-67 labeling index (LI), a tumour proliferation marker, in the same areas of the astrocytomas, and examined the statistical relationship between proton MRS and Ki-67 LI. The *N*-acetylaspartate (NAA)/creatine-phosphocreatine (Cr) and NAA/choline (Cho)-containing compound ratios were always significantly lower and the Cho/Cr ratios significantly higher than those for normal brain. The Cho/Cr ratio correlated positively and the NAA/Cho ratio inversely with Ki-67 LI. These findings suggest that the Cho signal in proton MRS reflects cellular proliferation. In Kaplan-Meier survival analysis, there was no significant difference between high (> 2.0, 14 cases) and low (< 2.0, 9 cases) Cho/cr ratio groups.

Key words Astrocytoma · Magnetic resonance spectroscopy · Ki-67

Introduction

Although CT and MRI can be used to solve many diagnostic problems related to brain tumours, they do not provide all the biological information required for the appropriate management of gliomas. Although positron emission tomography (PET) provides insights into tumour cell metabolism and biological behaviour [1], PET facilities are available in only a few specialised centres. The wider availability of single-photon emission CT (SPECT) scanners has intensified the search for SPECT tracers suitable for evaluation of gliomas. High-field MRI is present in most major medical centres and can be used for proton magnetic resonance spectroscopy (MRS) studies. A large number of reports indicate that proton MRS is useful in determination of histological type [2–5], benign or malignant transformation [6, 7] and recurrence or radiation necrosis [8–10].

The in vivo choline-containing compound (Cho) signal in proton MRS is reported to be predominantly attributed to cellular density and phospholipid metabolism [11, 12]. Clinically, increased choline signal coincides with malignant degeneration of cerebral gliomas [7]. In an attempt to determine accurate histological grading and cell proliferative activity of astrocytomas preoperatively, we performed proton MRS and Ki-67 labelling index (LI), a tumour proliferation marker, in the same areas of astrocytomas, and examined the relationships of MRS findings, Ki-67 LI and survival.

Materials and methods

All patients were studied using a protocol approved by Okayama University Medical School, and informed consent was obtained before inclusion in the study. We selected 23 patients with primary or recurrent astrocytomas: five grade II, 10 grade III and 8 gra-

Table 1 Clinical features and proton MRS data

Pathology	Number	Ki-67 LI (%)	NAA/Cr	Metabolic ratios NAA/Cho	Cho/Cr
Normal brain	14	Not done	2.15 ± 0.11	2.09 ± 0.13	1.07 ± 0.08
Astrocytomas II	5	4.7 ± 1.4	1.19 ± 0.28	0.66 ± 0.15	1.66 ± 0.44
Astrocytomas III	10	9.3 ± 2.2	0.81 ± 0.18	0.45 ± 0.13	2.42 ± 0.24
Astrocytomas IV	8	11.7 ± 2.8	1.07 ± 0.16	0.59 ± 0.15	2.31 ± 0.37
All astrocytomas	23	9.1 ± 1.5	0.98 ± 0.11[a]	0.54 ± 0.08[a]	2.22 ± 0.19[b]

[a] Mean values significantly lower than normal brains ($P < 0.01$)
[b] Mean values significantly higher than normal brains ($P < 0.01$). Results are indicated as mean ± standard deviation

de IV (glioblastoma multiforme), using the World Health Organization (WHO) criteria [13]. The patients were 11 men and 12 women, aged 15–68 years, mean 42.5 years.

We treated grade II astrocytomas by surgical resection, but almost all grade III and IV astrocytomas with a combination of surgery, radiotherapy and chemotherapy. Proton MRS was performed just before surgical resection, since the metabolic effects of surgery and radiation could then be ignored.

The site of highest malignancy, assumed to be that which exhibited contrast enhancement on T1-weighted images, was chosen for MRS and histological studies. In grade II astrocytomas exhibiting no contrast enhancement, the centre of the high-intensity area on T2-weighted images was chosen. We also examined 14 healthy volunteers as normal controls.

MRI was performed with a 1.5-T clinical system [9, 14]. T1-weighted images before and after an intravenous injection of 0.1 mmol/kg of gadopentetate dimeglumine were obtained using a spin-echo sequence of 500/13 ms/3 (repetition time/echo time/excitations). T2-weighted images were obtained with a fast spin-echo sequence of 4000/95 ms/2, echo-train length 8.

Proton MRS was performed on the same system, equipped with a bird-cage head coil. Field homogeneity was adjusted by shimming on the proton signal using an automated routine in the unit's research package. We used a point-resolved spectroscopy (PRESS) sequence with a chemical shift-selective (CHESS) pulse for water suppression, because this offers a gain of a factor of two in signal-to-noise ratio, less sensitivity to motion and diffusion, and no sensitivity to multiple-quantum effects when compared with other sequences, such as stimulated-echo or image-selected in vivo spectroscopy.

Regions of interest (ROI) containing a single spectroscopic voxel (1 cm^3) were selected from each study to include the area of highest Cho signal within the region of contrast enhancement. The spectra were processed on a work station with spectroscopic analysis software. The major metabolites examined included the CH_3 group of *N*-acetylaspartate (NAA) at 2.0 ppm, the CH_3 group of creatine-phosphocreatine (Cr) at 3.0 ppm, and the $(CH_3)_3$ group of Cho at 3.2 ppm. Metabolite signal intensity ratios (NAA/Cr, NAA/Cho, Cho/Cr) were calculated from the peak area for each ROI.

The patients underwent surgery using an image-guided stereotactic system to obtain specimens from the areas on which proton MRS was performed. For haematoxylin and eosin and Ki-67 immunostaining, the specimens were sliced from formalin-fixed, paraffin-embedded tissues [15, 16]. For immunohistochemistry, we used a mouse monoclonal antibody against Ki-67 (NCL-Ki67-MM1) diluted to 1:50 and the avidin-biotin complex method. Fields with the highest number of Ki-67-positive cells were identified at 100 × magnification and Ki-67 LI were calculated by counting 1000 tumour nuclei at 400 × magnification with a grid screen [15, 16].

All results are expressed as means ± standard errors (SE) and statistical differences were determined by analysis of variance (ANOVA). *P* values less than 0.05 were taken to indicate significance.

Results

Figures 1–3 show MRI, proton MRS and Ki-67 immunostaining in three representative cases. The clinical features, histological diagnoses, Ki-67 LI, the proton MRS findings and survival are summarised in Table 1. Ki-67 LI ranged from 1.1% to 24.8%: grade II astrocytomas from 1.6% to 9.8% (mean = 4.7 ± 1.4); grade III from 1.1% to 24.3% (9.3 ± 2.2) and grade IV from 1.8% to 24.8% (11.7 ± 2.8).

The NAA/Cr, NAA/Cho and Cho/Cr ratios for each astrocytoma and normal brains are shown in Table 1. The NAA/Cr and NAA/Cho ratios for normal brains of healthy volunteers were 2.15 ± 0.11 and 2.09 ± 0.13. Mean NAA/Cr and NAA/Cho ratios for the group of astrocytomas were 0.98 ± 0.11 ($P < 0.01$) and 0.54 ± 0.08 ($P < 0.01$), significantly lower than those for normal brains. The differences between grade II, grade III and grade IV astrocytomas as regards NAA/Cr or NAA/Cho ratio were not significant. The NAA/Cho ratio correlated inversely with Ki-67 LI (Spearman rank correlation test, $P = 0.015$), but the NAA/Cr ratio did not (Fig. 4).

Mean Cho/Cr ratios were 1.07 ± 0.08 for normal brains, 1.66 ± 0.44 for grade II astrocytomas, 2.42 ± 0.24 for grade III and 2.31 ± 0.37 for grade IV. There was a significant difference ($P < 0.01$) between the Cho/Cr ratio for the astrocytomas and that for normal brains. Although the Cho/Cr ratio was lower for grade II astrocytomas than for grades III and IV, this difference was not significant. However, the Cho/Cr ratio correlated positively with Ki-67 LI (Spearman rank correlation test, $P < 0.01$) (Fig. 4).

To determine whether the Cho/Cr affects survival, we divided our cases into high-ratio (> 2.0, 14) and low-ratio (< 2.0, 9 cases) groups and compared their survival; there was no significant difference in Kaplan-Meier survival analysis (Fig. 5 A). When tumours with high

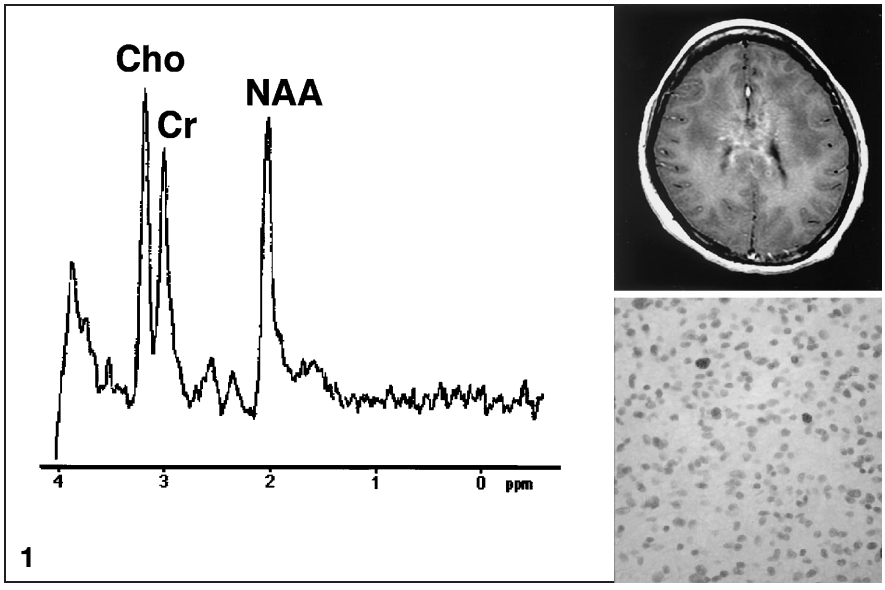

Fig. 1 Patient 2. Proton MRS, contract enhanced T1-weighted image and Ki-67 immunostaining of a 43-year-old woman with grade II astrocytoma in the corpus callosum. Ki-67 LI was 2.8 %, and the NAA/Cr, NAA/Cho and Cho/Cr ratios were 1.51, 1.26 and 1.20, respectively

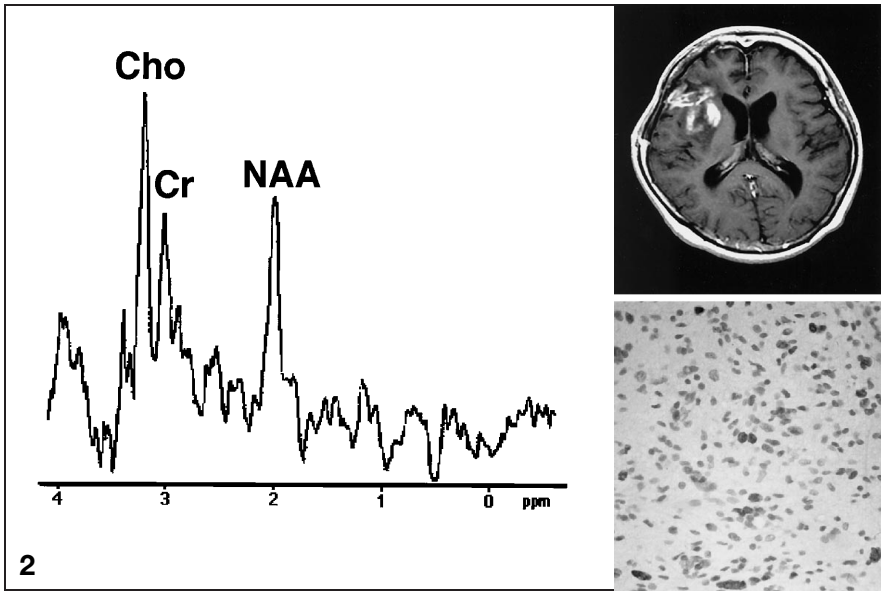

Fig. 2 Patient 11. As in Fig. 1: a 44-year-old woman with a grade III astrocytoma in the right frontal lobe. Ki-67 LI was 9.3 %, and the NAA/Cr, NAA/Cho and Cho/Cr ratios were 0.88, 0.86 and 2.59

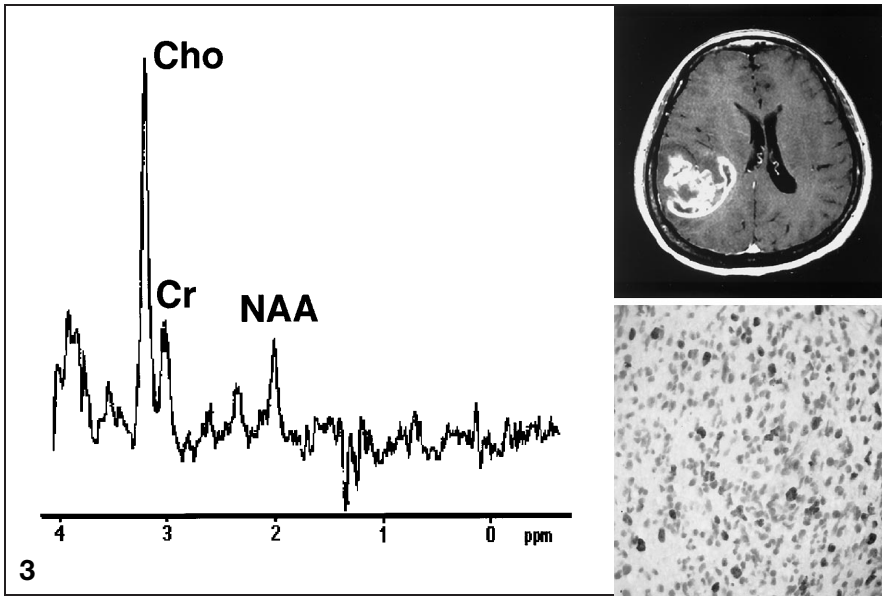

Fig. 3 Patient 20. As Fig. 1: a 62-year-old woman with a grade IV astrocytoma (glioblastoma multiforme) in the right parietal lobe. Ki-67 LI was 14.5 %, and the NAA/Cr, NAA/Cho and Cho/Cr ratios were 0.82, 0.26 and 3.54

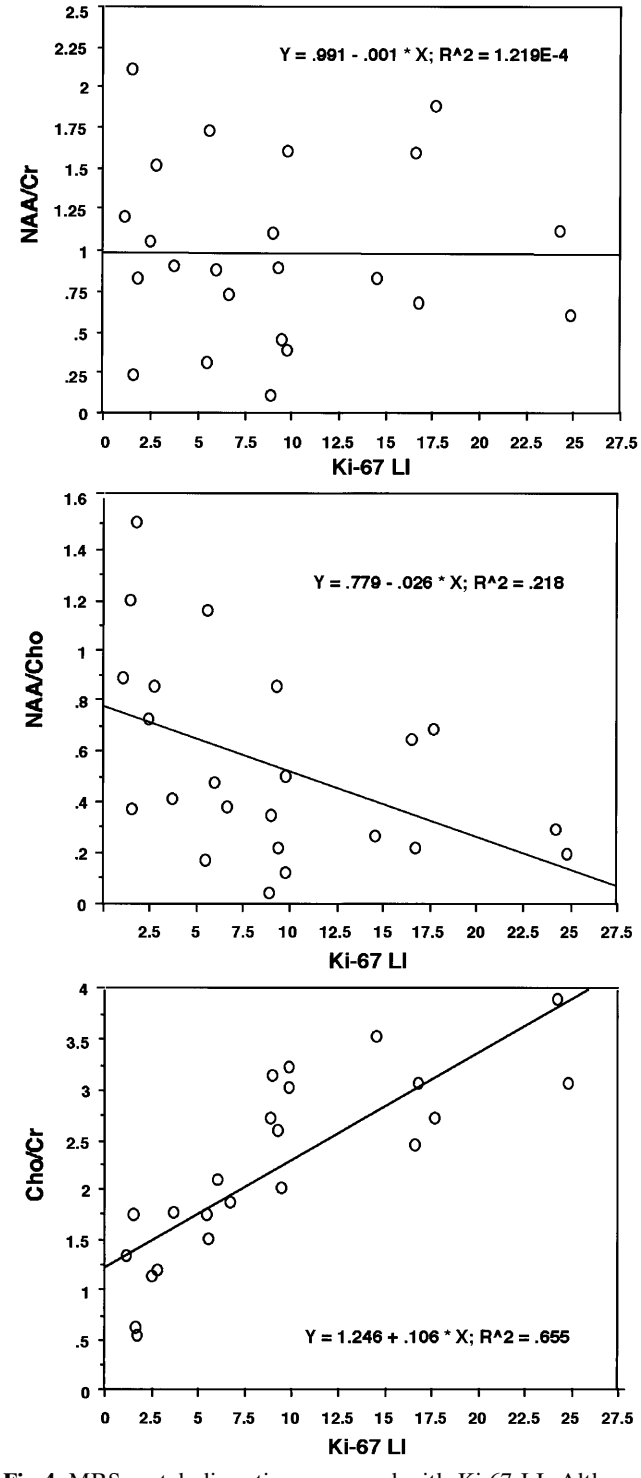

Fig. 4 MRS metabolic ratios compared with Ki-67 LI. Although the NAA/Cr ratio did not correlate with Ki-LI, the NAA/Cho ratio correlated inversely ($P = 0.015$), and the Cho/Cr ratio positively ($P < 0.01$)

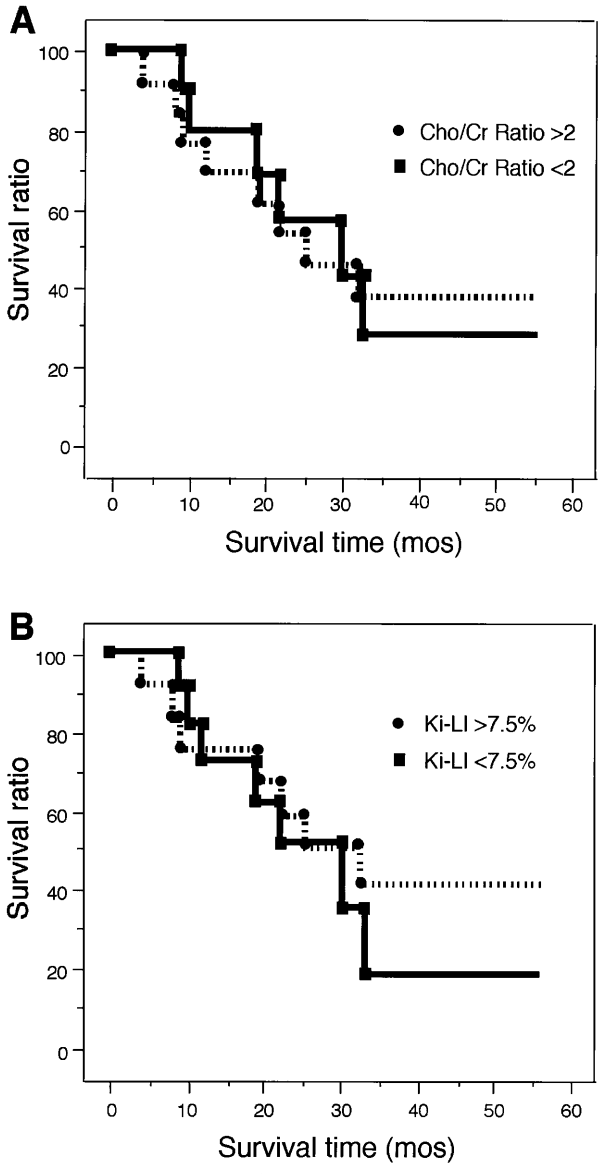

Fig. 5 **A** High (> 2) and low (< 2) Cho/Cr ratio groups and Kaplan-Meier survival analysis. Survival curves for the two groups did not differ. **B** High (> 7.5 %) and low (< 7.5 %) Ki-67 labelling index groups and Kaplan-Meier survival analysis. Survival curves for the two groups did not differ

(> 7.5 %) and low (< 7.5 %) Ki-67 LI were compared, again no difference was found on Kaplan-Meier analysis (Fig. 5 B).

Discussion

MRS is a noninvasive method for looking at intracellular pathophysiology. Proton MRS provides information about metabolic aspects of brain tumours. It

has recently been used to determine tumour histological type [2–5], to detect malignant transformation of brain tumours [6, 7] and to differentiate recurrent tumour from radiation necrosis [8–10]. In our study, the NAA/Cr and NAA/Cho ratios for all astrocytomas were significantly lower than those for normal brains, and the Cho/Cr ratios significantly higher. The Cho/Cr ratio for grade II astrocytomas tended to be lower than those for grades III and IV. These results agree with previous studies which showed an increase in the Cho/Cr ratio and a decrease in the NAA/Cr ratio with increasing histological grade of malignancy [7, 17]. The NAA signal in proton MRS is reduced for absent in brain tumours [2–4, 18–23], probably because the NAA molecule is neurone-specific [24, 25]. Low-grade hypothalamic/chiasmatic astrocytomas showed a Cho/NAA ratio higher than that of healthy brain [26]. In all published studies, however, metabolic signal intensities had large coefficients of variation within, and overlaps between, histological grades of glial tumours [21].

The number of proliferating cells in astrocytomas has been used to predict tumour growth and patient survival, and to determine more effective treatment. We used Ki-67 immunohistochemistry, which labelled cells in G1, S, G2 and M phases, but not G0, using a widely used antibody that recognises a proliferation-associated nuclear protein. The Ki-67 LI we obtained agreed with those in a number of previous studies, which have shown a general increase in LI with tumour grade, but also marked variation within each grade, particularly in higher-grade lesions [27].

We showed that the Cho/Cr ratio correlated positively and the NAA/Cho ratio inversely with Ki-67 LI, suggesting that the Cho signal in proton MRS adequately reflects cell proliferation in astrocytomas. Barbarella et al. [2] reported that the Cho/Cr of neoplastic tissues paralleled the Ki-67 LI, but no statistical analysis was performed. The Cho signal is thought to be attributable predominantly to water-soluble glycerophosphocholine and phosphocholine [11], and to correlate with in vitro measures of cell density and phospholipid metabolism [17, 28, 29]. Thus, the increased Cho peak found in most studies of brain tumours has been attributed to increased membrane synthesis, increased cellularity or rapid cell turnover [7].

Variations in treatment among our patients did not allow us to correlate the Cho/Cr ratio or Ki-67 LI with prognosis. Factors impacting on survival, including age, Karnofsky performance scale, tumour site, and treatment modality, varied between patients. This may explain why neither metabolic ratio nor proliferative activity was directly correlated with survival.

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