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Myoinositol to Total Choline Ratio in Glioblastomas as a Potential Prognostic Factor in Preoperative Magnetic Resonance Spectroscopy

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Abstract

***Isocitrate dehydrogenase* (*IDH*) wild-type diffuse astrocytic tumors tend to be pathologically diagnosed as glioblastomas (GBMs). We previously reported that myoinositol to total choline (Ins/Cho) ratio in GBMs on magnetic resonance (MR) spectroscopy was significantly lower than that in *IDH*-mutant gliomas. We then hypothesized that a low Ins/Cho ratio is a poor prognosis factor in patients with GBMs, *IDH*-wild-type. In the present study, we calculated the Ins/Cho ratios of patients with GBMs and investigated their progression-free survival (PFS) and overall survival (OS) to determine their utility as prognostic marker. We classified patients with GBMs harboring wild-type *IDH* (n = 27) into two groups based on the Ins/Cho ratio, and compared patient backgrounds, pathological findings, PFS, OS, and copy number aberrations between the high and low Ins/Cho groups. Patients with GBMs in the low Ins/Cho ratio group indicated shorter PFS (P = 0.021) and OS (P = 0.048) than those in the high Ins/Cho group. Multivariate analysis demonstrated that the Ins/Cho ratio was significantly correlated with PFS (hazard ratio 0.24, P = 0.028). In conclusion, the preoperative Ins/Cho ratio can be used as a novel potential prognostic factor for GBM, *IDH*-wild-type.**

Keywords: glioblastoma, magnetic resonance spectroscopy, myoinositol

Introduction

Gliomas, which are diagnosed based on pathological and genetic findings, are among the most common primary brain tumors.[1–5] Surgical resection is the preferred initial therapeutic strategy to improve prognosis[6,7]; however, it also increases the risk of brain dysfunction because either the border between the tumor and normal brain tissue is unclear or because the tumors infiltrate invasively into the adjacent healthy brain tissue. Therefore, it is helpful for surgeons to plan optimal surgical strategies if tumor malignancy is preoperatively predicted.

Magnetic resonance (MR) spectroscopy enables noninvasive quantification of tumor metabolites by analyzing their spectra; thus, it is widely used for the preoperative diagnosis of brain tumors.[8] We previously examined tumor metabolites in adult

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supratentorial diffuse astrocytic tumors and reported that the myoinositol to total choline (Ins/Cho) ratios in *isocitrate dehydrogenase* (*IDH*) wild-type gliomas, which are mostly defined as glioblastomas (GBMs), were significantly lower than those in the *IDH*-mutant gliomas.[9] Given that total choline is thought to represent cell density,[10] we presumed that lower ratios indicate myoinositol consumption in a glioma cell.

Myoinositols are sugar alcohols in the phospholipids of the intracellular membrane,[11] and are produced through food digestion or by hydrolysis or dephosphorylation of the intracellular membrane.[11–13] In astrocytes, myoinositols contribute to adjusting osmotic pressure in response to changes in intracranial pressure.[14] Most diffuse astrocytic tumors harboring wild-type *IDH* are classified as GBMs, which exhibit dismal prognoses.[1–3] Molecular factors that contribute to this poor prognosis have been identified based on genetic subtype, including the *TERT* promoter mutation status and *MGMT* promoter methylation status, copy number aberrations (e.g., +7, −10q, and −9p21), and epigenetic changes (e.g., histone H3).[15–20] However, such information is only available after surgical resection, and we ideally need a method for predicting outcomes in patients suspected of having GBMs, *IDH*-wild-type before surgery. Based on the observation that diffuse astrocytic tumors harboring mutant *IDH* have better outcomes than those harboring the wild-type *IDH*,[1–3] we hypothesized that low Ins/Cho ratios in GBMs, *IDH*-wild-type can be used as prognostic markers for this genetic subtype of glioma.

In the present study, we calculated Ins/Cho ratios by MR spectroscopy for patients with supratentorial GBMs harboring wild-type *IDH* to investigate the association of the ratio with prognosis and discuss whether it could be used as a preoperative prognostic factor.

Materials and Methods

Study design

We retrospectively investigated 27 cases of newly diagnosed GBMs or gliosarcoma harboring wild-type *IDH* in patients who underwent analyzed by MR spectroscopy and tumor resection between 2013 and 2019 at Fujita Health University Hospital. Patients who had already received treatment before MR spectroscopy, were younger than 20 years, GBM and gliosarcoma patients harboring *IDH* mutations, and showed high values of % SD in myoinositol and total choline (>50%) were excluded from this study. The primary outcomes were progression-free survival (PFS) and overall survival (OS). PFS was calculated from the date of the first operation to the date of the first of either confirmed recurrence, tumor relapse or death from any cause. OS was calculated from the date of the first resection to the date of death. Patient information was updated in October 2020 for follow-up purposes. All resected tissues were assessed by neuropathologists according to the World Health Organization (WHO) classification.[1] The study was approved by the local ethical review board of Fujita Health University (HG19-074).

MR spectroscopy

A single-voxel ^{1}H-MR spectroscopy with point-resolved spectroscopy sequence was performed with a 3 Tesla (T) scanner (Ingenia 3.0T; Philips Healthcare, Best, The Netherlands) using a dS Head Coil and Vantage Titan 3T (Canon Medical Systems Corporation, Otawara, Japan) using a 16 or a 32-channel coil. In each patient, ^{1}H-MR spectroscopy was performed using the following parameters: repetition time (TR)/echo time (TE), 2000/144 and 35 ms; number of excitations (NEX), 128; bandwidth, 1.61 HZ/point; and voxel of interest (VOI) size for metabolic measurements, 15 × 15 × 15 mm. T2-weighted images in three directions for setting the VOI were determined for each patient using the following parameters: TR/TE, 4250/82 ms; acquisition matrix size, 416 × 344; reconstruction matrix, 640 × 640; field of view (FOV), 230 × 230 mm; slice thickness, 4.0 mm; slice gap, 0.8 mm; NEX, 1; and reduction factor 1.9. tumor (K.M) with a 10- to 15-year experience including the VOIs inside the lesion based on T2-weighted images in three directions (Fig. 1). If tumors contained necrotic and cystic components, these components were included in the VOI to avoid selection bias and evaluate the whole tumor characteristics. In ^{1}H-MR spectroscopy, myoinositol and total choline were measured with short TE (TE = 35 ms) and long TE (TE = 144 ms), respectively[21–23] because of their relaxation time in T2, and the mean concentration of MR spectroscopy values were analyzed using an automatic quantification program (LCModel; Stephen Provencher, Oakville, Ontario, Canada).[24]

Evaluation of *IDH* mutation status

We evaluated *IDH* mutations using the Sanger method, with codon 132 in *IDH1* and codon 172 in *IDH 2*, and conducted analysis using by polymerase chain reaction (PCR). Briefly, DNA was extracted from resected frozen tissue and formalin-fixed and paraffin-embedded (FFPE), using DNeasy Blood and Tissue Kit (QIAGEN, Hulsterweg, The Netherlands) and REPLI-g FFPE Kit (QIAGEN).

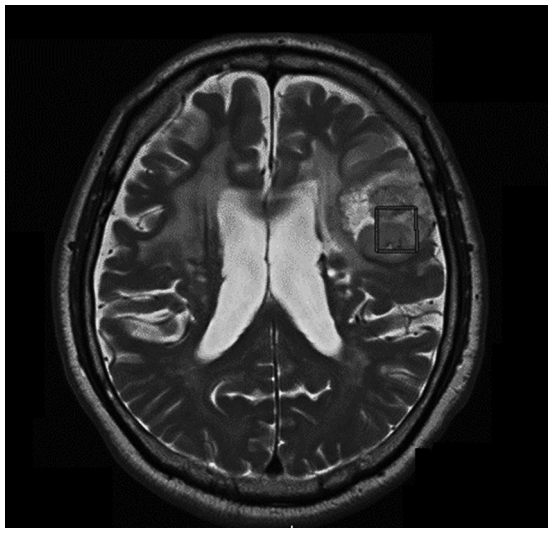

Fig. 1 Setting VOI of MR spectroscopy. VOI were single-voxel spectroscopy, and the size was set as 15 × 15 × 15 mm. VOI were selected by a board certified neuroradiologist, and set to include tumor as large as possible. It included necrotic and cystic components to evaluate tumor characteristics accurately. MR: magnetic resonance, VOI: volumes of interest.

The reaction mixtures for PCR comprised of DNA, primers, 10 × PCR buffer, 10 mM dNTP Mix (Thermo Fisher Scientific, Waltham, MA, USA), 50 mM $MGCL_2$, and PLATINUM Taq DNA polymerase (Thermo Fisher Scientific). After confirming the DNA bands of the PCR products by electrophoresis, we added BigDye Sequencing Buffer (Thermo Fisher Scientific), Ready Reaction Mix (Thermo Fisher Scientific), and the same primer to the PCR products, and repeated the PCR. Sequencing was performed with a BigDye Terminator version 3.1, Cycle Sequencing Kit (Thermo Fisher Scientific), and the results were analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA).

Next-generation sequencing analysis and comparison of copy number aberration

We analyzed samples that met our inclusion criteria and classified them according to the Ins/Cho ratio into high- and low-ratio groups. We prepared the diluted genomic DNA for subsequent experiments. After the whole-genome amplification using a SurePlex DNA Amplification System (Illumina, San Diego, CA, USA), library preparation was performed using a Nextera XT DNA Library Preparation Kit (Illumina). Shallow-whole-genome sequencing analysis was conducted with a VeriSeq PGS Kit-MiSeq (Illumina), and the results were analyzed using BlueFuse Multi Software (Illumina). In all cases, the chromosomes were divided into 2500 windows of approximately 1 Mb in size.

Statistical analysis

We used Fisher's exact test and Mann–Whitney *U* test to compare age at onset, sex, laterality, MIB-1 index, and pathology between the two groups. PFS and OS were analyzed using the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazards model was used to determine the relationship between the Ins/Cho ratio and prognosis.

We selected the following as explanatory factors in the multivariate analysis: status of *MGMT* promoter methylation; temozolomide and radiotherapy; gross total resection; subtotal resection, which was defined as >90% resection; Karnofsky performance status (KPS) which was greater than 70; and the Ins/Cho ratio. All statistical analyses were conducted using the EZR software.[25]

Results

Relation of patient and pathology characteristics with the Ins/Cho ratio

The mean and median values of the Ins/Cho ratios for the 27 patients included in this cohort were 1.09 and 0.81, respectively; therefore, we opted for a cutoff value of 0.9, and classified these patients into high (≥0.9) and low (<0.9) Ins/Cho ratio groups (n = 11 and 16, respectively). Tables 1 and 2 show the patient characteristics, pathologies, Ins/Cho ratios, and postoperative therapies. In all, 27 patients were histologically diagnosed (25 GBMs and 2 gliosarcomas). There were no statistically significant differences in sex, age at onset, laterality, MIB-1 index, or *MGMT* promoter methylation status between the two groups (Table 2). After recurrence, six patients underwent reoperation, six patients underwent additional chemotherapy other than temozolomide and bevacizumab, and seven patients received no treatment after recurrence.

PFS and OS analysis

We compared the prognosis between the high and low Ins/Cho ratio groups. During follow-up, tumors were recurrent in 94% and 91% of patients with a low Ins/Cho ratio (i.e., 15/16) and a high Ins/Cho ratio (i.e., 10/11), respectively. However, 81% (13/16) of patients with low ratios and 36% (4/11) with high ratios died. Figure 2a and 2b shows that the PFS and OS in patients with high Ins/Cho ratios were significantly shorter than those with low ratios, indicating that patients with low Ins/Cho ratios had higher recurrence and mortality rates (P = 0.021

Table 1 Characteristics and treatments of patients

Age Sex	Rec	PFS (mo)	Follow-up months	Outcome	*MGMT* promotor	Ins/Cho	EOR	Post-Ope therapies	Therapies after Rec
42F	Yes	9	22	Dead	M	0.573	GTR	TMZ, RT	Ope, TMZ, RT, BEV, CTx
43F	Yes	1	31	Dead	M	0.507	GTR	No treatment	Ope, TMZ, RT, BEV, CTx
43M	Yes	5	12	Dead	M	0.475	PR	TMZ, RT	Ope, BEV, CTx
44M	Yes	10	14	Dead	U	0.167	GTR	TMZ, RT, BEV	CTx
46F	Yes	3	12	Dead	M	0.450	GTR	TMZ, RT	TMZ, RT, BEV, CTx
62M	Yes	26	76	Alive	U	1.149	STR	TMZ, RT	Ope, TMZ, RT
63F	Yes	17	56	Alive	M	1.149	GTR	TMZ, RT	TMZ, RT, BEV
64M	No	19	19	Alive	ND	3.622	GTR	TMZ, RT, BEV	No recurrence
67M	Yes	9	11	Alive	U	0.816	PR	TMZ, RT, BEV	TMZ
68M	Yes	10	10	Alive	U	1.182	STR	TMZ, RT	No treatment
69F	Yes	1	12	Dead	U	2.054	GTR	No treatment	TMZ, RT, BEV
69F	Yes	12	20	Dead	M	0.655	GTR	TMZ, RT	TMZ, RT, BEV
70M	Yes	11	11	Dead	M	0.854	GTR	TMZ, RT	No treatment
71M	Yes	1	9	Dead	M	2.533	PR	TMZ, RT, BEV	No treatment
72M	Yes	1	22	Dead	M	0.451	GTR	No treatment	TMZ, RT, BEV
73F	Yes	1	23	Dead	U	0.405	STR	No treatment	Ope, TMZ, RT, BEV
73F	Yes	1	13	Dead	ND	1.128	PR	TMZ, RT	RT, BEV
74M	Yes	10	19	Alive	U	0.893	STR	TMZ, RT, BEV	TMZ, RT, BEV
74M	Yes	2	20	Dead	M	0.758	GTR	TMZ, RT	TMZ, BEV
74M	Yes	1	20	Dead	U	0.240	GTR	TMZ, RT	Ope, TMZ, BEV, CTx
75F	No	6	6	Alive	U	0.402	GTR	TMZ, RT	No recurrence
77M	Yes	13	14	Dead	M	0.695	GTR	TMZ, BEV	No treatment
77F	Yes	3	3	Dead	ND	0.439	GTR	TMZ, RT, BEV	No treatment
80F	No	39	39	Alive	M	1.176	PR	TMZ, RT	No recurrence
81M	Yes	4	4	Alive	U	1.397	GTR	No treatment	No treatment
86F	Yes	21	24	Dead	M	1.111	STR	TMZ	BEV
88F	Yes	12	12	Alive	ND	4.400	PR	TMZ, RT, BEV	No treatment

BEV: bevacizumab, CTx: other chemotherapies, GTR: gross total resection, Ins/Cho: ratio of myoinositol to total choline, M: methylated, mo: months, ND: no data, Ope: Operation, PFS: progression-free survival, PR: partial resection, Rec: recurrence, RT: radiotherapy, STR: subtotal resection, TMZ: temozolomide, U: Unmethylated.

and 0.048, respectively). In the multivariate analysis, Cox proportional hazards models revealed that the Ins/Cho ratio was significantly associated with PFS (hazard ratio 0.24, P = 0.028), suggesting that the Ins/Cho ratio was useful as a potential prognostic factor, especially for PFS. The data are summarized in Table 3.

Analysis of copy number aberrations by next-generation sequencing

We included 18 cases in the next-generation sequencing, analyzing 6 samples in the high Ins/Cho ratio (≥0.9) group and 12 samples in the low Ins/Cho ratio (<0.9) group. The overall noise value was <0.3. Analysis focused on regions with specific genes associated with poor prognosis in *IDH*-wild-type GBMs, including 7p11.2 (*EGFR*), 9p21.3 (*p16*), and 10q23.3 (*PTEN*).[17,18] Gain of 7p11.2 was detected in five cases with high ratios and in seven cases with low ratios, loss of 9p21.3 was detected in two cases with high ratios and in four cases with low ratios, and loss of 10q23.3 was detected in three cases with high ratios and in three cases with low ratios (Table 4). There were no significant

differences between the groups for any other window.

Discussion

In this study, we demonstrated that a high Ins/Cho ratio on MR spectroscopy was associated with a better prognosis than a low Ins/Cho ratio in patients with GBMs, harboring the wild-type *IDH*. The Ins/Cho ratio was found to be significantly associated with PFS by Kaplan–Meier and multivariate analysis. Fifteen of the 16 patients in the low Ins/Cho ratio group experienced tumor recurrence within 12 months, whereas only 6 of the 11 patients in the high Ins/Cho ratio group experienced recurrence in the same terms. Considering that total choline in MR spectroscopy reveals cell density,[10] low myoinositol levels per cell could contribute to poor prognosis. Indeed, a recent cohort study of recurrent GBMs treated with bevacizumab reported that a high myoinositol value on MR spectroscopy was associated with a good prognosis.[26] Given these results, we considered that myoinositol were consumed in the process of production of phosphatidylinositol 3-phosphate (PI3P) and has antitumor effects. Myoinositols are located in glial cells, especially in astrocytes,[27] and regulate intracranial osmotic pressure.[14] PI3P, which contains myoinositols in its structure, is produced by intracellular membrane metabolism[11] and activates the PI3K-Akt pathway as a second messenger.[28] Activation of this pathway may cause a poorer prognosis in patients with a low Ins/Cho ratio by promoting cell proliferation and myoinositol consumption. As shown in a previous study, oral administration of myoinositols can inhibit malignant transformation of tumor cells in patients with non-small-cell lung cancer, in which the PI3K-Akt pathway is important.[29] Other researchers also reported that myoinositols have antitumor effects that result from their phosphorylated metabolites, such as inositol 1,3,4,5,6-pentaphosphate and inositol hexaphosphate, which can induce tumor cell apoptosis.[30,31] These previous studies suggest that malignancy in *IDH* wild-type gliomas with low Ins/Cho ratios is associated with a simple reduction in antitumor effects.

Table 2 Comparison of patient characteristics by Ins/Cho ratio threshold

	Ins/Cho ratio ≥0.9 (n = 11)	Ins/Cho ratio <0.9 (n = 16)	P value
Male	5	9	0.70
Age at onset, median (years)	71	71	0.31
Left side	6	9	1.0
MIB-1 index, median	37.9	42.5	0.90
Use of BEV	7	14	0.19
>90% resection	7	14	0.19
MGMT promoter methylation	4/8	9/15	0.69
KPS ≥70	7	15	0.13

BEV: bevacizumab, Ins/Cho: ratio of myoinositol to total choline, KPS: Karnofsky performance status, n: number of patients.

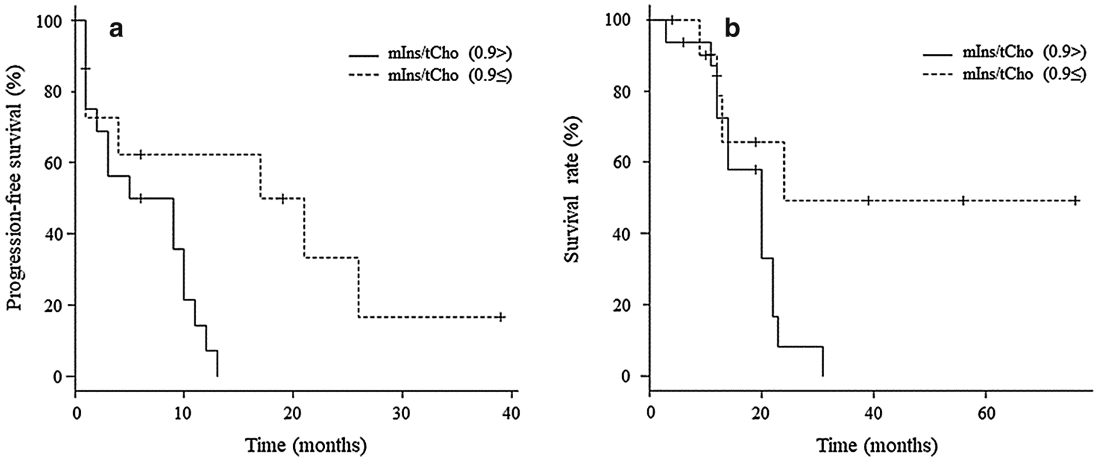

Fig. 2 Comparison of PFS and OS between the group with a high Ins/Cho ratio and a low Ins/Cho ratio. Comparison of PFS (a) and OS (b) between the group with a high Ins/Cho ratio (n = 11) and that with a low ratio (n = 16). Analysis was done by the Kaplan-Meier method, using the log-rank test (P = 0.021 and 0.048, respectively). Ins/Cho: ratio of myoinositol to total Choline, OS: overall survival, PFS: progression-free survival.

Table 3 Multivariate analysis of PFS and OS

	Explanatory factor	Hazard ratio (95% CI)	P value
PFS	Both use of TMZ and RT	0.31 (0.086–1.1)	0.069
	Ins/Cho ratio	0.24 (0.069–0.86)	0.028
	KPS ≥70	3.3 (0.51–21)	0.21
	MGMT promoter methylation	0.63 (0.24–1.7)	0.35
	>90% resection	0.89 (0.23–3.4)	0.87
OS	Both use of TMZ and RT	0.33 (0.023–4.8)	0.42
	Ins/Cho ratio	0.17 (0.028–1.1)	0.060
	KPS ≥70	1.3 (0.048–36)	0.87
	MGMT promoter methylation	0.97 (0.28–3.3)	0.97
	>90% resection	0.41 (0.068–2.5)	0.34

Ins/Cho: ratio of myoinositol to total choline, KPS: Karnofsky performance status, OS: overall survival, PFS: progression-free survival, RT: radiotherapy, TMZ: temozolomide.

Table 4 Comparison of copy number aberrations by Ins/Cho ratio

	Ins/Cho ratio ≥0.9 (n = 6)	Ins/Cho ratio <0.9 (n = 12)	P value
+7p11.2	5 (83%)	7 (58%)	0.60
−9p21.3	2 (33%)	4 (33%)	1.0
−10q23.3	3 (50%)	3 (25%)	0.34

Copy number aberrations identified by next-generation sequencing are compared by the Ins/Cho ratio. We defined an effective change of gain or loss when the average value of a window was >2.5 or <1.5. Ins/Cho: ratio of myoinositol to total choline.

However, multivariate analysis showed that the Ins/Cho ratio was significantly associated with PFS, but not OS (Fig. 2, Table 3). In this study, therapeutic strategies after recurrence were not uniform and could affect patient prognosis. For example, six patients (22%) selected surgical resection or other chemotherapies after recurrence, whereas seven patients (26%) selected no additional therapy after recurrence; however, four out of five patients whose survival time was more than 24 months were classified into the high Ins/Cho ratio group, suggesting that a high Ins/Cho ratio was strongly associated with good prognosis.

Copy number analysis by next-generation sequencing revealed no significant correlations between the Ins/Cho ratio and chromosomal areas, despite the association of specific copy number aberrations with GBMs (Table 4). Previous studies have reported that such copy number aberrations are in the early stage of tumorigenesis in GBM.[17,32,33] Therefore, our results suggest that low myoinositol levels are less likely to be caused by the early stage of tumorigenesis due to chromosomal changes and gene expression. Rather, it is more likely to be due to tumor progression, such as tumor growth or consumption of myoinositol.

There are some limitations to this study. First, because MRI studies, including MR spectroscopy, were performed as a preoperative evaluation, we retrospectively quantified tumor metabolites, which were analyzed using a single voxel. It might be more informative if the tumor metabolites were analyzed using a multi-voxel analysis. Second, there was lack of control of therapeutic strategies (e.g., resection extent by tumor location, postoperative bevacizumab use, and radiological dosages) or postoperative complications (e.g., delayed wound healing or high fever affected the start of treatments). These factors can affect the clinical course and outcomes of patients. Third, we analyzed the tumor lesion or necrotic and cystic components in the VOI to avoid selection bias. Considering that the necrotic and cystic components may show difference in Ins/Cho ratio compared to solid tumor lesion, further study, such as analysis of Ins/Cho ratio of solid tumor lesion, is also needed to investigate. Lastly, the small number of the cases may contribute to no significant difference in KPS or status of MGMT promoter methylation, which were known as potential prognostic factors of GBMs. Further study will need with a larger number of cases.

In conclusion, the noninvasive Ins/Cho ratio can serve as a novel potential prognostic marker for adults with supratentorial *IDH* wild-type GBM, providing useful preoperative information in patients with suspected GBMs. However, we can only speculate on why myoinositols are associated with patient outcomes, and questions around this will be targeted in future research.

Acknowledgments

MK was the author and conducted this study. TK, HS, and HK conducted the copy number analysis and drafted the manuscript. KM and YO analyzed the metabolites of gliomas in this study and drafted the manuscript. MA and SY pathologically diagnosed the tissues according to the WHO classification. SN, SO, JI, MH, and YH revised and edited the manuscript. We would like to thank Ms. Fujiko Sueishi for her technical support. The study was funded

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Conflicts of Interest Disclosure

The authors have no conflict of interest to declare.

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