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ORIGINAL ARTICLE

Reliable diagnosis of IDH-mutant glioblastoma by 2-hydroxyglutarate detection: a study by 3-T magnetic resonance spectroscopy

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Abstract We have previously reported that reliable detection of 2-hydroxyglutarate (2HG) in isocitrate dehydrogenase (IDH)-mutant WHO grade 2 and 3 gliomas is possible utilizing 3.0-T single-voxel magnetic resonance spectroscopy (SVMRS). We set out to determine whether the same method could be applied to detect 2HG in IDH-mutant glioblastoma. Forty-four patients harboring glioblastoma underwent pre-operative MRS evaluation to detect 2HG and other metabolites. Presence of IDH-mutations was determined by IDH1 R132H immunohistochemical analysis and DNA sequencing of surgically obtained tissues. Six out of 44 (13.6%) glioblastomas were IDH-mutant. IDH-mutant glioblastoma exhibited significantly higher accumulation of 2HG (median 3.191 vs. 0.000 mM, $p < 0.0001$, Mann-Whitney test). A cutoff of 2HG = 0.897 mM achieved high sensitivity (100.0%) and specificity (92.59%) in determining IDH-mutation in glioblastoma. Glioblastoma with high 2HG accumulation did not have significantly longer overall survival than glioblastoma with low 2HG accumulation ($p = 0.107$, log-rank test). Non-invasive and reliable detection of 2HG in IDH-mutant glioblastoma was possible by 3.0-T SVMRS.

Keywords Magnetic resonance spectroscopy · Glioblastoma · IDH mutation · 2-Hydroxyglutarate

Introduction

Glioblastoma is the most common and most malignant primary brain tumor. Despite recent advances, the median overall survival is 14.6 months. Recent whole-genome studies have shown that about 10% of glioblastoma harbor isocitrate dehydrogenase (IDH) mutations [1–4]. Glioblastomas with these mutations are known to have a better prognosis than their wildtype countertypes, and in the most recent, WHO classifications of tumors of the central nervous system [5] are distinguished from IDH-wildtype glioblastoma. In IDH-mutant gliomas, 2-hydroxyglutarate (2HG) is produced by conversion from α-ketoglutarate (α-KG). 2HG is known to competitively inhibit α-KG, causing DNA and histone hypermethylation, thus leading to glioma genesis [6]. We have previously shown that quantification of 2HG by single-voxel magnetic resonance spectroscopy (SVMRS) can be used to reliably distinguish between IDH-mutant and wildtype WHO grade 2 and 3 gliomas pre-operatively [7]. In that study, we excluded cases of WHO grade 4 glioblastomas from the analysis to minimize possible effects of necrosis on the spectra. In the current study, we analyzed whether glioblastoma could be pre-operatively divided into IDH-mutant and wildtype groups by SVMRS.

Manabu Natsumeda and Kunio Motohashi contributed equally.

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Materials and methods

Participants

Forty-seven consecutive patients harboring World Health Organization (WHO) grade IV gliomas, receiving magnetic resonance spectroscopy (MRS) evaluation at the Center for Integrated Brain Science, University of Niigata, before surgery and surgical treatment at the Department of Neurosurgery, University of Niigata, from July 2007 to September 2015 were included in the study. The patients underwent MRS evaluation a median of 6 days before surgery (range 1–22 days). A total of three patients were excluded from the study: two patients because of low signal-to-noise ratios of less than four and one patient because creatine (Cr) was not detected. Thus, a total of 44 patients were ultimately analyzed. Written informed consent was obtained from all of the participants in accordance with the human research guidelines of the Institutional Review Board of University of Niigata.

MRS analysis

MRI/^{1}H-MRS analysis was performed using a 3.0-T system (Signa LX, General Electric, Waukesha, WI) as previously described [7]. Briefly, proton density images (fast spin echo; TR/TE = 5000/40; FOV 20 × 20 mm; matrix 256 × 256; slice thickness 5 mm; interslice gap 2.5 mm) were taken. The slice with the largest depiction of tumor on proton density images was selected for SVMRS. A point-resolved spectroscopic sequence (PRESS), with chemical-shift-selective water suppression, was used with the following parameters: (TR 1.5 s; TE 30 ms; data point 512; spectral width 1000 Hz; number of acquisitions 128–196; volume of interest (VOI) 12–20 × 12–20 × 12–20 mm). The volume of interest (VOI) was designed to minimize suspected areas of necrosis and hemorrhage. Suspected glioblastoma cases with both large cystic components and no solid component or cases with intratumoral hemorrhage on pre- and post-contrast MR images were not assessed.

Spectral analysis was performed using LCModel version 6.3 (Stephen Provencher, Oakville, Ontario, Canada) [8]. Nineteen metabolites were included in this LCModel basis set, and glutathione (GSH), 2-hydroxyglutarate (2HG), myo-inositol (Ins), total NAA (tNAA: the sum of N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG)), total creatine (tCr: the sum of Cr and phosphocreatine (PCr)), and total choline (tCho: the sum of glycerophosphocholine (GPC) and phosphocholine (PC)) were noted. The following lipids and macromolecules were also noted: MM09, Lip09, and Lip 13.

Quantification estimates of metabolites were considered unreliable and excluded when Cramer-Rao lower bounds, returned as the percentage of standard deviation (%SD) by LCModel, were greater than 50% for 2HG [9, 10] and 35% for GSH and Ins, 30% for Glx, and 20% for tNAA, tCho, and tCr [7] as previously described. Because low estimates yielded large %SDs (i.e., when 2HG = 0, %SD = ∞), the above exclusion criteria were applied only when the estimated amount for metabolites was greater than 1.0 mM.

Pathological analysis

Surgical specimens were analyzed by two pathologists (H. T. and K. A.) according to the WHO classification 2016 [5]. IDH1 R132H (H09 clone, Dianova, Hamburg, Germany; 1:100) immunohistochemical analysis and DNA sequencing of *IDH1* and *IDH2* were performed as previously described [7].

Statistical analysis

Corrected metabolite concentrations of tumors in IDH-mutant glioma patients were compared those in IDH-wildtype glioma patients using the Mann-Whitney *U* test. Kaplan-Meier analysis was used to compare overall survival. Tests for associations between different parameters were carried out by the Fisher's exact test for 2 × 2 contingency tables. $p < 0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, http://www.graphpad.com). Receiver-operating characteristic (ROC) curve was used to determine a cutoff for 2HG concentration to obtain maximal sensitivity and specificity to identify IDH mutations was calculated using MedCalc (version 17.8) software. In order to obtain a robust 95% confidence interval, bootstrap method was employed using 1000 replications.

Results

A summary of the patient characteristics of mutant and wildtype IDH glioblastoma groups is provided in Table 1. Six out of 44 or 13.6% of glioblastoma were IDH mutant, comparable to the reported approximately 5–15% [1–4]. IDH-mutant glioblastoma patients were significantly younger (median 36 years) than IDH-wildtype glioblastoma patients (median 65 years) ($p < 0.0001$; Mann-Whitney test). There was a higher proportion of newly diagnosed glioblastoma patients in the IDH-wildtype group (34/38, 89.4%) compared to IDH-mutant (3/6, 50.0%) ($p = 0.0419$, Fisher's exact test). None of the three IDH-mutant glioblastoma patients underwent MRS before their initial surgery. 2HG values were consistent with IDH mutation status in all recurrent cases, whether IDH-mutant or wildtype. A higher proportion of patients were alive at

Table 1 Patient demographics of IDH-mutant and IDH-wildtype glioblastomas Results of unpaired *t* test (age) and chi-squared tests (others). The values inside parentheses represent percentage of patients within each group

Characteristic	Number of patients (%)		*p* value
	IDH-mutant GB	IDH-wildtype GB	
Number	6 (13.6)	38 (86.3)	
Men:women	5:1	27:11	
Age (years)			
Median	36	65	< 0.0001*
Range	29–40	12–81	
Newly diagnosed	3 (50.0)	34 (89.4)	0.0419*
Recurrent	3 (50.0)	4 (10.6)	
Outcome			
Alive	3 (50.0)	5 (13.2)	0.0632
Dead	3 (50.0)	33 (86.8)	

*$p < 0.05$

GB glioblastoma, *IDH* isocitrate dehydrogenase

follow-up in the IDH-mutant glioblastoma group (3/6, 50.0%) compared to IDH-wildtype glioblastoma group (5/38, 13.2%), although not statistically significant ($p = 0.0632$).

Representative SVMRS spectra of IDH-mutant and IDH-wildtype glioblastoma are provided in Fig. 1. Small peaks were detected at a chemical shift of about 2.25 ppm in IDH-mutant glioblastoma, reflecting the presence of 2HG. A higher lipid peak was noticed in both IDH-mutant and IDH-wildtype glioblastoma compared to those in WHO grade 2 and 3 gliomas [7].

IDH-mutant glioblastoma showed a significantly higher accumulation of 2HG (median 3.191 vs. 0.000 mM, $p < 0.0001$, Mann-Whitney test) (Fig. 2a). IDH mutant gliomas also expressed lower levels of Glx (median 7.090 vs. 9.393, $p = 0.033$), reflecting glutamine metabolism to produce 2HG in IDH-mutant gliomas. IDH-mutant glioblastoma exhibited less GSH accumulation, which is made from glutamine, although not statistically significant (median 1.653 vs. 1.924 mM, $p = 0.2977$). Levels of Ins, tNAA, tCho, and tCr were not significantly different between the two groups. 2HG was detected in all IDH-mutant glioblastoma, but not detected (2HG = 0) in 18 out of 38 (47.4%) ($p = 0.0668$, Fisher's exact test) IDH-wildtype glioblastomas (Fig. 3a). This data was remarkably consistent with our previous data in WHO grade 2 and 3 gliomas [7], further validating our methodology.

Glioblastoma is known to have prominent lipid peaks, as demonstrated in Fig. 1. We next compared metabolites, including lipid and macromolecules, between six IDH-mutant glioblastomas and eight IDH-mutant WHO grade 3 gliomas (Fig. 2b). Higher levels of Lip 13 were detected in IDH-mutant glioblastoma (median 25.420 vs. 7.061 mM, $p = 0.0173$).

ROC curve analysis obtained an optimal cutoff of 2HG = 0.897 mM, with a sensitivity of 100.0%, specificity of 92.6%, likelihood ratio of 13.5, and area under curve of

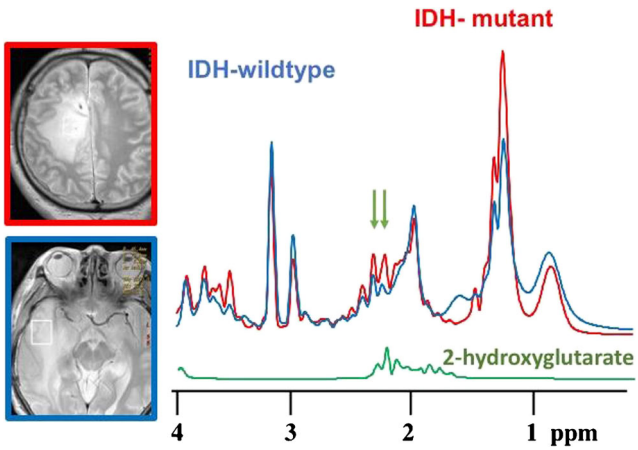

Fig. 1 SVMRS spectra of IDH-mutant (red) and IDH-wildtype (blue) glioblastoma. Small peaks were noted at a chemical shift of about 2.25 in IDH-mutant glioblastoma. Note the prominent lipid peaks

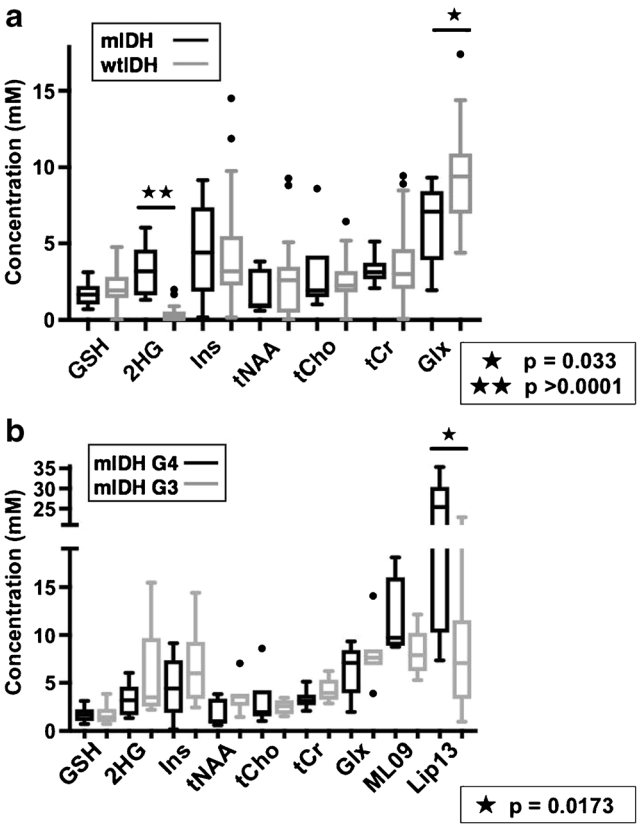

Fig. 2 **a** 2HG is elevated and Glx is decreased in IDH-mutant (mIDH) glioblastoma. **b** Lip13 is elevated in IDH-mutant glioblastoma (mIDH G4) compared to IDH-mutant WHO grade 3 (mIDH G3) gliomas

0.981 (Fig. 3a, b). A 95% confidence interval of 0.553 to 1.993 mM was obtained with the bootstrap method. A cutoff of 1.673 mM yielded the highest likelihood ratio, 22.5, with a sensitivity of 83.3% and specificity of 96.3%. At a cutoff of 1.489 mM, which was determined to be the optimal cutoff for grade 2 and 3 gliomas [7], sensitivity was 83.3%, specificity was 92.6%, and likelihood ratio was 11.25. Median overall survival was longer in glioblastoma with high accumulation of 2HG (2HG > 1.103 mM) (738 days), compared to low 2HG glioblastoma group (566 days). However, overall survival was not significantly longer in high 2HG group ($p = 0.17$, log-rank test, Fig. 4). All three recurrent IDH-mutant glioblastomas had poor prognoses with overall survival of 100 to 302 days; however, all three newly diagnosed IDH-mutant glioblastoma patients were alive at last follow-up (Table 1, Fig. 4).

These tumors included cases in which necrosis was evident on post-contrast MR images and Lip13 accumulation was high on MRS. Also, there were non-necrotic cases the VOI was selected to encompass a solid component or the lesion was invasive with heterogeneous enhancement on post-contrast MR images but without necrosis. The percentage of solid/invasive and necrotic lesions and median

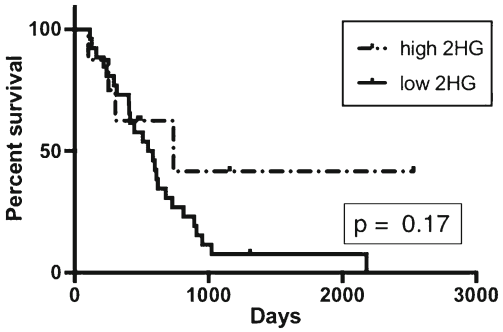

Fig. 4 Overall survival in IDH-mutant and IDH-wildtype glioblastoma patients

2HG and Lip13 values is summarized in Table 2. Somewhat surprisingly, median 2HG was less in solid/invasive IDH-mutant glioblastomas compared to IDH-wildtype glioblastomas (2.902 vs. 4.119 mM), probably reflecting the reduced cellularity in invasive lesions or secondary glioblastoma with solid low-grade components. As expected, Lip13 was much higher in the necrotic lesions both IDH-mutant and IDH-wildtype.

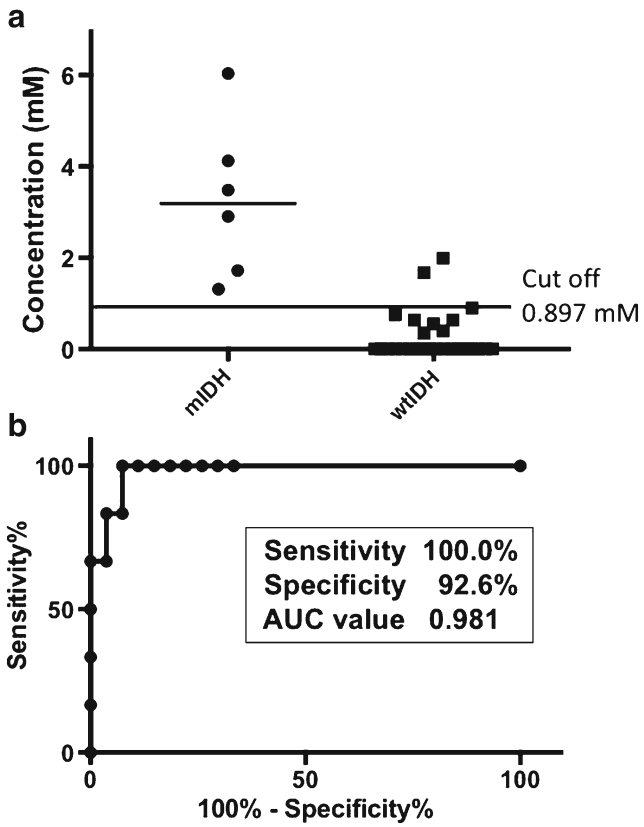

Fig. 3 **a** Determination of optimal cutoff of 2HG concentration to identify IDH-mutations by ROC analysis. **b** A cutoff of 2HG = 0.897 mM yielded a sensitivity of 100.0%, specificity of 92.8%, and area under curve (AUC) value of 0.981 in determining IDH-mutation by SVMRS

Discussion

Several studies, including ours, have shown that measurement of 2HG by MRS can be a reliable method to distinguish between IDH-mutant and IDH-wildtype gliomas [7, 11–13]. Almost all of these reports focus on grade 2 and grade 3 gliomas, partially because of the relative rarity of IDH-mutant glioblastoma. Over an 8-year period, we were able to assess six cases of IDH-mutant glioblastoma. In the current study, we have shown that by minimizing areas of necrosis by careful selection of VOI, 2HG can be reliably measured in WHO grade 4 glioblastomas as well.

We detected less Glx (the sum of glutamine and glutamate) in IDH-mutant glioblastoma. In vitro studies which placed isotope-labeled glutamine into media-growing IDH-mutant cells showed that the labeled carbon is ultimately used by 2HG [14], suggesting that 2HG is primarily derived from glutamine in mutant IDH gliomas. Glutamine is hydrolyzed by glutaminase to produce glutamate, which is subsequently converted to α-KG [14, 15]. Furthermore, a study injecting hyperpolarized ^{13}C α-KG into rats injected with IDH-mutant glioblastoma cells found that glutamine production is reduced in IDH-mutant gliomas, mainly due to the decrease of branched-chain amino acid transaminase 1 (BCAT1) enzyme, which catalyzes the transamination of branched-chain amino acids while converting α-KG to glutamate [16]. This decrease in Glx was consistent with findings of our previous report on

Table 2 Solid/invasive vs. necrotic tumors and effect on 2HG and Lip13 values

	Solid/invasive	Necrotic
IDH-mutant GB (%)	3 (50)	3 (50)
2HG (mM) median (range)	2.902 (1.309–3.479)	4.119 (1.718–6.041)
Lip13 (mM) median (range)	11.237 (7.356–23.174)	28.762 (27.662–35.355)
IDH-wildtype GB (%)	12 (31.6)	26 (68.4)
2HG (mM) median (range)	0.553 (0–1.993)	0 (0–1.786)
Lip13 (mM) median (range)	10.583 (1.710–37.333)	66.203 (17.989–244.293)

2HG 2-hydroxyglutarate, *GB* glioblastoma, *IDH* isocitrate dehydrogenase, *Lip13* lipid at 1.3 ppm

grade 2 and grade 3 gliomas, and also in line with a report from Nagashima et al. suggesting that elevated 2HG as well as decreased Glu is diagnostic for IDH-mutant gliomas [13]. Thus, the concentration of Glx was thought to be good surrogate marker to 2HG in determining possible false positive IDH-wildtype glioblastoma.

It is well known that glioblastomas have high lipid peaks, likely reflecting necrosis, and a previous report shows that glioblastomas have a higher lipid and macromolecule peaks at chemical shifts of 1.3 ppm (LM13) and 0.9 ppm (LM09) compared to normal brain [17]. We found the same tendency in IDH-mutant glioblastoma, as higher levels of Lip 13 (median 25.420 vs. 7.061 mM, $p = 0.0173$) were noted compared to IDH-mutant WHO grade 3 gliomas.

With careful selection of VOI and parameters, we were able to achieve a 100% specificity and over 92% specificity of 2HG detection by short-echo MRS with modulation of 2HG resonances by spectral fitting. During the study period, newer methods for detection of 2HG in IDH-mutant gliomas have emerged. Long-echo MRS with TE at 97 ms with the use of 3D volume-localized basis (VLB) spectra has been shown to be optimal for detection of 2HG [18, 19]. A comparative study of PRESS sequences at short- (35 ms) and long-TE (97 ms) found long-TE to be superior by minimizing the effect of macromolecule signals [19]. Unambiguous detection of 2HG in mutant IDH gliomas was achieved by 2D correlation spectroscopy (COSY) [12, 20, 21] and J-difference spectroscopy [20]. However, these methods are less available clinically and involve longer acquisition time; 2D correlation MRS involves complex quantification and has less sensitivity [22]. Pre-clinical trials involve intravenous injection of hyperpolarized ^{13}C-labeled α-KG to detect decreased glutamate production in IDH-mutant tumors [16] and hyperpolarized pyruvate to monitor the conversion of pyruvate to lactate, which is decreased in IDH-mutant tumors [23]. These newer techniques are outlined in two important review articles [22, 24].

In the new WHO classification [5], IDH-mutant glioblastomas are distinguished from IDH-wildtype glioblastoma. IDH-mutant glioblastomas occur in younger patients and carry a significant better prognosis than their IDH-wildtype counterparts [2, 25]. However, overall survival was not significantly longer in the high 2HG group in this study ($p = 0.17$, log-rank test, Fig. 4), partly due to the small number of IDH-mutant glioblastoma and because three out of six IDH-mutant cases were recurrent. Evidence suggests that IDH-mutant secondary glioblastomas are more susceptible to temozolomide [26], and IDH-mutant malignant astrocytomas benefit from radical surgical resection [27]. In vivo measurement of 2HG has many potential therapeutic implications in IDH-mutant gliomas, such as indication for radical surgery, indication for presurgical chemotherapy in select cases, determination of therapeutic response after treatments [28], and detection of rare *IDH1* and *IDH2* mutations. Pre-operative diagnosis of IDH-mutation by SVMRS also opens the door for intraoperative-targeted imaging and treatments of IDH-mutant gliomas.

This study was not designed to include a validation cohort. Thus, the cutoff may not be optimal when it comes to reproduction in an independent cohort. The same methods employed for WHO grade 2 and 3 gliomas were employed in the current study for glioblastoma, yielding a similar cutoff of 2HG = 1.489 mM. Ninety-five percent confidence interval determined by the bootstrap method was 0.553 to 1.993 mM. Careful selection of cases by discarding cystic lesions and lesions with marked intratumoral hemorrhage and by minimizing selection of necrosis when determining the VOI were essential to obtain reproducible results.

We have previously reported that quantification of 2HG by SVMRS can be used to reliably distinguish between IDH-mutant and IDH-wildtype WHO grade 2 and 3 gliomas pre-operatively. In the current study, we found that the same methods can be utilized to distinguish between IDH-mutant and IDH-wildtype glioblastoma. It has become increasingly clear that IDH-mutant gliomas have completely different biological backgrounds than IDH-wildtype gliomas. Thus, non-invasive diagnosis of IDH-mutation will increasingly become vital for the treatment of these tumors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was obtained in accordance with the human research guidelines of the Institutional Review Board of University of Niigata.

Informed consent Written informed consent was obtained from all of the participants.

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