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Intraoperative detection of isocitrate dehydrogenase mutations in human gliomas using a miniature mass spectrometer

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Abstract

Knowledge of the isocitrate dehydrogenase (IDH) mutation status of glioma patients could provide insights for decision-making during brain surgery. However, pathology is not able to provide such information intraoperatively. Here we describe the first application of a miniature mass spectrometer (MS) to the determination of IDH mutation status in gliomas intraoperatively. The instrumentation was modified to be compatible with use in the operating room. Tandem MS was performed on the oncometabolite, 2-hydroxyglutarate, and a reference metabolite, glutamate, which is not involved in the IDH mutation. Ratios of fragment ion intensities were measured to calculate an IDH mutation score, which was used to differentiate IDH mutant and wild-type tissues. The results of analyzing 25 biopsies from 13 patients indicate that reliable determination of IDH mutation status was achieved ($p = 0.0001$, using the Kruskal-Wallis non-parametric test). With its small footprint and low power consumption and noise level, this application of miniature mass spectrometers represents a simple and cost-effective platform for an important intraoperative measurement.

Keywords

Molecular cancer diagnostics; Ambient ionization mass spectrometry; Isocitrate dehydrogenase mutation; Miniature mass spectrometry; Point-of-care diagnostics

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Hannah Marie Brown and Fan Pu contributed equally to this work.

Conflict of interest Zheng Ouyang is the founder of PURSPEC Technologies Inc. All other authors declare that they have no conflict of interest.

Compliance with ethical standards Biopsies for tissue analysis were obtained from human subjects undergoing tumor resection for suspected glioma at IU Health Methodist Hospital, after they had provided written informed consent to participate in the research study, following an IUSMIRB approved protocol (IRB No. 1410342262). No results were shared with the neurosurgeons during the surgical resection so as not to affect the standard of care.

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Introduction

The accurate diagnosis of gliomas relies increasingly on molecular features [1]. However, the microscopic review of tissue biopsies, the principal source of intraoperative diagnostic information, does not provide any molecular or genetic information, including information on isocitrate dehydrogenase (IDH) mutation status. Conventionally, mutations in the enzyme IDH are determined using immunohistochemistry with results not available until some days after surgery (Fig. 1). Knowledge of IDH mutation status at the time of surgery could improve surgical outcomes because more aggressive tumor resection of IDH-mutated gliomas is associated with increased survival [2, 3]. IDH mutations alter enzymatic pathways and lead to the accumulation of the oncometabolite 2-hydroxyglutarate (2-HG) [4]. While 2-HG is present in very small concentrations in normal tissues, concentrations increase dramatically in tumors with mutations in IDH1 and IDH2, reaching levels up to 35 μmol per gram of tumor [5]. This feature can be used to assess mutations in IDH, as has been demonstrated using magnetic resonance spectroscopy and mass spectrometry (MS) [6, 7].

MS is a highly sensitive technique that is capable of the qualitative and quantitative analysis of complex samples, including biological tissues. This application is most accurately done in conjunction with liquid or gas chromatography but doing so requires tedious sample preparation. Matrix-assisted laser desorption ionization (MALDI) is an alternative method which is important in tissue analysis [8]. MALDI has been used for the detection of 2-HG in frozen sections; however, the required sample preparation and instrumentation make it incompatible with use in the operating room (OR) [9]. By contrast, ambient ionization is well suited for intraoperative use, as it allows chemical information to be obtained by MS at atmospheric pressure for a variety of matrices, including tissues, thereby eliminating sample pretreatment steps and making possible analysis in the OR. The first ambient methods were desorption electrospray ionization mass spectrometry (DESI-MS) and direct analysis in real time [10, 11]. These experiments have stimulated the development of numerous ambient ionization methods, including air flow–assisted desorption electrospray ionization, matrix-assisted laser desorption electrospray ionization, liquid micro junction-surface sampling, paper spray, MasSpec Pen, and rapid evaporative ionization MS [12–18]. They have also prompted a reexamination of conventional tissue diagnosis.

We have previously measured the abnormal accumulation of 2-HG for the intraoperative diagnosis of IDH mutation status using DESI-MS in near real time [19]. However, this intraoperative application requires expensive instrumentation. By contrast, miniature MS systems have small footprints, have low energy consumption, and produce little noise [20]. Their use in the OR offers the prospect of intraoperative genetic diagnosis in a cost-efficient manner. In an earlier study, we established the fact that a miniature mass spectrometer can be used to determine IDH mutation status in banked brain tissue samples [21]. In this small-scale clinical study with 13 subjects, we report the first application of a miniature mass spectrometer to determine IDH-mutation status of glioma tissue intraoperatively, building on prior laboratory-based work [21, 22].

Experimental

Sampling method

Extraction nanoelectrospray ionization (nESI), a method characterized by minimal sample preparation, was used to generate ions [23]. We have previously established this method for rapid diagnosis of IDH mutation status based on measurement of 2-HG using banked tissue samples [21], so similar procedures were carried out in the OR. Biopsied tissue specimens from surgeon-defined positions within the tumor and at the walls of the resection cavity were collected intraoperatively. The number and location of the biopsies were determined according to the medical judgment of the attending surgeon. The biopsies were touched with a thin strip of Whatman 1 filter paper (ca. 0.5 mm wide and 15 mm long) that was then inserted into a nanotip (i.d. 0.86 mm, length ~ 4 cm) pre-filled with 20 μL methanol/water (9:1, *v/v*). The nanotip was subsequently placed in a custom 3D printed sample holder for MS analysis.

MS analysis

The experiment used the ion trap based PURSPEC Mini β, a stand-alone system that does not require external pumps or gas tanks. The Mini β, which measures 55 cm × 24 cm × 31 cm (*L* × *W* × *H*), was placed on an aluminum cart with all equipment required for its operation in the OR (Fig. S1, see Electronic Supplementary Material, ESM). An enclosed volume was employed to minimize any material transfer from the instrument. Prior to each surgery, the instrument was rolled into the OR and operated within the surgical suite for the duration of the surgery. The method is not quantitative, but we carried out calibration of the instrument before and after every surgical case to establish mass accuracy. This was done using a solution composed of aspartic acid, glutamate (GLU), 2-HG, and N-acetylaspartate using nESI in the negative ion mode; their molecular ions were found at *m/z* 132, 146, 147, and 174, respectively (Fig. S2, ESM). Ionizing voltage (− 1.5 kV) was applied to initiate nESI from a disposable acupuncture needle (diameter 0.3 mm, length 4 cm).

Tandem MS analysis of 2-HG was performed in the negative ion mode with GLU, an abundant metabolite not specifically associated with glioma, serving as an internal standard. The product ion abundances for the transitions *m/z* 147 → 129 for 2-HG and *m/z* 146 → 128 for GLU provided a relative measure of the concentration of 2-HG in tissue. The precursor ions were selected using a 2-Th-wide mass window. For each sample, at least three product ion spectra were recorded, each recorded spectrum being the average of three scans. Each scan took ~ 2 s and spectra were saved manually, making the total data acquisition time per sample ca. 1 min and the total analysis time including sample manipulation ca. 3 min. Postoperative analysis of the same biopsies was performed using the extraction nESI method on a Thermo TSQ to confirm intraoperative IDH mutation predictions. The TSQ analysis was performed using multiple reaction monitoring (MRM) mode; transitions selected were 2-HG (*m/z* 147 → 129) and GLU (*m/z* 146 → 128) (Figs. S3 and S4, ESM). With respect to each transition, optimized tube lens values of 67 and 69 V and nominal collision energies of 12 and 13 were applied.

Data analysis

De-identified clinical data, consisting of patient demographics and radiology, surgical, and pathology reports, were obtained for each patient for correlation with the data produced by the Mini β system. The Mini β data were analyzed within 30 s postoperatively in MATLAB using a custom program to subtract background intensities and to calculate IDH mutation scores. This calculation can be done intraoperatively. The TSQ data were analyzed using Xcalibur. An IDH mutation score was calculated as the ratio of the two product ion intensities after correcting for the isotopic contributions of the ^{13}C glutamate fragment ion at *m/z* 129 (Eq. 1).

$$IDH \text{ mutation score } = \frac{I_{129} - (I_{128} \times 6.1\%)}{I_{128}} \quad (1)$$

Results and discussion

In this preliminary clinical study, we analyzed 25 biopsies from 13 subjects. The patient cohort is described in detail in Table 1. Of the 13 subjects, 4 were IDH mutant (7 biopsies) and 9 were IDH wild-type (18 biopsies), according to postoperative pathology reports based on immunohistochemistry. Representative product ion mass spectra from an IDH wild-type and an IDH mutant glioma are included in the ESM (Figs. S5 and S6). As shown in Fig. 2 (left), the IDH wild-type tissues all had IDH mutation status scores below 0.5, whereas all IDH mutant samples had scores above 1.7. The box represents the interquartile range with a median line and whiskers at ± 1.5 standard deviations. The clear differentiation between IDH wild-type and mutant ($p = 0.0001$) is consistent with our previous observations on banked tissue samples [21], which suggests that intraoperative diagnosis of IDH mutation status can be realized with the current methodology.

Postoperative analysis of the same biopsies was performed using extraction nESI on a Thermo TSQ. All IDH wild-type tissues had IDH mutation scores below 0.1 and all IDH mutant tissues had IDH mutation scores above 5.0 (Fig. 2, right) using this higher performance lab system. The higher sensitivity of the Thermo TSQ results in less noise and hence an increase in the absolute differences of the mutation scores. Identical conclusions can be drawn from both sets of results, as shown in Fig. 2. For both methods, the differences in population medians for the IDH mutation scores were statistically significant different (p values both 0.0001, using Kruskal-Wallis non-parametric test). Both instruments are capable of generating diagnostic information consistent with pathology. In this assay, the intraoperative application of a miniature mass spectrometer shows comparable performance with that of a conventional instrument.

Conclusion

We have successfully performed intraoperative diagnosis of IDH mutation using extraction nESI on Mini β. The instrumentation was modified to be compatible with use in the operating room. With its small footprint, low power consumption, minimal noise, and ability to generate reliable IDH mutation status predictions in near real time, the intraoperative use

of a miniature mass spectrometer represents an advance towards improved glioma patient treatment. Further studies will be carried out with protocol standardization and automation to validate these preliminary findings and reduce the barrier for broader application.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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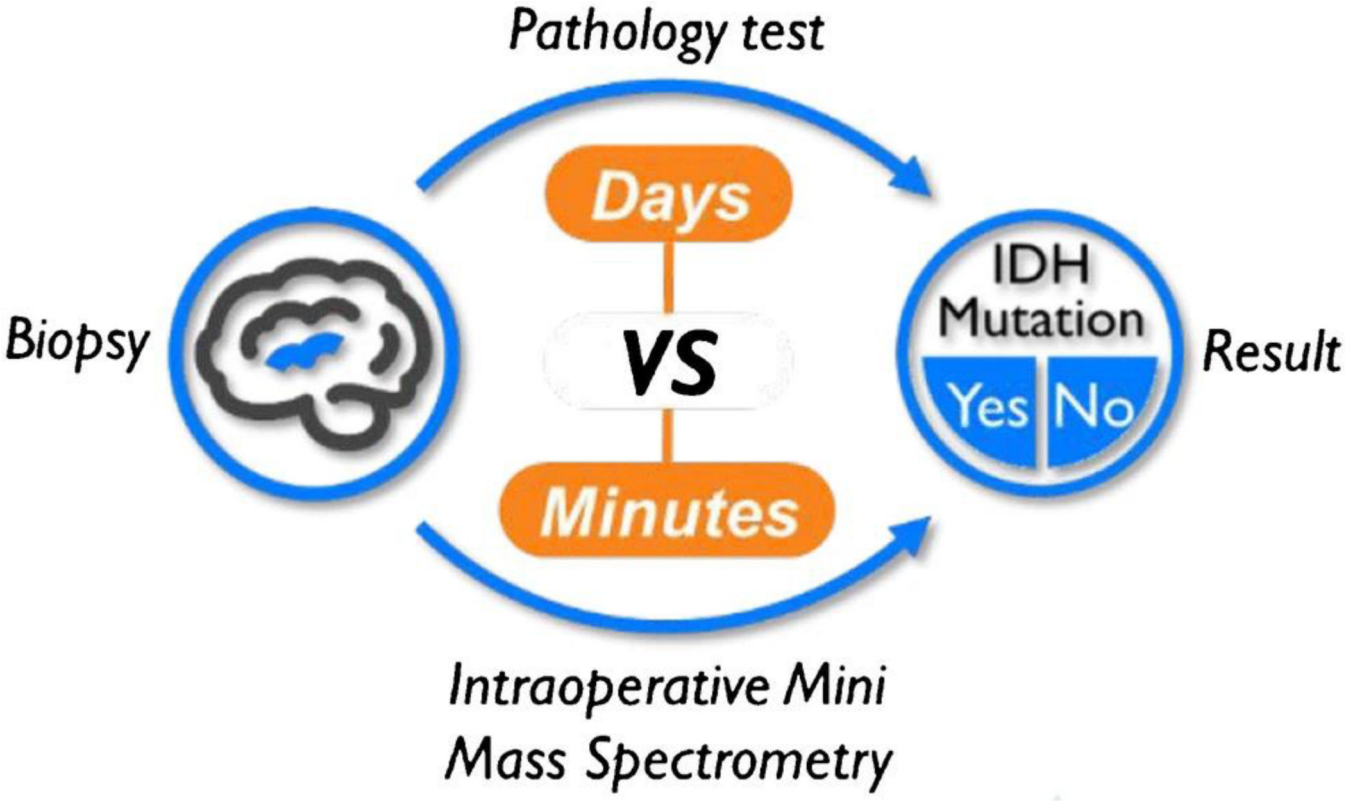

Fig. 1.

Intraoperative detection of IDH mutations with Mini MS determines mutation status in minutes as opposed to days by means of conventional genomic analysis or immunohistochemistry

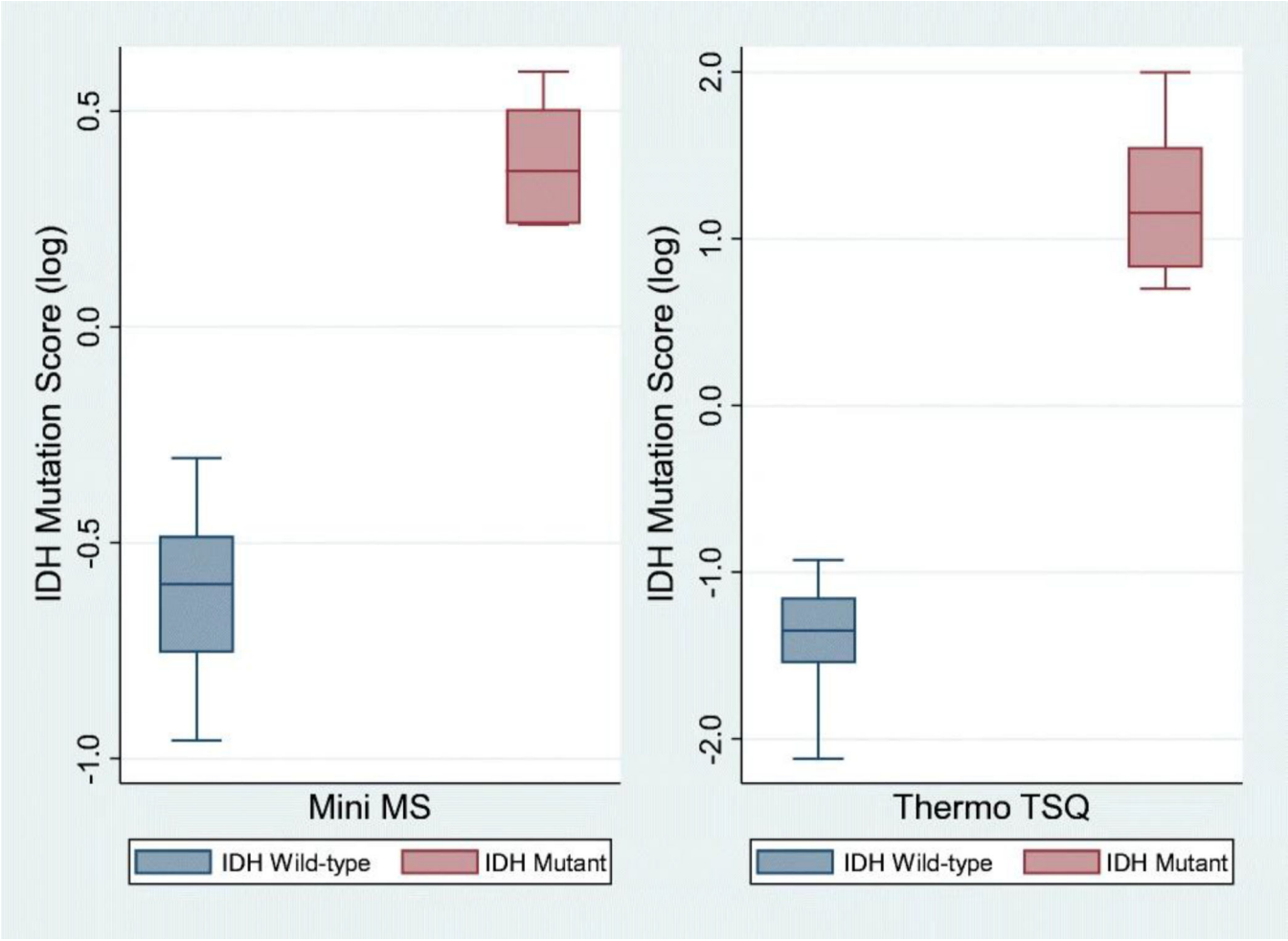

Fig. 2.

Box-and-whisker plot of IDH mutation scores in human glioma tissue specimens ($n = 25$) analyzed intraoperatively using a PURSPEC Mini β and in the laboratory using a Thermo Triple Quadruple TSQ. (The box represents the interquartile range with a median line and whiskers at ± 1.5 SD. Two outliers (IDH wild-type) with IDH mutation scores below 1.5 SD were excluded in the Mini MS plot. Population medians for IDH mutation scores for both methods were statistically different; p values for both are 0.0001, using the Kruskal-Wallis non-parametric test.)

Table 1

IDH mutation scores of tissue biopsies

Subject	Biopsy	Type of glioma	WHO grade	IDH mutation status	IDH mutation score (Mini MS)	IDH mutation score (TSQ)
1	1	Glioblastoma	IV	Wild-type	0.06	< 0.01
2	1	Glioblastoma	IV	Wild-type	0.02	0.02
3	1	Astrocytoma	II	Mutant	3.89	10.56
4	1	Pleomorphic xantoastrocytoma	II	Wild-type	0.33	0.03
	2				0.18	0.02
	3				0.35	0.07
5	1	Glioblastoma	IV	Wild-type	0.20	0.06
	2				0.24	0.06
	3				0.33	0.12
6	1	Glioblastoma	IV	Wild-type	0.35	0.04
	2				0.33	0.04
7	1	Glioblastoma	IV	Wild-type	0.28	0.07
	2				0.20	0.09
8	1	Anaplastic astrocytoma	III	Mutant	2.83	5.01
9	1	Glioblastoma	IV	Wild-type	0.11	0.05
10	1	Glioblastoma	IV	Mutant	3.20	35.51
	2				1.73	99.33
	3				1.73	14.34
11	1	Glioblastoma	IV	Wild-type	0.12	0.06
12	1		III	Mutant	2.07	6.73
	2				2.30	16.93
13	1	Glioblastoma	IV	Wild-type	0.25	0.04
	2				0.35	0.04
	3				0.50	0.07
	4				0.26	0.03