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Rapid determination of isocitrate dehydrogenase mutation status of human gliomas by extraction nanoelectrospray using a miniature mass spectrometer

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Abstract

Isocitrate dehydrogenase (IDH) I and II mutations in gliomas cause an abnormal accumulation of 2-hydroxyglutarate (2-HG) in these tumor cells. These mutations have potential prognostic value in that knowledge of the mutation status can lead to improved surgical resection. Information of mutation status obtained by immunohistochemistry or genomic analysis is not available during surgery. We report a rapid extraction nanoelectrospray ionization (nESI) method of determining 2-HG. This should allow the determination of IDH mutation status to be performed intraoperatively, within minutes, using a miniature mass spectrometer. This study demonstrates that the combination of tandem mass spectrometry with low resolution mass spectrometry allows this analysis to be performed with confidence.

Graphical Abstract

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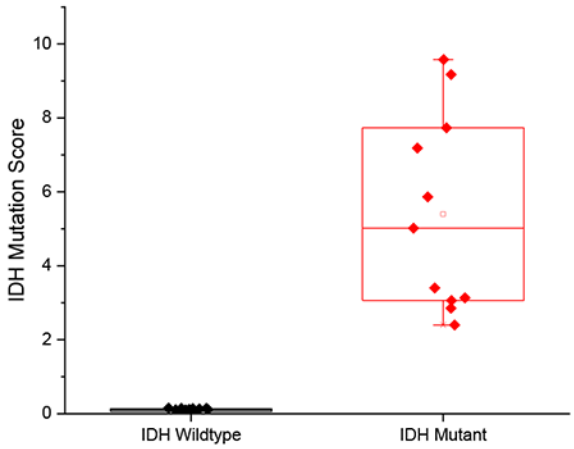
Compliance with Ethical Standards

Banked tissue samples were obtained from the Methodist Research Institute Biorepository in Indianapolis in accordance with approved Institutional Review Board (IRB) protocols at Indiana University School of Medicine (IUSM) (IRB #1410015344). Tissues for bulk tissue analysis were prospectively obtained from human subjects undergoing tumor resection for suspected glioma at Indiana University Department of Neurosurgery, Goodman Campbell Brain and Spine Institute, after they had provided written informed consent to participate in the research study, following an IUSM IRB approved protocol (IRB #1410342262).

Conflict of Interest

Zheng Ouyang is the founder of PURSPEC Technologies Inc.



Keywords

2-hydroxyglutarate; glutamate; IDH mutation; tandem mass spectrometry; clinical analysis

Introduction

Gliomas represent about 27% of all primary brain and other central nervous system tumors, while accounting for about 81% of all malignant brain tumors.[1] Patients with high grade gliomas have poor outcomes. Glioblastoma, the most invasive form of glioma, has a five-year survival rate of just 5.5% and there are projected to be 12,760 cases in the US in 2018.[1] Mutations of isocitrate dehydrogenase (IDH) I and II have been found in a majority of World Health Organization (WHO) grade II and III gliomas.[2, 3] Patients with IDH mutations have better outcomes than those with IDH wildtype.[4, 3] Clinical studies have shown that aggressive treatment with radiotherapy and chemotherapy could benefit patients with IDH mutant glioma.[5–7] Maximal surgical resection of IDH1 mutant malignant astrocytomas was also reported to benefit patients.[8] A rapid diagnostic method for intraoperative determination of IDH mutation status is much needed and could potentially improve resection outcome.

IDH 1 and IDH 2 enzymes catalyze the conversion of isocitrate to α-ketoglutarate. Mutant forms of the enzyme catalyze the further reduction of α-ketoglutarate to the oncometabolite 2-hydroxyglutarate (2-HG). The accumulation of 2-HG can reach up to 35 μmol per gram of tumor[9, 10] and the level of 2-HG in glioma can be indicative of IDH mutation status. Measurements of 2-HG in glioma have been demonstrated using magnetic resonance spectroscopy.[11–13] Magnetic resonance spectroscopy detection of 2-HG is non-invasive and can be performed *in vivo.* However, detection of 2-HG is challenging using conventional 1D magnetic resonance spectroscopy due to spectral overlap with glutamate (GLU) and glutamine, thus 1D-spectral editing or 2D correlation magnetic resonance spectroscopy is necessary to reduce number of false positives.[11]

Mass spectrometry (MS) is a highly sensitive technique for qualitative or quantitative analysis of complex samples. MS is conventionally used in conjunction with liquid or gas

chromatography although this requires tedious sample preparations including chromatographic separation. The direct MS analysis of complex samples without any sample preparation is first demonstrated with desorption electrospray ionization (DESI) MS[14] and direct analysis in real time (DART) MS[15]. Since then, numerous new ambient ionization methods have been reported, and comprehensive reviews are available[16–18]. DESI-MS has been applied to mapping of oncometabolites from tissue sections[19], discrimination of glioma, white matter and grey matter based on their lipid profiles[20], intraoperative assessment of tumor margins during glioma resection[21] and detection of microscopic skin cancer[22]. Tissue analysis has also been realized by other ambient ionization methods such as paper spray[23], needle biopsy spray[24], touch spray[25], swab touch spray[26], nano-DESI[27] and liquid microjunction surface sampling probe[28, 29].

Recently, we have developed ion trap based miniature mass spectrometer (Mini MS) systems[30–34] and demonstrated their use for therapeutic drug monitoring[33], illicit drug detection[34], and preclinical pharmacokinetics[35]. These miniature MS instruments take the form of standalone systems without external pumps or gas tanks and fit easily into point-of-care settings such as an operating room. The oncometabolite 2-HG has previously been measured intraoperatively by tandem mass spectrometry using a commercial benchtop mass spectrometer.[36, 19] Here we report using of a Mini MS with extraction nanoelectrospray ionization (extraction nESI) for 2-HG determination. The experiments were done with banked tissue samples as a preliminary study to assess performance of a miniature MS in intraoperative identification of IDH mutation status in glioma tissue biopsies. In this application, GLU and 2-HG were simultaneously isolated and the intensities of their fragments compared to provide a *relative* measure of 2-HG in tissue. The structures of the analytes and the MS/MS transitions of interest are given in Electronic Supplementary Material (ESM) Table S1.

Experimental

Tissue Samples

Tissue sections (15 μm thickness) were prepared using a cryotome and thaw mounted on superfrost microscope slides (Thermo Fisher Scientific, Waltham, MA). IDH mutation status was determined using immunohistochemistry and genetic sequencing at Indiana University Health Molecular Pathology Laboratory. Adjacent tissue sections and smears were H&E stained using a previously published protocol[20] and blindly evaluated by an expert neuropathologist to provide assessments of tumor cell percentage and diagnosis for each sample.

Extraction nESI

A narrow strip of paper (ca. 0.5 mm wide and 15 mm long) was cut from Whatman 1 filter paper. The paper strip was wiped over the tissue sample (thawed tissue section or intact tissue) to pick up small amounts of material. The sample strip was then inserted into a nanotip (i.d. 0.86 mm, length about 4 cm) preloaded with 10 μL methanol/water (9:1, v/v), which act as both extraction and spray solvent. An acupuncture needle (diameter 0.3 mm, length 4 cm) was inserted into the pulled nanotip to act as a disposable electrode to prevent

cross-contamination. Negative 1.3 kV was applied to the needle to initiate nanoelectrospray. Analytes were constantly extracted during the ionization process within the nanotip.

MS Analysis

A linear ion trap Mini MS, Mini β mass spectrometer (PURSPEC Technologies, West Lafayette, IN), was operated in the negative ion mode. The scan function was optimized to isolate and then fragment ions of both *m/z* 146 and 147 simultaneously by ramping RF voltage while a fixed AC frequency was applied. Ion abundances for the transitions of interest, *m/z* 147->129 for 2-HG and *m/z* 146->128 for glutamate (GLU), were recorded. For each sample, 5 spectra were recorded and each spectrum was an average of three scans. Each scan takes 1.7 second, and spectra were saved manually making the total analysis time per sample ca. 1 minute. The scan rate of the Mini MS was 3200 Da/s, ejection AC frequency was 330 kHz.

Additional details on materials, fabrication of nanotips, calculation of IDH mutation score and statistical analysis are given in the Supplementary Information.

Results and Discussion

Extraction nESI for Brain Tissue

Extraction nESI was reported previously for therapeutic drug monitoring in whole blood[37, 38], and we have adapted it here to direct tissue analysis. In this application, deprotonated forms of GLU and 2-HG were isolated simultaneously and the intensities of their fragments compared to provide a relative measure of 2-HG in tissue. The structures and MS/MS spectra of the individual compounds are shown in the ESM (Table S1 and Fig. S1, respectively). Fig. 1 illustrates tissue analysis performed using extraction nESI. On average, the time from sampling to result is ca. 5 min. Typical MS/MS spectra from tissue extracts recorded using the Mini MS are shown in Fig. 2. The scan function for these experiments utilized a wide precursor ion isolation window covering both *m/z* 146 and *m/z* 147, which correspond to the deprotonated forms of 2-HG and GLU, and which give product ions at *m/z* 129 and *m/z* 128, respectively. MS/MS of the extraction nESI from IDH mutant samples showed fragments from both 2-HG and GLU (Fig. 2 (a)), whereas MS/MS of the extraction from IDH wildtype samples showed only fragments from GLU (Fig. 2(b)).

GLU is an abundant brain metabolite and has been reported[39] to have lower levels in IDH mutant gliomas compared to IDH wild-type (3.23 ± 1.27 mM in IDH mutant and 5.22 ± 1.36 mM in IDH wildtype). The combined assessment of 2-HG and GLU for IDH mutation assessment improves prediction compared to using 2-HG alone.[39] Using endogenous GLU instead of isotope labeled internal standard as a reference for 2-HG simplifies the assay.

An IDH mutation score was obtained by calculating the ratio of product ion intensities, *m/z* 129 intensity divided by *m/z* 128 intensity, with isotopic corrections for contributions of the natural C_{13} glutamate fragment ion to signal at *m/z* 129. A higher IDH mutation score indicates more 2-HG relative to GLU. Since IDH mutations result in accumulation of 2-HG in tissue and decreased levels of GLU, we hypothesized that a high IDH mutation score would be indicative of an IDH mutant glioma.

Analysis of Tissue Sections

We analyzed 39 glioma tissue cryosections (29 IDH wild-type and 10 IDH mutant) prepared from banked glioma tissues, collected from 29 human subjects, as an initial evaluation of the method. The IDH mutation scores ranged from 0.04 to 0.16 for IDH wildtype and from 0.28 to 6.83 for IDH mutant samples (ESM Table S2). The distribution of IDH mutation scores was significantly higher in the IDH mutant glioma samples relative to the IDH wild-type gliomas, and the distributions were statistically different ($p=0.0025$, ESM Fig. S2). IDH mutant samples with low tumor cell percentage (TCP) had lower IDH mutation scores compared to IDH mutant samples with high TCP (ESM Fig. S2). This trend has been reported previously[19] and is recapitulated in our results.

Many of the tissue sections analyzed in this study are sections adjacent to those used in a previous study in which 2-HG was quantified using direct infusion ESI-MS/MS[40]. The IDH mutation scores obtained from the Mini MS samples are highly correlated to the previously published quantitative results. A coefficient of determination (R^2) of 0.9229 was obtained for the five IDH mutant tissue sections, plotted as red points in Fig. 3. Meanwhile, the 2-HG concentrations of IDH wildtypes were below limits of detection for both methods, hence the 23 blue points group at the lower left corner of the plot. The high correlation seen indicates that higher IDH mutation scores are related to higher concentrations of 2-HG in the tissue. The correlation is indicative only because the data are being compared with those of another study on adjacent tissue samples[40]. The comparison assumes that the distribution of 2-HG is homogeneous throughout the tissue and no sample deterioration occurred on storage.

Analysis of Bulk Tissues

To further evaluate the method for intraoperative diagnostic purposes, we analyzed frozen bulk tissue biopsies as an intermediate step to fresh tissue analysis. Tissue biopsies were originally collected during surgeries in 1.5 mL centrifuge tubes and kept frozen at −80 °C. After thawing, each tissue piece was touched using a paper strip and analyzed with extraction nESI using the Mini MS. Reproducibility was examined by sampling three times from the same position on the same tissue biopsy. The results are presented in ESM Table S3. The average relative standard deviation was 29.0 ± 3.6 % for three medium or high TCP IDH mutant tissues and 24.8 ± 11.4 % for five IDH wildtype tissues.

A total of 44 biopsies from 15 subjects were analyzed using extraction nESI with Mini MS. The IDH mutation status and IDH mutation scores are summarized in ESM Table S4. The IDH wildtype tissues had IDH mutation scores below 0.15 regardless of TCP, whereas all IDH mutant samples had IDH mutation scores above 0.40 ($p = 1.06$ E-6). Among the 28 IDH mutant tissue biopsies, 12 were low TCP tissues, 5 were medium TCP tissues and 11 were high TCP tissues. The IDH mutation scores of these samples are compared in ESM Fig. S3.

The TCP can significantly impact the diagnosis of IDH mutation; low TCP samples have fewer tumor cells and correspondingly lower quantities of 2-HG. In Fig. 4, IDH mutation scores of 11 IDH mutant with high TCP and 16 IDH wildtype tissue biopsies are compared.

The difference in IDH mutation scores between the highest-scored IDH wildtype and the lowest-scored IDH mutant with high TCP was 16 times (0.15 *vs.* 2.40). The difference in IDH mutation scores between the highest-scored IDH wildtype and the lowest-scored IDH mutant with low TCP was less than 3 times (0.15 *vs.* 0.40). Some low TCP samples have relatively high scores (ESM Fig. S3, up to 5.17), which may reflect TCP variations in tissue. However, discrimination of IDH mutants from IDH wildtypes is achieved regardless of their TCP.

Conclusions

We demonstrate a method for rapid assessment of IDH mutation status of banked human gliomas samples using extraction nESI with a portable Mini MS. The Mini MS instrument used in this study provided reliable diagnostic information regarding IDH mutation status with a much smaller footprint compared to conventional bench-top mass spectrometers, thus being more suitable for an operating room. The average sampling to result time was 5 minutes. Bulk tissues of IDH wildtypes and IDH mutants with high TCP were differentiated with a 16-fold difference in IDH mutation scores. These merits suggest that the methodology could enable assessment of IDH mutation status of glioma biopsies at point-of-care during brain surgery. In our follow-up study, we will evaluate this methodology in the intraoperative environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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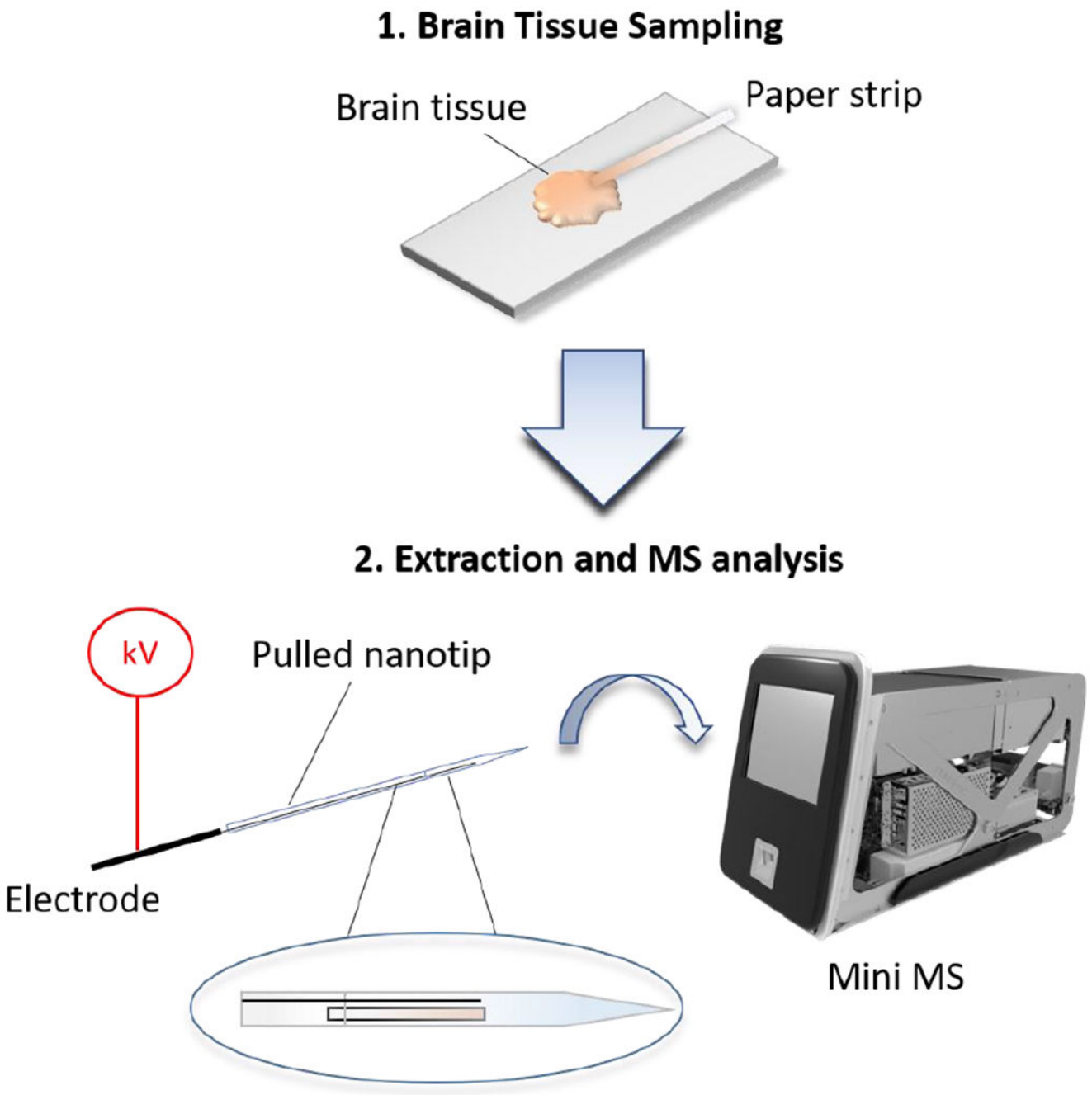

Fig. 1.
Extraction nanoelectrospray of brain tissue

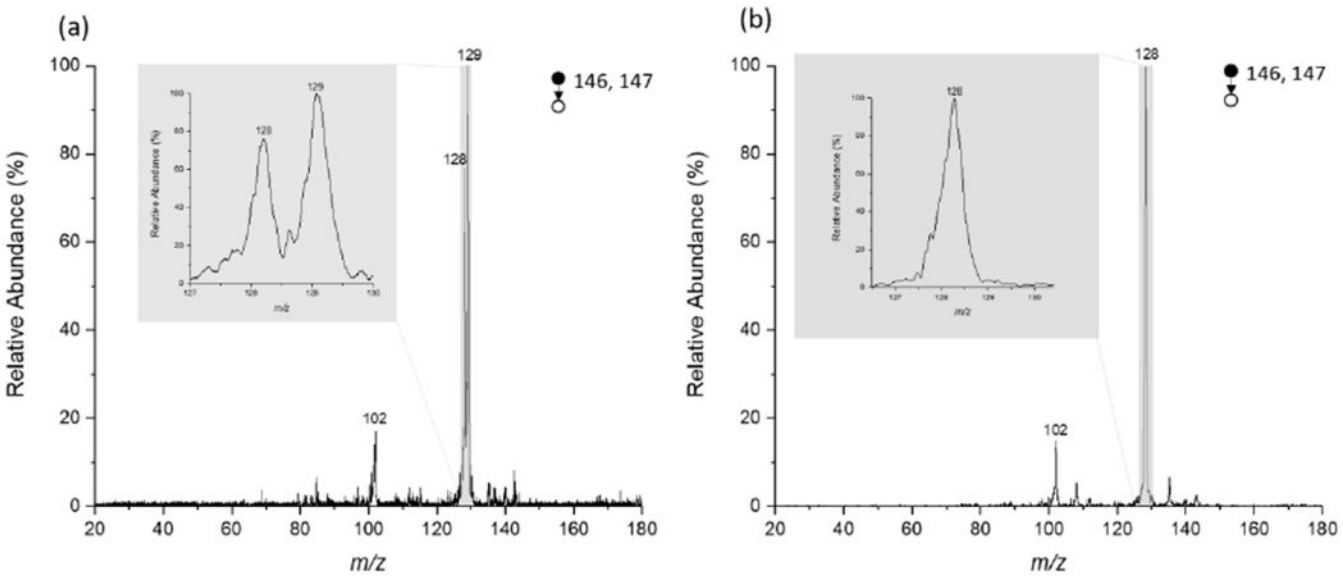

Fig. 2.

MS/MS spectra recorded using Mini MS (a) IDH mutant glioma and (b) IDH wildtype glioma. Note that peak at m/z 129 only occurs in IDH mutant tissue

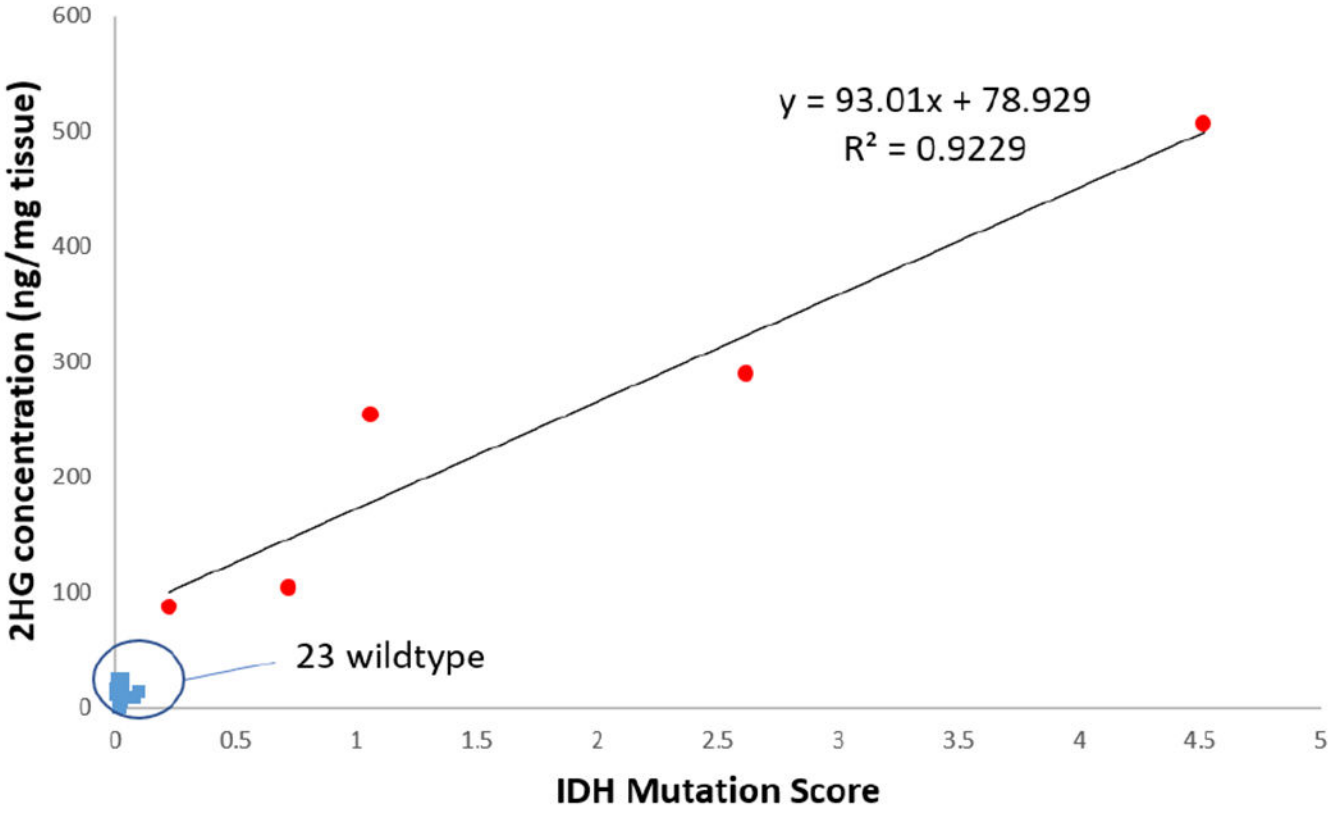

Fig. 3.

Correlation between concentration of 2-HG determined by triple quad MS analysis [40] and IDH mutation scores determined using the Mini MS [this work] from an adjacent tissue section

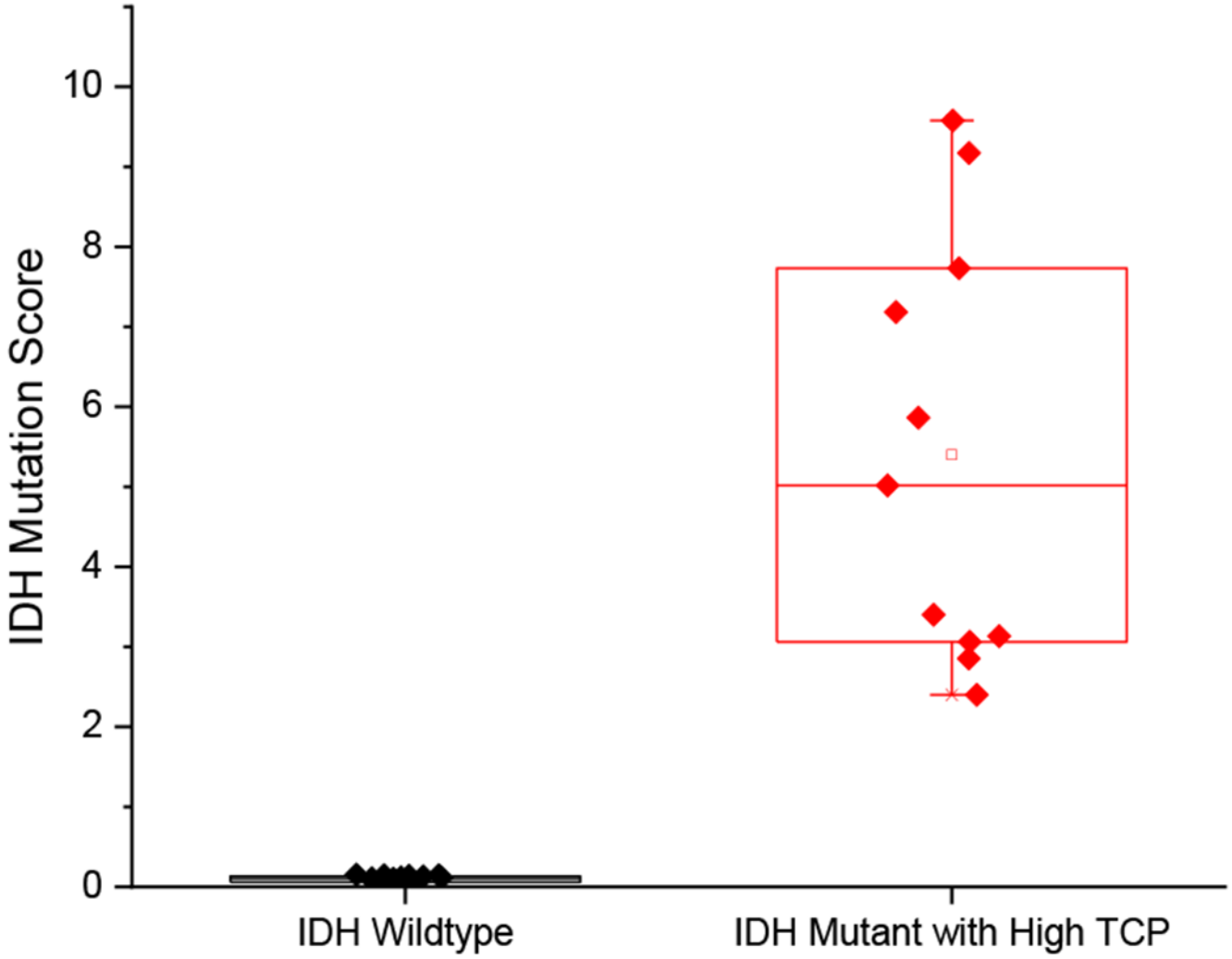

Fig. 4.
Comparison of IDH mutation scores of IDH wildtype (16 samples) and IDH mutant with high TCP (11 samples)