2-Hydroxyglutarate in Gliomas with IDH Gene Mutation Using High Resolution 1H-NMR Spectroscopy of Tissue Extracts

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Introduction

Somatic mutations in the enzyme isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) have been identified in astrocytic and oligodendroglial tumors of WHO grades II and III, and in secondary glioblastoma (GBM) [1]. The IDH1 and IDH2 mutations are associated with elevated levels of 2-hydroxyglutarate (2HG) and may serve as a clinical biomarker for disease stratification and prognosis [2]. We have been studying metabolism of glucose in the citric acid cycle of tumors by infusing [U-^{13}C]glucose and taking biopsies of tissue at the time of surgical resection for later analysis. These samples can also be used to investigate the presence of 2HG by ^{1}H-NMR methods. The aim of this study was to measure the concentration of 2HG by ^{1}H-NMR in the extracts.

Methods

Tissue samples from 22 patients with gliomas were examined: 7 IDH1/2 mutant (e.g., 3 WHO grade II and 4 WHO grade III) and 15 wild type tumors (e.g., 1 WHO grade I, 2 WHO grade II, 2 WHO grade III, and 10 WHO grade VI). These samples were taken from patients receiving an infusion of ^{13}C-enriched glucose. The samples ranged from 200 to 700 (mean ± SD, 490 ± 210) mg wet weight. Percholric acid extracts of the tissues were redissolved in 0.2 mL of deuterium oxide. ^{1}H-NMR spectroscopy was performed on a Varian 600MHz spectrometer using 3 mm broadband NMR probe. The spectral parameters were as follows: 90° pulse angle, 7225 Hz sweep width, 32K complex data points, relaxation delay 0.05 s and acquisition time 4.0 s, resulting in a repetition time of 4.05 s, 256 transients, and total acquisition time of 23 min. The absolute metabolite concentrations were calculated using equation: $[C]_i = (N_{TSP}/N_i) \times (S_i/S_{TSP}) \times (mol/M_{sample})$, where $[C]_i$ is the concentration of the metabolite (µmol/g), S_i is the amplitude of the metabolite and S_{TSP} is the signal amplitude of 3-(Trimethylsilyl)propionic acid (TSP), *mol* is the number of moles the TSP and M_{sample} is the weight of the sample. The terms N_i and N_{TSP} represent the number of ^{1}H nuclei contributing to the resonance of metabolites i(i = 2HG, Glu, Gln, and GABA) and TSP.

Results

Figures 1 and 2 demonstrate representative high resolution one-dimensional ^{1}H-NMR on glioma samples with IDH1/2 mutated and wild type tumors. 2HG signal was detected in the IDH mutated tumors but not wild type tumors. A 2HG molecule has two methylene groups ($^{4}CH_2$ and $^{3}CH_2$) and a methine group (^{2}CH) that give NMR signals at 4.03, 2.26, 1.99, and 1.84 ppm. Three of four multiplets of 2HG were observed (Figure 1). On the ^{1}H-NMR spectra, the 2HG resonances were partially overlapped with those of GABA, Glu, Gln, and NAA. In this study 2HG ($^{4}CH_2$ at 2.26 ppm), Glu ($^{4}CH_2$ at 2.36 ppm), Gln ($^{4}CH_2$ at 2.46 ppm), and GABA ($^{4}CH_2$ at 2.30 ppm) metabolites were measured by fitting a Voigt (Gauss and Lorentz) function. ^{1}H-NMR result was positive for 2HG in all tumors that contained IDH1/2 mutation but negative in all tumors with wild type . The measured 2HG levels ranged from 0.55 to 3.51 (mean ± SD, 2.00 ± 1.02) µmol/g. COSY spectroscopy and authentic solutions were used to confirm assignments.

Discussion

This study supports the use of 2HG as a biomarker of IDH1/2 mutation status in glioma [1, 3]. With the chemical shift dispersion that is available for analysis of tissue extracts, signals from Gln, Glu and GABA were easily resolved. The levels of 2HG in this work had a range of 0.55 - 3.51 µmol/g from seven glioma samples with IDH1/2 mutation (3 WHO grade II and 4 WHO grade III), which is consistent with the finding of previous *in vivo* ^{1}H-MRS study (e.g., 1.7 – 8.9 mM, Choi *et al*. [4]). Dang *et al*. [2] reported that the range of 2HG by mass spectroscopy was from 5 to 35 µmol/g. In addition, Glu levels were found to be lower in IDH1/2 mutant tumors compared to IDH1/2 wild type tumors (0.80 vs. 1.98 µmol/g, $P < 0.0001$). This result reflects that IDH mutations cause a decrease in Glu and/or α-ketoglutarate production and an increase in 2HG. Two-dimensional ^{1}H-NMR spectroscopy (e.g., COSY) may help detect and assign the resonances difficult to observe in the one-dimensional ^{1}H-NMR spectra (Figure 3).

References

[1] Yan, *et al.*, NEJM 2009;360-773. [2] Dang *et al*., Nature 2009;462: 739-743. [3] Parsons *et al*., Science 2008;321:1807-1812. [4]. Choi *et al*., Nat Med 2011; accepted.

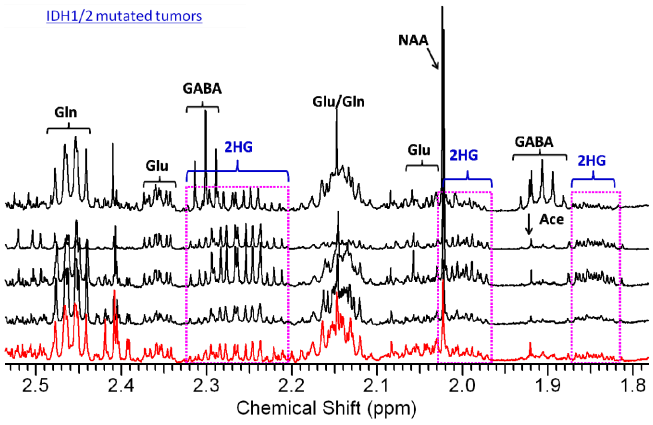

Fig. 1 *In vitro* ^{1}H-NMR spectra of 5 IDH1 mutated tumors. The increased 2HG levels were detected at 2.26, 1.99, and 1.84 ppm in all tumors.

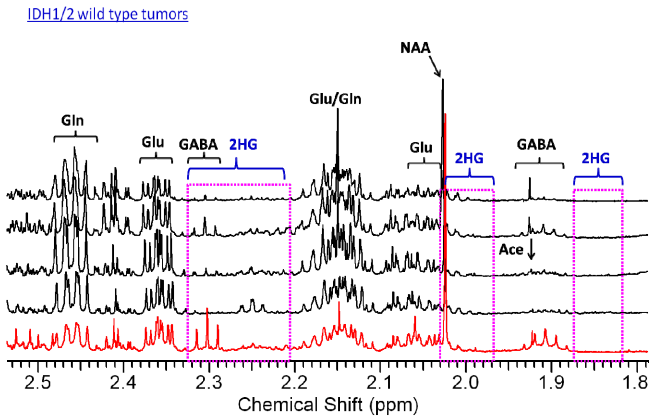

Fig. 2 I*n vitro* ^{1}H-NMR spectra of 5 IDH-wild type tumors. 2HG signals were not detected at 2.26, 1.99, and 1.84 ppm in all tumors.

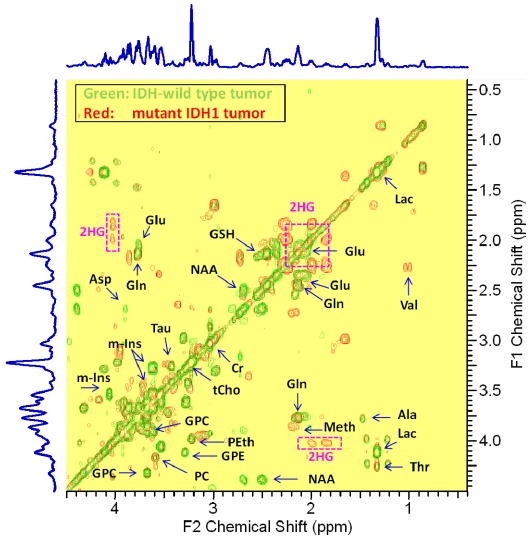

Fig. 3 2D-COSY overlaying spectra of IDH1 mutated and wild type tumors. 2HG was identified at 4.03, 2.26, 1.99, and 1.84 ppm.