Detection and Quantification of 2-Hydroxyglutarate in Gliomas with IDH Gene Mutation Using High Resolution 900MHz 1H-NMR Spectroscopy

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Introduction

Clinically, the presence of an IDH1 or IDH2 mutation has been shown to be associated with a better survival [1]. Several studies have shown that the elevated 2-hydroxyglutarate (2HG) can be used as a novel biomarker of IDH1/2 mutation status in glioma [2, 3]. Thus, the ability to detect and quantify the increased 2HG levels by using magnetic resonance techniques could have significant implications on patient care. The aim of this study was twofold, firstly to demonstrate the feasibility of *in vitro* high resolution 900MHz Cryo NMR spectroscopy for quantifying the 2HG concentrations in glioma samples with IDH1/2 mutation, and secondly to compare the range measured with the current method with previously published results.

Methods

Tissue samples from 20 patients with gliomas were examined: 7 IDH1/2 mutant and 13wild type tumors. The samples ranged from 80 to 950 (mean ± SD, 382 ± 310) mg wet weight. Percholric acid extracts of the tissues were redissolved in 0.28 mL of deuterium oxide. High resolution *in vitro* 1H-NMR spectroscopy was performed on a Bruker 900MHz spectrometer (Avance II 900) using 5 mm NMR probe. The spectral parameters were as follows: 90° pulse angle, 11718 Hz sweep width, 32K complex data points, relaxation delay 0.05s, acquisition time 2.8s, 64 transients, and total acquisition time of ~4 min. The absolute metabolite concentrations were calculated using equation: $[C]_i = (N_{TSP}/N_i) \times (S_i/S_{TSP}) \times [([C]_{TSP} \times V_{sample})/M_{sample}]$, where $[C]_i$ and $[C]_{TSP}$ are the concentration of the metabolite and 3-(Trimethylsilyl)propionic acid (TSP) (μmol/g), S_i is the amplitude of the metabolite and S_{TSP} is the signal amplitude of TSP, V_{sample} is sample volume 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, GABA, etc.) and TSP.

Results

Figure 1 shows high resolution one-dimensional 1H NMR spectrum of 100 mM 2HG solution scanned at 900MHz (21.1 Tesla) with corresponding chemical structure. A 2HG molecule has two methylene groups ($^4CH_2$ and $^3CH_2$) and a methine group (^2CH) that give NMR signals 1.84, 2.00, 2.24, 2.29, and 4.02 ppm. Five multiplets of relative intensities1:1:1:1:1 were observed (Figure 1). Figure 2 demonstrates representative high resolution one-dimensional 1H-NMR on glioma samples with IDH1 mutated (top) and wild type (bottom) tumors. 2HG signal was detected in the IDH mutated tumors but not wild type tumors. On the 900MHz 1H-NMR spectra, the five protons of 2HG gave five resonance multiplets, like house-made 2HG solution experiment (Figure 1). The 2HG resonances at 2.00 and 2.29 ppm were partially overlapped with those of GABA, Glu, and NAA. In this study 2HG ($^4CH_2$ at 2.24 and 2.29 ppm, $^3CH_2$ at 1.84 and 2.00 ppm, and $^2CH_2$ at 4.02) were measured by fitting a Voigt (Gauss and Lorentz) function. 1H-NMR result was positive for 2HG in all tumors that contained IDH1/2 mutation but negative in all tumors with wild type (e.g., 100% accuracy). The measured 2HG levels ranged from 0.29 to 5.79 (mean ± SD, 2.64 ± 1.94) μmol/g (Figure 3).

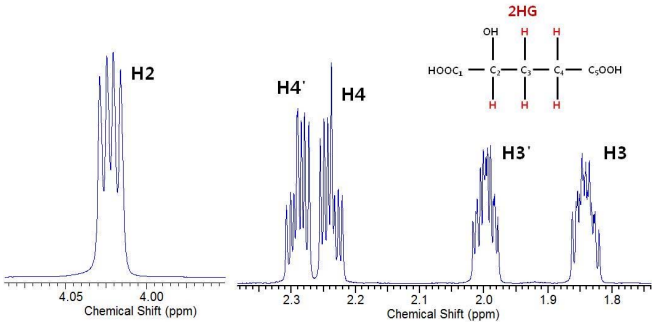

Fig. 1 900MHz 1H NMR spectrum of 100 mM 2-hydroxyglutaric acid (2HG) solution with corresponding chemical structure. 2HG resonances were detected at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm.

Discussion

The detection and quantification of 2HG in IDH1/2 mutated tumors is great interest because the elevated 2HG level has been linked to IDH1/2 gene mutation in glioma [2, 3]. This study represents, to our knowledge, the first *in vitro* measurements of absolute quantification of 2HG levels in IDH1/2 mutated tumors using high resolution 900MHz NMR spectroscopy. The levels of 2HG in this work had a range of 0.29 – 5.79 μmol/g from seven glioma samples with IDH1/2 mutation (4 WHO grade II and 3 WHO grade III), which is consistent with the finding of previous *in vitro* 600MHz NMR study (e.g., 0.55 – 3.51 μmol/g, Baek *et al*. [4]). In addition, other metabolite levels (e.g., Glu, GABA, NAA, Tau, Lac, PC, etc.) were found to be lower in IDH1/2 mutant tumors compared to IDH1/2 wild type tumors. This result reflects that IDH1/2 mutations cause metabolic alterations in gliomas with IDH mutants. In particular, Glu may be become depleted as it is converted first α-ketoglutarate and then to 2HG. Recent findings from Dang *et al*. [2] suggest that IDH1-R132 expression results in elevated flux from Gln to 2HG through Glu and α-ketoglutarate. Gln-to-Glu conversion could be a metabolic bottleneck for IDH mutated tumors [5].

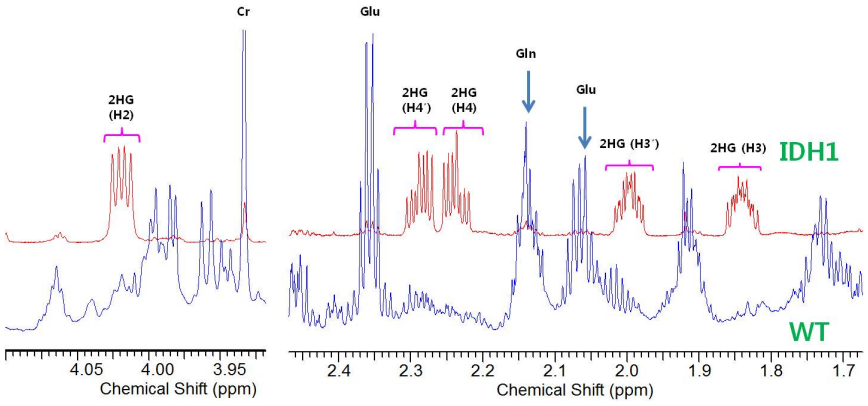

Fig. 2 900MHz 1H NMR spectra of human glioma samples with IDH1 mutated (top) and wild type (bottom) tumors. Elevated 2HG level is detected in the IDH1 mutant but not wild type tumors

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References

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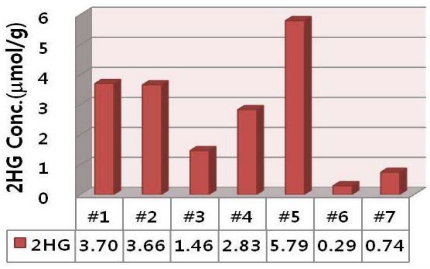

Fig. 3 Concentration level of 2HG in glioma tumors with IDH1/2 mutation.