Quantitative Two-Dimensional Correlated Spectroscopy in Gliomas with IDH Gene Mutation

Hyeon-Man Baek[1], Yun-Ju Lee[1], Eun-Hee Kim[1], Chaejoon Cheong[1], and Seung-Ho Yang[2]

[1]*Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Chungbuk, Korea,* [2]*Neurosurgery St. Vincent's Hospital, Catholic University, Suwon, Gyeonggi-do, Korea*

Introduction

Recently, *in vivo* 1H-MRS [1] and *ex vivo* 1H-HRMAS [2] have shown that onco-metabolite 2-hydroxyglutarate (2HG) could be detected in low grade gliomas and secondary GBMs with isocitrate dehydrogenase 1 or 2 (IDH1/2) gene mutation [3, 4]. However, the accurate estimation of 2HG levels by them is challenging due to significant overlap with neighboring metabolites such as GABA, Glu, Gln, and m-Ins. To our knowledge, quantitative two-dimensional correlated spectroscopy (2D-COSY) has not been applied to the characterization of metabolite patterns in mutant IDH1/2 brain tumor extracts. We present here that 2D-COSY can be used to obtain quantitative information of 2HG and to detect metabolic alterations in gliomas with IDH1/2 gene mutation.

Methods

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 (PCA) 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 using 5 mm NMR probe. Two-dimensional COSY spectra were acquired using a Bruker 900MHz spectrometer (Avance II 900) using 5 mm broadband NMR probe. The spectral parameters were as follows: 1024 data points with 4183 Hz sweep width in the F2 dimension, 512 data points with 4183 Hz sweep width in the F1 dimension, and 4 transients. For 2D-COSY data quantitation, the cross peak volumes of the metabolites were integrated, and then relative concentrations were calculated using equation: $[C]_i = (V_i/V_{Cr}) \times [C]_{Cr}$, where $[C]_i$ is the concentration of the metabolite (μmol/g), V_i is the cross peak volume of the metabolite and V_{Cr} is the diagonal peak volume of Cr. $[C]_{Cr}$ is the Cr concentration calculated from the one-dimensional 1H-NMR spectra using 3-(Trimethylsilyl)propionic acid (TSP) as a external reference.

Results

Figure 1 shows a two-dimensional COSY spectrum of the 100mM 2HG solution. 2HG cross peaks due to H2 methine and two (H3 and H3´) methylene protons are well isolated at (F1 = 4.02 ppm, F2 = 1.84 ppm) and (F1 = 4.02 ppm, F2 = 2.00 ppm). Other two methylene groups (e.g., H3-H3´ and H4-H4´) of 2HG were observed between 1.75 and 2.35 ppm. The following assignments of the cross peaks were made: H3´ and H4-H4´ at (F1 = 2.24 ppm, F2 = 2.00 ppm; F1 = 2.29 ppm, F2 = 2.00 ppm), H3 and H4-H4´ at (F1 = 2.24 ppm, F2 = 1.84 ppm; F1 = 2.29 ppm, F2 = 1.84 ppm), and H3-H3´at (F1 = 2.00 ppm, F2 = 1.84 ppm). Figure 2 shows overlaying COSY spectra of IDH1 mutated and wild type tumors. 2HG signal was detected in the IDH mutated tumor but not wild type tumor. The analysis of the 2D-COSY spectra of tumor extracts showed that in all cases with IDH1/2 mutation, the increased 2HG levels were detectable. The 2HG cross peaks were also clearly separated from other metabolites (e.g., Glu, Gln, NAA, GABA, GSH, etc.). The measured 2HG levels in this work ranged from 0.12 to 5.64 (mean ± SD, 2.38 ± 1.63) μmol/g, which is consistent with the separately measured one-dimensional 1H-NMR value (e.g., 0.29 – 5.79 μmol/g). These two measurements showed a strong correlation in 2HG levels between them (R^2 = 0.832, P = 0.005).

Discussion

The present study demonstrates the feasibility of multi-dimensional NMR technique in the detection and quantification of 2HG as a novel biomarker of IDH1/2 mutation status in glioma. In addition, Ala, Asp, Tau, m-Ins, PC, GPC, GPE, PE, Lac, and Thr cross peaks were well identified and quantified. Our analysis revealed that a significant decrease in the concentrations of Glu, PC and Tau, and a significant increase of GPC, were observed in the IDH1/2 mutated tumors (P < 0.05). This result reflects that levels of amino acids and choline derivatives were altered in the IDH1/2 mutated tumors, possibly associated with IDH gene mutation. Among metabolites with altered levels, Glu showed the biggest difference when comparing extracts of IDH1/2 mutated and wild type tumors (Figure 2). Glu levels may be reduced as it converted first to α-ketoglutarate and then to 2HG. Dang *et al*. [4] reported that IDH1 expression results in elevated flux from Gln to 2HG through Glu and α-ketoglutarate. Quantitative NMR spectroscopy may help characterize differences in metabolite levels between glioma types, and between glioma grades with IDH gene mutation.

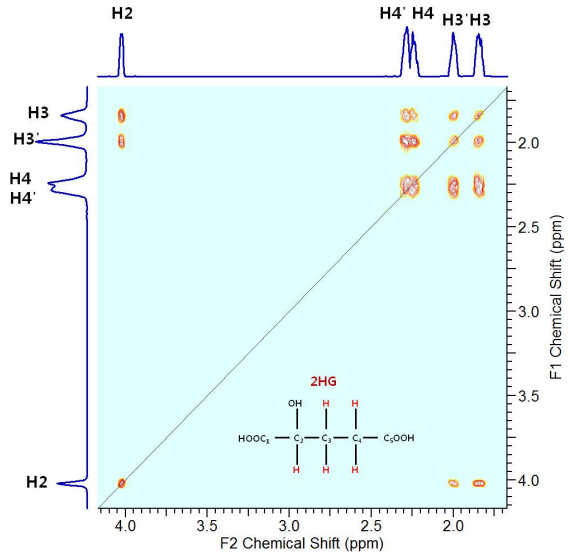

Fig. 1 900MHz 2D-COSY spectrum of 100 mM 2-hydroxyglutaric acid (2HG) solution with corresponding chemical structure. 2HG resonances were detected at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm.

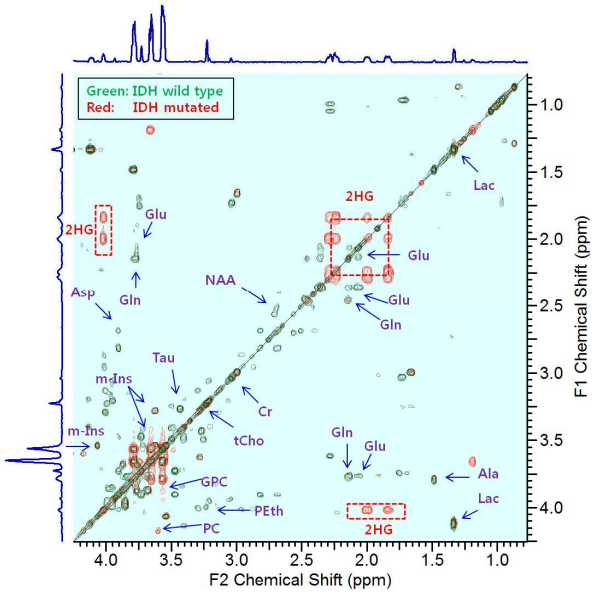

Fig. 2 2D-COSY overlaying spectra of IDH1 mutated (red) and wild type (green) tumors. Elevated 2HG level is detected in the IDH1 mutant but not wild type tumors

References

[1] Choi *et al*., Nat Med 2012; 624-629. [2] Jalbert *et al*., ISMRM 2011;183. [3] Yan, *et al.*, NEJM 2009;360-773. [4] Dang *et al*., Nature 2009;462: 739-743.

Acknowledgement: This work was supported in part by KBSI-#K32402 and - #T3222G.