13C-NMR Spectroscopy in the Detection of 2-Hydroxyglutarate as a Novel Biomarker of IDH Mutation Status in Glioma

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

Somatic mutations in isocitrate dehydrogenases (IDH1 or 2) have been identified in astrocytic and oligodendroglial tumors of WHO grades II and III and in secondary glioblastoma (GBM) [1]. The IDH1/2 mutation is associated with elevated levels of 2-hydroxyglutarate (2HG), which may serve as a clinical biomarker for disease stratification and prognosis [2, 3]. Recently, *in vivo* and *ex vivo* 1H-NMR studies have shown that onco-metabolite 2-hydroxyglutarate (2HG) could be detected in low grade gliomas and secondary GBMs with IDH1/2 gene mutation [4, 5]. However, the effects of IDH mutation or 2HG on cellular metabolism have not yet been elucidated [6]. The metabolic profiling analysis based on ^{13}C-NMR spectroscopy with stable ^{13}C-labeled isotope may give the possibility to evaluate the relative activities of metabolic pathways, as well as the metabolic phenotype of the analyzed system. In this study, the aim of this study was to demonstrate the feasibility of ^{1}H- and ^{13}C-NMR spectroscopy for the detection of 2HG in IDH-mutant and -wild type tumors.

Methods

Samples from 7 patients with gliomas were examined: 3 IDH1/2 mutant and 4 wild type tumors. The samples ranged from 110 to 950 (mean ± SD, 583 ± 345) mg wet weight. Percholric acid (PCA) extracts of the tissues were redissolved in 0.28 mL of deuterium oxide. High resolution *in vitro* ^{1}H- and ^{13}C-NMR spectroscopy was performed on a Bruker 900MHz spectrometer using 5 mm NMR probe. One dimensional ^{13}C spectra were acquired with NOE plus WALTZ-16 decoupling. The spectral parameters were as follows: 45° pulse angle, 56817 Hz sweep width, 32768 complex data points, acquisition time 0.58 s, and 544 transients. On the ^{1}H-NMR spectra, 2HG metabolite concentrations were calculated using an external reference, TSP (3-(Trimethylsilyl)propionic acid).

Results

Figure 1 shows a ^{1}H-decoupled ^{13}C-NMR of 100 mM 2HG solution scanned at 900MHz (21.1 Tesla) with corresponding chemical structure. A 2HG molecule has two methylene groups ($^{4}CH_2$ and $^{3}CH_2$) and a methine group (^{2}CH) that give ^{13}C-NMR signals at the following positions: C3 at 31.70 ppm, C4 at 34.18 ppm, C2 at 72.75 ppm, C1 at 181.94 ppm, and C5 at 183.55 ppm. Figure 2 shows high resolution one-dimensional ^{1}H- NMR spectrum of human glioma sample with IDH1 mutation. On the ^{1}H-NMR spectrum, five multiplets of relative intensities1:1:1:1:1 were observed at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm. 2HG signal was detected in the IDH mutated tumors but not wild type tumors. The measured 2HG levels ranged from 3.66 to 5.79 µmol/g. Figure 3 shows a ^{1}H-decoupled ^{13}C NMR spectrum obtained from the same glioma with IDH1 gene mutation. Based on the natural abundance ^{13}C, resonance locations of 2HG singlets in ^{13}C spectrum (C3 at 31.70 ppm, C4 at 34.18 ppm, C2 at 72.79 ppm, C1 at 181.95 ppm, and C5 183.56 ppm) were identified in the IDH1 mutated tumors.

Discussion

The data analysis of ^{1}H- and ^{13}C-NMR spectra of the tumor extracts demonstrated a significant increase in the concentration of the 2HG in IDH mutated tumors. In particular, 2HG peaks were well separated from other metabolites (e.g., Glu, Gln, etc.) in the 900MHz ^{1}H-NMR spectra. This result indicates that high magnetic NMR technique can be used in an aid in the detection and quantification of 2HG and other J-coupled metabolites. On ^{13}C-NMR spectra, 2HG peaks for each of the 5 carbons were detected in the IDH1 mutated but not IDH wild type tumors. It is expected that 2HG may be actively being produced during the period of ^{13}C-substrate infusion (e.g., [U-^{13}C]-glucose). Therefore, the present study demonstrates the feasibility of ^{1}H- and ^{13}C-NMR spectroscopy in the detection of 2HG as a novel biomarker of IDH mutation status in glioma.

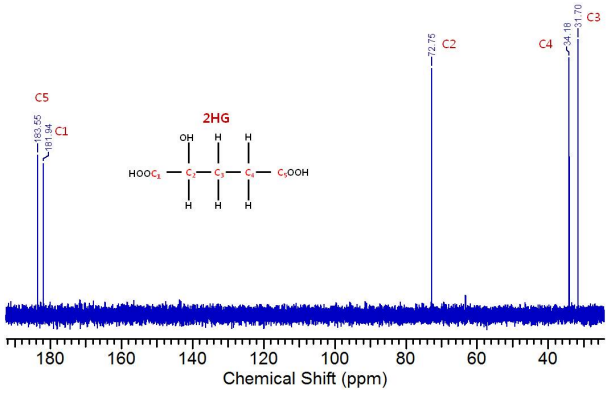

Fig. 1 900MHz ^{1}H-decoupled ^{13}C-NMR spectrum of 100 mM 2-hydroxyglutaric acid (2HG) solution with corresponding chemical structure.

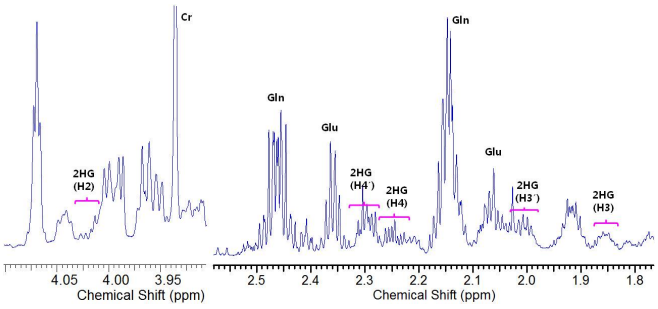

Fig. 2 900MHz ^{1}H-NMR spectrum of human glioma sample with IDH1 mutation. Elevated 2HG is detected at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm.

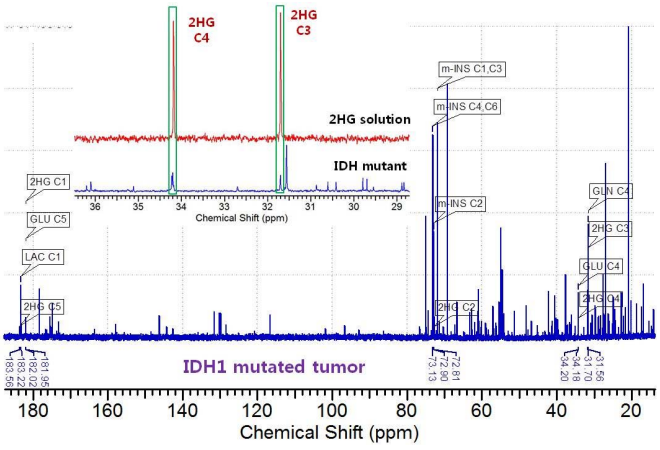

Fig. 3 900MHz ^{1}H-decoupled ^{13}C NMR spectrum of glioma sample with IDH1 gene mutation. 2HG ^{13}C-resonances (C3, C4, C2, C1, and C5) were detected at 31.70, 34.18, 72.79, 181.95, and 183.56 ppm.

References

[1] Yan, *et al.*, NEJM 2009;360-773. [2] Yan, *et al.*, NEJM 2009;360-773. [3] Dang *et al*., Nature 2009;462: 739-743. [4] Choi *et al*., Nat Med 2012; 624-629. [5] Jalbert *et al*., ISMRM 2011;183. [6] Reitman *et al*., PNAS 2011;3270-3275.

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