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

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**Introduction**

Somatic mutations in isocitrate dehydrogenases (IDH1 or 2) have been identified in astrocytic and oligodendrogial 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 13C-NMR spectroscopy with stable 13C-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 1H- and 13C-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. Perchloric acid (PCA) extracts of the tissues were redissolved in 0.28 mL of deuterium oxide. High resolution in vitro 1H- and 13C-NMR spectroscopy was performed on a Bruker 900MHz spectrometer using 5 mm NMR probe. One dimensional 13C 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 1H-NMR spectra, 2HG metabolite concentrations were calculated using an external reference, TSP (3-(Trimethylsilyl)propionic acid).

**Results**

Figure 1 shows a 1H-decoupled 13C-NMR spectrum of 100 nM 2-hydroxyglutaric acid (2HG) solution with corresponding chemical structure. Figure 2 shows high resolution one-dimensional 1H-NMR spectrum of human glioma sample with IDH1 mutation. On the 1H-NMR spectrum, five multiplets of relative intensities:1:1:1:1:1 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 1H-decoupled 13C NMR spectrum obtained from the same glioma with IDH1 gene mutation. Based on the natural abundance 13C resonance locations of 2HG singlets in 13C spectrum (C3 at 31.70 ppm, C4 at 34.18 ppm, C2 at 72.75 ppm, C1 at 181.95 ppm, and C5 at 183.56 ppm) were identified in the IDH1 mutated tumors.

**Discussion**

The data analysis of 1H- and 13C-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, Gin, etc.) in the 900MHz 1H-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 13C-NMR spectra, 2HG peaks for each of the 5 carbons were detected in the IDH1 mutated but not wild type tumors. It is expected that 2HG may be actively being produced during the period of 13C-substrate infusion (e.g., [U-13C]-glucose). Therefore, the present study demonstrates the feasibility of 1H- and 13C-NMR spectroscopy in the detection of 2HG as a novel biomarker of IDH mutation status in glioma.

**References**


**Acknowledgement**

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

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

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