Detection of 2-hydroxyglutarate and Metabolic Changes Associated with IDH1 and IDH2 Mutants Using NMR Spectroscopy

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Introduction

The detection and quantification of 2-hydroxyglutarate (2HG) in IDH1/2 mutated tumors is great interest because the elevated 2HG level has been linked to IDH1/2 gene mutation in glioma [1-3]. However, the downstream effects of IDH1/2 mutants or of increased 2HG on cellular metabolism are unknown. Recently, Reitman *et al* [4] reported that IDH mutants can induce multiple changes in the cellular metabolome based on mass spectroscopy. They found that 2HG-independent changes include reduction of glutamate and several metabolites. However, further investigations are required to determine whether the metabolic changes reported are reproducible in other techniques. The aim of this study was twofold, firstly to investigate the metabolic change of glioma cells with mutant IDH1/2 cells using high resolution NMR, and secondly to compare our findings with the previously published results.

Methods

U87MG cells were transfected with a gene vector coding for the wild type or IDH mutant enzyme (R132H, R172K). Both cell lines were incubated for 48h with DMEM containing 10mM [U-^{13}C]glucose with glutamate. The cells were washed twice with 0.9%(w/v) NaCl and extracted with 4% perchloric acid (PCA). IDH1/2 mutant (n=6) and wild type cell samples (n=6) were examined. ^{1}H-NMR was performed on a Bruker 900MHz spectrometer. The spectral parameters were as follows: 90° pulse angle, 11718 Hz sweep width, 32K complex data points, relaxation delay 0.05 s and acquisition time 2.8 s, resulting in a TR of 2.85 s, 64 transients, and total acquisition time of ~4 min. The concentrations were calculated using equation: $[C]_i = (N_{TSP}/N_i) \times (S_i/S_{TSP}) \times [([C]_{TSP} \times V_{sample})/M_{protein\ content}]$, where $[C]_i$ and $[C]_{TSP}$ are the concentration of the metabolite and TSP, S_i is the amplitude of the metabolite and S_{TSP} is the amplitude of TSP, V_{sample} is sample volume and M_{sample} is the protein content. 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.

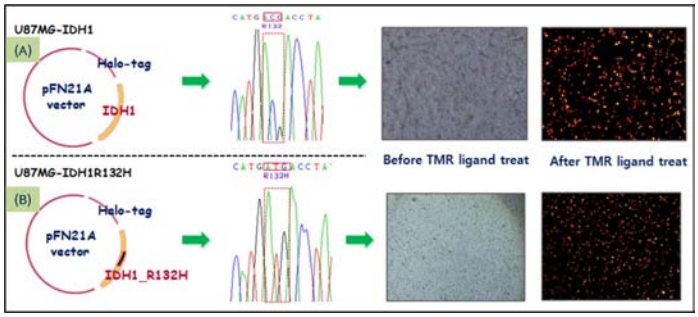

Figure 1- (A) U87MG cells transfected with IDH1 and (B) with IDH1-R132H: DNA sequencing results, and microscopic evaluation of the wild type and IDH mutant cells after TMR ligand treatment

Results and Discussion

In our study, IDH gene transfection efficiency was about 80~90% (Figure 1). Figure 2 shows representative 1D ^{1}H-NMR spectra of U87MG-IDH1 and -IDH1-R132H scanned at 900MHz (21.1 Tesla). Elevated 2HG signal was detected in the IDH mutated cells but not wild type cells (e.g., 100% accuracy). On the ^{1}H-NMR spectra, five multiplets of relative intensities1:1:1:1:1 were observed at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm. The 2HG peaks at 1.84 and 2.24 ppm were well separated from other metabolites (e.g., Glu, Gln, GABA, etc.), but other peaks of 2HG (at 2.00, 2.29, and 4.02 ppm) were partially overlapped with those of GABA, Glu, m-Ins, and Leu. In this work, 2HG ($^{4}CH_2$ at 2.24 ppm and $^{3}CH_2$ at 1.84 ppm) were measured by fitting a voigt function (Figure 2B).The measured 2HG levels ranged from 0.06 to 0.27 (mean ± SD, 0.12 ± 0.08) nmol/mg protein. This study represents, to our knowledge, the first *in vitro* quantification of 2HG levels in IDH1/2 mutated cells. In addition, Iso, Leu, Val, Thr, Lac, Ala, Ace, GABA, Glu, Gln, Cho, PC, GPC, s-Ins, Tau, m-Ins, and Gly were well identified and quantified (Figure 2). Our analysis revealed that a significant increase in the concentrations of 2HG, Iso, Leu, Ala, Glu, Gln, Tau, m-Ins, and Gly and Tau were observed in the IDH1/2 mutated cells ($P < 0.05$, in Figure 3). This result reflects that levels of amino acids and choline derivatives were altered in the IDH1/2 mutated cells, possibly associated with IDH gene mutation. However, our findings are not consistent with the previously published Mass Spectroscopy results by Reitman *et al*. [4]. Among metabolites with altered levels, Glu, Ala, m-Ins, and Gly showed big difference when comparing extracts of IDH1/2 mutated and wild type cells. Further studies are needed to determine whether the alterations reported here are reproducible in repeated NMR measurements.

References [1] Choi *et al*., Nat Med 2012; 624-629. [2] Baek *et al*., ISMRM 2012; 845. [3] Dang *et al*., Nature 2009;462: 739-743. [4] Reitman *et al*., PNAS 2011;3270-3275. **Acknowledgement:** This work was supported in part by KBSI- #T33416.

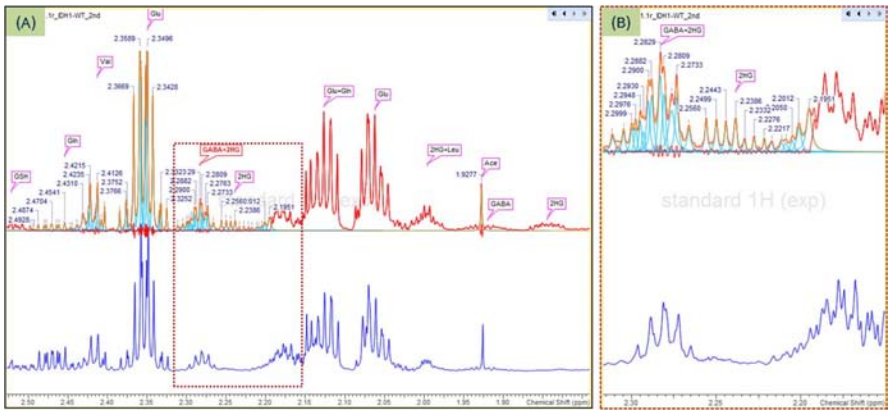

Figure 2 – (A) 900MHz ^{1}H-NMR spectra of U87MG glioma cells with the wild type and IDH1-R132H mutant (top) and (B) Elevated 2HG level observed in the IDH mutant but not wild type cells

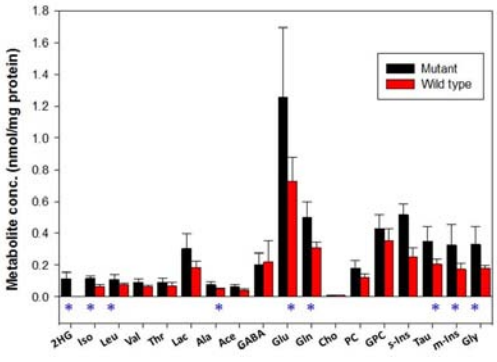

Figure 3 - Concentration of metabolites in U87MG glioma cells with IDH1/2 mutants