2-Hydroxyglutarate Labeling from [U-13C]glucose in Human Glioma Cells

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Introduction

Somatic mutations in isocitrate dehydrogenases (IDH1 or 2) are associated with elevated levels of 2-hydroxyglutarate (2HG), which may serve as a clinical biomarker for disease stratification and prognosis [1, 2]. However, the effects of IDH mutation or 2HG on cellular metabolism have not yet been elucidated [3]. Ex vivo and in vivo NMR spectroscopic methods using the stable ^{13}C-labeled isotope may offer a unique and novel approach to access the metabolite pools and fluxes associated with several metabolic pathways in human glioma cells [4]. The aim of the present study was two-fold: first, to investigate the identification of 2HG in 1D ^{1}H- and ^{13}C-NMR spectra of IDH mutated cells, and second, to demonstrate whether 2HG labeling from [U-^{13}C]glucose substrate feeding could be detected.

Materials and Methods

U87MG cells were transfected with a gene vector coding for IDH mutant enzyme (R132H). To investigate the identification of 2HG and labeling patterns on ^{13}C-NMR spectra, four different IDH transfection efficencies were evaluated (e.g., DNA : reagent = 1: 0.5, 1, 1.5, 3). Each cell line was 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). 1D-and 2D-NMR spectra were obtained on a Bruker 900MHz spectrometer, operating at 900 MHz for ^{1}H and at 226 MHz for ^{13}C-NMR measurements. The pool sizes of metabolites were determined from fully relaxed ^{1}H-NMR spectra of cell extracts using TSP as external reference. The concentrations were calculated with respect to the protein content.

Results

Microscopy, ^{1}H-NMR, and ^{13}C-NMR data from U87MG cells with different transfection efficiency of IDH1-R132H enzyme are shown in Figure 1. On the ^{1}H-NMR spectra, elevated 2HG level at 2.24 ppm was observed in the spectra of 3 of 4 IDH mutated cells (e.g., ratio of reagent/DNA ≥ 1), while the spectrum from a IDH mutated cell with the ratio of < 1.0 showed no detectable 2HG peak. Five multiplets of 2HG were clearly detected at 1.84, 2.00, 2.24, 2.29, and 4.02 ppm (e.g., 2D-COSY, not shown here). The measured 2HG levels were 0.038, 0.100, and 0.107 nmol/mg protein, respectively (Figure 2). In addition, other metabolite levels (e.g., Glu, Gln, etc.) were quantified and compared. On the ^{13}C-NMR spectra, 2HG peaks for each of the 5 carbons (C3, C4, C2, C1, and C5) were clearly identified at 31.70, 34.18, 72.79, 181.98, and 183.60 ppm (e.g., 2D-HSQC and HMBC, not included here). In Figure 3, 2HG C5-doublet (D) peaks at 183.60 ppm from [U-^{13}C]glucose were observed and quantified on the 1D ^{13}C-NMR spectra in 2 of 4 IDH mutated cells (e.g., the ratio ≥ 1.5). The carbon C5-D isotopomer of 2HG is a large peak that has no overlapping signals (e.g., lactate C1) in the IDH mutated cells.

Discussion

In this study, the ^{13}C-enrichment in C3 of alanine and lactate were measured form ^{1}H-NMR spectra by integration of peak areas of the ^{1}H-^{12}C signal and both ^{1}H-^{13}C satellite signals of the respective methyl groups. The fractional ^{13}C-enrichments were similar for each other in IDH mutant cells (e.g., range, 47-52% for lactate and 51-56% for alanine). The analysis of ^{1}H- and ^{13}C-NMR spectra of the cell extracts showed a significant increase in the concentration of the 2HG in IDH mutated cells, but not in IDH wild type and mutant IDH cells with low transfection efficiency. In particular, 2HG C5 labeling peaks from ^{13}C-substrate were well separated from other metabolites (e.g., glutamate C5 at 182.04, lactate C1 at 183.23 ppm, etc.) in the 900MHz ^{1}H-NMR spectra. This result demonstrates that 2HG can be actively being produced during the 24h period of [U-^{13}C]glucose substrate feeding. 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.

References

[1] Yan, *et al.*, NEJM 2009;360-773. [2] Dang *et al*., Nature 2009;462: 739-743. [3] Reitman *et al*., PNAS 2011;3270-3275. [4] Marin-Valencia *et al*., Cell Metabolism 2012; 827-837.

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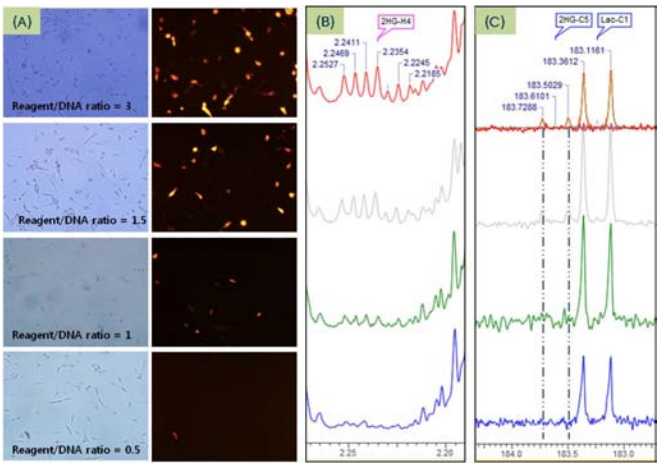

Figure 1- (A) Microscopic evaluation of IDH-R132H transfection efficiency in U87MG cells, (B) ^{1}H-NMR spectra, and (C) ^{13}C-NMR spectra after metabolic labeling with 10mM [U-^{13}C]glucose substrate

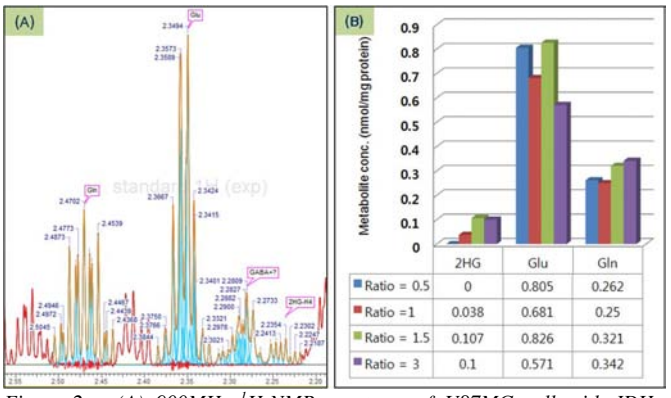

Figure 2 – (A) 900MHz ^{1}H-NMR spectrum of U87MG cell with IDH mutant, (B) Metabolic change (e.g., 2HG, Glu, Gln) according to IDH1-R132H transfection efficiency

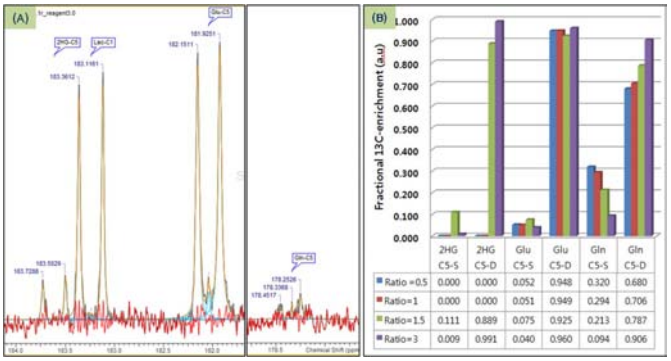

Figure 3 - 900MHz ^{1}H-decoupled ^{13}C NMR spectrum of U87MG cell with IDH mutant, (B) ^{13}C isotopomer change of metabolites (e.g., 2HG, Glu, Gln) according to IDH mutant transfection efficiency; S-singlet, D- doublet