2HG Metabolic Profiling Analysis based on 13C-NMR Spectroscopy with Stable13C-labeled Isotope

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Introduction

Clinically, the presence of somatic mutations in isocitrate dehydrogenases (IDH1 or 2) mutation has been shown to be associated with a better survival [1]. Patients with IDH1mutated tumor have a better prognosis than those without IDH1 mutations. The IDH1 and 2 mutations 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, 4]. The aim of this study was two-fold: first, to investigate the metabolic profiling analysis based on ^{13}C-NMR spectroscopy with stable ^{13}C-labeled isotope, and second, to demonstrate whether 2HG labeling from [U-^{13}C]glucose substrate feeding could be detected in IDH1 or 2 mutated cells.

Materials and Methods

For further studie, U87MG cells were transfected with a gene vector coding for the wild type or IDH mutant enzyme (R132H, R172K). To investigate the identification of 2HG and labeling patterns on ^{13}C-NMR spectra, different IDH transfection efficencies were also 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.

Results

U87MG cells with 80~90% transfection efficiency of IDH1-WT and IDH1-R132H enzyme are shown in Figure 1. On the high resolution ^{1}H-NMR spectra, elevated 2HG levels at 1.84 and 2.24 ppm were 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. As shown in Figure 2, 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. The range of the ^{13}C-enrichments was 48-53% for lactate and 51-56% for alanine. In Figure 3, 2HG C5-doublet (D) peaks at 183.60 ppm from [U-^{13}C]glucose were observed and quantified on the ^{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 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 (e.g., ratio of reagent/DNA < 1). This result demonstrates that 2HG can be actively being produced during the 24h period of [U-^{13}C]glucose substrate feeding. In addition, in our human tumor tissue study, on ^{13}C-NMR spectra, 2HG peaks for C5-D isotopomer were also 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 human gliomas or glioma cells.

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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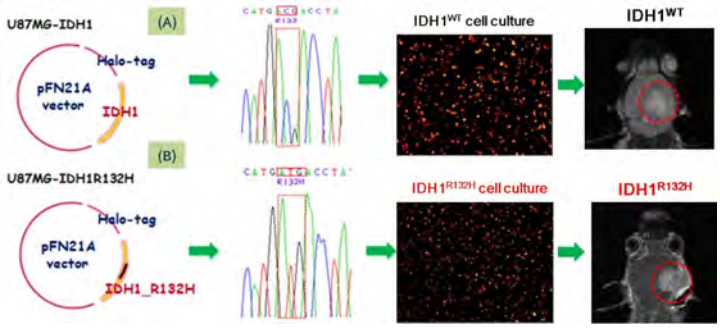

Figure 1. (A) U87MG cells transfected with IDH1-WT and (B) with IDH1-R132H: DNA sequence result, microscopic evaluation, and animal model development.

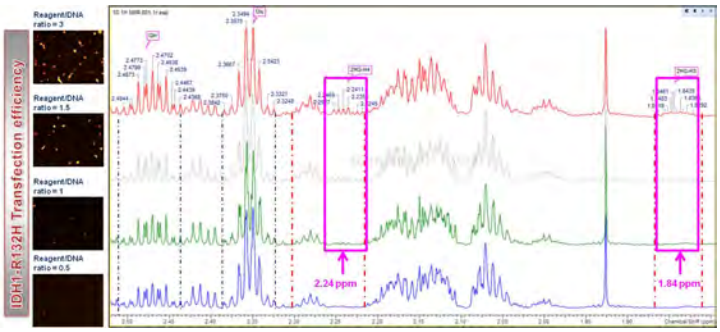

Figure 2. High resolution ^{1}H-NMR spectra from glioma cells with different IDH1-R132H transfection efficiency. 2HG onco-metabolite levels were quantified.

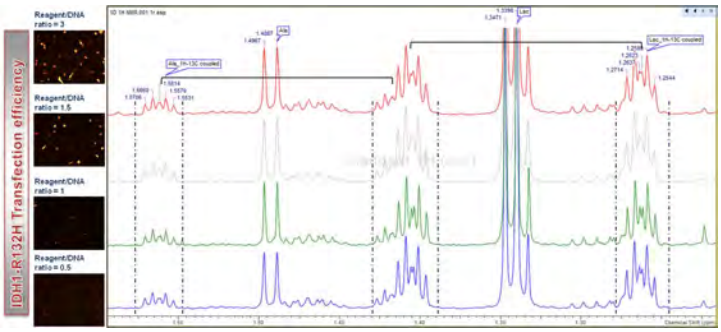

Figure 3. High resolution ^{1}H-NMR spectra showing ^{13}C-enrichment in alanine and lactate for each transfection efficiency case.

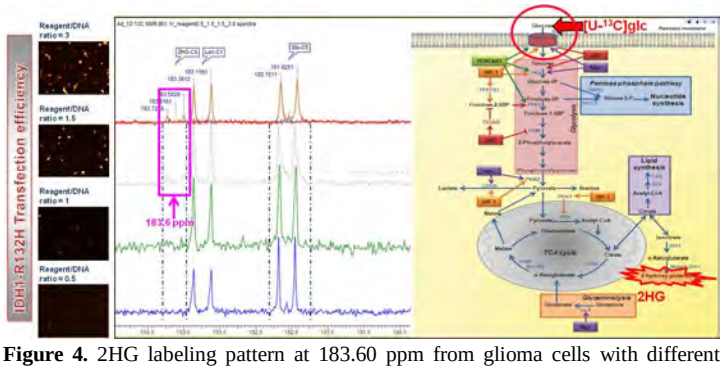

Figure 4. 2HG labeling pattern at 183.60 ppm from glioma cells with different IDH1-R132H transfection efficiency.