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

Hyun-Man Baek,1,2 Yun-Ju Lee,1 Gregory Hyung Jin Park,1 Eun-Hee Kim,1 Gyunggoo Cho,1 and Chaejoon Cheong1

1Division of MR Research, Korea Basic Science Institute, Ochang, Chungbuk, Korea, 2Department of Bio-Analytical Science, University of Science & Technology, Yuseong-gu, Korea

Introduction
The detection and quantification of 2-hydroxyglutarate (2HG) in IDH1/2 mutated tumors is of 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 spectrometry. 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 48 h with DMEM containing 10mM [U-13C]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. 1H-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_{i,SR}/N_i) \times (S_{TSP}/S_{i,SR}) \times \left( [i/(C)_{i,SR} \times V_{sample}/M_{protein} - M_{protein} \right) \times (S_{TSP}/S_{1H}) \times (i=2HG, Glu, Gln, GABA, etc.) \times TSP.

Results and Discussion
In our study, IDH gene transfection efficiency was about 80–90% (Figure 1). Figure 2 shows representative 1D 1H-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% ± 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, 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 Spectrometry 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