DETECTING AND QUANTIFYING 2HG AND ALTERATIONS IN METABOLITE PROFILES IN IDH1/2 MUTATION BEARING BRAIN TUMORS BY SOLID STATE HRMAS NMR

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INTRODUCTION

IDH1/2 mutations occur at a high frequency in diffusely infiltrating gliomas of the WHO grades II and III and were identified as a strong prognostic marker in all WHO grades of gliomas [1-2]. Mutated IDH1 or IDH2 protein leads to the elevated amount of the metabolite 2-hydroxyglutarate (2HG) in tumors [2]. This recent discovery has led to intensive research in investigating this new biomarker and developing approaches and non-invasive imaging methods for detection of IDH mutations. Magnetic Resonance Spectroscopy (MRS) is capable of detecting and quantifying metabolites in vivo. Since 2HG is elevated to the MRS detectable level [3], it is possible to detect 2HG in vivo. In this work, solid state high resolution (HR) magic angle spinning (MAS) NMR methods were used to identify the spectroscopic "finger print" of 2HG in brain tumor tissues to confirm that 2HG is the metabolite marker of IDH mutations. The metabolite profiles of gliomas with and without IDH mutations are investigated quantitatively.

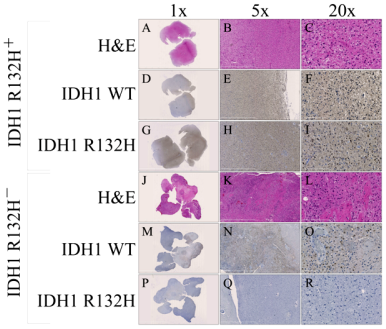

Fig.1 A-C: demonstrate images from a representative formalin fixed paraffin embedded glioma tissue. **D-F** show images from a representative glioma positive for *IDH1* R132H mutation.

MATERIALS AND METHODS

Brain tumor samples: 33 tissue samples with confirmed IDH1/2 mutation status were obtained for NMR analyses. Verification of *IDH1* R132H mutation-bearing tumor tissues was performed using immunohistochemistry (IHC) with IDH mutation specific antibodies. Representative immune-stained sections and either positive or negative for IDH1 R132H mutations are shown in **Fig. 1**. They were divided into the low grade group (WHO Grade II-III), which include IDH1/2 mutation positive (n = 14) and IDH1/2 mutation negative (n = 9), and glioblastoma (GBM) with IDH1/2 mutation positive (n = 4) or negative (n = 6).

HRMAS NMR Data Collection: HRMAS NMR experiments were conducted at 4 °C using a Bruker AVANCE 600 WB solid state NMR spectrometer (Bruker Instruments, Inc., Billerica, MA) with a dedicated 4 mm HRMAS probe. Two-dimensional (2D) 1H J-coupled Correlated SpectroscopY (COSY) was used with 6,000 Hz spectral width and 1.5 s relaxation delay. 32 transients were averaged for each of the 512 increments with a total acquisition time of ~6-8 hours depending on the sample. 2D J-coupling patterns of pure 2HG and a mixture of 2HG and glutamate (Glu) compounds were obtained at ~ 10 mM 2HG in D_2O, pH = 7.0. Utilizing this reference pattern, the unique cross-peaks arising from 2HG resonances (at δ = 4.1, 2.33, 2.05 and 1.88 ppm, at 4 °C, corresponding to the α, γ, β, and β' protons of 2HG, respectively) in the complex 2D COSY spectra of glioma tissues were identified.

Data Analysis: 2D COSY data were analyzed using Spinworks (University of Manitoba, Canada). Data were zero-filled to a 2k by 2k matrix and weighted with a shifted square sine bell function followed by Fourier transformation. The concentrations of 2HG and several clinically important metabolites related to gliomas in the tissue samples were estimated based on the amount of the external standard TSP added into the samples in different groups. Correlations 2HG levels in tissue samples with other brain metabolites, such as NAA, NAAG, Glu, Gln, Cr, Cho and myo-I, etc, were evaluated statistically.

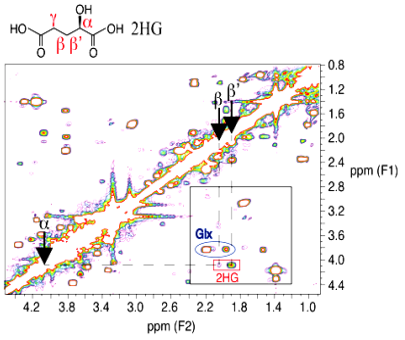

Fig. 2 2D COSY spectrum of HRMAS NMR of a representative *IDH1* mutation-positive glioma sample

RESULTS AND DISCUSSIONS

A 2HG specific J-coupling pattern, as showed in **Fig. 2**, was identified in brain tumors bearing IDH1 mutation using the 2D COSY method. The sensitivity and specificity of detecting 2HG and IDH1 mutation with 2D COSY NMR is 100% and 92%, respectively, confirming that IDH1 mutations result in production of the onco-metabolite 2HG [2]. Furthermore, quantifying 2HG in IDH1 mutation positive tumor tissues revealed that levels of 2HG were elevated to the range of 3-22 mM in the samples tested.

When comparing 2HG productions with other brain metabolites in the IDH1 mutation positive samples, we also found statistically significant reductions of NAAG, GABA, Glu, and MI in low grade tumors bearing with IDH1 mutation comparing to those of tumors without IDH1 mutation (**Fig. 3**). A similar pattern was found in the high grade GBM confirmed with IDH1 mutations vs. those without mutations. The observations can be related to previous study on amino acid and choline lipid metabolite levels in cells expressing IDH-Mutants [3]. To investigate whether differences in metabolism in gliomas harboring IDH1 mutations might relate to 2HG level, we evaluated if there is an association between 2HG level to and other brain metabolites. It is found that strong correlations between 2HG levels and concentrations of Lac, NAAG, Gln, Glu, Cr/PCr, and MI in low gliomas bearing IDH mutations [**Fig. 4**]. Those metabolites may be altered in lysates of cells expressing IDH mutations and are likely associated with the abnormal metabolic activities IDH mutations in glioma cells.

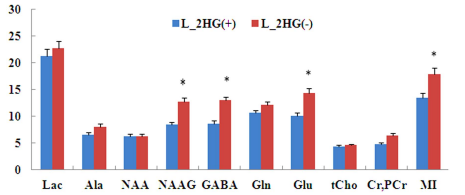

Fig. 3 Concentration of several metabolites in tumors with IDH1 mutation-bearing [2HG (+)] and mutation negative [2HG (-)].

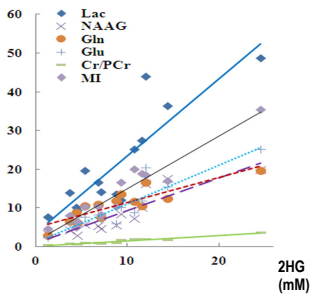

Fig. 4 Correlations of 2HG levels with other metabolites in glioma tissue samples with IDH1 mutations.

CONCLUSIONS

Using the solid state HRMAS NMR analysis, the current study confirmed the 2HG is an MRS detectable and highly specific metabolite marker of IDH mutations in gliomas. In addition, this quantitative metabolite analysis revealed the altered metabolite profile in tumors bearing with IDH mutations.

References: [1] Capper D, et al. Int J *Cancer,* 2011 [2] Lenny D, et al. *Nature,* 2009 [3] Zachary J. Reitman, et al. *PNAS*, 2011