INVESTIGATION OF THE CORRELATION OF MRS MEASURABLE 2-HYDROXYGLUTARATE (2HG) CONCENTRATION AND TUMOR PROGRESSION IN BRAIN TUMORS HARBORING IDH1/2 MUTATIONS

Liya Wang[1,2], Juliya Kalinina[3], Ruya Zhao[2], Run Lin[1,2], Shaoxiong Wu[4], Erwin Van Meir[3], and Hui Mao[1,2]

[1]Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA, United States, [2]Center for Systems Imaging, Emory University, Atlanta, GA, United States, [3]Department of Neurosurgery, Emory University, Atlanta, GA, United States, [4]Department of Chemistry, Emory University, Atlanta, GA, United States

INTRODUCTION

Mutations in isocitrate dehydrogenase 1/2 (IDH1/2) occur at high frequency in diffusely infiltrating gliomas of the WHO grades II and III and are considered as a strong prognostic marker [1-2]. The mutant enzyme gains a novel activity in producing the oncometabolite, R(-)-2-hydroxyglutarate (2HG), which can be detected in vivo and ex vivo by magnetic resonance spectroscopy. However, the role of 2HG remains to be better understood. This study attempted to investigate the relationships of 2HG concentrations obtained from ex vivo NMR analysis of tumor tissue samples with tumor progression characteristics observed in clinical pathology and radiology exams.

MATERIALS AND METHODS

Tumor tissue samples of 38 brain tumor patients were analyzed using high resolution magic angle spinning solid state NMR [3,4]. Verification of *IDH1* R132H mutation-bearing tumor tissues was performed using immunohistochemistry (IHC) with the IDH mutation specific antibody. Presences and concentrations of 2HG were determined by 2D Correlation Spectroscopy (COSY) [4]. All 38 brain tumor patients had routine clinical magnetic resonance imaging (MRI) exams to characterize brain tumors, including tumor core, edge, edema, volume and histopathological exams for tumor types and grade. The MRI protocol includes T2-weighted images (T2WI) and T1-weighted images (T1WI) taken both before and after the injection of a gadolinium, contrast agent. The tumor volume measurements were determined by manually tracking region of interest (ROI) based on the enhancing portion of the mass on each T1W image after injection of the contrast agent by using *ImageJ* program. Nonenhancing regions within the tumor, which can be visualized in both T1WI and T2WI, also were outlined and measured. The volume of the selected section of a tumor was calculated based the area from a ROI isolated on an individual slice multiplying it by the slice thickness plus the gap thickness. The total tumor volume is the sum of the volumes calculated for each of the multiple parallel slices. Cases with a cystic tumor were excluded when analyzing tumor size. All cases were divided into the three grade groups, which include grade II (n = 8), grade III (n = 19), and grade IV (n = 11) based on WHO grade. 2HG concentrations were also correlated with WHO grades and the Ki-67 proliferation index (MIB) of the tumors (n=17). Two-tailed nonparametric Mann–Whitney test for comparison and Spearman for correlation were used with $P < 0.05$ considered as statistically significant. Two patients were specially followed up for two years.

RESULTS AND DISCUSSIONS

Significantly elevated levels of 2HG were found in all gliomas carrying IDH1 mutations comparing to the group without IDH1 mutations, consistent with the earlier reports that IDH1 mutations result in production of the onco-metabolite 2HG. Furthermore, we found the 2HG level is higher in the tumors at higher grades (Grade I-II vs. Grade III). However, in IDH mutation positive Grade IV GBM, 2HG level is not significantly higher than those at a lower grade. When comparing the 2HG level with tumor volume and tumor proliferation measurements of MIB index, it is found that the 2HG level is associated with the increased the tumor volume (**Fig. 1A**). 2HG level is positively correlated to the MIB index (**Fig. 2B**).
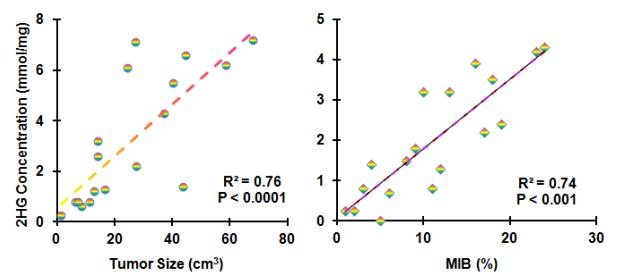
Among 38 patients, we also followed up two cases of IDH mutation positive tumors, given the availability of the tissue samples. In first case, initial MRI exam at the diagnosis showed that there was a 4.2 x 5.1 x 4.2 cm³ mass in the right frontal lobe involved the right frontal cortex. The mass demonstrated T1 hypointensity (**Fig. 2a**) and T2 hyperintensity. There was no appreciable post contrast enhancement (**Fig. 2b**), suggesting a low grade tumor which is confirmed with pathology classification of Grade II oligodendroglioma. The NMR

Fig. 1: There was a positive correration between 2HG level and tumor size from 18 available data sets (left panel) and MIB from 17 available data sets (right panel).

analysis of tumor tissue collected at the initial diagnosis reported a 2HG level of 3.5 mM. Two years later, MRI exam showed interval development of significant T1 hypointensity (**Fig. 2c**) and mass-effect centered in the right frontal lobe with increased tumor size. The overall abnormal T1-hypointensity increased to approximately 9.0 x 6.3 x 6.0 cm. Furthermore, post-contrast MRI showed markedly nodular, irregular enhancements (**Fig. 2d**). NMR analysis of the tissue from the 2nd biopsy indicated that 2HG level was elevated to 8.5 mM. Changes of 2HG levels indicated by α-H of 2HG were shown in the NMR spectra (**Fig. 3A**).Pathology analysis confirmed that the tumor has developed to advance anaplastic oligodendroglioma (Grade III). Similarly in the 2nd case, the patient was diagnosed as oligoastrocytoma (Grade II) with no visible contrast enhancement (**Fig. 2e-f**). NMR analysis showed a low 2HG level at 1.2 mM (**Fig. 3B**).

Fig. 2: Contrast enhanced MRI of two examples of low grade tumors harboring IDH mutation progressed to GBM (Grade IV) revealed the low signal lesion on pre T1WI (a, e) and no enhancement on post contrast T1WI (b, f). Two years later, same case showed the increased tumor size on T1WI (c, g), and strong contrast enhancement on post T1WI (d, h).

However, when the tumor recurred two years later, increase tumor volume (**Fig. 2g-h**) and Grade IV (glioblastoma) were reported. This was companied with a drastically elevated 2HG level. These two cases thus provided patient specific examples to support the general finding that the 2HG level is increasing with the tumor grade. The observations expand the findings from previous study about IDH-Mutants as a strong prognostic marker in all WHO grades of gliomas [5]. The 2HG level associated with tumor size and proliferation index provides additional information to the pathogenesis of tumors associated with IDH gene mutations.

Fig. 3: The 2HG level increased after the tumors progressed to higher grade after two years. Case one (A) presented 2HG at 5.5 mM in the initial diagnosis but increased to 7.2 mM two years later showed 2HG increased to 20 mM after recurrence from 2.3 mM at the 1st exam (B).
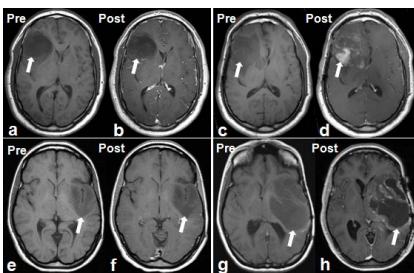
CONCLUSIONS

The findings of this study provide the evidence that 2HG level has a strong correlation with several clinically important prognostic index, such as tumor size and MIB value. The excess 2HG accumulated in tumors may contribute to the formation and malignant progression of gliomas [5]. Additional studies are needed to further investigate the relationships of 2HG concentrations and tumor progression features to establish the use of 2HG levels as a biomarker for predicting brain tumor prognosis and responses to the treatment.
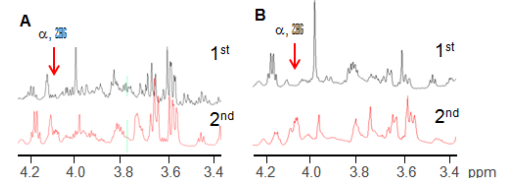
References: [1] Parsons, D.W., et al. Science, 2008. [2] Yan H., et al. N Engl J Med, 2009. [3] Guo, C., et al. Curr Opin Neurol, 2011.[4] Kalinina, J. et al. J Mol Med, 2012. [5] Dang L., et al. Nature, 2009.