Detection of 2-hydroxyglutarate in Mutant Brain Tumors in vivo using Proton Magnetic Resonance Spectroscopy

R. Nagarajan[1], M. A. Thomas[1], W. B. Pope[1], R. M. Prins[2], N. Wilson[1], N. Salamon[1], and L. M. Liau[2]
[1]Radiological Sciences, University of California Los Angeles, Los Angeles, California, United States, [2]Neurosurgery, University of California Los Angeles

Introduction: Several years ago, Frahm and co-workers reported reduced NAA, choline (Cho), creatine (Cr) and increased myo-inositol (mI) in a patient with L-2-hydroxyglutaric acidemia (2HG) (1). Excess accumulation of 2-HG has been shown to lead to an elevated risk of malignant brain tumors in patients with inborn errors of 2HG metabolism. Barbot et al. reported persistent high concentrations of 2-hydroxy glutarate (2HG) in urine, plasma and CSF samples of six pediatric patients with 2HG determined by gas chromatography-mass spectrometry (GC-MS) (2). A recent work by Dang and co-workers reported a mutation observed in the isocitrate dehydrogenase1 (IDH1*)* gene, which occurs in the majority of grade II and grade III gliomas and secondary glioblastomas, resulting in significant elevation of 2HG in these tumors (3). These tumors also show secreted 2HG levels in culture media as measured using liquid chromatography- mass spectrometry (LC-MS), having mean concentration of approximately 10 μM/g, which is well within the detectability of proton (^{1}H) magnetic resonance spectroscopy (MRS). This compares to a few hundred nM/g in wild type tumors. A major goal of this study was to investigate if LC-Model processed MRS using prior knowledge can detect 2HG in mutant brain tumors *in vivo*.

Methods: The PRESS (4) localized 1D MRS sequence was used on a 3T Trio-Tim MRI/MRS scanner (Siemens Medical Systems, Erlangen, Germany) running on the VB15 platform. A Siemens 12 channel "receive" phased-array head coil was used for this study. The entire protocol was approved by the institutional review board (IRB), and informed consent was obtained from each human subject. All patients had measurable disease on magnetic resonance imaging (MRI) for which surgical resection was warranted. Clinical classification and grading of the tissue was performed by a board-certified neuropathologist. Twenty seven brain tumor patients have been scanned so far: 16 primary GBM (grade IV), seven oligodendroglioma (grade III), and four low grade (grade II). Within one week prior to surgery, patients with newly diagnosed or recurrent gliomas underwent pre-operative MRI and MRS scans. The majority of IDH1 mutant patients belonged to low grade (grade II and III) whereas the wild type belonged to higher grade (grade IV). The following parameters were used for 1D MRS: TR/TE=2.0s/30ms, 128 averages and the voxel size for PRESS localization was 2x2x2 cm^3. As described by Bal et al (5), the spectral peaks of 2HG can be identified at different locations very close to glutamate (Glu) and glutamine (Gln), a phantom containing 2mM 2HG was prepared to verify the MRS detectability on the 3T MRI scanner. The prior knowledge for 2HG was home developed for processing the MRS data using the LC-Model algorithm (6).

Results and Discussion: Figure 1A shows a PRESS localized spectrum recorded in a 41 y.o. mutant brain tumor patient using the 3T MRI/MRS scanner. Figure 2 shows a 1D MRS recorded in a 45 yo. brain tumor (wild type) patient. As confirmed by the phantom scans, the 2HG peaks were overlapping with that of Glu and Gln. Fig.3. shows creatine-normalized ratios of 2HG, glycerylphosphocholine (GPC), Glu and Glu+Gln in wild type and mutant tumors. In brain tumor MRS data, 2-HG was elevated in gliomas with the IDH1 mutation compared to wild-type tumors (p=0.0013). The lowest Cramer-Rao lower bound (CRLB) values for 2HG in the mutant brain tumors was 11% as reported by the LC-Model algorithm, where as a wide range of CRLB values (40-999%) was reported in the wild type indicating the concentrations of 2HG below MRS detectability. Also, significantly elevated glycerophosphocholine (GPC) ratios were seen in the IDH1mutants (p=0.010). In the wild-type tumors, on the other hand, there were elevated levels of glutamate/glutamine (Glu+Gln) compared to IDH1 mutations (p=0.006). We found increased 2-HG levels in human malignant gliomas that contained the R132 IDH1 mutation. Increased choline in IDH1 mutants could reflect increased cell density due to IDH1-mutation-mediated cellular proliferation. The quantitative levels of 2-HG within IDH1 mutant tumors may provide some mechanistic insight into the role of IDH1 mutations in gliomagenesis and tumor progression.

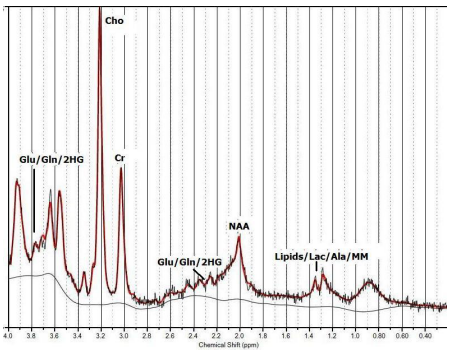

Fig.1. 1D MR spectrum recorded in a 41yo mutant patient

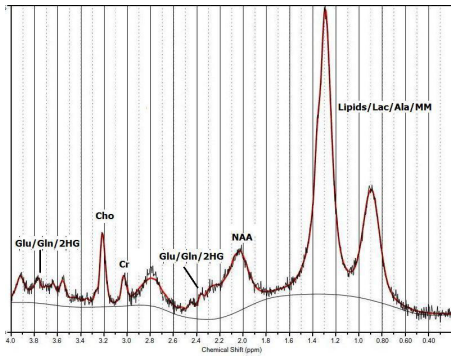

Fig.2. 1D MR Spectrum recorded in a 45yo wild type patient.

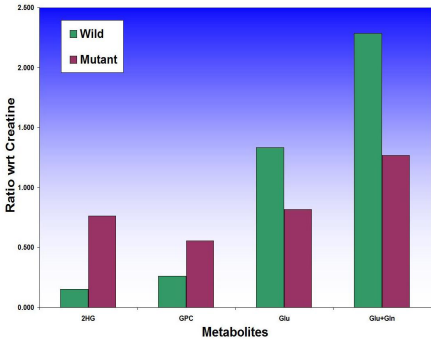

Fig.3. Metabolite ratios of 2HG, GPC, Glu and Glu+Gln in wild and mutant type tumors.

Conclusion: Our pilot results show that the MRS findings are in agreement with that of the LC-MS data acquired using resected tumors. MRS imaging provides a non-invasive measure of 2-HG in gliomas, which may serve as a potential biomarker for monitoring patients with IDH1 mutant brain tumors.

References

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