In vivo spectroscopic imaging of 2-hydroxyglutarate in human gliomas at 7.0 T

Sandeep Ganji[1], Keith M Hulsey[1], Elizabeth A Maher[2,3], and Changho Choi[1]

[1]*Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, Texas, United States,* [2]*Harold C. Simmons Cancer Center, UT Southwestern Medical Center, Dallas, Texas, United States,* [3]*Department of Internal Medicine, UT Southwestern Medical Center, Dallas, Texas, United States*

TARGET AUDIENCE: Neuro-oncologists, MR spectroscopists.

INTRODUCTION Isocitrate dehyrogenase mutations (IDH1 and IDH2) are present in the majority of low grade gliomas (~60-90%) [1]. The mutations impart a functional gain and convert α-ketoglutarate to 2-hydroxyglutarate (2HG, an oncometabolite), which is otherwise only present in vanishing small amounts [2-3], leading to elevated 2HG levels. Under physiological pH 2HG gives multiplets at 2.25 (H4, H4′), ~1.9 (H3, H3′), and 4.0 (H2) ppm [3], however, precise measurement of 2HG is difficult due to overlapping multiplets from glutamate (Glu), glutamine (Gln) and γ-aminobutyric acid (GABA). Here we report for the first time ^{1}H spectroscopic imaging (SI) of elevated 2HG levels in brain gliomas with robust separation from Glu, Gln and GABA signals at 7T. Our method uses PRESS volume localization in an SI sequence to acquire *in vivo* data. We present phantom validation data and *in vivo* measurements in patients with gliomas.

METHODS To improve the differentiation of 2HG signal in the presence of overlapping signals from Glu, Gln and GABA, numerical optimization of PRESS echo time was carried out. At TE = 78 ms (TE_1 = 58 ms and TE_2 = 20 ms) 2HG signal appears as an inverted peak at 2.25 ppm, well separated from Glu (2.35 ppm), Gln (2.45 ppm) and GABA (2.28 ppm) signals which appear as positive peaks. The performance was validated in a phantom containing 2HG (8mM) and glycine (Gly) (10mM). For *in vivo* acquisition, T_{2w} FLAIR images were acquired for positioning the spectroscopic imaging slice on the tumor mass. Water suppressed metabolite and unsuppressed water data were acquired with a TR of 1.8 sec, a spectral width of 5000 Hz and 2048 complex points per FID. A four-pulse scheme was used for water suppression. The PRESS RF carrier was set to 2.6 ppm. The PRESS 90˚ and 180˚ RF pulses had bandwidth of 4.2 kHz (9.8 ms) and 1.3 kHz (13.2 ms), respectively. The volume of interest (VOI) was positioned to cover FLAIR enhancing tumor regions and if possible normal brain regions for comparison. The SI grid typically covered an area of 200 x 160 mm^2 (field of view, FOV), of a 15 mm thick slice along the head-foot direction. Regional saturation bands were used to reduce the signals from subcutaneous and outside VOI regions. Residual water signal in water suppressed metabolite data was removed using the HL-SVD algorithm of the JMRUI [4]. Residual eddy current artifacts were corrected using unsuppressed water data. Frequency-drifts were corrected using in-house Matlab programs. LCModel software [5] was used for metabolite signal estimation. Basis-sets for LCModel analysis were created using published chemical shift and coupling constants [6-7]. Absolute quantification of the metabolites was performed using creatine (Cr) in gray matter at 8 mM. Written informed consent was obtained from subjects prior to the scans.

RESULTS AND DISCUSSION Figure 1 (**a**) shows the simulated spectral pattern of 2HG, Glu and Gln at PRESS TE = 78ms. The Glu and Gln signals at 2.35 and 2.45 ppm, respectively, appear as positive peaks, whereas 2HG appears as a negative peak at 2.25 ppm. Spectra from phantom experiments show an inverted peak of 2HG at 2.25 ppm (Fig 1 (**b**)). Spectra and LCModel fits were consistent with the simulated 2HG spectral pattern. Figure 2 shows *in vivo* SI data from a subject with brain tumor in the left-medial fronto-parietal lobe. Figure 2 (**a, b**) shows spectra from the FLAIR enhancing region with LCModel spectral fitting, individual metabolite signals and residuals obtained using basis-sets with 2HG and without 2HG simulated spectra. The residuals obtained using basis-sets without 2HG simulated spectra in figure 2 (**b**) show an inverted signal at 2.25 ppm which is not present in the residuals obtained using basis-sets with 2HG simulated spectra (Fig 2 (**a**)), no differences were observed in other spectral regions. Spectral fitting without 2HG signal also changed the metabolite estimates of Glu and Gln (estimates in Fig 2 (**a**, **b**)). The 2HG, Glu and Gln estimates were 4 mM, 2 mM and 5.8 mM, respectively in the tumor spectra (Fig 2 (**a**)). GABA was not measurable in either tumor or normal brain regions. Figure 2 (**c, d**) shows spectra from a normal brain region with LCModel spectral fitting, individual metabolite signals and residuals obtained using basis-sets with 2HG and without 2HG simulated spectra. The 2HG signal was not observed in either of the LCModel fits, whereas the Glu and Gln concentrations remained the same. No differences were observed in the residuals across entire spectral region (Fig 2 (**c**, **d**)) and Glu and Gln estimates were not affected by removing the 2HG signal from the basis-set. 2HG, Glu and Gln concentration maps of the same subject are shown in figure 3. The regions of elevated 2HG and reduced Glu are consistent with the FLAIR enhancing location. Figure 4 shows the concentration maps of 2HG, choline (Cho), and N-acetylaspartate + N-acetylaspartylglutamic acid (tNAA) from a subject with a diffuse glioma. Elevated Cho region and reduced tNAA region correlate with each other. However tumor specific marker 2HG showed different spatial distribution compared to Cho. In conclusion, the present study reports the first *in vivo* spectroscopic imaging of oncometabolite 2HG, without contamination from Glu and Gln signals at 7T.

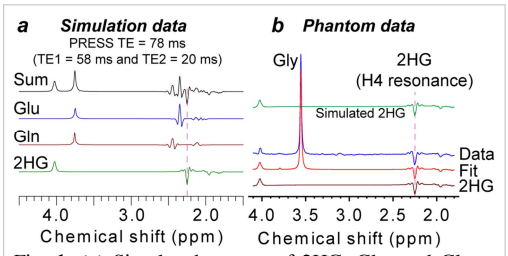

Fig. **1**: (**a**) Simulated spectra of 2HG, Glu, and Gln at PRESS echo time of 78 ms. (**b**) SI data from a phantom containing 2HG (8 mM) and Gly (10 mM). Spectra (blue), LCModel fit (red) are consistent with the simulated data (green).

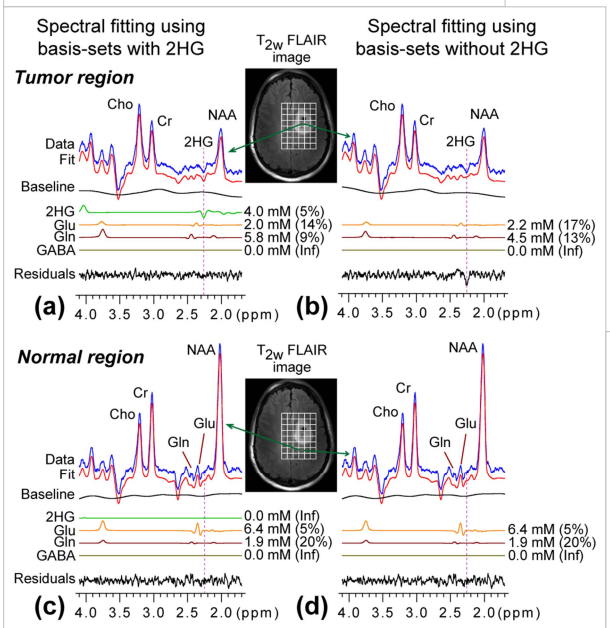

Fig. **2**: *In vivo* data from patient with glioma. (**a**) and (**b**) Tumor region spectra (blue), LCModel metabolite fits (red) and residuals (black) using basis-sets with and without 2HG signal. (**c**) and (**d**) Normal brain region spectra (blue), LCModel metabolite fits (red) and residuals (black) using basis-sets with and without 2HG signal. 2HG, Glu, Gln and GABA estimates in mM are also shown (CRLB values in parentheses).

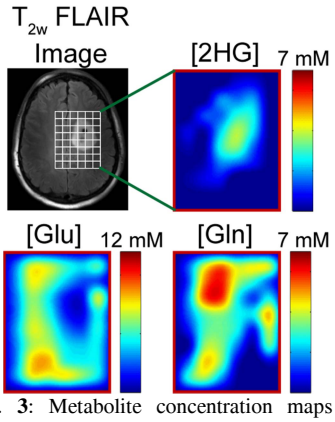

Fig. **3**: Metabolite concentration maps of 2HG, Glu and Gln for subject shown in Fig 2.

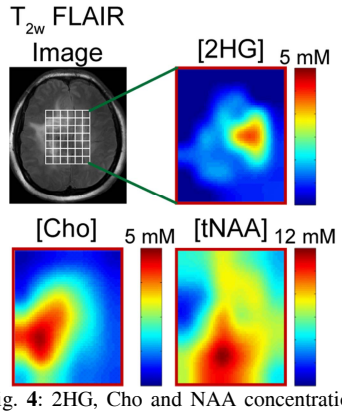

Fig. **4**: 2HG, Cho and NAA concentration maps in a glioma patient. 2HG shows regional variation different from Cho.

REFERENCES **1**. Yan, H. et al. N. Engl. J. Med. 360, 765–773 (2009). **2**. Dang L. et al. Nature 4602:739-44 (2009). **3**. Choi C. et al. Nat Med 18(4): 624-629 (2012). **4**. Naressi A. et al. MAGMA 12:141–152 (2001). **5**. Provencher S.W. Magn Res Med 30:672-679 (1993). **6**. Bal D. and Gryff-Keller A. Magn Res Chem 40:533-536 (2002). **7**. Govindaraju V. et al. NMR Biomed 13:129-153 (2000).

ACKNOWLEDGEMENTS This study was supported by NIH CA159128 and CPRIT RP101243.