

In vivo detection of IDH mutations in gliomas by 1H-MRS

C. Choi¹, S. Ganji¹, R. DeBerardinis¹, Z. Kovacs¹, R. Bachoo¹, J. Pascual¹, I. Dimitrov^{1,2}, B. Mickey¹, C. Malloy^{1,3}, and E. Maher¹
¹University of Texas Southwestern Medical Center, Dallas, Texas, United States, ²Philips Medical Systems, ³VA North Texas Health Care System

INTRODUCTION A number of recent seminal studies have reported that the unusual metabolic activities observed in tumors are driven by oncogenic mutations. A high fraction of gliomas contain mutations in the metabolic enzymes, isocitrate dehydrogenase-1 and -2, IDH1 and IDH2 (1,2). These sporadic, heterozygous, gain-of-function mutations are confined to the active site of the enzyme and result in a neomorphic activity that causes the mutant enzyme to produce an "onco-metabolite", 2-hydroxyglutarate (2HG) (3). As a result, this metabolite, which is normally present in vanishingly small quantities, can be elevated by orders of magnitude in gliomas bearing IDH1 or IDH2 mutations (3,4). In a large study of more than 400 central nervous system tumors, Yan *et al.* identified IDH1 mutations in over 70% of grade II and III astrocytomas and oligodendrogliomas, with a sizable fraction of the remainder containing analogous mutations in IDH2 (2). By contrast, less than 5% of primary glioblastomas (GBM) contained these mutations. Furthermore, among all patients with high-grade gliomas, presence of the most common IDH1 mutation, R132H, was associated with a three-fold increase in the duration of survival after diagnosis (1). Therefore, noninvasive methods for 2HG measures have outstanding potential and could allow the clinicians to predict tumor genetics, stage, likelihood of therapeutic response and other crucial parameters that have direct bearing on patient care. To our best knowledge, *in vivo* detection of this onco-metabolite in human brain tumors has not been reported to date. Here, we are presenting *in vivo* ¹H-MRS data that indicate the accumulations of 2HG in patients with gliomas.

METHODS An MRS study was carried out in patients with glioblastoma (GBM), anaplastic astrocytoma (AA) and low-grade glioma (LGG), determined by histological analyses of biopsies according to the World Health Organization criteria. Written informed consent was obtained prior to the scans. Experiments were conducted in a whole-body 3T scanner (Philips Medical Systems). A body coil was used for RF transmission and an 8-channel phased-array coil for reception. Following the survey, T₂w-FLAIR images (both axial and sagittal) were acquired to identify tumor masses. Water-suppressed PRESS spectra were obtained at (TE₁, TE₂) = (32, 80) ms from a 2×2×2 cm³ voxel positioned at the center of the enhanced FLAIR. Single-voxel localization was obtained with a 9.8-ms 90° RF pulse (BW = 4.2 KHz) and a 13.2-ms 180° RF pulse (BW = 1.3 KHz). Data acquisition parameters included; TR = 2 s, sw = 2.5 KHz, 2048 sampling points, and 64 signal averages (scan time 2.1 min). An unsuppressed water signal was acquired with the PRESS sequence and a STEAM sequence ((TE, TM) = (18, 21) ms; TR = 20 s). Data were corrected for frequency drifts and eddy current artifacts during the post-data processing. LCModel software was used for spectral analysis (5). 3D volume localized spectra of 25 metabolites (including 2HG) were calculated with published chemical shift and J coupling constants (6,7) and used as basis sets for the spectral fitting. Metabolite concentrations in tumors were estimated using the STEAM water signal as a normalization reference and assuming a normal-brain creatine (Cr) concentration at 8 mM and identical relaxation effects between normal brain and tumors.

RESULTS and DISCUSSION The five non-exchangeable protons of 2HG are J coupled, giving rise to multiplets at 4.02, ~2.25, and ~1.9 ppm at pH neutral [6], as shown in Fig. 1. Although the H4 and H4' multiplet at ~2.25 ppm is proximate to the glutamate (Glu), GABA and NAAG multiplets, the signal may be detectable using PRESS TE = 112 ms at 3T in tumors. Figure 2 presents *in vivo* brain spectra obtained with this PRESS from three tumor patients; secondary GBM, AA, and LGG. In addition to the well-known spectral abnormalities commonly observed in tumors (high Cho and low Cr and NAA), the tumor data showed abnormalities between 2.2 - 2.3 ppm. The signal amplitude at 2.25 ppm was abnormally high in tumor spectra compared to the normal-brain spectrum. An LCModel fitting using a basis set with 2HG reproduced the spectra well, giving enhanced 2HG levels, as shown by individual spectral components. The 2HG levels were estimated to be 0, 8.5, 4.5 and 3.4 mM in spectra a, b, c and d, with CRLBs of 2HG at ∞, 5, 7 and 8%, respectively. The Glu levels were lower in tumors than in normal brain. The GABA levels were estimated as 0.8, 0.8, 0.6 and 1.3 mM, respectively, while the multiplet of the NAAG glutamate moiety was negligible in all tumor spectra with infinite CRLBs. The tumor GABA estimates comparable to or higher than the normal GABA level may be due to potential experimental errors since GABA is known to be decreased in tumors. Fitting without GABA, assuming negligible GABA levels in tumors, gave 2HG level of 9.8, 4.3 and 4.3 mM, with CRLB at 4, 6 and 5%, for spectra b, c and d, respectively. The AA patient had three scans with 3-month intervals, and showed elevated 2HG levels constantly. The LGG patient was scanned twice with a 3-month interval, and showed increased 2HG levels in both data. In total, 49 tumor patients were scanned with the PRESS sequence (29 GBM, 9 AA, and 11 LGG), some of which had several MR scans between radiation and/or chemotherapy treatments. 2HG was detected with CRLB < 20% in 39 spectra out of 123, the concentrations being 2 - 8 mM.

REFERENCES 1. Parsons DW *et al.* Science 2008;321:1807-1812. 2. Yan H *et al.* N Engl J Med. 2009;360:765-773. 3. Dang L *et al.* Nature. 2009;462:739-744. 4. Ward PS *et al.* Cancer Cell. 2010;17:225-234. 5. Provender SW. Magn Reson Med. 1993;30:672-679. 6. Bal D and Gryff-Keller A. Magn Reson Chem. 2002;40:533-536. 7. Govindaraju V *et al.* NMR Biomed. 2000;13:129-153.

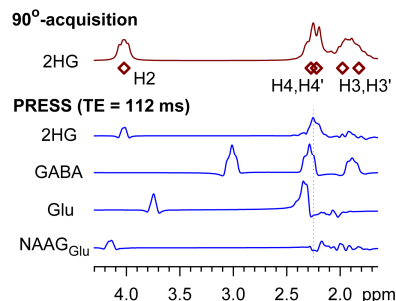


FIG. 1. Calculated spectra of 2HG, GABA, Glu and NAAG_{Glu} at equal concentrations for 90°-acquisition and PRESS (TE₁, TE₂) = (32, 80) ms. Spectra were broadened to singlet FWHM of 5 Hz. A vertical dotted line is drawn at 2.25 ppm.

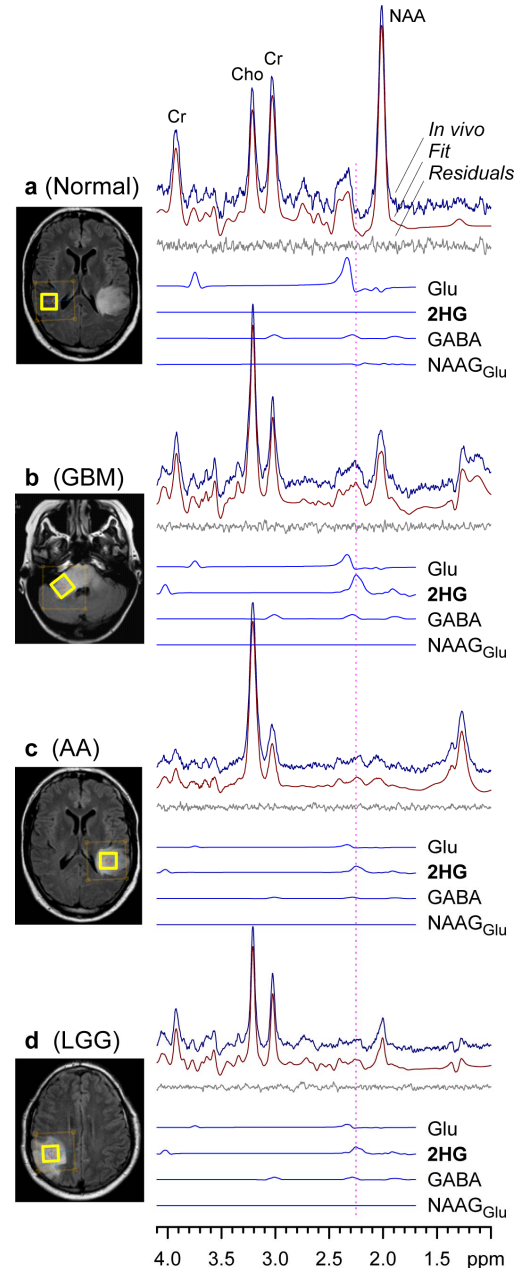


FIG. 2. *In vivo* brain spectra and fitting results are shown together with individual components of Glu, 2HG and GABA. The spectra were obtained from three patients; secondary GBM, anaplastic astrocytoma (AA), and low-grade glioma (LGG). A normal-brain spectrum in (a) was obtained from a contra-lateral region in the AA patient. Voxel positioning (2×2×2 cm³) is shown in the T2w FLAIR images. Spectra were acquired using PRESS TE = 112 ms, TR = 2 s, and NEX = 64. Spectra are scaled relative to the brain water signal (TE = 18 ms; TR = 20 s). A vertical dotted line indicates the 2HG peak location (2.25 ppm).