Measurement of elevated 2-hydroxyglutaric acid in brain tumors by difference editing at 3T in vivo

C. Choi[1], S. Ganji[1], Z. Kovacs[1], R. DeBerardinis[1], and E. Maher[1]
[1]University of Texas Southwestern Medical Center, Dallas, Texas, United States

INTRODUCTION Recent years have seen a resurgence of interest in the clinical potential of tumor metabolism. This interest stems from a number of seminal studies documenting that the abnormal metabolic activities in tumors are driven by oncogenic mutations. A high fraction of gliomas contain mutations in isocitrate dehydrogenase-1 and -2, IDH1 and IDH2 [1,2]. The mutations produce an "onco-metabolite", 2-hydroxyglutaric acid (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]. The studies indicated that 2HG is elevated mostly in low-grade gliomas and secondary glioblastomas (GBM), as measured by mass spectroscopy *in vitro* and *ex-vivo*. Therefore, noninvasive methods for 2HG measures have outstanding potential and could allow the clinicians to predict tumor genetics, stage, and likelihood of therapeutic response. 2HG has five non-exchangeable J-coupled protons [5], resonating at 4.02 (H2), 1.83 (H3), 1.98 (H3′), 2.22 (H4), and 2.27 (H4′), as shown in Fig. 1. Precise measurement of 2HG by standard ^{1}H-MRS may not be straightforward due to the spectral overlap with neighboring resonances. Here, we report *in vivo* measurement of 2HG in human brain tumors for the first time, achieved by means of difference editing.

METHODS Single-voxel localized difference editing (MEGA) was employed for measurement of 2HG in brain tumors at 3T (Philips Medical Systems). The H2 resonance (4.02 ppm) was edited using a 20-ms Gaussian RF pulse (truncated at 10%; BW = 58 Hz), tuned to 1.9 ppm, for selective 180° rotation of the coupling partners, H3 and H3′ resonances, Fig. 1. The editing RF pulses were switched on and off in alternate scans. Volume 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). Echo time was optimized to 106 ms, using density matrix simulations that incorporated the shaped RF and gradient pulses. *In vivo* data were obtained from patients with oligodendroglioma (low-grade) and primary GBM (high-grade). Written informed consent was obtained prior to the scans. T_2w-FLAIR images were acquired to identify tumor masses. Data were obtained from a 2×2×2 cm^3 voxel, positioned within the enhanced FLAIR regions. Data acquisition parameters included; TR = 2 s, sw = 2.5 KHz, 2048 sampling points, and 256 signal averages (scan time 8.5 min). An unsuppressed water signal was recorded with a STEAM sequence (TE = 18 ms; TR = 20 s). In addition, a PRESS spectrum was acquired at TE = 97 ms for comparison. Data were analyzed with LCModel, using basis sets calculated with published chemical shift and coupling constants [5,6]. Metabolite concentrations in tumors were estimated using the STEAM water signal as a normalization reference and assuming a normal-brain creatine (Cr) level at 8 mM and identical relaxation times between normal brain and tumors.

RESULTS and DISCUSSION For 2HG difference editing, the H2 resonance was chosen as a target since this resonance is weakly coupled (J = 4.6 and 8.4 Hz, with δ ≈ 2 ppm) to the H3/H3′ resonances and the H3/H3′ resonances are additionally coupled to the H4/H4′ resonances, as shown in Fig. 1. The Cr-CH_2 resonance, the major obstacle in terms of signal intensity, was eliminated *via* subtraction. The 4.1 ppm resonance of lactate (Lac) was canceled since its coupling partner at 1.31 ppm was unaffected by the editing 180° pulse (BW = 58 Hz). The NAAG 2.06 and 1.9 ppm resonances were affected by the editing pulse, resulting in a coediting of the coupling partner at 4.15 ppm. However, since this NAAG resonance is fairly distant from the 2HG H2 resonance, a potentially-coedited NAAG signal may not interfere with 2HG editing. Figures 2 and 3 present numerical-calculation and *in vivo* results of the 2HG editing. A 2HG doublet was clearly detected at 4.02 ppm in the difference spectrum from the oligodendroglioma patient (Fig. 2d), while no signal was discernible at 4.02 ppm in primary GBM (Fig. 2b). The difference spectrum from primary GBM showed a coedited signal at 3.75 ppm, mainly attributed to Glu. In contrast, a coedited Glu signal was not discernible in oligodendroglioma, indicating decreased Glu in this patient. The NAA singlet (2.01 ppm) was affected by the editing pulse and thus coedited, appearing as a negative peak in the difference spectrum. The elevated and normal levels of 2HG in these tumor patients were also indicated from the spectral patterns at 2.2 - 2.3 ppm in the edit-off subspectra (Figs. 2a and 2c). Although the 2HG and Glu signals may be separable, this region is further complicated by the GABA 2.28 ppm resonance, as illustrated in Fig. 3a. Figure 2e shows a PRESS spectrum obtained at TE = 97 ms, at which the 2HG multiplet at 2.25 ppm was maximum (as indicated by simulation). LCModel analyses of Fig. 2d resulted in 2HG estimates higher by ~50% than Figs. 2c and 2e, giving non-negligible GABA estimates in these PRESS spectra. Given that a coedited GABA signal was not discernible in Fig. 2d, the GABA estimates from Figs. 2c and 2e are most likely overestimation. Thus, the difference editing may be advantageous over the PRESS methods for precise measurement of 2HG although the 2HG signal yield of the editing is less (~50%) than that of the PRESS (see Fig. 3). For Fig. 2d, 2HG was estimated as 7.2 mM with CRLB of 4%. In addition, 2HG was detected in other patients with astrocytomas (low-grade) and secondary GBM, the concentrations being at 2 - 8 mM. The *in vivo* results of the present study are in excellent agreement with the prior *in-vitro* and *ex-vivo* studies [1-4].

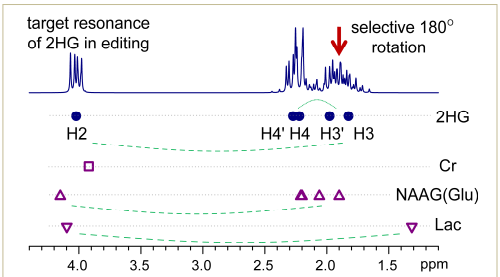

FIG. 1. A calculated 90°-acquired ^{1}H spectrum of 2HG at 3T, broadened to singlet FWHM of 1 Hz, is shown together with the resonances of 2HG and potential obstacles in 2HG editing; Cr-CH_2, NAAG glutamate moiety, and lactate. In the present study, editing RF pulses were tuned at 1.9 ppm to induce selective 180° rotations of the 2HG H3/H3′ resonances and consequently bring about an edited H2 doublet at 4.02 ppm. Green (dashed) lines indicate J coupling connections.

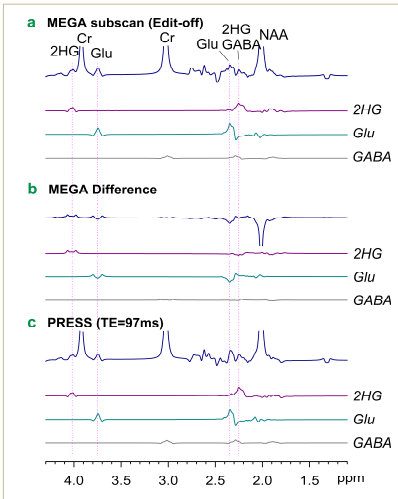

FIG. 3. Calculated spectra for MEGA edit-off subscan (a), difference (b), and PRESS TE = 97 ms. Metabolites include 2HG (5mM), Glu (5 mM), GABA (1mM), Cr (8mM), NAA (10 mM), NAAG (2mM), and Lac (1mM). Spectra are broadened to singlet FWHM of 5 Hz.

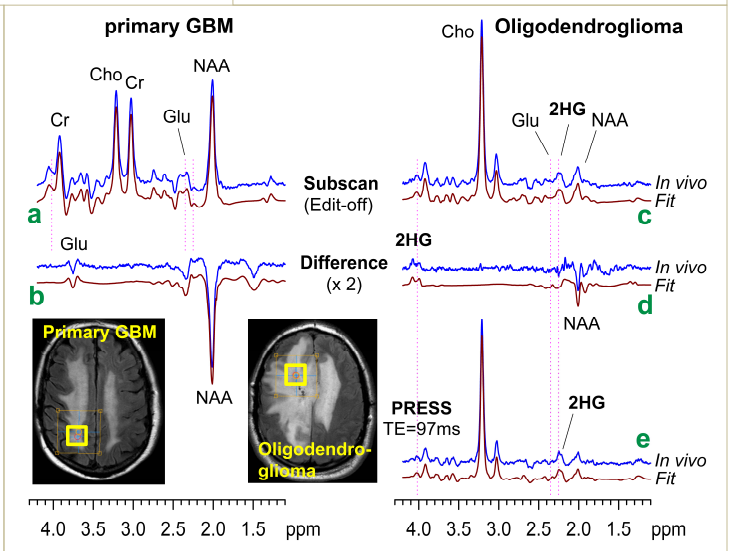

FIG. 2. Edit-off subspectra and difference spectra from a GBM (left) and a oligodendroglioma (right) patients are shown together with the voxel positioning. Also, a PRESS spectrum from the oligodendroglioma patient is shown at the bottom. Difference spectra are 2-fold magnified. Vertical dotted lines are drawn at 4.02, 2.35 and 2.25 ppm.

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.** Bal D and Gryff-Keller A. Magn Reson Chem. 2002;40:533-536. **6.** Govindaraju V *et al*. NMR Biomed. 2000;13:129-153.