Enhancement of 2-hydroxyglutarate detectability by triple-refocusing difference editing at 3T in vivo

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TARGET AUDIENCE: MR spectroscopists and neuro-radiologists in brain tumors.

PURPOSE: With the discovery of IDH1 and IDH2 mutations [1,2] and the resulting production of 2-hydroxyglutarate (2HG) in gliomas [3-5], the role of the altered metabolism as a driver in malignant transformation has become a central focus in understanding the biology of these tumors and as a possible target for therapy [6,7]. Given the great potential of 2HG as a biomarker in the diagnosis and management of glioma patients as well as the workup of an undiagnosed mass or abnormality in neurology, the capability of detecting 2HG noninvasively and precisely is urgently needed. In ^{1}H-MRS, the 2HG signals are overlapped with other metabolite signals, making it difficult to measure 2HG reliably. Specifically, because the C4 proton resonances of 2HG at ~2.25 ppm, which give rise to the strongest 2HG signal in standard MRS, is proximate to the GABA 2.29 ppm resonance, 2HG estimation is extensively interfered by the GABA signal. *Ex-vivo* high-resolution NMR data from resected tumors (Fig. 1) indicate that GABA may be decreased in many tumors, but higher than normal levels are present in some tumors, concentrations ranging from 0 to ~2 mM. When the 2HG levels are comparable to or lower than GABA levels, 2HG estimation is elusive, requiring an MRS strategy that affords good selectivity of 2HG against GABA.

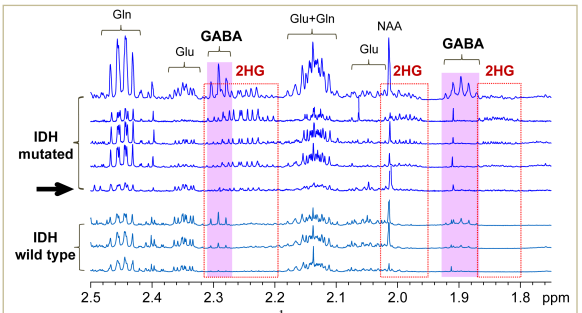

FIG 1. *Ex-vivo* high-resolution ^{1}H spectra (600 MHz) from tumor tissues (5 IDH mutated and 3 IDH WT). 2HG multiplets are indicated by red boxes. GABA signals are shaded (pink). The spectrum at the fifth row (indicated by an arrow) was obtained from a patient whose *in-vivo* spectra are shown in Fig. 4.

METHODS: In multiple refocusing, the signals from strongly-coupled spins are substantively modulated by subecho times for a constant total TE, while weakly-coupled spin signals are largely governed by the total TE only. This affords an opportunity of difference editing for separating strongly-coupled resonances from weakly-coupled or uncoupled resonances. We investigated the feasibility of triple-refocusing difference/summing editing for separation between the strongly-coupled 2.25-ppm resonance of 2HG and the weakly-coupled 2.29 ppm resonance of GABA at 3T. A non-slice selective 180° pulse (E180) was implemented between the two 180° pulses (13.2 ms long) of PRESS (Fig. 2). A pair of subecho time sets was searched for, with numerical analyses, using the following criteria: 1) large 2HG 2.25-ppm signal *via* subtraction, 2) small GABA 2.29-ppm signal *via* subtraction, and 3) small 2HG 2.25-ppm signal *via* summing. T_2 relaxation effects were taken into account using T_2 = 180 ms for both 2HG and GABA. Two subecho time sets were obtained at a total TE of 99 ms; one was (TE_1, TE_2, TE_3) = (26, 20, 53) ms with 14 ms long E180, and the other was (34, 37, 28) ms with 32 ms long E180. Following phantom validation, the editing method was tested in 5 patients with IDH mutations. Experiments were conducted in a whole-body Philips 3T scanner. Data were collected from 8 mM volumes using 96 averages per each subscan (TR = 2 s). Edited spectra were obtained *via* subtraction and summing of the subspectra, and were analyzed with LCModel using numerically-calculated spectra as basis functions. Metabolite concentrations were estimated with reference to water at 42 M.

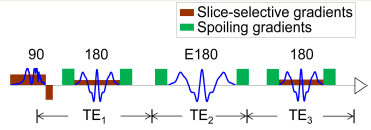

FIG 2. Triple-refocusing sequence scheme used for difference/summing editing of 2HG. Two subspectra were acquired using (TE_1, TE_2, TE_3) = (26, 20, 53) and (34, 37, 28) ms (total TE = 99 ms). The E180 durations were 14 and 32 ms, respectively.

RESULTS and DISCUSSION: Figure 3 illustrates the performance of triple refocusing difference/summing editing using the two subecho time sets. 2HG exhibited a positive signal in subspectrum-A and an inverted signal in subspectrum-B, thereby leading to an edited 2HG signal at 2.25 ppm following subtraction and null 2HG following summing. Due to the weak coupling effects, the GABA signal was similar in the subspectra, thus subtraction and summing gave null GABA and edited GABA signals, respectively. Following phantom validation, the triple-refocusing editing was applied in patients with IDH mutations. Figure 4 shows *in-vivo* results from an IDH-mutated glioma. The PRESS spectrum showed a signal at 2.2 - 2.3 ppm (Fig. 4c), which was analyzed, by spectral fitting, to a composite signal of 2HG 0.5 mM and GABA 0.7 mM. The correlation coefficient between 2HG and GABA, returned by LCModel, was quite large (-0.39). In contrast, triple-refocusing difference editing (Fig. 4a) resulted in 2HG level at 1.9 mM. GABA was measured as zero in the difference spectrum. This is as expected since GABA signal was canceled *via* subtraction. Summing editing, which should contain edited GABA without 2HG signal, resulted in zero concentrations of both 2HG and GABA (Fig. 4b). Of note, we obtained a high-resolution ^{1}H spectrum from a biopsy of this patient, in which GABA was not detected (Fig. 1, spectrum indicated by an arrow). For the 5 patients who were scanned with both the triple-refocusing editing and PRESS, the 2HG estimates from the editing and PRESS were different (similarly to Fig. 4) when the 2HG levels were relatively low (< 3 mM) (3 patients), but when 2HG levels were high (> 4 mM) the 2HG estimates from the two methods were about the same (2 patients).

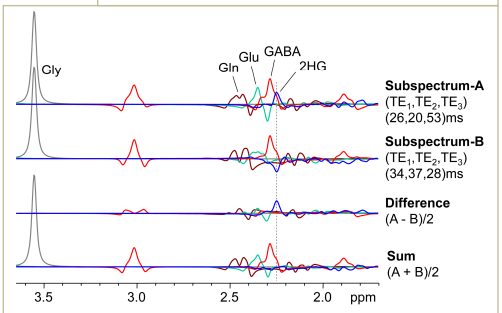

FIG 3. Theoretical illustration of constant-TE triple refocusing difference/summing editing for separation between 2HG and GABA at 3T. Subtraction between subspectra-A and -B leads to edited 2HG and null GABA, while summing gives null 2HG and edited GABA. Spectra were calculated for equal concentrations and broadened to glycine FWHM of 0.03 ppm (3.8 Hz). A vertical line is drawn at 2.25 ppm.

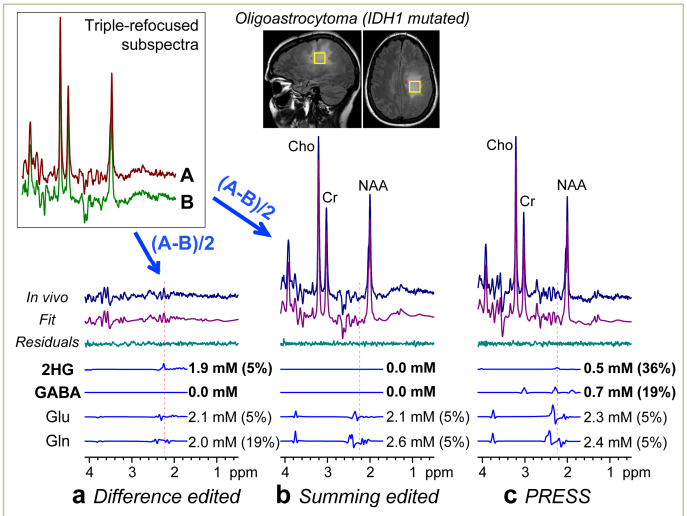

FIG 4. *In-vivo* demonstration of triple-refocusing difference/summing editing of 2HG and GABA at 3T. Spectra from an IDH-mutated glioma, obtained with triple-refocusing difference editing (a), summing editing (b), and PRESS (TE=97ms) (c), are shown with LCModel outputs and individual components of 2HG, GABA, Glu and Gln. CRLBs are shown in brackets. Vertical dotted lines are drawn at 2.25 ppm. *Ex-vivo* high-resolution ^{1}H NMR on a biopsy from this patient did not show measurable GABA (see Fig. 1).

CONCLUSION: 2HG and GABA can be separated without considerable interferences, using constant-TE triple-refocusing difference/summing editing at 3T. Further study will be required to evaluate the capability of the summing editing for detection of GABA, which is also an important metabolite in tumors as a marker of neuronal disruption by infiltrating glioma cells and is associated with high incidence of seizure activity.

REFERENCES: **1.** Parsons et al. Science 2008;321:1807-12. **2.** Yan et al. N Engl J Med 2009;360:765-73. **3.** Dang et al. Nature 2009;462:739-44. **4.** Figueroa et al. Cancer Cell 2010;18:553-67. **5.** Ward et al. Cancer Cell 2010;17:225-34. **6.** Prensner *et al*. Nat Med 2011;17:291-293. **7.** Metallo *et al*. Nature. 2011;481:380-384.

This study was supported by NIH CA159128 and CPRIT RP101243.