1H-MRS study of neurochemical profiles of human primary glioblastoma in mouse models at 9.4T

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INTRODUCTION:

High field MRS is gaining popularity in clinical and preclinical studies due to its enhanced spectral and spatial resolution and hence reliable quantification of metabolites is feasible [1, 2]. The aim of present study is to investigate and quantify biomarkers (such as *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), glutamate (Glu), glutamine (Gln), glycine (Gly), myo-inositol (mIns), γ-aminobutyrate (GABA), glycerophosphocholine and phosphocholine (GPCPC), Taurine (Tau) etc.) in various types of tumors (Glioblastoma (GBM), Lung metastasis and Breast metastasis). Preliminary results on human primary GBM implanted in mouse brain at 9.4T are reported.

METHODS:

Animals: Human primary GBM tumor was collected and was gently dissociated into single cells within 2-3 hours of surgical resection. $5x10^4$ viable tumor cells were injected stereotactically into the right caudate of NOD-SCID mouse brain. The mice were anesthetized using isoflurane (1%-2%) with oxygen throughout the experiments. The body temperature and respiration were monitored and a hot air blower was used to maintain the temperature. ***Experiments***: All *in vivo* (5 GBM mice, 4 normal mice) experiments were performed at 9.4T (Varian horizontal-bore animal scanner) using a home-built 15 mm diameter transmit/receive surface coil. T_2-weighted images (both axial and sagittal) were acquired using fast spin echo sequence (TR = 4 s, TE = 9 ms, effective TE = 27 ms) to identify the tumor masses. Single-voxel water-suppressed ^{1}H NMR spectra were obtained, using a PRESS sequence at TE = 19 ms, from a 3×3×3 mm^3 voxel positioned within the tumor. Single-voxel localization was obtained with a 550 μs 90° RF pulse (BW = 10.7 kHz) and 3 ms 180° RF pulses (BW = 5.4 kHz). Data acquisition parameters: TR = 3 s, SW = 5 kHz, 4096 sampling points, and 256-320 averages. An unsuppressed water signal was also acquired using the PRESS sequence from the same voxel. ***Data analysis***: Residual water suppression was performed using HLSVD algorithm of jMRUI [3] and data were corrected for frequency drift and eddy current artifacts during the post-processing using in-house MATLAB program. LCModel software [4] was used for data analysis. Unsuppressed water signals were used as reference for metabolite quantification. All results were presented as mean ± standard deviation (SD). Unpaired t-test (two ways) was performed to compare metabolite estimates between normal and GBM mice. The level of significance (p) was set to 0.05.

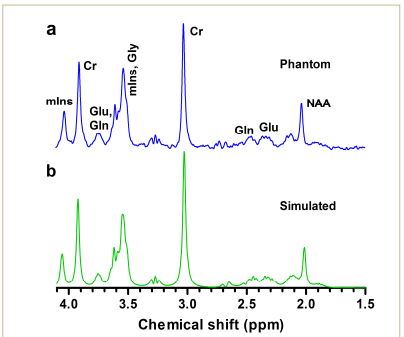

FIG. 1. (a) Single voxel ^{1}H MR spectra obtained from a phantom (NAA, Cr, Glu, Gln, Gly, GABA and mIns). (b) Simulated spectra (LB = 0.03 ppm, without T_2 effect) with identical metabolite concentrations.

RESULTS AND DISCUSSION:

Benefit of higher spectral resolution at high magnetic field was reflected from the clear separation between C4-proton resonances of glutamate (2.35 ppm) and glutamine (2.45 ppm) in both phantom (Fig.1a) and simulation (Fig.1b). At 9.4T these two resonances were separated by 40 Hz. Figures 2a and 2b show in vivo ^{1}H MR spectra obtained from normal mice and mice with GBM respectively. The spectral pattern at the Glu-Gln region (2.3-2.5 ppm, Fig. 2b) was in agreement with simulated and phantom spectra. The CRLBs of both Glu and Gln were less than 7%. ^{1}H MRS of GBM mice had elevated Gly ($p < 0.01$), mIns ($p < 0.01$), Tau ($p < 0.05$), CRLBs of all three metabolites being less than 5%. Also, reduced concentrations of Glu, GABA, NAA+NAAG ($p < 0.01$) compared with normal mice (Fig. 3a) were observed. Elevation of Gly implies malignancy of the glioma, as reported in several *in vivo* and *in vitro* studies [5-8]. Gln/Glu and GPCPC/NAA ratios were substantially increased in GBM mice compared to normal mice (Fig. 3b). Reduction in NAA, GABA and Glu/Gln ratio ($p < 0.01$) in the GBM may reflect neuronal loss. These observations are consistent with previous studies [10, 11]. Tau was elevated and alanine was not altered significantly, as opposed to prior report [11]. Our mice model, which was prepared by injecting whole tumor representative population in the mice brain unlike isolated human glioma initiating population [11] or tumor cell line [12], may represent more accurate scenario of human glioblastoma.

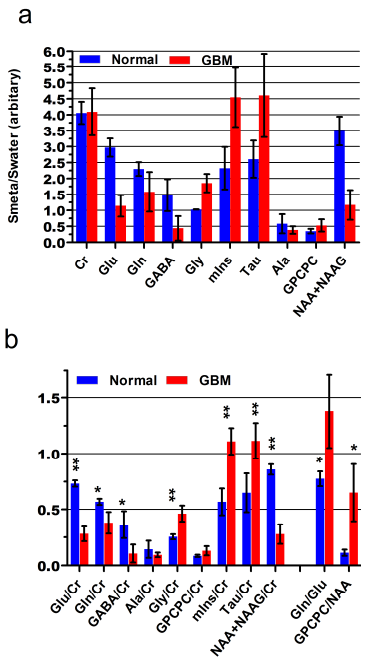

FIG. 3. Normalized mean (±SD) metabolite estimates from LCModel (a) and ratios (b) in normal mice brain and mice with GBM. p-value: * < 0.01, ** < 0.001

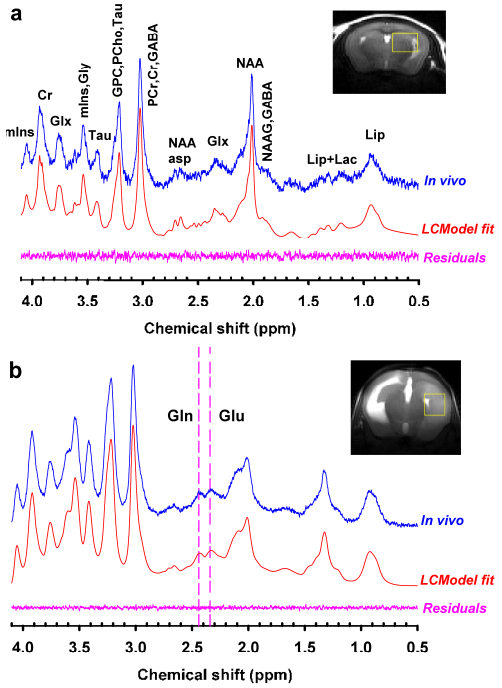

FIG. 2. Single voxel (27 μL) *in vivo* ^{1}H MR spectra obtained from normal mice brain (a) and mice with GBM (b) together with LCModel fit and residuals.

ACKNOWLEDGEMENT:

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REFERENCES:

[1] Tkáč I *et al*. Magn Reson Med 2004;52:478-484. [2] Choi I-Y *et al*. Neurochem Res 2003;28:987-1001. [3] Naressi A *et al*. MAGMA. 2001;12:141–152. [4] Provencher SW Magn Reson Med 1993;30:672-679. [5] Carpinelli G *et al*. Anticancer Res 1996;16:1559-1563. [6] Gyngell ML *et al*. NMR Biomed 1992;5:335-340. [7] Choi C *et al*. Magn Reson Med 2011;66:609-618. [8] Kinoshita Y and Yokota A. NMR Biomed 1997;10:2-12. [9] Mader I et al. MAGMA 1996;4:139-150. [10] Thorsen F *et al*. NMR Biomed 2008;21:830-838. [11] Mlynárik V *et al*. NMR Biomed 2011;DOI:10.1002/nbm. 1763. [12] Doblas S *et al*. NMR Biomed 2011:DOI:10.1002/nbm.1785.