

Metabolic Characterization of Recurrent Grade 2 Glioma using Proton HR-MAS Spectroscopy

L. Jalbert¹, A. Elkhaled¹, R. Srinivasan¹, H. Yoshihara¹, C. Cloyd^{1,2}, G. Bourne¹, S. M. Chang³, S. Cha¹, J. Kurhanewicz^{1,4}, and S. J. Nelson^{1,4}

¹Department of Radiology & Biomedical Imaging, University of California - San Francisco, San Francisco, CA, United States, ²School of Pharmacy, University of California - San Francisco, San Francisco, CA, United States, ³Department of Neurological Surgery, University of California - San Francisco, San Francisco, CA, United States, ⁴Department of Bioengineering & Therapeutic Sciences, University of California - San Francisco, San Francisco, CA, United States

Introduction: Recent advances in MR spectroscopy have offered new insight into tumor physiology that may be valuable in understanding the process of glial tumorigenesis. Proton High Resolution Magic Angle Spectroscopy (¹H HR-MAS) is a technique that can predict cell physiology through accurate characterization of tissue metabolism¹. This technology has previously been applied to evaluate high-grade gliomas², but there have been fewer reports concerning the physiological processes that occur in treated lesions and the ability to differentiate between changes associated with different histological subtypes of low-grade glioma. This study aims to improve our understanding of the mechanisms involved in transformation from grade 2 to higher-grade malignancy, as well as providing fundamental metabolic information differentiating astrocytoma and oligodendrogloma histological subtypes using ¹H HR-MAS.

Methods: Fifty-four patients who had pathologically confirmed WHO Grade 2 recurrent glioma were included in our study. These patients had received prior treatment with surgical resection and/or radiation and chemotherapy and were enrolled at time of suspected recurrence.

In vivo MR Scans: Preoperative MR studies were conducted at either 1.5 or 3 Tesla. The scans included 6 directional axial Diffusion Weighted Imaging (DWI) with b=1000s/mm²; lactate-edited 3D MRSI with PRESS volume localization; and dynamic Perfusion Weighted Imaging (PWI) with an injection of 0.1mmol/kg body weight Gd-DTPA contrast agent at 5mL/s.

Data Analysis: In house software was used to quantify DWI, MRSI, and PWI parameters. Tissue sample locations were selected based on surgically accessible areas with low ADC, elevated Choline/N-Acetylaspartate index (CNI), or elevated PWI peak height and low recovery. Visualization of sample locations and image-guided tissue acquisition were performed using BrainLAB surgical navigation software (BrainLAB Inc.). Upon surgical excision, the tissue samples were immediately snap frozen in liquid nitrogen and stored at -80°C. 110 tissue samples were collected and 48 samples have been analyzed so far.

Ex vivo ¹H HR-MAS: Tissue samples were placed in a 35µl zirconium rotor custom designed by Varian with 3µl 99.9% atom-D deuterium oxide containing .75 wt% 3-(Trimethylsilyl)propionic acid (TSP) from Sigma Aldrich. Samples were scanned at 11.7 Tesla, 1° C, 2250Hz spin rate in a 4mm gHz nanoprobe with a Varian INOVA 500 MHz multi-nuclear spectrometer. A 1D Carr-Purcell-Meiboom-Gill (CPMG) Sequence was run with TR/TE=4s/144ms, 512 scans, 40,000 acquired points, 90° pulse angle, 20000Hz spectral width, with an acquisition time of 35 minutes. To estimate In-vivo Concentrations, the ERETIC method was utilized for quantification³. 2D Total Correlation Spectroscopy (TOCSY)⁴ was performed to further resolve choline head groups phosphocholine (PC) and glycerophosphocholine (GPC). Post processing was performed using jMRUI and a customized HR-QUEST algorithm to extract metabolite concentrations⁵. Parameter fits with less than 13% Cramer-Rao error estimates were included for analysis with a Wilcoxon rank sum test to assess statistical significance (p <0.05).

Results: Approximately half of the patients had tumors that were assessed as upgrading to WHO Grade 3. The 48 tissue samples analyzed so far have included 19 oligodendroglomas (11 Upgraded/8 Non-upgraded), 23 astrocytomas (10 Upgraded/13 Non-upgraded), and 6 oligoastrocytomas (1 Upgraded/5 Non-upgraded). Results shown are from 1D CPMG spectra, which successfully resolved PC and GPC head groups (Figure 1). Figure 2 shows differences for tumors that were assessed as upgrading at the time of recurrence versus those that did not. The upgraded lesions exhibited higher levels of PC (p=.008), GPC (p=.049), and glucose (p=.002), with free choline (p=.188) being elevated but not statistical significant. Total choline was also higher in the upgraded samples (p=.01). Metabolite concentrations that differentiated between oligodendrogloma and astrocytoma histological subtypes are shown in Figure 3. Creatine (p=.1), glutathione (p=.1), and total choline (p=.11) were also elevated in astrocytoma but did not reach statistical significance. Astrocytomas that upgraded showed increased levels of PC (p=.049) and GPC (p=.02) compared to non-upgraded lesions, while the upgraded oligodendroglomas showed elevated glucose (p=.02) compared to non-upgraded lesions. Future studies will not only increase the number of samples but will also be used to more fully analyze the difference in metabolite levels based upon the 2D TOCSY data.

Conclusions: The ex vivo ¹HR-MAS results from our study suggest that higher PC, GPC, total choline, and glucose concentrations may contribute in identifying low-grade glioma patients whose tumors have become more aggressive. This is vitally important for treatment planning and selection. The results also suggest that ¹HR-MAS can reflect differences in the biological parameters associated with different histological subtypes of glial tumors. Future studies will compare the ex vivo data with corresponding in vivo parameters to identify noninvasive parameters that could also assess whether a lesion has upgraded.

References: [1] Sitter et al. (2009). *PNMR* 54(3): 239-254 [2] Righi et al. (2009). *NMR Biomed* 22(6): 629-37. [3] Albers et al. (2009). *Mag Reson Med* 61(3): 525-32. [4] Swanson et al. (2008). *Magn Reson Med* 60(1):33-40[5] Stefan et al. (2009). *Meas. Sci. Technol* 20(10)

This study was supported by the NIH Brain Tumor SP0RE Grant P50CA097257

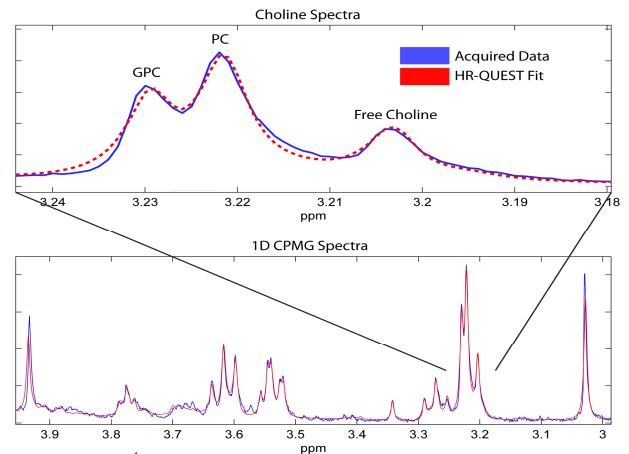


Figure 1. 1D ¹H HR-MAS CPMG Spectra of an upgraded astrocytoma

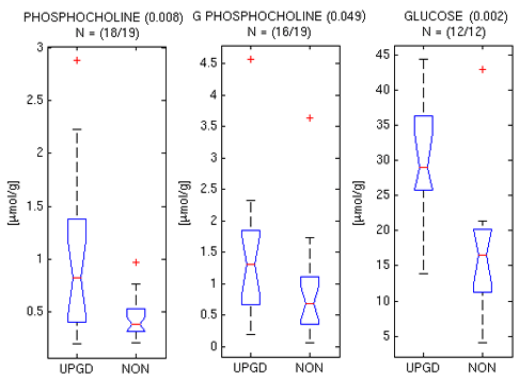


Figure 2. Patients who upgraded from WHO Grade 2 (UPGD) to Grade 3 showed elevated concentrations of phosphocholine, glycerophosphocholine, and glucose

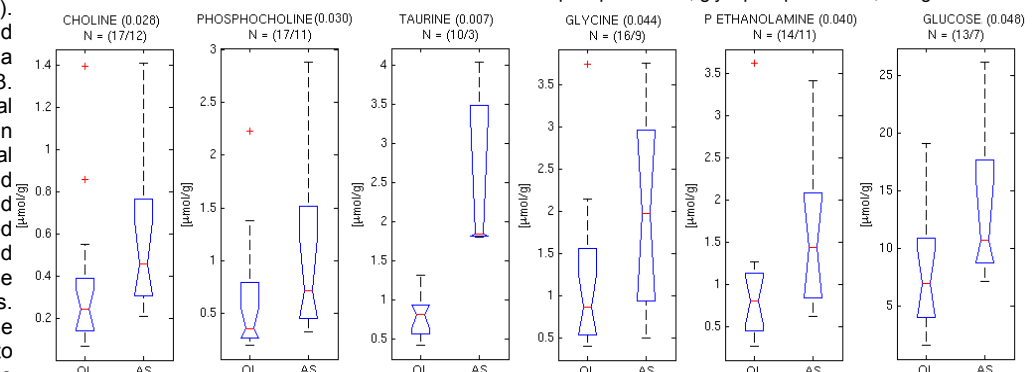


Figure 3. Differences between astrocytoma (AS) and oligodendrogloma (OL) histological subtypes