Evaluation of metabolic heterogeneity in regions of contrast-enhancing versus non-enhancing GBM using ^{1}H HR-MAS spectroscopy

Adam Elkhaled[1], Joanna J Phillips[2], Susan M Chang[3], Soonmee Cha[1], and Sarah J Nelson[1,4]

[1]*Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, California, United States,* [2]*Department of Pathology, University of California, San Francisco, San Francisco, CA, United States,* [3]*Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA, United States,* [4]*Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, California, United States*

Introduction: Glioblastoma multiforme (GBM) is the most common and malignant type of primary brain tumor, characterized by the formation of highly permeable vasculature, which can be interrogated radiologically via the leakage of T1-sensitive (Gd-based) contrast agents. While regions of contrast-enhancing tissue are considered to be tumor in patients with GBM, the full extent of infiltrative disease remains difficult to assess. The goal of this study was to characterize the metabolic profile of tissue samples from non-enhancing tumor using the *ex vivo* NMR technique of proton high-resolution magic-angle-spinning (^{1}H HR-MAS) spectroscopy.

Methods: Our institutionally-approved study enrolled a total of fifty-one treatment-naive patients who were presenting for surgical resection owing to a suspected diagnosis of GBM. Patients received a pre-surgical MR examination on a 3T GE scanner that included pre- and post-contrast T1-weighted 3D IRSPGR images (TR/TE/TI =8/3/400ms, 1.5mm slices, 15° flip angle, 256x256 matrix), 3D lactate-edited ^{1}H spectroscopy (MRSI), 6-direction diffusion-weighted imaging (DWI), and perfusion-weighted imaging. Automated algorithms generated maps of the choline-to-N-acetylaspartate index (CNI), apparent diffusion coefficient (ADC) and perfusion curves.

Image-Guided Tissue Samples: Regions of suspected tumor were identified as having elevated CNI values, low ADC values, or elevated perfusion peak height/reduced recovery, and were designated for tissue sampling using surgical navigation software (BrainLab). 1-4 tissue samples were removed during surgery, and divided into two parts: one was fixed for evaluation by a pathologist for confirmation of GBM, and the other flash-frozen in liquid nitrogen to be analyzed via ^{1}H HR-MAS.

***Ex-vivo* ^{1}H HR-MAS**: Tissue samples (~5-20mg) were loaded into a 35µL Varian zirconium rotor with 3µL 99.9% atom-D deuterium oxide containing 0.75 wt% 3-(Trimethylsilyl)propionic acid (TSP) prepared by Sigma Aldrich. ^{1}H HR-MAS spectroscopy was performed at 11.7 Tesla, 1° C, 2250Hz spin rate in a 4mm gHZ nanoprobe using a 500MHz Varian INOVA spectrometer. A 1D Carr-Purcell-Meiboom-Gill (CPMG) sequence was acquired with TR/TE=4s/144ms, 512 scans, 40,000 points, 90° pulse angle, and 20,000Hz spectral width. The Electronic Reference To access In-vivo Concentrations (ERETIC) method provided an external standard for quantification[1].

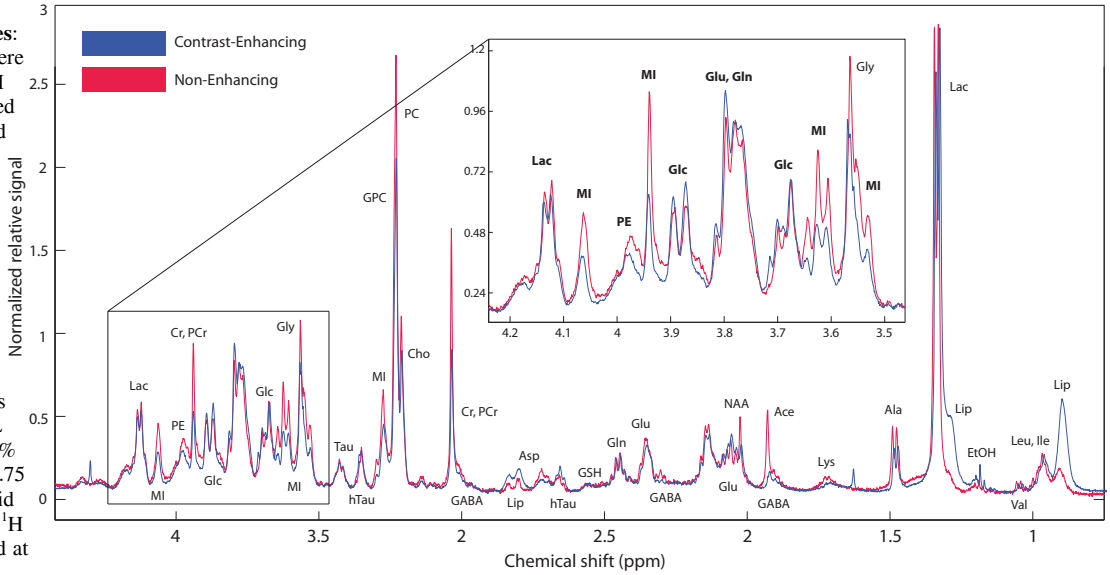

Figure 1: Mean CPMG spectra for contrast-enhancing (n = 83) and non-enhancing (n = 40) GBM tissue samples

Metabolites	Median levels ± SE (N° spectra)		*P* value
	CE	NE	
(P)Cr	0.84 ± 0.09 (57)	1.27 ± 0.02 (25)	0.009
MI	1.27 ± 0.15 (53)	1.94 ± 0.34 (24)	0.006
Cho	0.21 ± 0.06 (55)	0.37 ± 0.24 (26)	0.004
PE	1.24 ± 0.13 (54)	1.74 ± 0.29 (25)	0.04
Ala	0.75 ± 0.12 (43)	1.56 ± 0.41 (13)	0.02

Table 1: Relative melabolite levels of CE *vs* NE

Analysis:
The location of acquired tissue was referenced on T1-weighted images using 5mm-diameter ROIs generated from surgical coordinates. Individual samples were visually defined as contrast-enhancing (CE) or non-enhancing (NE) based on the intensity of the ROIs relative to normal-appearing tissue. jMRUI permitted the pre-processing of *ex vivo* spectra in the time domain, and the semi-parametric fitting algorithm HR-QUEST was used to quantify 26 metabolites from known concentrations of stock solutions. Analysis was performed on pathologically confirmed GBM samples whose metabolites were fit with Cramer-Rao error bounds ≤11%. The Wilcoxon ranked-sum test enabled a statistical comparison of CE vs NE metabolite profiles; statistical significance was defined as $p<0.05$.

Results:
A total of 123 image-guided tissue samples were pathologically confirmed as GBM. Of these samples, 83 demonstrated T1 contrast enhancement from imaging, and the remaining 40 were non-enhancing. Figure 1 contains the mean CPMG spectra obtained from the respective CE and NE samples, normalized by sample weight and ERETIC signal area. Within the magnified spectral inset, levels of creatine (Cr, PCr), myo-inositol (MI), and phospho-ethanlolamine (PE) are shown to be elevated in the NE relative to CE tumor (Fig.1, Table 1). Although all of the choline-containing species were found to be elevated above normal –as expected with highly cellular tumor – only the free choline (Cho) was statistically significant between the two groups, being more abundant in NE tumor. There was also considerable variation in phosphocholine (PC) and glycerophosphocholine (GPC) levels, which prevented any distinguishable difference in the measure of total choline (tCHO). Alanine was elevated in the NE tumor. To further investigate an apparent elevation of lipid (Lip) in CE versus NE samples, normalized spectra were compared according to peak heights at several known Lip resonances[2]. The Wilcoxon ranked-sum test revealed that CE samples demonstrate a significant increase in mobile Lip at ~ 0.9 ppm (CH_3-) and 1.3 ppm ($-CH_2-$), respectively, as well as an increase in the polyunsaturated Lip peak at ~2.8 ppm ($=CH-CH_2-CH=$) ($p < 0.001$).

Conclusions:

That spectra obtained from CE and NE tumor showed similar elevations in tCHO provides evidence that the samples originated in regions of relatively similar cellularity. Inherent differences in metabolite expression between CE and NE tumor, however, indicate that there is considerable heterogeneity within the lesion which is related to the micro-environment of GBM. Elevations of Cr in NE tumor are consistent with improved oxygenation relative to more hypoxic conditions experienced in the CE tumor that borders necrosis. The heightened levels of Lip in CE tumor may reflect ongoing apoptosis in an oxygen-deprived environment[3]. Elevations of Ala, PE, and MI suggest that the infiltrating margin of GBM has a metabolic profile that is more similar to that of grade III gliomas, which have less vasculature and necrosis than GBM. Information derived from this study may be helpful in determining *in vivo* markers of NE tumor that will assist in defining surgical margins and residual tumor.

References: [1] Albers et al. *Magn Reson Med* 2009;61(3): 525-32. [2] Zietkowski et. al. *NMR Biomed.* 2010;23(4):382-90, [3] Opstad, et. al. *NMR Biomed.* 2008;21(7):677-85.

Acknowledgements: Funding for this study was provided by NIH RO1 CA127612 and P01 CA118816 grants.