Comparison of glioma sub-populations using in-vivo ^{1}H HR-MAS spectroscopy

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Introduction: Many studies have evaluated the differences in metabolite levels between gliomas of different grades using proton HR-MAS spectroscopy, but there has not previously been a comparison of metabolic profiles for patients with newly diagnosed versus treated lesions[1]. This project compares ex vivo metabolic, pathologic and in vivo ADC parameters for tissue samples derived from patients with gliomas of varying grade and recurrence status.

Methods: Patients diagnosed with new or recurrent WHO grade IV and recurrent WHO grade II glioma (N=43,29,54) received a pre-surgical MR examination on a 1.5 or 3 Tesla magnet. Functional imaging included 3D lactate-edited ^{1}H spectroscopy (MRSI), 6-directional diffusion-weighted imaging (DWI), and perfusion-weighted imaging. Automated algorithms generated maps of the choline-to-N-acetyl-aspartate index (CNI) and apparent diffusion coefficient (ADC), along with perfusion curves.

Image-Guided Tissue Samples: Regions of suspected tumor were identified from elevated CNI values, low ADC values, or elevated perfusion peak height/reduced recovery and were designated as targets for tissue sampling using surgical navigation software (BrainLab, Inc.). Tissue samples were divided into two parts and flash-frozen in liquid nitrogen. A pathologist scored one part of the sample for tumor cellularity(*I-III,* in order of increasing cell density) and evaluated the MIB-1 index based on the number of cells stained with KI-67 antibody relative to the total cell count. The second part of the sample was analyzed with ex vivo HR-MAS. The median in vivo ADC value within the 5mm diameter region from which the sample was removed was determined using registered biopsy coordinates. These values were then normalized by normal-appearing white matter (NAWM).

Ex-vivo ^{1}H HR-MAS: Tissue samples (~5-20mg) were loaded into a 35μL Varian zirconia 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 20000Hz spectral width. The Electronic Reference To access In-vivo Concentrations (ERETIC) method provided an external standard for quantification[2]. Acquisition of 2D Total Correlation Spectroscopy (TOCSY)[3] data allowed for improved separation of choline moieties with overlapping spectra. Results presented here reflect 1D spectra.

Analysis: Post-processing of the ex vivo spectra utilized jMRUI and a customized HR-QUEST fitting algorithm to measure metabolite concentrations[4]. A data visualization/summary program compared group-specific metabolite concentrations with < 13% Cramer-Rao error bounds using the Wilcoxon ranked-sum test and evaluated correlations between continuous variables with the Spearman rank order test ($p<0.05$ considered statistically significant). Only samples classified as containing tumor by a pathologist were included in the final analysis.

Results:

A comparison of the metabolite levels in samples from the patients with newly diagnosed (ND) and recurrent grade IV glioma revealed no significant differences, with the following observations being noted: glutamine levels trended toward higher values in newly diagnosed patients (P=0.08) and the detection of N-acetyl-aspartate (NAA) was less frequent in the patients with recurrent lesions. Preliminary analysis of the pathologic data for the ND and recurrent grade IV samples suggested that increased tumor cellularity was associated with higher MIB-1 indices (median MIB-1 indices for ND samples per tumor cellularity score were: *I*=4.43%, *II*=16.06%, *III*=19.34%). The ND samples with increased tumor cellularity had higher total[Cho]/[Cre] (median values per tumor cellularity score: *I*=0.72, *II*=1.41, *III*=2.07). Glutathione (GSH) from ND samples showed a modest negative correlation with nADC values (R=-0.42, P=0.05). Samples from patients with recurrent low-grade glioma were separated according to whether histological analysis indicated that they had upgraded at the time of recurrence to grade III (Upgraded, N=21) or remained grade II (Non-Upgraded, N=21). As is shown in Figure 1, both non-upgraded and upgraded samples demonstrated a statistically significant elevation in *myo*-inositol (P<0.00001/0.0002) and creatine (P=0.0003/0.003), when compared with recurrent grade IV samples. Total choline [free choline + phosphocholine (PC) + glycerophosphocholine (GPC)] was higher in the upgraded samples compared to grade IV samples (P=0.005) and the in-vivo nADC increased with decreasing grade (rec. IV vs. III, P=0.02; rec. IV vs. II, P=0.002). The [*myo*-I]/total[Cho] ratio was highly significant in distinguishing non-upgraded glioma from all other sub-classifications of glioma(II vs. rec./ND/upgraded, $P=0.001/4x10^{-7}/3x10^{-4}$)

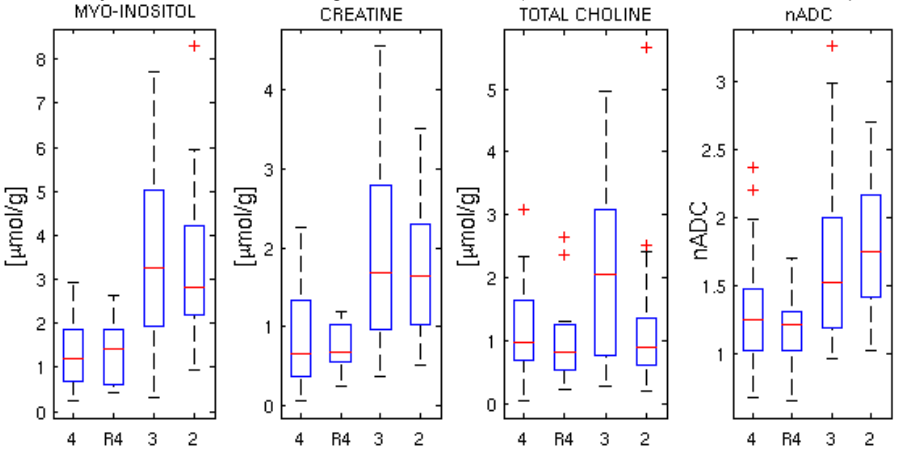

Figure 1: Comparison of parameters according to tumor grade and recurrence status: New (4) & Recurrent (R4) high grades; Upgraded (3) & Non-Upgraded (2) low grades.

Discussion: Newly diagnosed grade IV gliomas were metabolically indistinguishable from their recurrent counterparts despite the disparity in treatment. Of interest is that there was significant difference in several of the metabolic parameters between the low grade gliomas that had upgraded to grade III and the recurrent grade IV lesions. This implies that the current practice of treating grade III and grade IV glioma as the same disease may not be the best strategy and that metabolic parameters may be relevant in stratifying patients for their response to different therapies. The observed differences in myo-inositol and total choline suggest that these parameters may also have clinical utility. One possible application for the use of the ratio of [*myo*-I]/total[Cho] [5] is in the detection of characteristically high-grade regions within low-grade glioma. The in-vivo data confirmed previous reports concerning the relationship between ADC values and tumor grade[6]. The heightened expression of glutathione in newly diagnosed samples with low ADC values is of interest for describing metabolic processes that are associated with differences in tissue architecture and/or tumor cellularity.

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