EFFECTS OF TREATMENT ON THE METABOLIC CHARACTERISTICS OF GRADE 2 AND GRADE 3 GLIOMAS

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Introduction: Recent research has shown that the evaluation of cellular metabolism can help with the diagnosis and assessment of treatment effects in patients with brain tumors. High-resolution magic angle spinning (HRMAS) spectroscopy provides metabolic data from whole tissue samples for investigating tumor biology. In this study, we used HRMAS to compare the differences in metabolism between newly diagnosed gliomas of grades 2 and 3, and recurrent gliomas of grades 2 and 3 who were treated using standard-of-care. Identifying differences in metabolic parameters between these two groups is important in understanding how tumor progression affects the pathways of glioma growth and invasion, and in identifying biomarkers that may be associated with treatment effects. In order to assess whether treated and untreated gliomas of the same grade are different entities, and thus might have different responses to subsequent therapy, we used a multivariate pattern recognition method to discriminate between them based on their metabolic profiles.

Data Acquisition: Our study included 137 patients: 86 newly diagnosed non-enhancing grade 2 (n=55) and grade 3 (n=31) gliomas, and 51 recurrent grade 2 gliomas that recurred as grade 2 (n=22) or grade 3 (n=29). A total of 349 image-guided biopsy samples were obtained from the 137 patients; the samples were then analyzed using HRMAS spectroscopy and histology. HRMAS data was obtained by scanning the biopsy tissues with a Varian 500 MHz spectrometer, equipped with a gHX gradient nanoprobe. Samples were evaluated at 1° C while the tissue was spun at 2250 Hz at the magic angle (theta = 54.7 degrees). The fully relaxed water presaturation sequence parameters were pulse width=7.8 mgrs, transients=128, sweep width=40 kHz, and 40,000 points. The ERETIC method was used to provide a constant reference for quantification.

Methods: The *ex vivo* spectra were processed using jMRUI and a customized QUEST fitting algorithm to measure metabolite concentrations. A random effects model was used to compare metabolite concentrations in treated and untreated lesions, by grade. P-values were adjusted for multiple comparisons using Holm's method. A supervised learning algorithm was used to determine whether it is possible to accurately discriminate between the recurrent and newly diagnosed gliomas based on their metabolic profiles. Spectral parameters obtained from small chemical shift bins formed the input vectors, and the associated categories (newly diagnosed or recurrent) were the desired outputs or classes. The supervised learning method we used was multivariate logistic ridge regression with automatic wrapper-based feature selection. This method treated all biopsy samples as being independent. The supervised learning models were validated using 0.632 bootstrap accuracy estimates.

Results: A comparison of the metabolite levels in samples from patients with treated and newly diagnosed gliomas of grades 2 and 3 revealed significant differences in the myo-inositol to total choline ratio (MCI) ($p=<0.001$). The supervised learning model for distinguishing between these lesions had 90.25% accuracy (CI=[89.69%, 90.81%]) and was based on features of the spectra corresponding to myo-inositol (My-I), choline, aspartate (ASP), glutathione (GSH), creatine (Cre), and lactate.

Lower MCI levels ($p=0.004$) and higher levels of taurine ($p=0.002$) and aspartate ($p<0.001$) were detected in recurrent grade 3 gliomas, compared to newly diagnosed grade 3 gliomas. The multivariate supervised learning model for distinguishing between recurrent and newly diagnosed grade 3 gliomas based on features of the spectra corresponding to My-I, choline, GSH, N-acetyl-aspartate (NAA), and lactate had 92.45% accuracy (CI=[89.75%, 95.15%]).

Lower MCI levels ($p=0.003$) and higher GSH levels ($p=0.014$) were detected in recurrent grade 2 gliomas compared to their newly diagnosed counterparts. The supervised learning model for distinguishing between recurrent and newly diagnosed grade 3 gliomas based on features of the spectra corresponding to choline, GSH, glucose, ASP, 2HG, phosphoethanolamine (PE), and lactate had 94.44% accuracy (CI=[92.2%, 96.68%]).

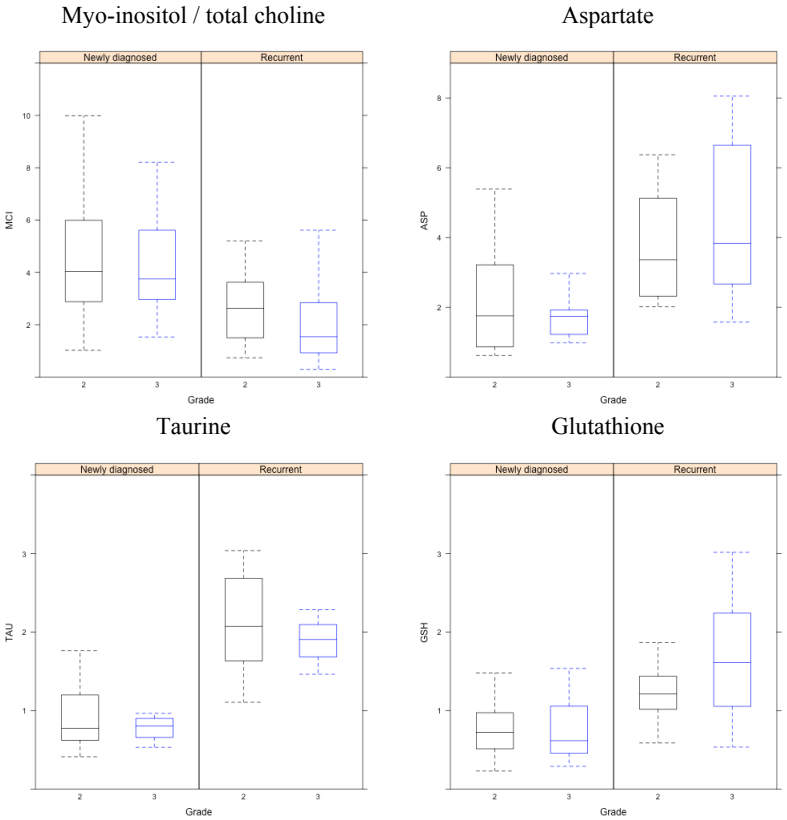

Figure 1. Metabolite levels in recurrent and newly diagnosed tumors, by grade.

Conclusion: Grade 2 and 3 newly diagnosed gliomas had significantly different metabolic profiles than recurrent gliomas of the same grade. MCI, a biomarker associated with treatment effect [1], was significantly lower in recurrent lesions, regardless of grade. Multivariate supervised learning models were able to distinguish between treated and recurrent gliomas with more than 90% accuracy, suggesting that the metabolic profiles of these tumors are significantly different.

References: [1] Srinivasan (2010), *Neuro-Onc.* 12(11):1152–1161. [2] McKnight (2011), *JMRI* 33:808–816. [3] Witten and Frank (2005), Data Mining.

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