MULTI-VARIATE PATTERN ANALYSIS FOR IDENTIFICATION OF METABOLITES THAT ARE PREDICTIVE OF MALIGNANT TRANSFORMATION IN GLIOMAS USING HRMAS SPECTRA FROM IMAGE GUIDED TISSUE SAMPLES

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Introduction: Recent oncology research shows that the evaluation of cellular metabolism can be very helpful for the diagnosis and assessment of treatment effects for patients with brain tumors. High-resolution magic angle spinning (HRMAS) spectroscopy provides detailed metabolic data of whole biopsy samples for investigating tumor biology. Analysis of such data can lead to identification of metabolites that may be used as biomarkers for discriminating different types of cancer, for grading tumors, and for assessing their evolution. The identification of *ex vivo* metabolites can also inform the acquisition of *in vivo* magnetic resonance spectroscopy (MRS), which can lead to a non-invasive assessment of tumor biology. In this study, we applied multivariate pattern recognition methods to HRMAS spectra in order to identify metabolites that are predictive of malignant transformations of gliomas and to accurately detect those patients exhibiting malignant transformations. Our method identifies regions in the HRMAS spectrum that can be used to accurately discriminate between different tumor grades in patients with recurrent or newly diagnosed gliomas, without making any prior assumptions about which metabolites are present. We begin by identifying all the regions in the HRMAS spectrum that have a mutual association with tumor grade. Then, we select a small subset of these features to build a parsimonious model that is capable of diagnosing new patients based on their HRMAS spectra. These features are then traced back to metabolites that are known to appear in the chemical shift range corresponding to the regions that were identified. These metabolites represent the best set of discriminatory features and would therefore also be of interest for acquiring *in vivo* data that would contribute to assessing glioma grade.

Data Acquisition: Our study included 53 patients with recurrent WHO grade 2 gliomas, and 36 patients with newly diagnosed WHO grade 4 gliomas (GBM). Among the recurrent grade 2 gliomas, at the time of recurrence, seven tumors had upgraded to grade 4, 24 had upgraded to grade 3, and 22 had remained grade 2. The newly diagnosed patients with GBM had not received any prior treatment. The patients with recurrent low-grade glioma had received prior treatment with surgical resection, radiation, or chemotherapy. Tissue samples of 10-40 mg were obtained from regions of the brain that were suspected of containing tumor based on pre-operative MR examinations. The tissue samples were divided into two parts. The first part was examined by a pathologist for histological features and for consistency with the tumor grade diagnosis. Only tissue samples consistent with the diagnosis were included in the analysis. The second part of the sample was analyzed with *ex vivo* HRMAS, performed at 11.7 Tesla, 1° C, 2.25 MHz spin rate, in a 4 mm GHz nanoprobe using a 500 mHz 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 MHz spectral width. The ERETIC method provided an external standard for quantification.

Analysis: Each data sample was normalized using the ERETIC concentration and the tissue weight of each sample. The data was then grouped into frequency bins of widths 5, 10, 15, 20, and 25 to account for the fact that different metabolites have different linewidths. This resulted in an input vector with 18,266 dimensions. To reduce the dimensionality of the input space, we applied a conditional probability-based feature selection technique that calculates the mutual association between class decisions and feature values based on conditional probabilities [1]. We selected only those features that had more than a 0.5 mutual association with the output. This resulted in between 30 and 40 features for each classification problem that we performed. In the classification step, we used a Functional Tree model, which was trained on paired examples of inputs and associated classes [3, 4]. The model was tested using cross-validation and bootstrapping [2]. We further performed a Genetic Search [5] of the reduced feature space in order to obtain a parsimonious, stable subset of the features, which could accurately distinguish the categories of interest.

Results: We performed four classification experiments. In the first, we built a classifier for distinguishing between recurrent low-grade gliomas that had upgraded to a higher tumor grade from those which had not. We performed a second experiment in which we modeled the difference between patients who had upgraded to grade 3 and those who had remained grade 2. Next, we built a model capable of distinguishing between newly diagnosed GBMs and recurrent low-grade gliomas. We also compared the differences between newly diagnosed GBMs and recurrent gliomas that had upgraded to grade 3. The classification accuracy of our experiments is summarized in Table 1. We discriminate between tumors of different grades with more than 90% accuracy. Table 1 also shows the subsets of metabolites that are best at discriminating between various groups of tumors. Discrimination is possible with a very small number of spectral areas (4-8). Myo-inositol is one of the most important metabolites in terms of its discriminating power. Low levels of myo-inositol are indicative of high grade. Figure 1 shows a scatterplot of the tissue samples plotted against chemical shifts of 3.52 and 3.03 ppm, corresponding to myo-inositol and creatine. In this figure, we can see that the GBMs form a distinct group in this two-dimensional space. The grade 2 and grade 3 gliomas are harder to distinguish, but differences between them are nevertheless evident.

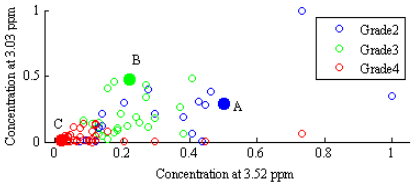

Figure 1 Tissue samples from patients with tumor grades 2, 3, and 4, plotted against concentrations at 3.52 ppm (Myo-I) and 3.03 ppm (Cre).

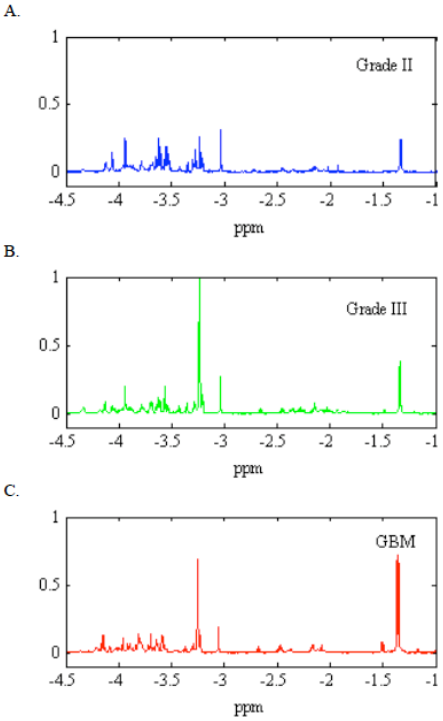

Figure 2. HRMAS spectra for samples A (grade 2), B (grade 3), and C (grade 4) in Figure 1.

Table 1. Classification accuracy and features selected for the classification models of different groups.

Class 1	Class 2	Training Accuracy	Cross-Validation	Boot-strapping	Higher for Class 1	Lower for Class 1
upgrade	non-upgrade	94%	94%	91%	**2.64**/H-Tau; **3.23**/GPC; **2.26**/2HG	**3.52**, **3.68**/Myo-I; **2.30**/GABA
grade 3	grade 2	91%	91%	89%	**2.65**/H-Tau; **3.20**/Cho; **3.24**,**3.69**/GPC	**3.21**(pres. of GPC); **3.77**, **3.52** (Myo-I);
GBM	low grade	100%	98%	98%	**1.37**/Lac; **1.46**/Ala; **1.28**/Lip	**3.62**, **4.03**/Myo-I; **3.93**/Cr; **2.28**/2HG; **1.91**/GABA
GBM	grade 3	98%	95%	95%	**3.55**/Gly	**3. 29** (Myo-I); **1.84** (2HG); **3.03** (Cr)

Discussion: The method we present in this study has identified a small set of metabolites that were used to accurately discriminate between gliomas of different grades and, most importantly, to distinguish upgraded versus non-upgraded lesions. GBMs are very different from low-grade gliomas, so identifying these tumors is relatively easy. Distinguishing between grade 2 and grade 3 gliomas is more challenging, but can still be done with high accuracy using areas of the HRMAS spectrum corresponding to Cho, GPC, Myo-I, and H-Tau. We also found that regions corresponding to 2HG were useful for discriminating between patients with recurrent low-grade gliomas who had upgraded from grade 2 to higher grade and were relatively low in primary GBMs. This is consistent with the hypothesis that 2HG is useful in discriminating between primary and secondary GBMs. Another interesting finding was a combination of high intensities at 3.20, 3,24 and 3.69 ppm and low intensity at 3.21 ppm were useful in discriminating between grade 3 and grade 2 glioma. This region of the spectrum contains choline, PC, and GPC. After examining the data, we noticed that for samples containing high GPC, the region at 3.21 ppm represents a valley between different peaks. Several of the metabolites selected by our method for their discriminating power—choline, lactate, lipid, myo-inositol and creatine—are accessible using current *in vivo* MRS methods, implying that these may be able to determine whether patients with recurrent low grade glioma have upgraded to a more malignant phenotype without the need for a tissue diagnosis.

References: [1] Ahmad et al. (2005) Pattern Recog. Letters 26(1) :43–56. [2] Efron et al. (1997) JASA 92(438): 548-560. [3] Gama (2004) Machine Learning 55(3): 219-250 (2004). . [4] Landwehr et al. (2005) Machine Learning 59 (1-2):161-205. [5] Sivanandam et al. (2007) Springer.

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