Combined Quantitative Approach for Brain Tumor Fingerprinting

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Introduction

Magnetic Resonance Spectroscopic (MRS) studies of brain biomarkers have been proven to provide statistically significant biomarkers for tumor grade differentiation and improved predictors of survival for cancer patients [1]. *Ex-vivo* HRMAS high-resolution MR spectra of unprocessed tissue [2] are ideal for illuminating *in vivo* ^{1}H MRS observations, allowing the investigation of high-grade tumors micro-heterogeneity [3], and the relationship between metabolites and cell processes, such as Cho-containing compounds involved in phospholipid metabolism, and lipids involved in apoptosis leading to necrosis [4]; further, two-dimensional ^{1}H HRMAS MRS enables more detailed and unequivocal assignments of biologically important metabolites in intact tissue samples [5]. In addition, a major focus in cancer research is identifying genetic markers that can be used for precise diagnosis or therapy. Microarray-DNA gene expression studies in human cancer can identify genetic markers of malignant transformation, using expression profiling as a screen to identify differentially expressed genes in malignant tissue; the comprehensive understanding of the genetic alterations present in all tumors could greatly improve tumor analysis and treatment. We introduce a combined quantitative approach for brain tumor fingerprinting together with machine-learning sensor-fusion methods to combine ^{1}H HRMAS MRS and gene-expression data, to robustly identify diagnostic biomarker profiles which can serve as surrogate markers important for monitoring efficiency of anticancer therapies to improve survival and quality of life of cancer patients.

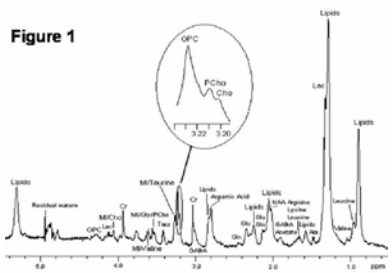

Figure 1: *Ex-vivo* HRMAS proton spectra of pediatric brain tumor 5.2mg sample (CPMG, TE=25ms);

Materials and Methods

^{1}H HRMAS MRS: Ex-vivo proton HRMAS studies of pediatric anaplastic ganglioglioma with grade-4 glial component changes were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HRMAS probe (Bruker). The temperature was controlled at 4 ^{o}C. Specimen samples were placed into 4mm zirconium oxide (Zirconia, Bruker) rotors with spherical inserts. 10 μl D_2O containing 50 mM TSP (trimethylsilyl propionic-2,2,3,3-d_4 acid, M_w=172, δ=0ppm) served as the deuterium lock reference and external chemical shift reference respectively. MAS rotation frequency was 4.0 ± 0.001 kHz. One-dimensional ^{1}H NMR spectra (Figure 1) were acquired on all samples using a rotor-synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90^o-(τ-180^o-τ)$_n$-acquisition], which works as a T_2 filter to remove the spectral broadening, with interpulse delay (τ = 250μs) synchronized to the MAS rotation frequency. Typical acquisition parameters were 256 transients with 32,768 (32k) data points, 100ms ≤ TE ≤ 500ms. A line-broadening apodization function of 1.0Hz was applied to all HRMAS ^{1}H FIDs prior to Fourier transformation. Eight tissue samples were measured with weights 1.9mg – 12mg. Good-quality spectra were acquired even with the smallest samples. 1D spectra were quantified after correcting for T2-decay and for the number of protons in the metabolite [6]. Two-dimensional ^{1}H-^{1}H TOCSY spectra (Figure 2) were acquired using a MLEV-17 mixing sequence for homonuclear Hartman-Hahn transfer, and water suppression (spectral bandwidth 14ppm; 256 increments along the first axis, 4K data points along the second axis, 80ms mixing time, N_{scans} = 8, repetition time = 2s, total acquisition time = 80 min). Data were weighted with a square sine bell function before Fourier transformation. 2D spectra were quantified following [7]; glycine (Gly) at 3.56ppm was used as internal standard. Both 1D and 2D spectra were manually phased, and referenced with respect to TSP. **Genomics:** mRNA extraction and microarray-DNA gene-expression analysis was done on two groups of samples from each specimen, (a) samples that have not been subjected to HRMAS NMR and (b) samples that have. **Data fusion:** Classifiers were first constructed independently for MRS and genomics data. Genes of interest were identified using 'gene ranking' and 'gene clustering' methods, and MRS features of interest such as areas, peak heights, chemical shifts etc. were also identified. Data were discretized using threshold values. A combined classifier was developed.

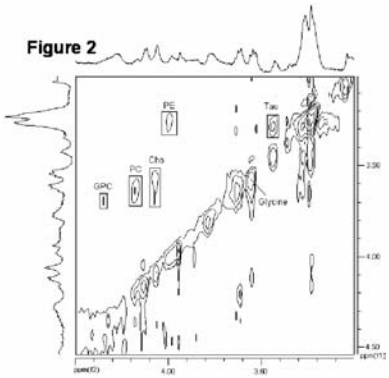

Figure 2: Typical TOCSY spectrum

Results

^{1}H HRMAS MRS: Principal metabolites are assigned by comparison to literature data (Figure 1). Metabolites that could not be directly quantified from 1D spectra are quantified using 2D TOCSY spectra (Figure 2). The relationship between 1D- and 2D - quantification results was linear: the average slope of the calibration curve of TOCSY measurements against 1D fully relaxed measurements was found to be 0.13±0.05. **Genomics:** Comparison between the group of samples that were not subjected to HRMAS NMR and the group of samples that were, has validated that the HRMAS ^{1}H MRS procedure did not degrade mRNA and that the genomic data, even with minute amounts of mRNA, were of good quality. The RNA integrity number (RIN: 1 = poor, 10 = excellent), was 7.2 ± 0.7. Analysis of our microarray-DNA data using 5% false discovery rate and above 1.4 folds change as threshold, revealed a total of 3,614 significantly overexpressed and 929 significantly under-expressed genes in tumors versus control brain. **Data fusion:** We have successfully attempted to identify complementary information between the two data sets using the feature fusion approach [8] and feature extraction methods using t-test ranking, mutual information / information gain, ReliefF to select the most informative features from the MRS-based and genomics-based classifiers. Further, we are developing a combined classifier using the decision fusion approach [9] to directly combine the MRS-based and genomics-based classifiers. We have analyzed a total of 43 samples to-date : Normal from epileptic surgeries 2y (10) and 17y (7); low grade: meningioma 21y (6), pilocytic astrocytoma 21y (6), high grade: GBM 23y (10); and metastatic tumor 40 y (7).

Discussion

Ex-vivo HRMAS MR spectra are of high quality even with small (2mg – 5mg) tumor samples. mRNA can be extracted from such samples, and mRNA quality of such samples subjected to NMR is good. Our combined MRS and genomic-based classifiers using feature-fusion and decision-fusion approaches to robustly identify diagnostic biomarker profiles, can serve as surrogate markers to monitor the efficiency of anticancer therapies.

References

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