Combining High Resolution Magic Angle Spinning H1 NMR and Molecular Genomics Predicts Survival in Brain Tumor Patients Better than Either Methodology Alone

L. G. Astrakas1,2, K. D. Blekas1, O. C. Andronesi3,4, M. N. Mindrinos5, P. M. Black6, L. G. Rahme1, and A. A. Tzika1,4

1NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA, United States; 2Department of Medical Physics, University of Ioannina, Ioannina, Greece; 3Department of Computer Science, University of Ioannina, Ioannina, Greece; 4Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Athinoula A. Martinos Center of Biomedical Imaging, Boston, MA, United States; 5Stanford Genome Technology Center, Department of Biochemistry, Stanford University School of Medicine, Palo Alto, CA, United States; 6Neurosurgery, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, United States; 7Molecular Surgery Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States

Introduction: Our objective was to develop and optimize a novel approach that combines biomarkers detected with high-resolution magic angle spinning (HRMAS) 1H NMR and molecular genomics to improve prognostication of brain tumors. Our hypothesis is that current tissue typing can be enhanced by developing and applying a classification strategy analysis algorithm that produces unique tumor fingerprints by combining biomarker profiles from MRS and whole-genome expression profiling performed on microscale brain tumor biopsies. Fusion of different sources of information can be used to improve system performance and facilitate detection, recognition, identification, tracking, change detection, and decision making in defense, robotics, and medicine [1, 2]. Few previously described classifiers have attempted to combine data from different sources [3-6]. An efficient fusion scheme using complementary information can improve confidence and accuracy. Here, we focus on fusing HRMAS 1H NMR data of brain tumor biopsies and gene expression data received from the same brain tumor biopsies.

Methods: We used a previously designed 2D adiabatic Total through Bond correlation SpectroscopY (TOBSY) HRMAS 1H NMR pulse sequence, based on novel concepts rooted in solid-state NMR spectroscopy (7). All HRMAS experiments were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (1H, 13C, 2H) HRMAS probe. After HRMAS, RNA was extracted, purified, and quantified and genomic analysis was performed following standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes. A standard Affymetrix protocols (Affymetrix, CA, USA). We then employed a Support Vector Machine (SVM) classifier [8] with linear kernels, implemented in the LIBSVM environment for multi-class SMV [9]. The original feature space comprised 54,675 genes.

Results: The architecture of our classification system is shown in Fig. 1. A typical ex vivo HRMAS 1H NMR spectra using TOBSY in anaplastic astrocytoma is shown in Fig. 2. We performed multiple stepwise logistic regression analysis to evaluate how gene expression values, HRMAS NMR data, and their combination predict survival. We chose the 15 best genes according to their MRMR algorithm rank and 15 metabolite values (Ala, Asp, alanine (Ala), polyunsaturated fatty acids (PUFA), glutamine (Gln), glutamate (Glu), lactate (Lac), taurine (Tau) and lipids (Lip).

Discussion: Although we believe that these promising results are affected by sample size, they clearly demonstrate that the combination of NMR gene and expression data predict a clinically meaningful parameter such as survival better than either technique alone. Our results, using adult brain tumor biopsies, demonstrate that with appropriate quality control, we are able to produce meaningful data and introduce a novel classification scheme that complements and substantiates the current hypothesis of cancer stem cells [11] as a means of determining brain tumor classification and treatment. The data suggest that clinical MRI, MRS and MR imaging of gene expression in ex vivo can be combined to produce improved combined images, which could then be used to readily discriminate between different tumor types as well as metastasis and high-grade gliomas, a distinction not made adequately at present [3].

References: