Using paired tissue and serum samples to characterize human lung cancer metabolomics with ex vivo 1H HRMAS MRS.

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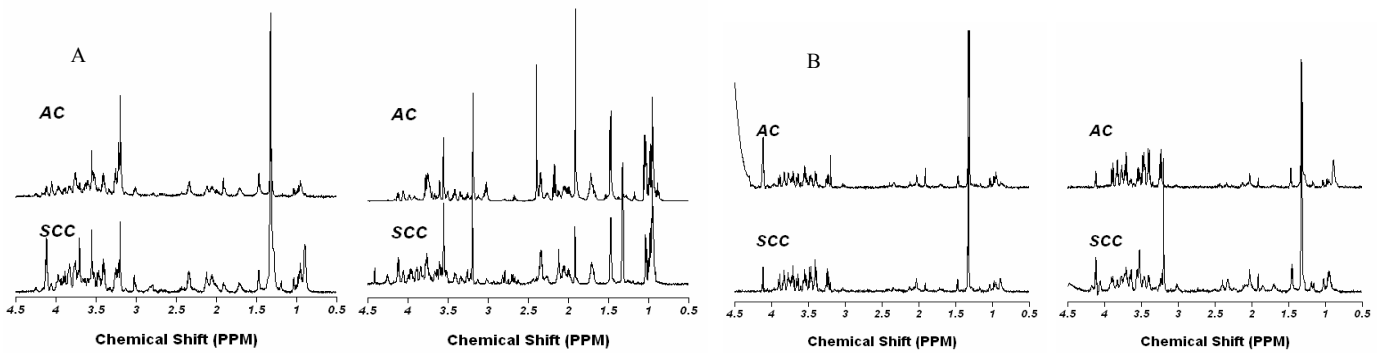
Introduction: Lung cancer is the primary cause of cancer related death in both men and women in the United States, with a 5-year survival rate of less than 16 percent. Although tests such as CT and PET can identify cancerous legions, their radiation hazards and high costs exclude them from feasible screening tools for the general population. In this study we are using High Resolution Magic Angle Spinning (HRMAS) proton MRS (1HMRS) to identify metabolomic profiles of lung cancer tissue and matched serum samples. By correlating this metabolomic data with traditional histopathology from the same tissue samples, we hope to identify serum lung cancer markers, as well as glean more information about aggressiveness of the lung cancer.

Methods: Paired tissue and serum samples from 80 patients of adenocarcinoma (AC) and squamous cell carcinoma (SCC) without identifiers were analyzed. *MR Spectroscopy*. MR experiments were carried out on a Bruker AVANCE spectrometer operating at 600 MHz (14.1T). A 4mm zirconia rotor was used with Kel-F inserts to create a 10μl sample space. For both tissue and sera, 1.0μl of D_2O was added for ^2H field locking. All measurements were carried out at 4°C for better tissue metabolite preservation. Rotor spinning rate was regulated by a MAS controller, and verified by measuring the inter-SSB distances from spectra with an accuracy of 1.0Hz. A repetition time of 5s and 128 transients were used to acquire each spectrum. Spectra were collected with a spinning rate of 3600Hz, with a rotor synchronized CPMG filter to reduce broad resonances; 360 CPMG cycles were applied with one π-pulse between two rotor cycles in each CPMG cycle to result in a filter time of 200ms. Spectroscopic data were processed with the MatLab based software developed in the Lab. *Histopathology*. After spectroscopy, tissue samples were fixed in formalin, embedded in paraffin, cut into sets of 5μm sections at 100μm intervals, and stained with hematoxylin and eosin. Volume percentages of histological features (cancer, stroma, necrosis, lymphatic structures, and cartilage) were analyzed and quantified by a pathologist.

Results: Spectroscopy and histology analyses of tissue specimens are designed to discover lung cancer metabolomic profiles that are closely associated with lung cancer pathologies. These discovered profiles will then be tested with serum spectroscopy measurements. While the data analyses procedures are currently underway, the spectral differences can be appreciated through visual evaluations of the spectra in panel A. The spectra on the left were obtained from tissue samples containing ~50%Vol cancer cells each, while the right spectra were from benign lung tissue samples of AC and SCC patients, respectively. Examining these spectra, it is clear that while cancer spectra for AC and SCC (left) are very different, particularly in the regions of 0.8-1.0 and 3.0-4.0 ppm, they are drastically different from benign tissues (right).



However, as expected the drastic changes in tissue spectral profiles were not prominently displayed in the paired corresponding serum spectra presented in panel B. Thus, the procedures of statistical analyses outlined in the proposal will be necessary to generate numerical profiles values that can predict patient status from serum analyses. These analyses are currently underway in our laboratory.

Conclusions: The advantages of using HRMAS technique to analyze intact tissue samples non-destructively have been demonstrated by numerous studies. When applying this method on analyses of serum samples, we can routinely obtain high resolution spectra with minimal amount of samples (10μl) as previously demonstrated and also shown here. With these measured high spectral resolution and their relationship with histology, the potential metabolomic profiles for tissue can be investigated and further the values of these tissue metabolomic profiles for establishing serum metabolomic markers for lung cancer can be evaluated.

Acknowledgements: Authors acknowledge partial support by NIH grants: CA095624, CA115746, CA115746S2, CA141139 and the A. A. Martinos Center for Biomedical Imaging.