Detection of Breast cancer aggressiveness with metabolomic profiles

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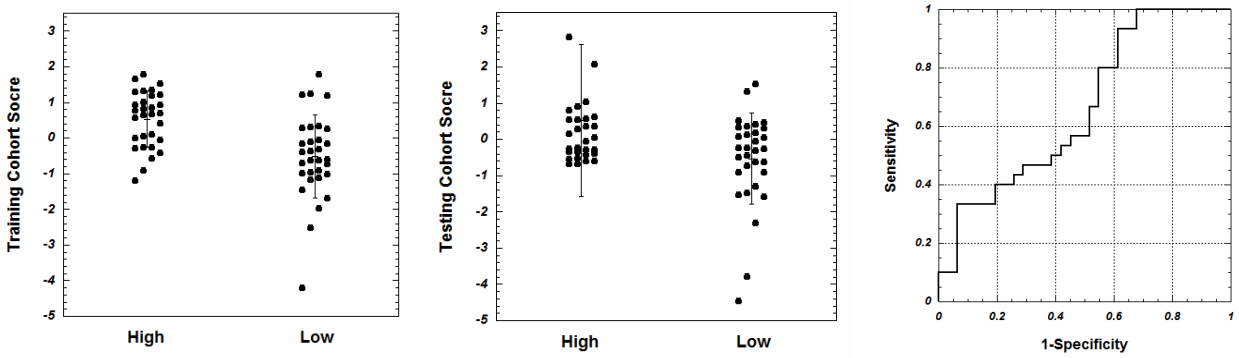
Introduction: At present, the diagnosis of breast cancer and prognostication of patient outcome rely exclusively upon pathological evaluation of morphological changes in biopsy and/or surgery specimens according to criteria established prior to the era of imaging-based cancer screening. With the implementation of annual screening, these diagnostic and prognostic paradigms are challenged by the new patient populations of early disease stages. The uncertainty of the current criteria of breast cancer pathology in assessing the characteristics of new patient populations of early diseases has become controversial since the current criteria established with symptomatic patient populations prior to the screening era may not accurately reflect the status of progression and the course of development of these early diseases. Applying the high-resolution magic angle spinning proton magnetic resonance spectroscopy (HRMAS 1HMRS) technique that we developed for intact tissue analysis on human breast cancer samples; we can quantify individual metabolites from native tissues and correlate those metabolites with quantitative pathology measured from the same samples, and with patient clinical statuses. In this project, we will seek to establish breast cancer metabolomic profiles that can improve the accuracy of breast cancer pathological evaluations and the assessment of patient outcome.

Methods: In this study, we analyzed 132 human breast cancer intact tissue samples with high resolution magic angle spinning (HRMAS) followed by pathology. *MR Spectroscopy*. MR experiments were carried out on a Bruker AVANCE spectrometer operating at 600 MHz (14.1 T) A 4mm zirconia rotor was used with Kel-F inserts to create a 10µl sample space; 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 3600 Hz, 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, ductal structures, and necrosis) were analyzed and quantified by a pathologist.

Results: Using statistical analyses we were able to differentiate samples into groups of high and low potential of unfavorable outcome. High potential of unfavorable outcome is defined as ER, PR and Her2 triple negative, or T≥3, or N>1, or with recurrence. The 132 cases were randomly split into two groups of 61 cases for the training cohort and testing cohort. A student's t test for both the training and testing cohorts were able to separate the group with high potential from the group with low potential with respective p values of p=0.0001 and p=0.0203, and with an overall accuracy of 66% for the testing cohort.



Conclusions: The advantages of using the HRMAS technique to analyze intact tissue samples non-destructively have been demonstrated by numerous studies. We have been able to obtain high resolution spectra with the tissue samples, while subsequently being able to perform the histopathology on the same piece of tissue. With these measured metabolic profiles and their relationship with histology and clinical outcome, there is potential for the tissue metabolic profiles to provide valuable biological information in the clinic at the early stage of treatment, such as at the time of biopsy, instead of measuring these parameters after surgery and through long term follow-up. This ultimately can contribute to more accurate diagnoses and the best prognoses while preserving the patient quality of life to the highest degree.

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