Metabolomic fields of human prostate cancer

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Target Audience: Prostate cancer (PCa) is the most frequently diagnosed malignancy in men worldwide, and the second leading cause of cancer death for men in the United States. If new PCa biomarkers and their imaging applications could predict tumor stage and location, and estimate malignant potentials, treatments and prognoses for patients would be drastically improved.

Purpose: Our study of human PCa metabolomics using intact tissue high resolution magic angle spinning (HRMAS) MRS showed the potential to improve sensitivity in PCa detection and characterization, including identifying cancer recurrence status. In these studies, we discovered that PCa metabolomic information could delocalize from cancer glands to surrounding histologically benign (Hb) structures to generate PCa metabolomic fields. The current study is designed to evaluate the ability of metabolomics to reveal PCa clinical and pathological status through the presence of PCa metabolomic fields.

Methods: A total of 386 samples from 173 PCa patients were divided into training and testing cohorts according to the chronological order in which they were analyzed. The first 199 samples from 82 patients were grouped to form the training cohort, while the remaining 187 samples from 91 patients formed the testing cohort. Following MRS, histology examinations of every tissue revealed that only 43 of the 386 samples studied contained PCa glands; the remaining 343 samples were benign tissues. *MR Spectroscopy.* A Bruker (Billerica, MA) AVANCE spectrometer operating at 600MHz (14.1T) was used for all MR experiments. Tissue samples were placed into a 4mm rotor with 10μl plastic inserts and 1.0μl D_2O was added for field locking. Spectra were recorded at 3ºC with the spectrometer frequency set on the water resonance, and rotor-synchronized experimental protocols with or without DANTE sequence with spinning at 600 and 700Hz (±1.0Hz). Thirty-two transients were averaged at a repetition time of 5s. Spectra were measured with a rotor-synchronized DANTE experimental protocol or Min(A,B) protocol and analyzed using AcornNMR-Nuts. The relative intensities of metabolic resonances were obtained, normalized by the total spectral intensity between 0.5 and 4.5 ppm. *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 were analyzed and quantified by a pathologist.

Results: Various spectral regions measured from Hb samples, and the metabolomic profiles calculated according to principal component analysis (PCA), can differentiate PCa clinical and pathological statuses with statistical significance. For instance, ANOVA analyses revealed increases in 3.60ppm spectral intensities can differentiate the Gleason score (GS)7 group from GS6 and GS5. Spectral region 0.93-0.96ppm, measured from Hb samples, was able to differentiate pathological stage (pT) (T2ab, T2c, and T3). To assess PCa aggressive potential, patient population was divided into two groups of "Low", GS≤6 and T2; and "High", GS>6 or T3, aggressive potential. Summed spectral intensities between 3.63 and 3.60ppm measured from Hb samples can identify cases of low aggressive potential using a threshold of one standard deviation below the mean (M-SD), as calculated from the entire cohort. The metabolomic profile represented by PC4 from Hb samples can also identify less aggressive PCa cases with pre-operative serum PSA density (DPSA)<0.3 ng/ml/g and GS<8. Using a threshold M-SD, the 3.63-3.60ppm region can identify 10 out of 68 (15%) "Low" cases individually for each cohort, and with PC4, 6/68 (9%) and 8/68 (12%) for the training and testing cohorts, respectively.

Figure 1. Means and standard errors for "High" and "Low" populations are presented in solid and open squares, respectively. Means and standard deviations for the entire cohort are presented as solid diamonds. Individual cases with values below the M-SD for "High" and "Low" populations are presented in solid and open circles, respectively.

Discussion: The results of this study demonstrate that PCa metabolomics can represent PCa status delocalized beyond PCa glands. This suggests the existence of metabolomic field effects, or metabolomic fields, in which metabolomic profiles acquired from Hb tissues of PCa patients, can indicate disease condition and status. While the measured parameters present significant overlaps among the measured groups, PCa metabolomic data are still able to isolate a sub-group of patients, not currently achievable in the PCa clinic.

Conclusions: The observed phenomenon of cancer metabolite delocalization beyond the histological lesion may provide the means for rectifying the acknowledged limits imposed by histological sampling error. Through applications in metabolomic imaging, cancer metabolite delocalization may be particularly valuable for the detection and diagnosis of early stage PCa, characterized by small and heterogeneously distributed lesions. Thus, the existence of MRS-detectable cancer metabolomic fields may impact diagnostic imaging beyond the PCa clinic. In particular, our demonstration of the ability of PCa metabolomics measured from Hb tissues of cancerous prostates to identify 18~19% of less aggressive PCa from the majority (>70%) of PSA screening detected new PCa cases, may provide biochemical assurance for the reduction of overtreatments for more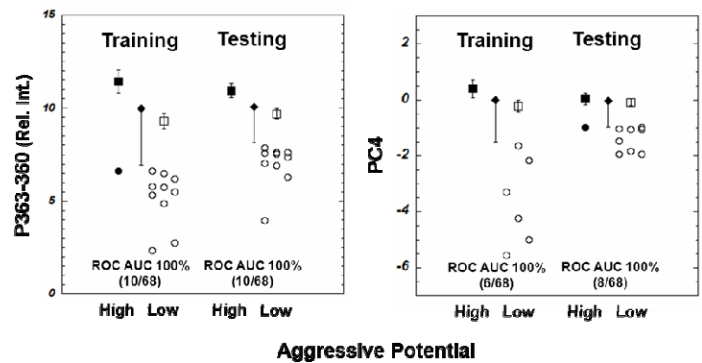 than 33,000 PCa patients annually in the USA alone.