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Text Transcript - Chemometric Analysis of Urinary Volatile Organic Compounds Discriminates Murine Mammary Tumor Presence and Progression Over Time

Hi, I'm Paul Grocki and today I am going to be discussing chemometric analysis of urinary volatile organic compounds discriminates murine mammary tumor presence and progression overtime. This problem was explored as current screening methods for breast cancer often produce false positive results and are invasive. Previous literature published by the authors indicates volatile organic compounds as potential biomarkers for breast cancer in mice as they are by or end products of metabolic pathways dysregulated by cancer. Additionally, our studies in mouse models show VOCs can determine treatment efficacy noninvasively. Therefore, if VOCs can distinguish cancer and determine treatment efficacy, we posed the question, can they also be used to distinguish cancer presence and monitor cancer progression. To explore this question, tumor cells were injected in the iliac artery of a subset of female mice and their urine was collected over the course of three weeks with urine samples being collected prior to injection to serve as a healthy control class. The urine was collected over dry ice in 10 mL headspace vials. Guanidine hydrochloride was added to denature major urinary proteins that bind to nonpolar analytes in hydrophobic pockets. Upon denaturation, solid phase micro extraction was employed to concentrate and extract volatiles which were then insert into a GCMS QTOF to separate an identify these volatiles. The data matrix generated through GC-MS analysis is subject to data treatment and screening. All matrix peaks were spectrally aligned. Features appearing as silanes or siloxanes, which are degradation products of the GCMS, along with features not present in at least half of either Control or Cancer samples classes were removed. The data was then normalized through MSTUS and auto scaling to remove non biological intraclass variation and obtain a matrix of volatiles with a similar signal range. This normalized data set was then subject to univariate and multivariate statistical analysis. Prior to tumor injection 20 urine samples were collected from healthy mice. Samples were then collected over the course of three weeks with 12 samples being collected in week 1, 15 in Week 2 and 18 in week three. Data screening left a matrix of 250 qualifying VOCs. Students T test between control and all cancer (Cancer weeks 1,2, and 3) samples identified there were 44 VOCs deemed statistically significantly differentially excreted between the two classes by a P-value of less than 0.05 and 37 VOCs when run between Cancer Weeks one and 3. Heat maps generated using VOCs identified with P-value less than 0.05 are seen here in Figure 3 and demonstrate that most VOCs are down regulated by cancer and then as cancer progresses from Week one to Week three. Principal component analysis or PCA was used to visualize global data patterns. PCA on 10 VOCs with P value less than 0.05 between all Cancer versus Control and the Week one versus Week three comparisons separated Cancer from Control with 97% sensitivity and 90% specificity and additionally distinguished Cancer week one from Week three with 93% classification accuracy. To find a single, smaller panel of VOCs with the ability to distinguish Control from Cancer Week one from Cancer Week three we employed supervised statistics through linear discriminant analysis (LDA). A small panel of five VOCs had the ability to distinguish Cancer

Weeks one and three from Control with 100% sensitivity at 95% specificity and that same panel can distinguish Cancer Week one from Week three with 100% sensitivity and 92% specificity. This study has further confirmed the identity and concentration of VOCs changes upon cancer injection and its progression. Supervised statistical analysis using a single panel of five VOCs can distinguish the healthy, Control class from Cancer while simultaneously having the ability to classify Week one from Week three. Hence, this panel of the VOC's can detect cancer and accurately distinguish early cancer from late cancer. To further this study, and determine the human translatability of this model, urine samples will be collected from humans with different grades of breast cancer. I would like to thank the National Science foundation for their financial contribution and my PI, Dr. Mangilal Agarwal for the opportunity to do and present undergraduate research. Thank you very much for listening, I will be monitoring the comment section for questions.