Identifying Novel Biomarkers in Urine from Canine Transitional Cell Carcinoma Patients

1George Pope, 2Liping Yang, 3Cheri Goodall, 4Andrew Schlueter, 5Shay Bracha
1College of Agricultural Sciences, Department of BioResource Research, 2Department of Chemistry, 3College of Veterinary Medicine, Department of Clinical Sciences Oregon State University, Corvallis, OR

Introduction

• Bladder Cancer in the United States has progressively been one of the more common cancers and is estimated to have 74,000 new cases as well as 16,000 deaths in 2015.1

• This type of cancer also has one of the highest rates of recurrence with an average of 60% within 5 years and 90% after 15 years.2

• Canine TCC was found to resemble the same malignancy in humans when comparing histopathological characteristics, molecular features, and biological behavior.3

• Previous research has shown that the lipid profiles between canine TCC bladder tissues and normal bladder tissues differ. This discovery was then applied to human bladder cancer tissues and similarities of the lipid profiles between human bladder cancer and canine TCC were identified.4

• We hypothesized that the lipidomic profiles of the urine extracted from canines with TCC, UTI, and healthy bladders will exhibit different signatures.

• The following study had two objectives. The first, to identify an ideal method for lipid extraction from urine samples. The second, to determine the differences in the lipid profiles extracted from the urine of the three cohorts.

Methods

Animals. The study included 15 dogs assigned to three cohorts: healthy (4), UTI (2), and TCC (9) based on their diagnosis. Recruitment was done with written consent from the dogs’ owners and in accordance with IACUC guidelines of Oregon State University (OSU).

Urine Collection. Urinary tracts of UTI and TCC dogs were prescreened with ultrasound imaging machine. Urine was collected in an aseptic manner (trans-abdominal cystocentesis for UTI and healthy dogs, urinary catheter for TCC dogs) and evaluated by urinary analysis and bacterial culture and sensitivity test. Diagnosis of TCC was confirmed via cytology or histology.

Extraction of Lipids.

A) SpeedVac Extraction: 1 mL of sample was dried through the use of a speedvac (Image D). This powder was then re-suspended in a buffer solution of dichloromethane / isopropanol : methanol and was further subjected to mass spectrometry analysis.

B) HP20 Extraction: Lipids were extracted by filtration through a resin, known as HP20 (Image A). Five resin beads were submerged into 50uL of urine (Image B). The beads were then washed three times with 20 μL of H2O. Following the washing step, the beads were submerged in 100 μL of methanol for 10 minutes. The liquid was then transferred to a new container (Image C) and stored at -80°C until analysis by mass spectrometry (LC-MS/MS).

C) Lyophilization Extraction: 1 mL of each sample (Image D) were removed for lyophilization. Each cohort was lyophilized for a minimum of twelve hours in separate containers (Image E). 600 uL of chloroform and 300 μL of methanol was then added and then vortexed. The solution was incubated for 1 hour at room temperature. 180 μL of double-distilled H2O was then added followed by centrifugation at 15,700 RCF for 5 minutes at room temperature (Image F). The lower phase was then dried through the use of a speedvac (Image G) while the top phase was recovered and stored at -20°C. The extracted lipid powder was then dissolved in 250 μL of methanol and 250 μL of acetone/IR. This sample was then stored at -20°C. When ready, the sample was analyzed for lipids by mass spectrometry (LC—MS/MS).5

Lipid Extraction Results

• The SpeedVac extraction, the HP20 extraction, and the Lyophilization Extraction methods were all successful at extracting lipids from the urine. The SpeedVac extraction method and the HP20 extraction method, both, showed similar quantities of lipid yield.

• The Lyophilization extraction method was the most efficient and resulted over 116 readable peaks on the Mass Spectrometry analysis. These peaks also had the highest intensities.

Urine Lipidomic Profiling

The Principle Component Analysis showed the clustering of these cohorts to have clear separation based on lipidomic profiles. These results produced a robust model to classify the lipidomic signature into TCC, UTI, and Healthy Bladder cases. This body of results was statistically significant and confirmed the hypotheses of this research project.

Conclusions

• Lyophilization Extraction was most effective method for the extraction of lipids from urine samples.

• Based on the differences in categories between the cohorts of TCC, UTI, and normal urine, it can be proposed that each cohort can be segregated from one another based on its lipidomic profile.

Future Directions

• Following the annotation of the lipidomic profiles, a list of potential biomarkers for TCC will be validated on a larger cohorts of dogs. These lipids can serve as potential biomarkers for disease presence and progression.

• Comparative studies will be performed on human patients for the identification of similarities in disease etiology and identification of novel cross species biomarkers and therapeutic targets.

• A recent study from our lab has shown that proteomic analysis with similar cohorts could predict presence of TCC with 90% confidence through a multiplex protein analysis.6 Our future research plan will examine via multi variant analysis if subsidizing our proteomic data with the lipidomic analysis will increase sensitivity and specificity of a potential diagnostic test.

References