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Deconvolution of Human Urine across the Transcriptome and Metabolome

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Guests: Dr. Sevahn Vorperian received a PhD from Stanford University and is now a postdoctoral fellow at Genentech. Dr. Stephen Quake from Stanford University and the Chan Zuckerberg Initiative.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. The measurement of DNA fragments circulating in plasma after release from cells, known as cell-free DNA, has revolutionized many fields of medicine, from identification of fetal aneuploidies to the detection of certain tumors. Cell-free DNA testing has many advantages over alternative approaches, perhaps the most notable being the avoidance of an invasive tissue biopsy, which carries some risk of downstream adverse events. While cell-free DNA evaluates cells' static genetic code, recent efforts have turned to cell-free RNA as it provides more dynamic information, allowing researchers to identify the genes getting turned on and off as part of a cell's response to disease and other environmental changes.

Most studies evaluating cell-free RNA have been performed in plasma but this has limited utility for the evaluation of certain tissues that are underrepresented in this sample type. The genitourinary tract is a prime example. Genitourinary diseases are marked by distinct cellular changes, but these are difficult to detect in plasma as these tissues contribute little to the cell-free RNA population circulating in whole blood. A new research article appearing in the November 2024 issue of *Clinical Chemistry* proposes a novel solution to this problem by exploring cell-free RNA in urine. What information can be found in the urine transcriptome and how will this impact the diagnosis of genitourinary diseases?

In this podcast, we're joined by the article's lead and senior authors. Dr. Sevahn Vorperian received a PhD in Chemical Engineering from Stanford University and is now a Postdoctoral Fellow at Genentech. Dr. Stephen Quake is the Lee Otterson Professor of Bioengineering and Applied Physics at Stanford University and Head of Science at the Chan Zuckerberg Initiative.

Dr. Quake, let's start with the basics. What is a liquid biopsy and what are cell-free nucleic acids?

Stephen Quake: Yeah. Liquid biopsy is a way to measure what's going on inside your body without having to physically invade the body, by taking a bodily fluid that's easy to access like blood or urine. That's why they're called liquid biopsies, and obviously, urine is even easier than blood. Cell-free nucleic acids are the DNA and RNA from cells that are circulate in your body through your various bodily fluids and they've been very informative in helping us understand the physiology of what's going on in different tissues and cell types.

Bob Barrett: To date, cell-free nucleic acid liquid biopsies, especially those measuring cell-free RNA, largely focus on plasma or serum. Dr. Vorperian what inspired this study in urine?

Sevahn Vorperian: Yeah. So, the initial inspiration for the study actually came after drinking a glass of water while I was writing the manuscript from the first part of my doctoral work. Kind of as you just highlighted and what Steve was just describing, cell-free RNA and cell-free DNA liquid biopsies have largely considered blood, plasma, and serum to date, where there was a primary focus on identifying gene sets to discriminate between cases and controls to facilitate disease diagnosis. So, I spent the first part of my PhD working to resolve blood-based cell-free RNA liquid biopsies at cell type resolution using single-cell transcriptomic data.

The idea here is that single-cell transcriptomics offers a very powerful molecular reference of the messenger RNAs a given cell type in the body expresses to perform its specialized functions. So, prior to this work, the paradigm for liquid biopsy was largely tissue of origin, which can fall short of the cell type resolution that's typically afforded by tissue histology following an invasive needle biopsy. So, a primary finding from the first part of my PhD was that in the plasma cell-free RNA of healthy individuals, around 10% of the cell-free transcriptome originated from cell types in solid tissues and organs.

So, there was one day that I was very deep in thought about how I could start to see these small fractions of cell type specific RNA from kidney and cell types, and prostate cell types in men. In that moment that I had stood up to go to the bathroom after drinking that glass of water, I realized that if I could measure cell-free RNA in urine, I could perhaps see more of these cell types than in plasma cell-free RNA. So, I began looking at the literature and had seen that while some work had been done studying extracellular vesicles or small RNA, in comparison, very little work that had been done studying messenger RNA, which is super important from this perspective of cell type deconvolution, given the molecular reference afforded by single-cell transcriptomic data. So, it was pretty clear that there was this gap in the literature that needed to be filled, and at that point, Steve and I started

looking for samples to see if we can try out some of these ideas.

Bob Barrett: It seems like that was a well-timed glass of water.

Sevahn Vorperian: Yes.

Bob Barrett: So, tell us about the highlights when you measured the urine transcriptome.

Sevahn Vorperian: First and foremost, I think prior to describing our findings on the urine transcriptome, we want to emphasize that we observed that for a successful RNA isolation, collecting a clean urine sample is very important. So, patients collecting urine specimens with a clean touch procedure, and subsequent refrigeration until processing, rather than just letting a collect a sample sit out on the bench was very important. And also, moreover, that urine can vary in solute concentration. So, we had addressed this, both by measuring urine spot creatinine as well as in how we analyzed our data.

To discuss our findings measuring the urine transcriptome, urine can generally be regarded as having two separate fractions upon centrifugation. You have the sediment, which can include cells or cast and other debris if a patient is sick, and supernatant, which is where the cell-free RNA is. In our study, we sequenced RNA isolated from both the sediment as well as the cell-free RNA in the supernatant from a cohort of healthy controls and patients with kidney stones. Upon performing cell type deconvolution on the sequenced RNA that we isolated, we observed predominant relative fractional contributions from genitourinary cell types, including the kidney and the prostate cell type specific RNA, as well as bladder and secretory cells types.

We further observed some small fractional contributions of cell types from high turnover tissues such as the intestine, which were reflected with known marker genes. So, we observed an increase in prostate cell type specific RNA in the urine cell-free RNA relative to urine sediment RNA, and moreover that for patients with inflammation as measured by urine dipstick, that urine cell-free RNA reflected more cell type specific signal than from non-immune cell types, where immune cell type specific RNA dominated the urine sediment of those patients. So, collectively, depending on what cell types you are interested in profiling, urine cell-free RNA may be more desirable to measure, and no matter what, you want to make sure that you're working with as clean of a urine sample as possible.

Bob Barrett: While in the study, you compared the urine transcriptomes to plasma cell-free RNA. What did you find there?

Sevahn Vorperian: We found that urine exhibits a very distinct transcriptional landscape relative to blood. This part of our study was motivated by the fact that urine and blood interface with distinct sets of tissues, and we wanted to see if this was reflected at the transcriptional level. So, in healthy individuals in either transcriptome, we had observed enrichment for genitourinary cell type specific RNA, including prostate epithelial cell types, and prostate epithelial cell types and kidney epithelia as well as secretory cell types in ciliated cells and urine cell-free RNA. In plasma cell-free RNA, we observed an increase in cell type specific RNA originating from more so hematopoietic cell types such as erythrocytes and platelets.

We observed non-genitourinary contributions of cell-type specific RNA, including intestinal enterocytes enriched in both urine fractions, and endothelial cells enriched in plasma cell-free RNA, and that at a bulk level, both urine transcriptomes were enriched in metabolic signal relative to plasma cell-free RNA.

Bob Barrett: You also measure the urine metabolome, where your study is among the first to measure cell-free RNA alongside another "ome." What did you observe there?

Sevahn Vorperian: This aspect of our study was really motivated by the fact that urine reflects metabolic waste and if we could start to take or couple gene expression measurement with a more functional readout such as the metabolome. Using untargeted metabolomics, we identified a broad chemical spectrum from several distinct molecule classes, including amino acids and derivatives, carbohydrates, fatty acids and conjugates, nucleic acids and exogenous compounds, and enrichment across urine transcriptome and metabolome had identified a joint enrichment for various metabolic pathways, especially those involved in amino acid metabolism from these healthy individuals and patients with kidney stones.

Genes and metabolites, a subset of these, further corresponded to metabolic substances associated with the renal proximal tubule, which is a highly metabolic cell type. So, the primary takeaway here is that in the future a possible integration across urine cell-free RNA and metabolomics could potentially offer more so functional insights on the gene expression measurements that we're getting from nucleic acid liquid biopsy.

Bob Barrett: Well, finally, Professor Quake, two long independent lines of your academic research program over the past decade have been liquid biopsy and single-cell genomics. Whereas Dr. Vorperian, you developed ways to bring these fields together during your PhD in the Quake Lab. Looking forward, what are each of you most excited by?

Sevahn Vorperian: I think, first off, I am really excited about the translational implications from the ability to begin to resolve a liquid biopsy at cell type resolutions, especially where invasive biopsies are hard to perform or infeasible. Already, we've begun to see a broad range of diverse contexts from pregnancy complications or multi-system inflammatory syndrome in children to astronauts in space flight, or even studying additional new biofluids, such as our study here or another one looking at cerebrospinal fluid. So, I'm also very excited by the opportunity afforded by integrating multiple measurement modalities such as proteomics and metabolomics to begin deriving more specific biomarker panels for disease diagnosis and subtyping.

Stephen Quake: So, for my part, I'm just thrilled to see that work we began purely out of basic science curiosity, which is to say, using single-cell transcriptomics to make cell atlases of the human body, is now finding applications that will hopefully, ultimately, affect human health. It's just a lovely thing to see that interplay between translational research and basic science discovery, and Sevahn's work had that elements in it and brought these two threads together in a way that I'm just so pleased and proud of.

Bob Barrett: That was Dr. Stephen Quake from Stanford University and Dr. Sevahn Vorperian from Genentech. They wrote a research article exploring the transcriptome and metabolome in human urine in the November 2024 issue of *Clinical Chemistry*, and they've been our guests in this podcast on that topic. I'm Bob Barrett. Thanks for listening.