Title: Development of next generation extracellular vesicle based urine tests for prostate cancer

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Abstract:

Introduction and Objective: Non-invasive urine and blood biomarkers are commercially available to assess the risk of clinically significant prostate cancer (csPCa) and the need for a biopsy. However, these tests are limited in their specificity resulting in a significant number of men still undergoing biopsy to avoid missing csPCa. Exosomes and other extracellular vesicles (EVs) provide a platform for blood and urine biomarkers since they are released by all types of cells and preserve molecular constituents from their cells of origin within lipid membranes. The EPI urine test from Exosome Diagnostics (ExoDx) is currently the only commercially available EV based cancer diagnostic test; it uses three well established EV RNA markers to estimate the risk of csPCa. Studies by us and others have shown that an expanded urine EV PCa marker panel would improve disease stratification if these markers could be incorporated into a clinical grade assay. Our objective in this current study is to identify and characterize the EV RNA transcripts that are enriched by using antibodies for prostate specific membrane antigen (PSMA) to capture urinary EVs that originated from PCa cells. This is the first step in the development of a next generation urinary EV test with increased specificity for csPCa within a rigorous and reproducible assay format.

Methods: Urine EVs from patients with csPCa and from healthy controls were prepared using the ExoDx clinical platform. These total EV samples were then compared with EVs enriched by immunocapture either with a PSMA specific antibody or with an isotype IgG control reagent. RNA was prepared from each of these three types of urinary EV samples and analyzed using RNAseq.

Results: RNAseq analysis of urinary total EVs, PSMA-antibody captured EVs and isotype IgG control captured EVs showed excellent mapping to transcriptome regions. We detected over 600 genes differentially enriched by PSMA antibody vs. isotype IgG EV capture and identified 18 genes from a candidate list of prostate biomarkers that have been assembled based on previous studies. A principal component analysis of 196 differentially expressed transcripts obtained from EVs enriched by PSMA immunocapture vs IgG controls showed excellent discrimination between prostate cancer patients and healthy controls. By using a feature selection approach to optimize for discrimination between prostate cancer patients and healthy controls we identified a novel 6-gene signature that, when derived from a PSMA capture EV dataset, shows a superior AUC for the accuracy of cancer detection (greater than 0.95) than that obtained from applying the same 6-gene signature on total EVs or on isotype IgG controls.

Conclusions: EV PSMA immuno-capture provides a robust approach to expanding the panel of PCa biomarkers that can be incorporated into clinical grade PCa urine EV assays, supporting the translation of pre-clinical discovery into clinical practice.