Integrated Liquid Biopsy for Prostate Cancer: Capturing Circulating Tumor Cells and Urinary Cell-Free Total Nucleic Acid

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INTRODUCTION

Prostate cancer remains a leading cause of cancer-related mortality worldwide. Early detection and precise disease monitoring are vital for better patient outcomes. Liquid biopsy, a minimally invasive approach for analyzing tumor-derived biomarkers in bodily fluids, holds promise for Prostate cancer detection and monitoring. We assessed the effectiveness of an integrated liquid biopsy system for capturing circulating tumor cells (CTCs) from blood and cell-free Total Nucleic Acids (cfTNA) from urine.

MATERIALS & METHODS

Whole Blood and urine was collected from healthy donors. Four 10 mL whole blood samples were spiked with 50 LNCaP cells each and the CTCs were isolated using the Revolution CTC Enrichment Kit (Epithelial Origin). Molecular profiling of CTCs was carried out using immunocytochemistry (ICC) using the primary antibody EpCAM. High-resolution images of the stained slides were obtained using an LSM900 microscope.

Ten 20 mL urine samples were spiked with an RNA fusion reference standard containing the known prostate cancer mutation TMPRSS2-ERG to a concentration of 10 ng/mL. Isolation of the RNA fusion reference standard was performed using the Revolution cfTNA Max20 Kit. All samples were eluted in 50 uL. RT-qPCR was performed on the eluants for Prostate cancer-associated genomic alterations. The quality of extracted RNA was determined using the Agilent High Sensitivity RNA ScreenTape®.

RESULTS

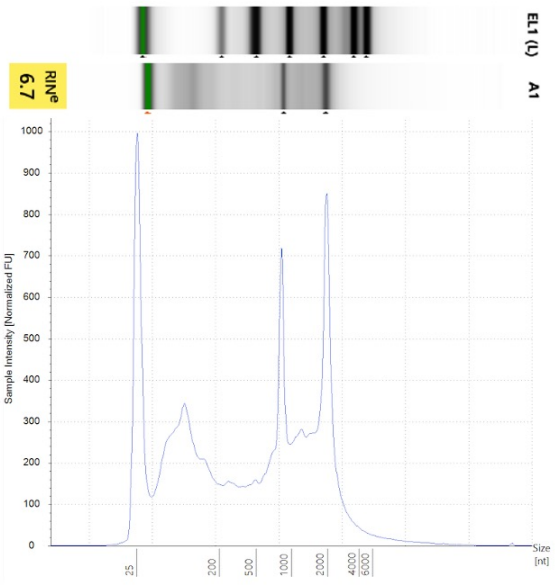

Figure 1. Electropherogram tracing of total nucleic acid on Agilent TapeStation. The prominence of the 18S and 28S peaks indicates the successful isolation of high-quality RNA for the samples spiked with TMPRSS2-ERG.

	Mean Copy Number	Mean Ct Value
TMPRSS2-ERG (10ng/mL) n=10	1,765 ± 450 copies/µL	26.3 ± 0.6

Table 1. Reverse Transcriptase PCR demonstrates that TMPRSS2-ERG fusion RNA was detected in eluates after extraction with the Revolution cfTNA Max20 Kit.

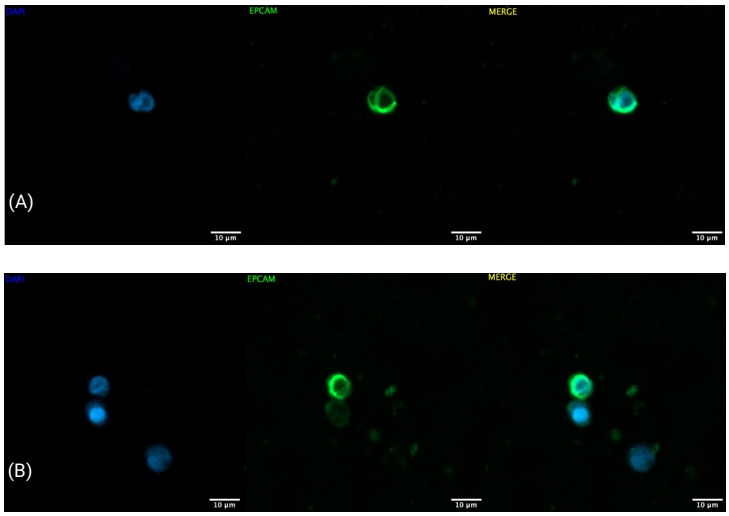

Figure 2. Immuno-stained images of captured LNCaP cells in whole blood. The cells were isolated using the Revolution CTC Enrichment Kit and stained for EpCAM (Green) to confirm epithelial cell origin and DAPI (blue) to highlight cell nuclei. The overlay of EpCAM and DAPI staining confirms the presence of LNCaP cells in whole blood.

CONCLUSION

LNCaP cells and the TMPRSS2-ERG RNA fusion were efficiently extracted using the Revolution cfTNA Max20 Kit and the Revolution CTC Enrichment Kit. The TapeStation electropherogram revealed distinct 18S and 28S peaks, with a RIN score 6.7. RT-qPCR analysis yielded a mean Ct value of 26.3±0.6 from the extracted samples indicating the successful isolation of the TMPRSS2-ERG prostate mutation. Immunocytochemistry confirmed the successful isolation of prostate cancer cells, which was evident through positive EpCAM and DAPI fluorescence.

Our findings highlight the efficacy of an integrated liquid biopsy for capturing CTCs from whole blood and cfTNA from urine to detect Prostate cancer. This non-invasive approach holds promise for improving early detection, prognostication, and personalized management of Prostate cancer patients. Further validation in larger cohorts and longitudinal studies is warranted to establish the clinical utility of this integrated liquid biopsy strategy in Prostate cancer diagnosis and monitoring.