Exploring Liquid Biopsy for the Detection of Bladder Cancer: Unraveling RNA Fusions

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INTRODUCTION

This study is in the emerging area of using liquid biopsy for the non-invasive detection of bladder cancer. Our investigation interrogates urine samples for specific RNA fusions associated with bladder cancer, specifically ETV6-NTRK3 and PRCC-TFE3. By employing liquid biopsy methods, we aim to offer a less intrusive and more accessible means of diagnosing bladder cancer, potentially transforming cancer diagnostics. Specific RNA fusions detected in readily available patient urine samples hold promise as dependable biomarkers for early detection and continuous monitoring of bladder cancer and contribute to efforts to refine better diagnostic methodologies. Ultimately, the aim is to enhance patient outcomes by streamlining cancer management through more effective and patient-friendly approaches to combating bladder cancer.

MATERIALS & METHODS

Urine was collected from healthy male donors and preserved with nRichDX urine preservative. The urine samples were centrifuged at 16,000 x g for 10 min to obtain cell-free urine. Four 20 mL aliquots of the precleared supernatant urine samples were spiked with an RNA fusion reference standard from Anchor Molecular containing ETV6-NTRK3 at a concentration of 1 ng/mL and PRCC-TFE3RNA at a concentration of 10 ng/mL. All samples were extracted using the nRichDX Revolution Max20 cfTNA Isolation Kit. The eluants from the extracted samples were treated with DNase. The cfTNA profile was assessed on an Agilent 4150 TapeStation System using a high-sensitivity RNA ScreenTape. Extracted cfTNA from urine were evaluated using an RT-qPCR assay to quantify the number of copies of the fusion RNA.

RESULTS

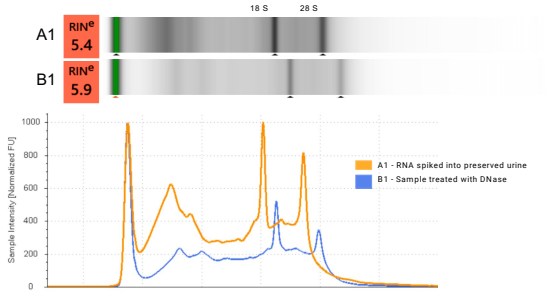

Figure 1. Electropherogram of total RNA on an Agilent TapeStation. The prominence of the 18S and 28S peaks indicates the isolation of high-quality RNA for the samples spiked with ETV6-NTRK3.

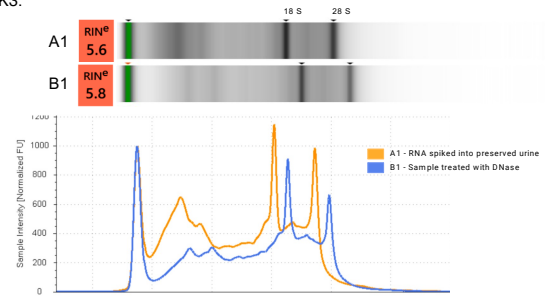

Figure 2. Electropherogram of total RNA on an Agilent TapeStation. The prominence of the 18S and 28S peaks indicates the isolation of high-quality RNA for the samples spiked with PRCC-TFE3.

The renal RNA fusions ETV6-NTRK3 and PRCC-TFE3 RNA were successfully extracted using the nRichDX Revolution cfTNA Isolation Kit. TapeStation analysis demonstrated prominent 18S and 28S peaks, confirming the successful extraction of RNA from 20 mL urine samples.

	Mean Copy Number	Mean recovery
ETV6-NTRK3 (1 ng/ml)	120.6 ± 22.7 copies/uL	51.69% ±9.74%
PRCC-TFE3 (10 ng/ml)	12001 ± 2424.9 copies/uL	52.15% ±10.54%

Figure 3. Reverse Transcriptase PCR demonstrates that both ETV6-NTRK3 and PRCC-TFE3 fusion RNA was detected in eluates after extraction with the Revolution cfTNA Max 20 Kit.

CONCLUSION

Our recent findings represent a significant advancement in bladder cancer diagnostics. We've uncovered the consistent recovery of renal RNA fusion ETV6-NTRK3 and PRCC-TFE3RNA across all samples. This discovery not only sheds light on specific RNA fusions as potential biomarkers but also underscores the profound impact of our methodology. We've achieved unprecedented extraction efficiency by leveraging our ability to extract RNA from up to 20 mL of whole urine in a single extraction, coupled with an RNA-focused preservative solution. This heightened sensitivity in fusion RNA detection holds promise for enhancing diagnostic accuracy and early detection rates in bladder cancer. Our research stands poised to revolutionize the landscape of bladder cancer diagnostics by offering a dual advantage of reliability and convenience. With our method, clinicians gain a powerful tool for early diagnosis and monitoring, empowering them to intervene proactively and improve patient outcomes. As we continue to pioneer innovative diagnostic approaches, our overarching goal remains steadfast: to transform bladder cancer management and improve the lives of those affected by the disease.