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Urine-based Molecular Profiling of Bladder Cancer

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Urine

INTRODUCTION

Current diagnosis and monitoring methods of bladder cancer patients are often invasive and/or lack sensitivity and specificity. The tremendous success in cell-free DNA (cfDNA) blood tests provides a strong rationale of using body fluids such as blood and urine as minimally invasive “liquid biopsies” to identify DNA-based molecular biomarkers in bladder cancer. Urinary cfDNA as a liquid biopsy holds great potential for a more sensitive alternative to blood-based liquid biopsy testing for clinical use in bladder cancer due to its noninvasiveness and convenient home access.

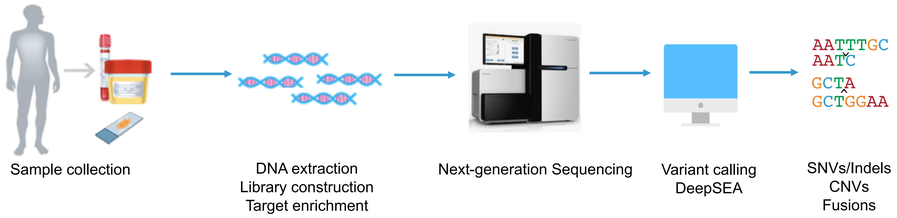
Here, we report the development of urinary cfDNA (ucfDNA)-based targeted next generation sequencing (NGS) assay for integrated molecular profiling, including single nucleotide variations (SNVs), insertions or deletions (Indels), gene fusions, and copy number variations (CNV), as well as its clinical application in molecular profiling in bladder cancer.

MATERIAL AND METHODS

Urine cfDNA-based targeted NGS assay

Urine samples were collected from patients using Predicine in-house developed Urine Collection Kit which contains urine preservation buffer to maintain the integrity of cfDNA in urine. The sequencing library was constructed with the urine cfDNA extracted from urine samples. Then, the library is hybridized with the PredicineCARE™ panel and sequenced to a median depth > 20000x using Illumina platform. NGS data is analyzed using in-house NGS analysis pipeline, which starts from the raw sequencing data (BCL files) and outputs the final variants.

Figure 1. PredicineCARE™ assay, a targeted NGS assay for tissue, urine, and blood.



Assay validation

To assess the sensitivity of SNVs detection in the urine specimen, spiked urine cfDNA were used to generate a series of testing materials with variable mutation allele frequencies (MAF) from 0.125%-1%. To evaluate the sensitivity of Indel, CNV, and fusion detection in urine cfDNA, we fragmented reference gDNA (HD753) to urine cfDNA size and spiked it into urine cfDNA from healthy donors, which generated samples with pre-defined AFs.

Molecular profiling of bladder cancer

Urine, tumor tissue, and blood samples were collected from bladder cancer patients and processed with the PredicineCARE™ assay (Table 1). SNVs, Indels, fusions, and CNVs were called using proprietary DeepSEA NGS analysis pipeline.

RESULTS

Validation of urine cfDNA-based NGS assay

Table 1. Specification of PredicineCARE™ assay

Parameter	PredicineCARE™ ctDNA Panel
Regions Analyzed	152 genes
Panel Size	582 kb
Assay Sensitivity	Urine - 0.5%, report down to 0.25% Plasma - 0.25%, report down to 0.1%
Sequencing Depth	>20,000X
Turnaround Time	10 days
Input Sample Type	Plasma 2-5ml Whole blood (5-10ml) Urine - 40ml (minimum 10ml)

Table 2. Analytical sensitivity for SNV detection

Expected MAF (%)	PPA (%)	PPA 95% CI (%)	PPV (%)	PPV 95% CI (%)
0.125	66.7	51-80	96.77	83.3-99.9
0.25	92.2	84.6-96.8	94.32	87.2-98.1
0.5	98.9	94-100	100	95.9-100
0.75	100	96-100	100	96-100
1	100	92.1-100	100	92.1-100

Table 3. Analytical sensitivity for Indel detection

Expected MAF (%)	PPA (%)	PPA 95% CI (%)	PPV (%)	PPV 95% CI (%)
0-0.5	79.4	62.1-91.3	77.14	59.9-89.6
0.5-1	97.2	85.5-99.9	100	90-100
1-100	100	47.8-100	100	47.8-100

Table 4. Analytical sensitivity for fusion detection

Expected CN	PPA (%)	PPA 95% CI (%)	PPV (%)	PPV 95% CI (%)
0-0.25	60	14.7-94.7	100	29.2-100
0.25-0.55	100	78.2-100	100	78.2-100
0.55-100	100	69.2-100	100	69.2-100

Table 5. Analytical sensitivity for CNV detection

Expected MAF (%)	PPA (%)	PPA 95% CI (%)	PPV (%)	PPV 95% CI (%)
2.375	100	69.2-100	100	69.2-100
2.75	100	69.2-100	100	69.2-100
3.125	100	63.1-100	100	63.1-100

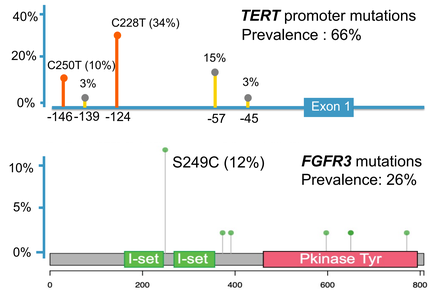
MAF: mutation allele frequency, PPA: positive percent agreement
PPV: positive predict value, CI: confidence interval

The assay reached 98.9% sensitivity at 0.5% MAF for SNV detection, 97.2% sensitivity in 0.5-1% MAF for indel detection, 100% sensitivity in 0.25-0.55% MAF for fusion detection, and 100% sensitivity at 2.375 CN for CNV detection (Table 2-5).

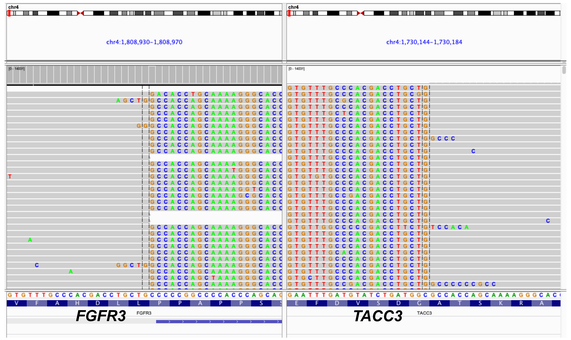
Molecular profiling of bladder cancer patients

Figure 2. Showcase of SNV/Indel (A), Fusion (B), and CNV (C) detected in the urine sample of bladder cancer patients. Segments in red and blue color in C indicate copy number gain and loss, respectively. *PIK3CA* and *PIK3CB* copy number gain, and chromosome 9 and 11 loss were detected in this urine sample.

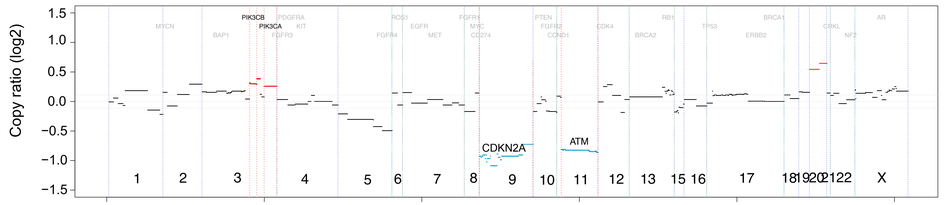
A. *TERT* and *FGFR3* mutations detected in urine



B. *FGFR3-TACC3* fusions detected in urine



C. CNVs detected in urine, including *PIK3CA* and *PIK3CB* copy number gain, and CDKN2A and ATM copy number loss



CONCLUSIONS

- Predicine has developed a proprietary urine cfDNA-based targeted NGS assay to detect SNVs, Indels, CNVs, and fusions in urine samples.
- Preliminary data suggest that urine-based cfDNA testing provides a completely noninvasive approach for detection of genomic biomarkers in bladder cancer, providing the foundations for early cancer detection, therapeutic monitoring, and minimal residual disease assessment.