Detection of early-stage urothelial cancers using methylation patterns in urine cell-free DNA

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INTRODUCTION

- Plasma cell-free DNA (cfDNA) has emerged as a promising non-invasive method for multi-cancer early detection (MCED). However, the detection of early-stage urological cancers remains challenging due to their low tumor fraction in plasma, which complicates accurate identification.[1,2]
- Urine cfDNA (ucfDNA) offers a promising method for enhancing the detection and monitoring of early-stage urothelial cancers due to its close proximity to the affected organs and the simplicity of the urine collection process.[3]
- In prior research, we established the effectiveness of ucfDNA methylation patterns in detecting non-muscle invasive bladder cancer (NMIBC) in patients who presented with suspicious bladder lesions, as identified through cystoscopy or imaging.[4]
- To advance this research, we developed a urine classifier to evaluate the effectiveness of targeted methylation pattern analysis in detecting bladder cancer and upper tract urothelial carcinoma (UTUC) using ucfDNA.

OBJECTIVE

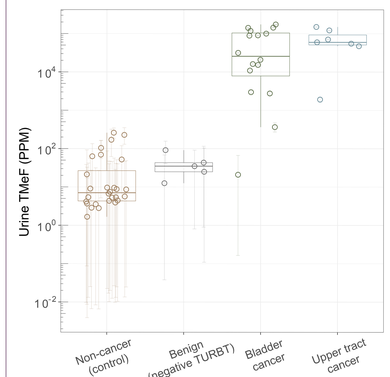
- Train a biopsy-free urine classifier for bladder cancer and UTUC detection, and evaluate the performance of the urine classifier in an independent test set comprised of patients with early-stage bladder or upper tract cancer.

KEY RESULT: METHYLATION PATTERNS IN URINE CELL-FREE DNA IDENTIFY EARLY-STAGE UROTHELIAL CANCERS

UCSD samples show elevated TMeF compared to non-cancer and benign samples.

- Patients with confirmed cancer had higher tumor methylated fraction (TMeF; a methylation-based quantification of tumor allele fraction) levels compared to non-cancer samples (Wilcoxon-test p-value=6.81e-13). Benign patients who were suspected of new localized bladder cancer but found to be benign by TURBT displayed TMeF levels similar to those of non-cancer samples (Wilcoxon-test p-value = 0.0657) (**Figure 2**).

Figure 2. Biopsy-free TMeF estimates for UCSD cohort



Each point represents a study sample. X-axis represents the UCSD cohort. Error bars represent the 95% credible interval of the posterior distribution.

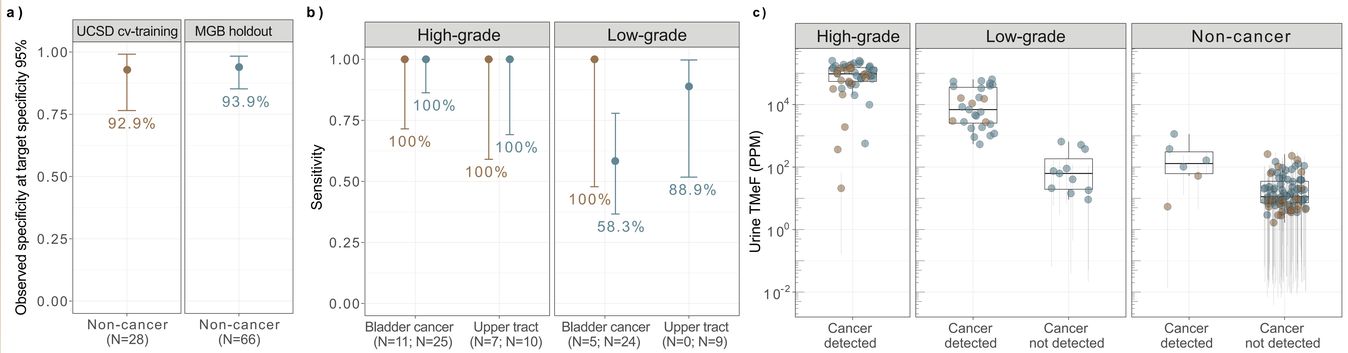
Urine classifier detects early-stage urothelial cancer with high sensitivity and specificity in training and holdout cohorts.

UCSD cv-training:
- Observed specificities were 92.9% (26/28; 95% CI: 76.5-99.1%) at a target specificity of 95% (**Figure 3a**), and 100% (28/28; 95% CI: 87.7-100.0%) at a target specificity of 98%.
- At a target specificity of 95%, 100% sensitivity was observed for low-grade NMIBC (5/5; 95% CI: 47.8-100.0%) and high-grade NMIBC (11/11; 95% CI: 71.5-100.0%) (**Figure 3b**).
- At 98% specificity, the locked classifier had 100.0% sensitivity for low-grade NMIBC (5/5; 95% CI: 47.8-100.0%), and 90.9% sensitivity (10/11; 95% CI: 58.7-99.8%) for high-grade NMIBC.
- For UTUC cases, both 95% and 98% locked classifiers had 100% sensitivity in detecting high-grade UTUC (7/7; 95% CI: 59.0-100.0%) (**Figure 3b**).

MGB holdout:
- Observed specificities were 93.9% (62/66; 95% CI: 85.2-98.3%) at a target specificity of 95% (**Figure 3a**), and 97.0% (64/66; 95% CI: 89.5-99.6%) at a target specificity of 98%.
- Both locked classifiers demonstrated 58.3% sensitivity for low-grade NMIBC (14/24; 95% CI: 36.6-77.9%) and 100% sensitivity for high-grade NMIBC (25/25; 95% CI: 86.3-100%) (**Figure 3b**).
- For UTUC cases, both locked classifiers had sensitivity of 88.9% for low-grade UTUC (8/9; 95% CI: 51.8-99.7%) and 100% for high-grade UTUC (10/10; 95% CI: 69.2-100%) (**Figure 3b**).

- Cancer samples detected by the urine classifier had higher TMeF compared to undetected cancer samples (**Figure 3c**, p-value = 2.075e-07).
- High-grade cancers detected by the classifier had significantly greater TMeF than low-grade cancers detected by the classifier (**Figure 3c**, p-value = 2.93e-08).

Figure 3. Classifier performance across high-grade and low-grade urothelial cancers



a) Dot plot depicts classifier's observed specificity in UCSD cv-training and MGB holdout cohort. b) Cancer detection sensitivity at target specificity 95% across low-grade and high-grade bladder cancer and upper tract cancer, with the color representing the cohort. c) TMeF level shown by grade with color representing the cohort.

CONCLUSIONS

- A biopsy-free urine classifier based on ucfDNA methylation patterns can detect NMIBC and UTUC with high sensitivity at high specificity in patients with high-grade disease.
- Urine classifier performance was validated in an independent test set and did not require matched blood or tissue.
- Analysis of urine TMeF, an estimate of tumor allele fraction, demonstrated that undetected cancer samples have lower TMeF compared to detected samples.
- Current efforts are focused on evaluating performance for NMIBC recurrence monitoring and minimal residual disease assessment before cystectomy.

References

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Disclosures

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METHODS

Patient selection

- UCSD patient selection was performed as described previously.[4] Here we report on a UCSD cohort of patients with no prior history of cancer and with a new NMIBC diagnosis (N=16, with 5 low-grade and 11 high-grade cases) and UTUC (N=7, all high-grade), as well as age and gender-matched non-cancer controls (N=28) (**Table 1**). UcfDNA methlyation data from non-cancer and confirmed cancer participants were used for training of the urine classifier and evaluated in a cross-validation framework.
- The McCain GU BioBank (MGB) cohort was comprised of patients with a new diagnosis of NMIBC (N=49, 24 low-grade, 25 high-grade) and UTUC (N=19, 9 low-grade, 10 high-grade), as well as age and gender-matched non-cancer controls (N=66) (**Table 1**). UcfDNA methylation data from the MGB cohort were used as an independent holdout set to evaluate classifier performance.

Table 1. Patient cohort

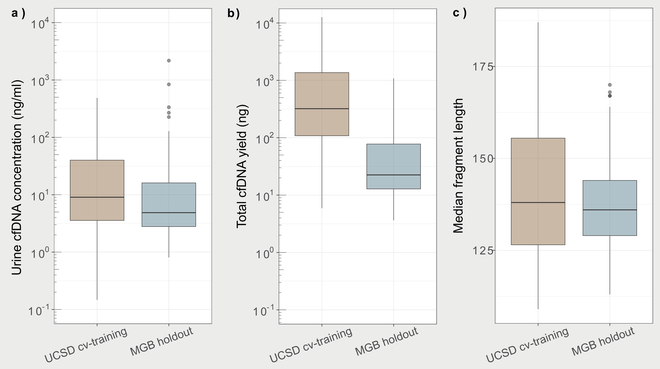
	UCSD cohort				MGB cohort		
	Non-cancer (N=28)*	Confirmed cancer (N=23)		Suspected of cancer with negative TURBT			
		Bladder cancer (N=16)	UTUC (N=7)	Benign (N=5)	Non-cancer (N=66)*	Bladder cancer (N=49)	UTUC (N=19)
Age (years, median)	66.5	70	66	55	67	69	61
Sex (% male, [n/N])	82.1% (23/28)	62.5% (10/16)	57.1% (4/7)	20% (1/5)	68.2% (45/66)	71.4% (35/49)	57.9% (11/19)
Stage (%, [n/N])							
0	–	62.5% (10/16)	14.3% (1/7)	–	–	75.5% (37/49)	89.5% (17/19)
I	–	31.2% (5/16)	28.6% (2/7)	–	–	24.5% (12/49)	10.5% (2/19)
>I	–	6.2% (1/16)	57.1% (4/7)	–	–	–	–
Grade (%, [n/N])							
High grade	–	68.8% (11/16)	100% (7/7)	–	–	51.0% (25/49)	52.6% (10/19)
Low grade	–	31.2% (5/16)	–	–	–	49.0% (24/49)	47.4% (9/19)

**The non-cancer cohort was age and gender-matched to the confirmed cancer cohort.*

Sample processing

- For the UCSD cohort, prospectively collected urine samples were processed as previously described.[4] Briefly, up to 50 mL of urine supernatant preserved with Streck Urine Preserve (median 30 mL, sd 11.66) was concentrated, extracted using a modified version of the MagMax Cell-Free DNA Isolation Kit (ThermoFisher Scientific), and then quantified. Up to 75 ng of ucfDNA was subjected to bisulfite conversion and enrichment with a custom panel, then sequenced to a target depth of 50-100 million reads.
- For the MGB cohort, urine supernatant samples previously stored at -80°C were thawed in the presence of Streck Urine Preserve. The final volume of preserved urine (median 4.7 mL, sd 0.97) was then processed in the same manner as described above.[4]
- Despite differences in pre-analytical conditions and lower input volume leading to lower ucfDNA yields, normalized yield of ucfDNA per mL of urine and fragment size were similar between the two cohorts (**Figure 1**).

Figure 1. Characterization of extracted urine cfDNA



Data analysis

- **TMeF generation:** Biopsy-free TMeF estimates in urine cfDNA were generated as described previously.[4]
- **Classifier training:** The urine classifier was developed using the Galleri classifier[5] infrastructure. The classifier was trained using 6-fold cross-validation (cv) with GRAIL's sample biobank, which included plasma and cancer tissue, along with prospectively collected urine samples from both cancer (N=179) and non-cancer participants (N=289). Only urine samples were used for cancer/no-cancer signal training. Classifier performance was evaluated in the UCSD cohort (UCSD cv-training), and a classifier trained on the full data set was locked at 95% and 98% target specificities. Desired specificity thresholds were set using the full set of N=289 non-cancers. The locked classifier was then evaluated on an independent test set (MGB holdout) in two batches and results were aggregated.