Bladder Cancer Early Recurrence Diagnosis through Detection of Cancer Cell-Derived DNA in Patient Urine

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Researchers from Iwate Medical University and other institutions (Iwate Prefectural Central Hospital; Geninus, Inc.; Sapporo Medical University) have revealed that monitoring cancer cell-derived DNA in the urine (here namely “urine DNA”) of bladder cancer patients using a highly sensitive nucleic acid quantification technique called Digital PCR (dPCR), with their proprietary technology (patent pending), allows for early prediction of bladder cancer recurrence and evaluation of treatment effectiveness.

Highlights

- In order to develop useful biomarkers for bladder cancer recurrence diagnosis, this study tracked cancer cell-derived DNA in the urine of bladder cancer patients and verified its potential as a recurrence diagnostic biomarker for bladder cancer.
- This study is the world's first to demonstrate that the examination of cancer cell-derived DNA in the urine of bladder cancer patients using dPCR can sensitively detect postoperative recurrence.
- Predicting bladder cancer recurrence through urine collection may reduce the burden of invasive tests such as cystoscopy.

Background

Traditional methods for diagnosing bladder cancer recurrence, such as cystoscopy, impose a significant burden on patients, and urine cytology tests have been criticized for their low sensitivity. Additionally, there is a lack of effective blood and urine biomarkers for diagnosing bladder cancer recurrence and evaluating treatment effectiveness, emphasizing the need for the development of low-burden and highly sensitive biomarkers. Recent studies have shown that genetic mutations derived from cancer cells can be detected in the urine of bladder cancer patients, making it a promising diagnostic biomarker. However, there has been no previous report verifying the validity of monitoring urine DNA mutations from bladder cancer patients in context of treatment as a "recurrence diagnostic biomarker." In this study, the researchers used dPCR, a highly sensitive nucleic acid quantification technique, to monitor genetic mutations in DNA extracted from urine sediments before and after treatment in bladder cancer patients and assessed its validity as an early recurrence diagnostic

biomarker (Figure 1). Since urine contains a large number of normal cells, the proportion of genetic mutations derived from bladder cancer cells is extremely low. Previously, researchers at Iwate Medical University have developed a primer/probe library that facilitates dPCR to measure such low-frequency genetic mutations in blood (patent pending), leading to the initiation of this research. In bladder cancer patients, cancer cell-derived DNA can also be detected in the blood. In this study, the researchers measured bladder cancer cell-derived DNA in urine using the originally developed dPCR primer/probe library, in addition to genetic mutations in blood.

Methods

The study included a total of 32 cases, consisting of 15 cases with a history of surgery for bladder cancer and 17 cases treated with a new therapy. DNA was extracted from cancer tissues obtained through transurethral resection of bladder tumors (TURBT) performed as initial treatment for non-metastatic bladder cancer. Panel sequence analysis and mutation analysis of the TERT promoter region were conducted on the cancer tissue DNA. The detected genetic mutations for each case were used as biomarkers in urine and blood. Blood and urine samples were collected before and after various treatments, as well as during regular follow-ups. The allelic frequency of genetic mutations in DNA in urine and blood was monitored using dPCR analysis for two years after registration. Primer probe sets for dPCR analysis were mainly selected from libraries developed at Iwate Medical University. Finally, the monitoring of urine and blood allelic frequency (VAF) was compared with existing diagnostic methods (cystoscopy, urine cytology, CT) to assess whether it could "early recurrence detection" and "evaluate treatment efficacy."

Results

Genetic mutation analysis of cancer tissue DNA detected genetic mutations that would be appropriate for monitoring in 30 out of 32 cases (93.8%). The average number of monitored mutations per case was 2.3 (1-4), and approximately 90% of cases were analyzable using the probe library developed at Iwate Medical University. Among the seven cases that experienced recurrence during the observation period, five cases showed urine DNA mutations with a VAF of 1% or higher more than seven months earlier than conventional recurrence diagnosis methods. In the remaining two cases, the VAF of urine genetic mutations remained low, below 1%. It may be explained by pyuria due to inflammation, causing dilution of cancer cell-derived DNA by normal DNA derived from white blood cells. Moreover, the remaining 23 cases without recurrence all showed consistently low VAF in urine DNA, below 1%. Among the 19 cases that underwent TURBT during the observation period, two of the four cases with postoperative recurrence showed a persistent increase in VAF in urine DNA with 1% or higher, while the other two cases had VAF below 1% (Figure 1A). These two cases with low VAF due to inflammation. In contrast, the VAF of the 15 cases that underwent TURBT without recurrence

remained consistently below 1% (Figure 1B). Among the 13 cases that underwent BCG therapy during the observation period, all three cases that experienced recurrence after BCG therapy showed a persistent increase in VAF in urine DNA with 1% or higher (Figure 2C), while the remaining 10 cases without recurrence showed consistently low VAF in urine DNA, below 1% (Figure 2D). Therefore, this study demonstrated the potential clinical validity of urine DNA as a biomarker for bladder cancer, providing early detection of recurrence and evaluation of treatment effectiveness.

Conclusion

Monitoring genetic mutations in urine DNA allows for the early detection of recurrence and evaluation of treatment effectiveness for bladder cancer, surpassing existing tests such as cystoscopy and urine cytology. This suggests that urine DNA is a valid biomarker for bladder cancer.

Prospective

• Urine DNA monitoring tests may confirm the absence of recurrence, potentially reducing the frequency of stressful cystoscopy examinations.

• Evaluating treatment effectiveness through this method may aid in predicting recurrence, assisting in deciding the need for additional treatment after the initial therapy.

• While blood-based DNA monitoring for cancer, known as the "OTS-Assay," is currently available at Iwate Medical University, ongoing research and development will explore the possibility of performing the same assay using urine DNA.

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Glossary

1. Digital PCR(dPCR): Digital PCR is a technique that quantifies individual molecules in a sample by counting them, allowing the identification and quantification of rare molecules present in a normal sample, such as around 0.01%, which is difficult to achieve with conventional PCR methods.
2. Biomarker: Biomarkers are substances in body fluids or tissues, such as proteins or genes, that serve as indicators of physiological conditions or diseases.
3. Cystoscopy: A diagnostic and postoperative periodic examination for bladder cancer. This procedure involves inserting a thin camera, approximately 7mm in diameter, through the urethra, inflating the bladder with water, and observing the presence of tumors within the bladder.
4. Urine Cytology: An examination where cells extracted from urine are observed under a

microscope to check for the presence of cancer cells.

5. TURBT: Transurethral Resection of Bladder Tumor (TURBT) is a standard surgical procedure performed for non-metastatic local bladder cancer. A camera with a diameter of 7mm is inserted into the bladder, and tumor tissue within the bladder is cut and removed using an electric knife.
6. Panel Sequencing: A method of sequencing specific gene regions in the DNA base sequence. When using a cancer panel, it can help identify gene mutations associated with cancer.
7. TERT Promoter: The promoter region of the TERT gene, which is involved in the elongation of telomeres at the end of genes. In bladder cancer, approximately 70% of cases are known to have gene mutations in the TERT promoter region.
8. VAF: When multiple types of genes exist at the same position, each individual gene is called an allele, and the mutated allele is referred to as the mutant allele. The frequency of mutant alleles within the entire tissue is called the Variant Allele Frequency (VAF), and VAF in circulating tumor DNA (ctDNA) is known to be associated with the amount of tumor in the body.
9. Primers and Probes : Reagents used in performing digital PCR. One probe is required for each mutation. Iwate Medical University has designed over 1000 probes for frequent gene mutations in various cancers (patented).
10. BCG Therapy: A treatment method for bladder cancer where the BCG (Bacillus Calmette-Guérin) vaccine is injected into the bladder. This therapy is performed for aggressive cancers or those prone to recurrence.

Original paper

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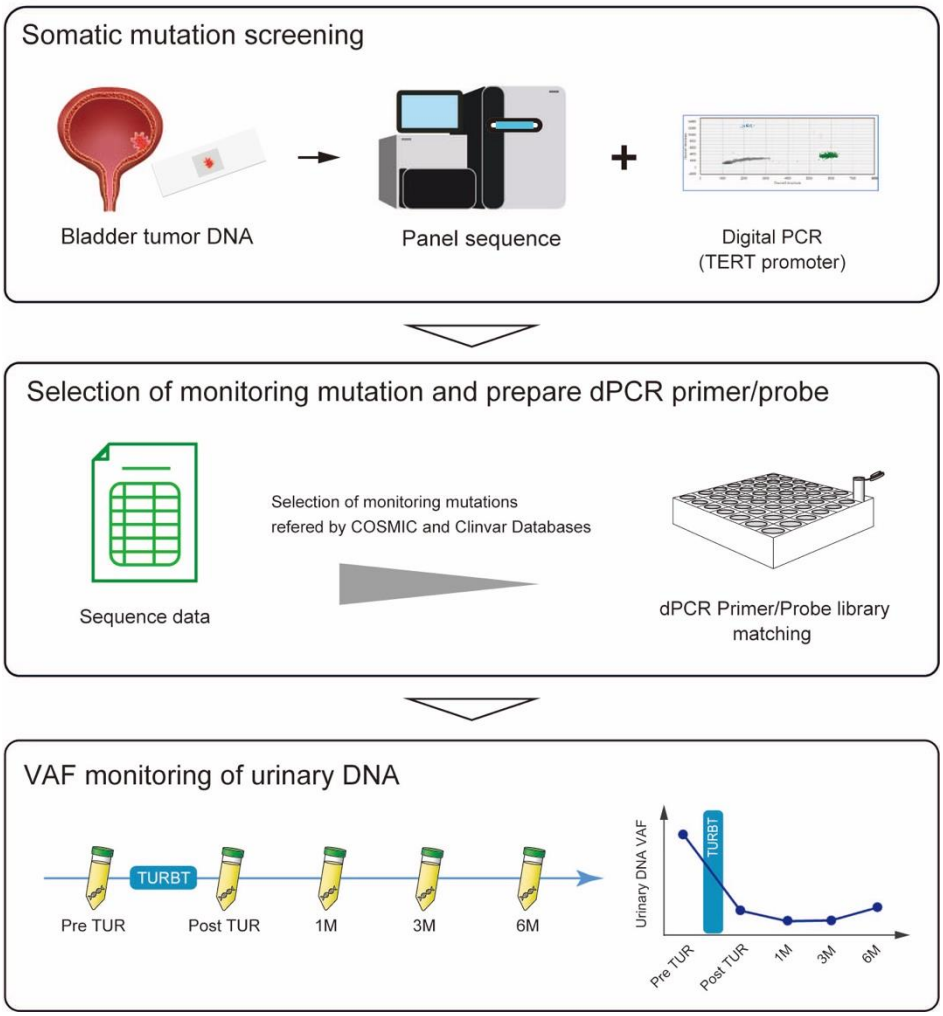

Figure 1: Method for Gene Mutation Detection and Monitoring of Urinary DNA Variant Allele Frequency (VAF)

1. Somatic Mutation Detection: DNA extracted from cancer tissue obtained during bladder cancer surgery was subjected to analysis using panel sequencing and digital PCR analysis of mutations in the TERT promoter region, which is found in approximately 70% of bladder cancer cases. Gene mutations for each case were measured.

2. Selection of Tracked Gene Mutations: From the sequencing results, gene mutations that serve as the origin of the tracked cancer were selected based on various databases. The selected mutations were cross-referenced with the digital PCR library corresponding to pan-cancer mutations held at our facility.

3. Monitoring Gene Mutations in Urinary DNA: Urine samples were collected before and after surgery, as well as during subsequent regular examinations. The Variant Allele Frequency (VAF) of gene mutations in urinary DNA was monitored for 2 years using digital PCR.

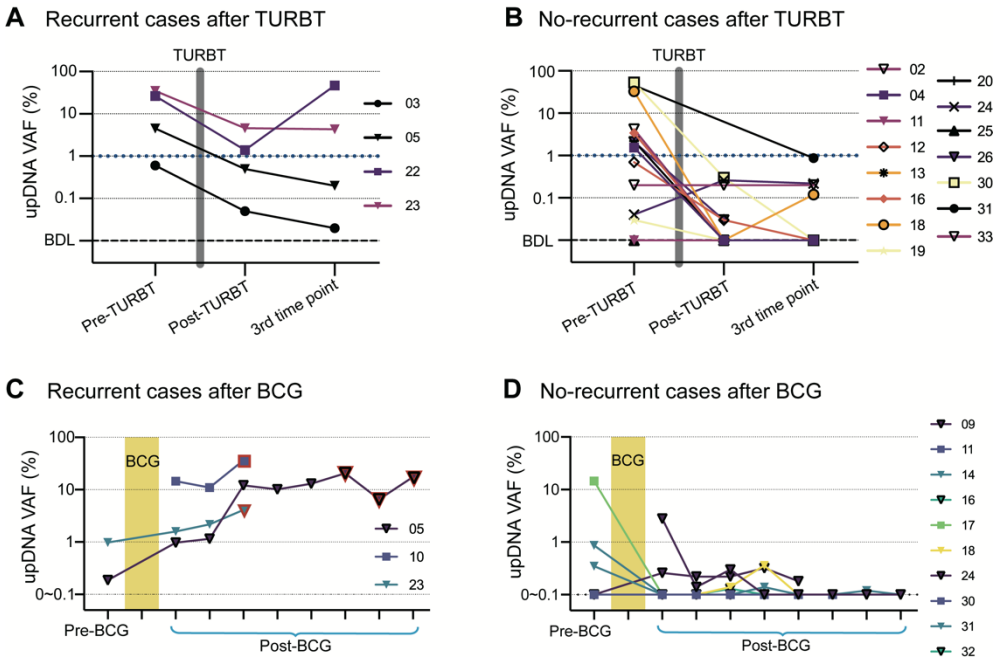

Figure 2: Dynamics of Urinary pellet DNA (upDNA) Variant Allele Frequency (VAF) before and after TURBT and BCG Therapy

(A, B) The dynamics of VAF were evaluated at three points: before and after TURBT, and at the nearest follow-up observation point after TURBT. (A) Among the four cases that recurred after TURBT, VAF in two cases continued or increased to 1% or more after TURBT, while in the remaining two cases, it decreased to 1% or less. (B) Among the 15 cases that did not recur after TURBT, VAF decreased to 1% or less in all cases. (C, D) The dynamics of VAF in urinary DNA before and after BCG therapy and during subsequent follow-up observations are shown. (C) Among the three cases that recurred after BCG therapy, all cases showed VAF of 1% or more and an increasing trend. (D) Among the 10 cases that did not recur after BCG therapy, VAF remained at 1% or less in all cases and continued to stay low thereafter.