

Emerging role of liquid biopsy of cell-free tumor DNA for bladder cancer surveillance

Minyong Kang, Ja Hyeon Ku

Department of Urology, Seoul National University Hospital, Seoul, Republic of Korea

Correspondence to: Ja Hyeon Ku, MD, PhD. Department of Urology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Republic of Korea. Email: kuuro70@snu.ac.kr.

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Urothelial bladder cancer (UBC) is the second most common type of malignancy in the urinary tract with a high frequency of recurrence and a high progression rate (1). Prior to deciding on a therapeutic strategy, appropriate risk assessment and outcome prediction are critical for achieving better prognosis in patients with UBC who required adjuvant treatments (2). The European Organization for Research and Treatment of Cancer (EORTC) risk tables and the American Joint Committee on Cancer (AJCC) TNM staging system have been validated and are widely used to estimate the risk of disease recurrence and progression in patients with non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC), respectively (3,4). Despite the simple and practical potential, current staging systems may be less accurate at risk assessment than novel prognostic models that incorporate more comprehensive information, such as molecular markers and genetic signatures.

Along with recent technological breakthroughs, such as next-generation sequencing (NGS), which have been made in the era of molecular diagnosis over the last 10 years, liquid biopsy has received a great deal of attention as a non-invasive and simple method of molecular diagnosis and monitoring in patients with various type of malignancies (5). This novel technology can provide comprehensive genomic information about disease conditions through analysis of cell-free tumor DNA (ctDNA) from peripheral blood or urine samples of cancer patients, with the aim

of improving oncological outcomes (6). The concept of ctDNA was first reported in 1977 by Leon and colleagues, who suggested that persistently increased levels of ctDNA can be a pathognomonic sign of disease relapse and poor prognosis (7). Mechanistically, DNA fragments that flow out of a broken cell membrane are shed into the circulation during cell death (8). Therefore, cancer patients may have higher ctDNA levels than healthy people because of the rapid turnover rate of malignant cells and because there are many necrotic cells in cancerous tissue compared to normal cells in benign tissues (8). In fact, cancer patients usually have a three-fold higher level of median ctDNA in the bloodstream than do normal individuals, and the quantity of ctDNA in a liquid biopsy is dependent on tumor burden, aggressiveness, and location (8).

Although liquid biopsy has not achieved widespread clinical use in UBC in contrast to other cancers, Christensen *et al.* (9) recently published promising results of a liquid biopsy analysis of plasma and urine specimens in the journal *European Urology*; those authors focused in particular on FGFR3 and PIK3CA hotspot mutations as disease surveillance tools in patients with UBC. While previous studies on liquid biopsy in various types of malignancies have critical pitfalls, such as that they require a time-consuming diagnostic process and are associated with high economic cost, this study suggests a faster and cheaper method of disease surveillance that uses droplet digital polymerase chain reaction (ddPCR) assays. Last year,

the same research group reported very attractive results of liquid biopsy analysis generated using ddPCR technology to detect circulating ctDNA in patients with non-muscle invasive bladder cancer (NMIBC) (10). To the best of our knowledge, this research group first suggested that ctDNA measured in plasma and urine is a promising tool for monitoring disease course in bladder cancer patients, particularly by using NGS and ddPCR technologies.

In the current study, tissues from two patient cohorts, the first with NMIBC (n=363) and the second with bladder cancer undergoing radical cystectomy (RC; n=468) were initially screened to detect hotspot mutations in FGFR3 (S249C, Y373C) and PIK3CA (E545K). Notably, FGFR3 and/or PIK3CA mutations were detected in 36% of NMIBC patients (n=129/363) and 11% of RC patients (n=44/403). In patients harboring these mutations, the association between ctDNA levels in liquid biopsies and tumor characteristics as well as oncological outcomes during the course of disease were subsequently analyzed. Moreover, ctDNA levels in urine specimens were significantly associated with pathologic features seen later in disease progression in NMIBC patients. Levels of ctDNA in blood samples were also significantly associated with later disease recurrence in RC patients. For instance, 89% of patients with detectable plasma ctDNA experienced recurrence, while only 33% of patients without plasma ctDNA developed disease recurrence. Additionally, there was a positive correlation between ctDNA levels measured in urine supernatant and plasma. Considering these results, the authors suggest that elevated levels of urine and plasma ctDNA with FGFR3 and PIK3CA mutations can be significant indicators of disease progression and metastasis in patients with bladder cancer.

Although the study by Christensen *et al.* gave promising results of liquid biopsy for disease surveillance in patients with bladder cancer, particularly focusing on hotspot mutations in ctDNA, the retrospective design and low volume of the plasma samples obtained during the study period were critical limitations (9). Moreover, there are no standard methods of pre-analytical and analytical methods for liquid biopsy, including sample collection, processing, and storage, or of ctDNA extraction, processing and quantification (11). Because all steps in the liquid biopsy process can influence the quality and accuracy of the final results, these technical issues must be addressed before the routine use of the liquid biopsy in a clinical setting for cancer patients.

In summary, liquid biopsy for detecting ctDNA based on

ddPCR from urine and plasma specimens may be a practical and powerful non-invasive surveillance tool for monitoring of disease recurrence and progression in bladder cancer patients, potentially improving clinical outcomes in the near future.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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