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Urinary Cell-Free DNA in Liquid Biopsy and Cancer Management

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Abstract

BACKGROUND: The current methodology used to detect, diagnose, and monitor many types of cancers requires invasive tissue biopsy testing. Recently, liquid biopsy using blood, plasma, urine, saliva, and various other bodily fluids has shown utility to solve many issues associated with tissue biopsy. Blood/plasma has received most of the attention within the liquid biopsy field, however, obtaining blood samples from patients is still somewhat invasive and requires trained professionals. Using urine to detect cell-free DNA cancer biomarkers offers a truly non-invasive sampling method that can be easily and reproducibly conducted by patients.

CONTENT: Novel technologies and approaches have made the detection of small quantities of cell-free tumor DNA of varying lengths possible. Recent studies using urine circulating tumor DNA to detect cancer mutations and other biomarkers have shown sensitivity comparable to blood/plasma cell-free DNA liquid biopsy for many cancer types. Thus, urine cell-free DNA liquid biopsy may replace or provide supplementary information to tissue/blood biopsies. Further investigation with larger patient cohorts and standardization of pre-analytical factors is necessary to determine the utility of urine cell-free DNA liquid biopsy for cancer detection, diagnosis, and monitoring in a clinical setting.

SUMMARY: In this mini-review we discuss the biological aspects of cell-free DNA in urine, numerous studies using urine cell-free DNA to detect urological cancers, and recent studies

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using urine cell-free DNA to detect and monitor non-urological cancers including lung, breast, colorectal, and other cancers.

Introduction

Following various imaging tests, tissue biopsy is the standard approach for cancer diagnosis and aids in treatment planning. However, tissue biopsy requires invasive procedures; it only provides a snapshot of the molecular aberration in the tumor, which does not reflect tumor heterogeneity. Multiple tissue biopsies are impractical for disease monitoring (1). Liquid biopsy, an emerging field in the past decade, has the potential to solve many of the problems associated with tissue biopsy and could be a better method for cancer screening. Many promising cancer biomarkers are being investigated that may be applicable for liquid biopsy testing. Cell-free DNA (cfDNA) and circulating tumor cells (CTCs) have been the primary biomarker targets that are present in different bodily fluids such as blood/plasma, urine, saliva, cerebral spinal fluid (CSF), and more (2). While CTCs can provide DNA, RNA, and protein for analysis, they are very rare, lack cancer heterogeneity, and there is not a standardized isolation method. Although circulating tumor DNA (ctDNA) can be contaminated with DNA from normal cells including circulating blood cells, it is easier to isolate, represents tumor heterogeneity, and is more sensitive for treatment monitoring and determining tumor burden (1). Due to these factors, ctDNA seems to have greater clinical utility and ease of access than cellular tumor DNA from CTCs. So far, blood has been studied most, but acquiring this source of liquid biopsy material is invasive and requires a trained professional to obtain samples from patients (3). Urine samples are easily accessible, have high patient compliance, and represent the body's health due to the constant filtration of blood via the kidneys (4). Thus, liquid biopsy using urine cell-free DNA (ucfDNA) is less invasive, and patients can frequently collect large volumes of urine at home (3). Since 2005, the total number of Food and Drug Administration (FDA) and European Medicines Agency (EMA) clinical trials involving ucfDNA analysis is only 20 compared to 404 for serum/plasma cfDNA (5). Overall, cfDNA analysis in urine has received far less attention than in blood, yet some recent studies show similar cancer mutation detection rates between blood and urine cfDNA (6, 7). With continued ucfDNA research and the standardization of pre-analytical and analytical factors for urine processing, this form of liquid biopsy could increase early cancer detection, improve disease monitoring, and provide real-time treatment efficacy to physicians. In this review, we highlight the current understanding of the biology of ucfDNA and recent studies using ucfDNA as a biomarker for urological and non-urological cancers.

THE CHARACTERISTICS AND BIOLOGY OF URINE CFDNA

Urine contains many compounds such as cells, salts, and cell-free nucleic acids like DNA, mRNA, micro-RNAs (miRNAs), and long non-coding RNAs (lncRNAs) (8). These circulating cell-free nucleic acids are found at various concentrations in the urine. For example, the concentration of RNA is reported to be 20–140 ng/mL and miRNAs may be more resistant to nucleases because of their short size, but exact concentrations are unknown (9). Thus, additional studies are needed to evaluate the concentrations of various RNAs in urine. Cell-free DNA concentrations range from 1 to 200 ng/mL (10) and they

can accumulate in the urine via multiple processes (Fig. 1). The cfDNA can be directly released into the urine by necrotic and apoptotic cells of the urogenital system. Additionally, it can be transported through the blood and filtered into the urine by the kidneys, and cells can secrete them in exosomes. However, the exact biological mechanisms are still unclear and may differ between cancer types, prompting further investigation (9). In healthy individuals, circulating cfDNA concentration is relatively low, but this concentration can increase when the body undergoes various forms of stress such as cancer, resulting in up to a 10 times increase of cfDNA compared to healthy control levels (11). Urine cfDNA has 2 distinct size groups: high molecular weight, greater than 1000 bp, and low molecular weight, typically from 40 to 250 bp (12). Su and coworkers isolated both sizes of urine cfDNA while studying samples from colorectal carcinoma patients and concluded that the 150 to 250 bp DNA originated, at least in part, from circulation, while the large fragments originated from urinary tract cells (13). Because urine can contain both local ctDNA from urological cancer cells and ctDNA in circulation from non-urological cancers, it may be a better alternative for disease monitoring than blood/tissue testing (11). For many non-urological cancers, most urine ctDNA (uctDNA) is below 100 bp in size, likely because glomerular filtration within the kidneys restricts ctDNA fragments greater than 70 kDa (double stranded of 107 bp in size) from passing through the nephron (12). Markus et al. confirmed this by performing whole-genome sequencing and found the modal size of urine cfDNA to be 80 to 81 bp and successive peaks at 10 bp intervals, indicating some form of cfDNA protection via protein/histone association in urine (14). Therefore, because only these limited-sized compounds can pass the barrier, urine is considered “cleaner” than plasma/serum due to lower concentrations of proteins and cells, making ucfDNA isolation less complex (13). However, in urine, nucleic acids are subject to higher levels of DNase activity, which breaks down the cfDNA fragments (8). However, the amount of nucleases present in the urine may provide us with another helpful biomarker. Zhou and coworkers found a greater concentration of ucfDNA with jagged ends (double-stranded DNA with single-stranded overhangs) than plasma cfDNA, likely due to different DNase activity levels. In urine samples from bladder cancer patients, they found lower levels of ucfDNA with jagged ends than healthy volunteers, potentially due to cancer-induced decreased nuclease activity (15). Also, Yao and colleagues studied the half-life of cfDNA in urine, serum, and saliva and found the half-life to be extremely short and immeasurable for urine cfDNA (16). Thus, pre-analytical factors such as sample collection, sample volume, processing time, preservatives, and first void urine may have a large impact on the quantity of cfDNA recovered and thus affect sensitivity and specificity of analytical assays. In a pilot study, Augustus and coworkers have studied many of these pre-analytical variables and offer recommendations to maximize ucfDNA quantity. They found that the first void urine is not the only portion with a high cfDNA concentration, that fresh urine should be processed right away or Streck preservative should be added, and urine samples should be taken more than 1.5 h apart, among other findings (17). Another important pre-analytical factor for ucfDNA liquid biopsy is the method of DNA isolation. Oreskovic and colleagues have studied different cfDNA extraction methods and found the Qiagen QIAamp Circulating Nucleic Acid Kit to poorly recover ucfDNA fragments below 150 bp. They also concluded that the Q Sepharose extraction method can recover ucfDNA over 40 bp and is the best method for next-generation sequencing (NGS) and single-stranded library preparation (10).

For ucfDNA isolation in bladder cancer, the QIAamp Circulating Nucleic Acid Kit is the most used, although no consensus has been made on the optimal kit yet (18). This is concerning because the Qiagen kit may not detect ultra-short ucfDNA in non-urological cancer studies. New analytical technologies such as quantitative PCR (qPCR), droplet digital PCR (ddPCR), and NGS have allowed for more accurate detection of smaller cfDNA quantities and fragments, leading to advancements in this field (19). Further standardization of pre-analytical and analytical techniques will allow for the continuation of advances within the ucfDNA analysis field for both urological and non-urological cancers.

UROLOGICAL CANCERS

Bladder cancer.—Most ucfDNA liquid biopsy studies have focused on urological cancers because the majority of ucfDNA comes from dying cells of the urogenital system (20). These cancers include bladder and kidney, male reproductive organs, and prostate cancers (Table 1). Bladder cancer cells are in direct contact with urine; therefore, cellular necrosis introduces longer cfDNA fragments that current analytical techniques can more easily detect (31). Casadio and coworkers assessed the integrity of >250 bp ucfDNA in early-stage bladder cancer patients by measuring 3 common bladder cancer gene biomarkers from the urine supernatant: *MYC*, *ERBB2*, and *BCAS1*. DNA integrity is a marker for necrotic cancer cells because DNA from normal apoptotic cells is highly fragmented (typically shorter than 250 bp), while necrotic cancer cells release long, unfragmented DNA. Integrity is a calculated ratio comparing the concentrations of long ucfDNA to short ucfDNA, and in this study, the DNA integrity ratio increased 40-fold in cancer patients. Analysis was performed using qPCR and resulted in a sensitivity of 73% with a specificity of 84% for symptomatic bladder cancer patients, showing promising results for early bladder cancer diagnosis using ucfDNA integrity analysis (21).

Other studies have analyzed common genomic alterations in bladder cancer and shown that ucfDNA mutation analysis may have clinical relevance. Russo and colleagues attempted to detect *TERT* promoter mutations in ucfDNA using ddPCR and targeted NGS on matched tumor specimens. This mutation is found in over 75% of bladder tumors, which likely contributed to the observed 92% concordance between tissue samples and ucfDNA having the *TERT*228 G > A/T mutation (22). In another study, Ou and coworkers collected 10 to 50 mL of urine from patients and developed a 5 gene panel for detecting bladder cancer from ucfDNA present in 2 mL of urine supernatant using a NGS assay. This pilot study showed a better concordance between this 5 gene ucfDNA panel (*TERT*, *FGFR3*, *TP53*, *PIK3CA*, and *KRAS*) and cancer tissue than plasma's concordance with cancer tissue. This is an important step in the development in a non-invasive diagnostic clinical test because other urine liquid biopsy tests such as bladder tumor antigen, cytology, and fluorescence in situ hybridization show limited sensitivity/specificity and are not accepted for clinical diagnosis (23). Additionally, Hayashi and colleagues had analyzed *TERT* promoter and *FGFR3* mutations in ucfDNA using ddPCR and found a sensitivity of 78% in combination with cytology results for diagnosing patients with upper tract urothelial carcinoma (UTUC). Urine cytology is the only non-invasive diagnostic method that is currently recommended for UTUC detection, yet it has only a 40% sensitivity (27). These findings show promising

potential for using non-invasive ucfDNA genomic alteration analysis to detect bladder cancers.

Also, some researchers have developed novel approaches to detect bladder cancers in ucfDNA. For example, Cheng and colleagues used a novel technology called shallow-depth genome-wide bisulfite sequencing to detect copy number alterations and ucfDNA methylation at a 93.5% sensitivity from 20 mL of morning voided urine from bladder cancer patients. These results show an increased sensitivity compared to conventional urine cytology, especially for tumors of low grade (24). Another novel technology designed by Dudley and coworkers for early-stage non-muscle-invasive bladder cancer diagnosis is a high-throughput sequencing method to detect urine tumor DNA, referred to as utDNA cancer personalized profiling by deep sequencing (uCAPP-Seq). This targeted-sequencing, mutation-informed approach utilizes prior sequencing knowledge from patients' tumors and germline tissue and tests urine samples for these mutations. Conversely, the tumor-naïve approach detects driver mutations without knowing the patient's tumor genotype. The targeted-sequencing method detected 93% of cancer cases before treatment and the tumor-naïve approach detected 84%. They also found *PLEKHS1* gene promoter mutations in 46% of the patients with bladder cancer, indicating the potential clinical utility of these mutations as bladder cancer biomarkers (25). Another method, cell-free single-molecule unique primer extension resequencing (cf-SUPER), was developed by Zhao and coworkers to look for mutation-harboring ucfDNA fragments, using as little as 1 ng of DNA. They used 22 bladder cancer-related genes and analyzed 740 mutation hotspots to detect mutations in ucfDNA and tissue samples with over 82% sensitivity (26). These approaches have attempted to solve problems with the more conventional methods described previously. While they show promise, further validation with independent, larger patient cohorts needs to be conducted to confirm the performance of these advanced approaches.

Renal cell cancer.—A few studies have investigated kidney cancers in the ucfDNA liquid biopsy field. Smith and coworkers used both targeted and untargeted sequencing methods to detect renal cell carcinoma (RCC) from plasma and urine cfDNA. The targeted approach sequenced a 10-gene panel consisting of the most common mutated genes in RCC patients while the untargeted approach employed genome-wide sequencing. Overall, they found that there are low levels of ctDNA for RCC, yet there is some evidence that ucfDNA may be better than tissue biopsy at representing tumor heterogeneity (28). Another assay designed by Nuzzo and colleagues called cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) can detect early-stage RCC from plasma and urine samples using a small amount of DNA (10 ng). The ucfDNA from samples was used to correctly classify RCC patients and controls with area under the receiver operating characteristic (AUROC) of 0.858, which is comparable to the 0.99 AUROC shown for plasma cfDNA (29). These preliminary studies show some promise for ucfDNA in RCC detection, however, there was a small number of participants in these studies, and the evidence for clinical usage is not overwhelming.

Prostate cancer.—In prostate cancer, Salvi and coworkers evaluated cfDNA integrity like Casadio and colleagues did in their study on bladder cancer patients and found that ucfDNA

integrity only had a 58% sensitivity compared to 95% sensitivity for prostate-specific antigen levels (30). Their results indicate that ucfDNA integrity is not a reliable biomarker for prostate cancer diagnosis compared to conventional methods. Very few studies have tried using ucfDNA as a biomarker for prostate cancer, so further investigation is required to determine if there is a clinical utility for a ucfDNA liquid biopsy detection approach for prostate cancers.

NON-UROLOGICAL CANCERS

Lung cancer.—As the field of urine liquid biopsy expands, researchers have begun exploring the presence of ctDNA in urine for non-urological cancers (Table 2). Interest in detecting ucfDNA mutations in lung cancer patients has grown due to the prevalence of lung cancer. For example, Reckamp and coworkers detected *EGFR* L858R and T790M mutations from ctDNA in the urine of patients with metastatic non-small cell lung cancer (NSCLC) at 75% sensitivity and 72% sensitivity, respectively, compared to tissue samples. This study marks the first use of ucfDNA to detect *EGFR* mutations in NSCLC patients. Additionally, they found increased sensitivity when urine volumes of 90 to 100 mL were collected, opposed to less than 90 mL, showing the need for pre-analytical standardization of urine collection (32). Husain and colleagues also detected T790M mutant DNA fragments and monitored ucfDNA levels after the administration of osimertinib, an anti-epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Their findings showed that ucfDNA could make the assessment of patient drug response more accessible and could assist in future drug development studies (33). In another lung cancer study, Xie and group detected *KRAS* mutations in advanced-stage NSCLC patients using ucfDNA at a 93% concordance with tumor tissue and found significantly higher overall cfDNA levels in the *KRAS*-mutated patients vs the wild-type *KRAS* patients (34). This study reports one of the highest concordances with tumor tissue for urine cfDNA. Another more recent pilot study conducted by Satapathy and coworkers found a reduced sensitivity of 60% when performing ddPCR analysis of urine samples from adenocarcinoma patients compared to tissue analysis. There is a chance this lower sensitivity could be due to pre-analytical variation because small urine volumes were used (30 mL), and the samples were without preservatives, such as EDTA, yet, testing multiple liquid biopsy samples increased overall sensitivity (35). These preliminary studies show the possible utility of using ucfDNA for lung cancer detection and monitoring in future patients, but further clinical studies are needed to solidify the efficacy of ucfDNA liquid biopsy.

Breast cancer.—Urinary cfDNA in breast cancer patients has received increasing attention, with a few recent studies testing ucfDNA liquid biopsy in early breast cancer patients. Zuo and colleagues used ddPCR to detect *PIK3CA* mutations in ucfDNA with a sensitivity of 91% compared to tissue biopsy analysis, while plasma showed a 93% sensitivity. Thus, urine and plasma samples showed similar rates of ctDNA mutation detection, and 240 of the 250 early-stage breast patients showed identical results between urine and plasma (7). Zhang and coworkers also tested urine and plasma ctDNA *PIK3CA* mutations with ddPCR and concluded over 77% sensitivity compared to tissue biopsy. Additionally, they showed a significant drop in ucfDNA after receiving treatment (36). Guan et al. showed a concordance of over 97% with tissue biopsy samples and early breast cancer

patients have higher ucfDNA levels than the healthy controls (37). These studies indicate that ucfDNA quantity and mutations could be useful biomarkers in the early detection of breast cancer and monitoring of disease progression.

Colorectal cancer.—Also, urine cfDNA studies have identified a few biomarkers for colorectal cancer (CRC) detection and have shown promising results. Su and group compared detection rates of *KRAS*-mutated cfDNA in urine, plasma, and serum of colorectal carcinoma patients and found significantly higher levels of low molecular weight cfDNA in urine and serum compared to plasma. When using 200 μ L of bodily fluid, urine had a 95% sensitivity which was significantly higher than serum (35%) and plasma (40%), but when using only 10 μ L of bodily fluid, all fluids showed comparable sensitivity. Long circulating DNA and proteins in the blood may account for the lack of sensitivity increase when using greater bodily fluid concentrations, possibly because of PCR amplification inhibition. Another interesting finding was that most of the detected mutated ucfDNA molecules were smaller than 700 bp in length (6). In a more recent study, Bach and coworkers measured the cfDNA methylation levels of 6 CRC-associated markers in 40 mL of urine from colorectal cancer patients and healthy volunteers. Using the *SEPTIN9* and *SDC2* methylation markers, detection of 70% of CRC cases was possible with 86% specificity from the urine supernatant. These levels of detection came close to the levels seen for *SEPTIN9* methylation CRC detection in plasma (75% to 81%), for which there is an FDA-approved test available (38). With further research, a similar, less invasive, urine-based test may soon be available as well.

Other cancers.—Other cancer types, such as brain cancer, have received attention in the urine liquid biopsy field recently. Mouliere and colleagues investigated 35 glioma patients' ctDNA in CSF, plasma, and urine using shallow whole-genome sequencing and found over a 2-fold increase in ucfDNA concentration for glioma patients vs controls and concluded that ucfDNA is significantly shorter (101 bp) and more fragmented than in healthy (137 bp) controls. While specific mutation detection was not spectacular, the fragmentation pattern seen in ucfDNA may be an important diagnostic biomarker for potential glioma patients (39). In pancreatic cancer, Terasawa and group used ddPCR to detect cfDNA *KRAS* mutations from urine and plasma samples of 56 patients with pancreatic ductal adenocarcinoma. *KRAS* mutations were found in 42% of cases for both urine and plasma and urine proved to have higher sensitivity in patients suffering from renal function degeneracy (40). This is one of the only studies using ucfDNA to detect pancreatic cancers, yet the evidence shows substituting urine for blood as a liquid biopsy sample may be as sensitive, if not more sensitive. Further investigation is required to determine if there is clinical utility for a urine cfDNA liquid biopsy detection approach of pancreatic cancers.

Conclusion

For both urological and non-urological cancers, urine liquid biopsy using cfDNA has shown its value and promise for cancer detection, monitoring cancer progression, and development of metastases. Compared to blood/plasma cfDNA liquid biopsy, much research still needs to be conducted to fully understand the utility and intricacies of this relatively new liquid biopsy field. For example, the mechanism by which non-urological circulating

cfDNA passes through the pores of the glomerulus is still unclear and requires further exploration. Urine liquid biopsy concordance with tissue has shown similar results to blood liquid biopsy, thus warranting the continuation of research because of the non-invasive nature and ease of urine collection as opposed to blood. However, while urine sample collection is simple compared to other bodily fluids, this may result in more pre-analytical variability. Pre-analytical variability coupled with rapid degradation of ucfDNA from high nuclease activity may pose challenges in reproducibility between urine liquid biopsy studies. Exploration and standardization of the best collection devices, preservatives, and extraction methods are still needed to limit this variability. Further, increasing the size of patient cohorts and using new, sensitive genetic analysis technologies may help urine cfDNA liquid biopsy become a regular clinical testing procedure. For example, single-strand library preparation could help us further investigate the newfound jagged ends present in ucfDNA. With supplemental research, new biomarkers and technologies may be discovered, such as cf-SUPER, which has allowed for high-sensitivity detection of bladder uctDNA at low DNA input concentrations. Novel methods designed to extract and evaluate the low molecular weight, ultra-short cfDNA may advance the ucfDNA liquid biopsy field for non-urological cancers as well. In summary, we believe urinary cfDNA is a promising biomarker for liquid biopsy in cancer management.

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Nonstandard Abbreviations:

cfDNA	cell-free DNA
CTCs	circulating tumor cells
ctDNA	circulating tumor DNA
ucfDNA	urine cell-free DNA
uctDNA	urine circulating tumor DNA
NGS	next-generation sequencing
ddPCR	droplet digital PCR
RCC	renal cell carcinoma
CRC	colorectal cancer

Human Genes

MYC	myc proto-oncogene bHLH transcription factor
ERBB2	erb-b2 receptor tyrosine kinase 2
BCAS1	brain enriched myelin associated protein 1

TERT	telomerase reverse transcriptase
FGFR3	fibroblast growth factor receptor 3
TP53	tumor protein p53
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
KRAS	<i>KRAS</i> proto-oncogene, GTPase
PLEKHS1	pleckstrin homology domain containing S1
EGFR	epidermal growth factor receptor
SEPTIN9	septin 9
SDC2	syndecan 2

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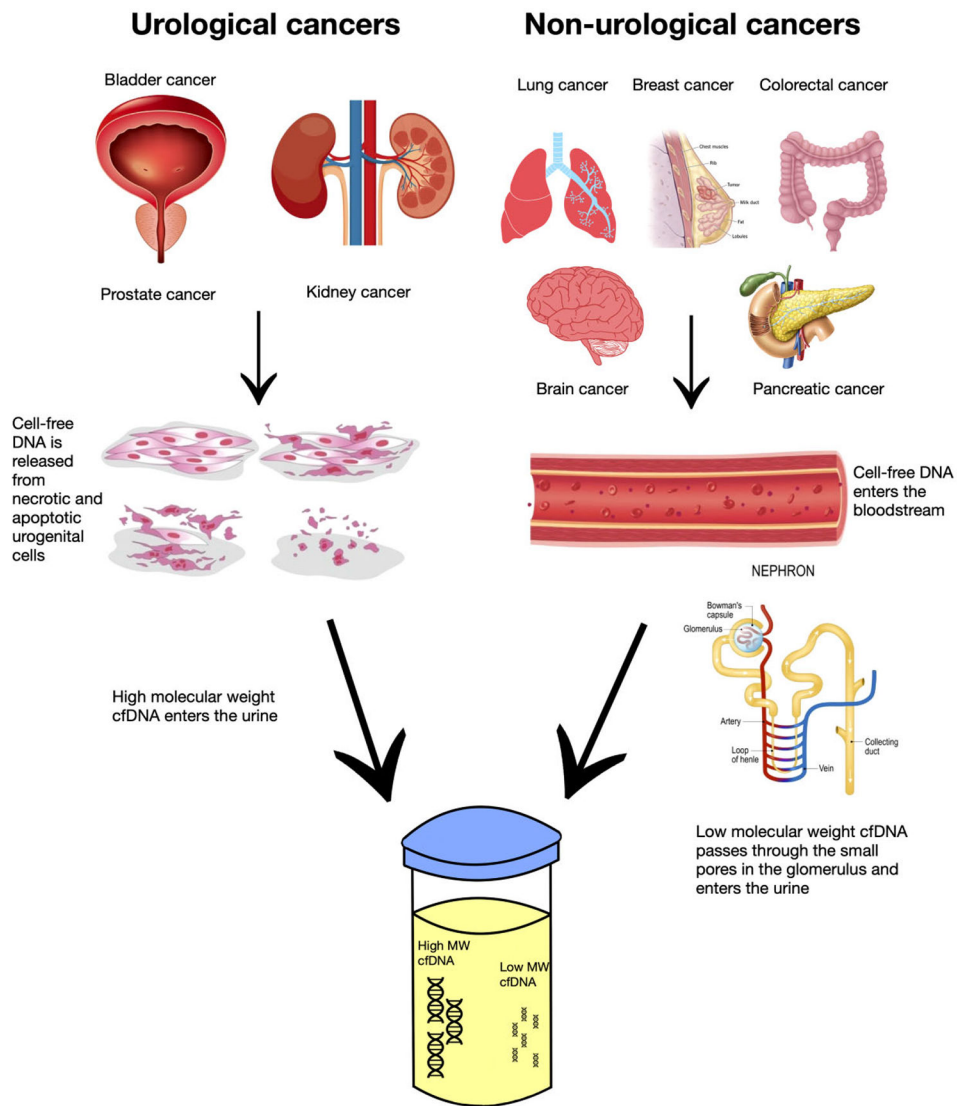


Fig. 1. Urinary cell-free DNA enters the urine through different processes and in various sizes. Cell-free DNA from non-urological cancers enters the bloodstream and passes through the kidneys. Only low molecular weight (typically 40 to 250 bp) cfDNA can pass through the small pores during glomerular filtration into the urine. High molecular weight (typically greater than 1000 bp) cfDNA from urological cancers originates from necrotic and apoptotic cells in the urogenital system and is released directly into the urine.

Table 1.

Urine cell-free DNA liquid biopsy studies for urological cancers.^a

Cancer type	Cancer patients enrolled	Assessed biomarkers	ucfDNA analytical technique	Study purpose	Tumor mutation profiling comparison	Year	Reference
Bladder	51	ucfDNA integrity	qPCR	Cancer diagnosis	73% sensitivity and 84% specificity with healthy patients	2013	Casadio et al. (21)
Bladder	104	<i>TERT</i> 228 G > A/T mutation	ddPCR	Cancer detection	92% concordance with tissue, 96% specificity	2018	Russo et al.(22)
Bladder	92	48 bladder-cancer specific genes	NGS	Cancer detection	N/A	2020	Ou et al.(23)
Bladder	46	ucfDNA methylation and copy number alterations	Shallow-depth genome-wide bisulfite sequencing	Cancer detection, diagnosis, monitoring	93.5% sensitivity and 95.8% specificity	2019	Cheng et al. (24)
Bladder	54	utDNA mutations	Novel high-throughput sequencing method (uCAPP-Seq)	Cancer detection & monitoring	93% sensitivity and 100% specificity for tumor mutation-informed approach, 84% sensitivity and 96% specificity when blinded	2019	Dudley et al. (25)
Bladder	47	22 bladder cancer-related genes, 740 mutation hotspots	Cell-free single-molecule unique primer extension resequencing (cf-SUPER)	Cancer detection	82.7% sensitivity and 89.6% specificity	2020	Zhao et al.(26)
Bladder	56	<i>TERT</i> promoter and <i>FGFR3</i> mutations	ddPCR	Cancer diagnosis	55.4% sensitivity and 100% specificity	2019	Hayashi et al. (27)
Renal	91	Common renal cancer mutations	sWGS	Characterization of urine cell-free tumor DNA	Roughly 50% for a targeted approach using plasma and urine	2020	Smith et al.(28)
Renal and Bladder	120	cfDNA differentially methylated regions	Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq)	Cancer detection	0.858 AUROC	2020	Nuzzo et al. (29)
Prostate	67	ucfDNA integrity	qPCR	Cancer detection	58% sensitivity and 44% specificity	2015	Salvi et al. (30)

^a Abbreviations: cfDNA, cell-free DNA; ddPCR, droplet digital PCR; NGS, next-generation sequencing; qPCR, quantitative PCR; sWGS, shallow whole genome sequencing; ucfDNA, urinary cell-free DNA; utDNA, urine tumor DNA; AUROC, area under the receiver operating characteristic; N/A, not performed.

Table 2.

Non-urological cancer urine cell-free DNA liquid biopsy studies.^a

Cancer type	Cancer patients enrolled	Assessed biomarkers	ucfDNA analytical technique	Study purpose	Tumor mutation profiling comparison	Year	Reference
NSCLC	63	<i>EGFR</i> (L858R, T790M) mutations	ddPCR, NGS	Rociletinib (EGFR TKI) response	L858R: 75% sensitivity and 100% specificity T790M: 72% sensitivity and 96% specificity	2016	Reckamp et al. (32)
NSCLC	9	<i>EGFR</i> mutations	ddPCR, NGS	Osimertinib (EGFR TKI) response	N/A	2017	Husain et al. (33)
NSCLC	150	<i>KRAS</i> mutation	ddPCR	Predicting mutations and outcomes	93% concordance with tissue	2018	Xie et al. (34)
NSCLC	81	<i>EGFR</i> mutations	ddPCR	Diagnosis and management	60% sensitivity and 100% specificity	2021	Satapathy et al. (35)
Breast	250	<i>PIK3CA</i> mutations	ddPCR	Detection, monitoring, disease relapse	91% sensitivity and 100% specificity	2020	Zuo et al. (7)
Breast	200	<i>PIK3CA</i> mutations	ddPCR	Treatment and disease relapse	77% sensitivity	2020	Zhang et al. (36)
Breast	300	<i>PIK3CA</i> and <i>TP53</i> mutations	N/A	Cancer monitoring	97% concordance with tissue biopsy	2020	Guan et al.(37)
Colorectal	20	<i>KRAS</i> mutations	qPCR, RE-PCR	Cancer detection	95% sensitivity (200 µL fluid used)	2008	Su et al. (6)
Colorectal	92	cfDNA (<i>SEPTIN9</i> & <i>SDC2</i>) methylation levels	qMSP	Cancer detection	70% sensitivity and 86% specificity	2021	Bach et al.(38)
Glioma	35	52 commonly mutated glioma genes, fragmentation patterns	WES, sWGS	Cancer detection	N/A	2021	Mouliere et al. (39)
Pancreatic	56	<i>KRAS</i> mutations	ddPCR	Cancer detection	42% sensitivity	2019	Terasawa et al. (40)

^a Abbreviations: NSCLC, non-small cell lung cancer; ddPCR, droplet digital PCR; NGS, next-generation sequencing; EGFR, epidermal growth factor receptor; cfDNA, cell-free DNA; qPCR, quantitative PCR; RE-PCR, restriction enriched polymerase chain reaction; qMSP, quantitative methylation specific PCR; WES, whole-exome sequencing; sWGS, shallow whole-genome sequencing; N/A, not performed.