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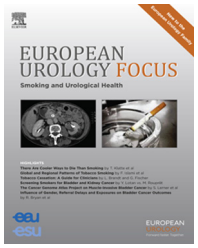

Review – From Lab to Clinic

Emerging Utility of Urinary Cell-free Nucleic Acid Biomarkers for Prostate, Bladder, and Renal Cancers

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Abstract

Context: By 2020 the estimated incidence of genitourinary (GU) cancers (prostate, bladder, and kidney) will be over 2 million worldwide and responsible for ~800 000 deaths. Current diagnosis and monitoring methods of GU cancer patients are often invasive and/or lack sensitivity and specificity. Given the utility of blood-based cell-free nucleic acid (cfNA) biomarkers, the development of urinary cfNA biomarkers may improve the sensitivity of urine assays utilizing urine sediment for GU cancers. This review of urinary cfNA in GU cancers identifies the current stage of research, potential clinical utility, and the next steps needed to enter clinical use.
Objective: To critically evaluate the literature of urinary cfNA in GU cancers for clinical utility in diagnosis, screening, and precision medicine. Furthermore, the strategy for future efforts to discover potential new urinary cfNA biomarkers will be described.
Evidence acquisition: A PubMed database (2006 to current) search was performed according to Preferred Reporting Items for Systemic Review and Meta-analysis using key Medical Subject Headings terms. Additional studies were obtained by cross-referencing from the literature.
Evidence synthesis: The collective research publications in urinary cfNA of GU cancers present a promising alternative *liquid biopsy* approach compared with blood biopsies and urine sediment, particularly for early-stage GU diseases.
Conclusions: Urinary cfNA as a liquid biopsy holds potential for a more sensitive alternative to blood biopsies and urine sediment-based tests for clinical use in GU cancers. Not only does urinary cfNA offer advantages including the potential for more frequent testing, monitoring, and home use, but also has applications in early-stage GU cancers.
Patient summary: In this review, we evaluated the current status of urinary cell-free nucleic acid in genitourinary cancers. We identified the potential advantages of urinary cell-free nucleic acid over blood and urine sediment and its clinical use in genitourinary cancer.

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1. Introduction

Liquid biopsies offer a powerful and minimally-invasive alternative for tissue biopsies, but have yet to be translated to routine clinical practice. Circulating tumor-derived cell-free nucleic acid (cfNA) biomarkers hold great promise as minimally-invasive blood tests for cancer diagnosis and precision medicine [1]. However, blood contains proteins,

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inhibitors, nucleic acids derived from normal cells, and potentially infectious agents that can negatively affect circulating tumor DNA (ctDNA) biomarker detection [1]. Furthermore, due to the anatomical site of genitourinary (GU) cancers blood-based tests may not readily detect ctDNA from the peripheral blood, particularly in early-stage GU cancers. Urine is a particularly attractive alternative due to the direct contact of urinary flow-through with draining of GU organs. Urine sampling is noninvasive, yields large volumes compared to blood, permits routine collection, and improves patient compliance.

Urine has been found to be a comparable, if not a better body fluid source for GU-derived biomarker detection to serum/plasma [2]. A majority of biomarker studies in GU cancers utilize urine sediment (cells that shed into urine). This has limited biomarker sensitivity due to the paucity of GU-derived cells present in urine [3,4], presence of urinary crystals [5], and concentration of inhibitors upon urinary cell sedimentation [6]. The sensitivity of GU cancer urinary biomarkers, particularly for early-stage, low-grade, noninvasive tumors, may be better if the cfNA fraction in urine is used. Tumor-derived cell-free DNA (cfDNA) has been found to be enriched in urine of liver [7], colon [8–10], and lung cancers [11]. Moreover, the processing of urinary cfNA is simpler as prior manipulation is unnecessary.

Here, we review studies of urinary cfNA as biomarkers in prostate, bladder, and kidney cancers and discuss the potential clinical utility. In particular, cfDNA, cell-free RNA (cfRNA), and cell-free microRNA (cfmiR) biomarkers will be evaluated.

1.1. *Current biomarker landscape for GU cancers*

GU cancer patient outcome would be greatly improved by identification of robust biomarkers for early detection of new and recurrent GU cancers, prognostication, and treatment stratification. In renal cell carcinomas (RCCs), biomarker development is not well established. Diagnosis often occurs at advanced stages resulting in a poor 5-yr survival rate of <8% [12], Furthermore, imaging, fine needle aspiration, and core biopsy are not sensitive in providing a definitive diagnosis [13]. Thus, there is a distinct need for better screening and diagnostic assays to allow for early intervention. In addition, patient stratification for targeted therapies is critical in high-risk, metastatic RCCs evident by stable/long-lasting responses [14]. Being able to monitor patient treatment efficacy with a simple and sensitive urine-based assay is highly needed.

Bladder cancer (BC) has the highest treatment cost due to frequent recurrence and the costly gold-standard screening methods (cystoscopy and cytology) [15]. Cystoscopy, which has 90% sensitivity [16], is invasive with low patient compliance (< 40%) [15]. Urine cytology suffers from low sensitivity, particularly in low-grade BC disease (4–31%) [17]. Other available screening tests (ie, bladder tumor antigen, nuclear matrix protein 22 [NMP22], fluorescence in situ hybridization, etc.) also lack sensitivity and do not replace cytoscopy/cytology. The high BC recurrence rate (60–80%) results in recommended routine life-time screenings or definitive treatment such as cystectomy depending on cancer grade and risk [16]. In addition, the value of cystoscopy/cytology to monitor treatment efficacy is limited [18]. Thus, development of sensitive urinary cfNA-based diagnostic and screening tests could improve identification of patients with recurrence.

Prostate-specific antigen (PSA) has poor specificity in differentiating indolent and malignant prostate diseases, leading to many unnecessary invasive biopsies [19]. Low-risk prostate cancer (PC) patients are placed on active surveillance which relies on annual biopsies that carries a morbidity risk. Multiple tests (ie, Prolaris, Decipher) have been useful in identifying high-risk PC patients, but are costly and invasive as tissue is required. Encouragingly, urinary prostate cancer antigen 3 has been a promising biomarker for identifying patients that would benefit from a biopsy [20], but still remains strongly PSA-dependent limiting the biomarker for PC screening. More definitive PC biomarkers are needed to better monitor these patients for risk assessment and identify targeted therapy [19]. Urine-based liquid biopsy for repeat monitoring would offer better patient compliance and reduce morbidity risk.

Overall, there is a great need to develop efficient body fluid biomarkers for early detection, prognosis prediction, and monitoring for recurrence/treatment efficacy. Given the increasing availability of new therapies for GU cancers, monitoring of urinary cfNA biomarkers as a representative tumor biopsy becomes increasingly important.

2. Evidence acquisition

The PubMed database was searched for pertinent articles using Medical Subject Headings terms “urine,” “biomarker,” and “cell-free” in different combinations with “PC,” “BC,” “transitional cell carcinoma,” “kidney cancer,” or “RCC” published between 2006 to December 2016, and updated on January 31, 2017. Only original articles published in English and related to human individuals were included in the initial search. Relevant articles were selected accordingly (Fig. 1). Specific studies on the improvement of urinary cfNA isolation were also taken into account. Relevant references cited in the retrieved full-text articles were also assessed. Articles were filtered based on the criteria that urine cfNA biomarkers were evaluated in comparison to matched-tumor, matched-blood, or clinically-available GU cancer screening biomarkers (ie, NMP22).

3. Evidence synthesis

3.1. *Types of urinary cfNA*

Most GU cancer studies have focused on biomarker development utilizing urine sediment, which is extracted post-centrifugation from whole urine. However, evaluating solely urine sediment ignores the presence of cfNA in urine obtained postcentrifugation as urine supernatant or in whole urine. In this review, we highlight studies of cfNA-derived from urine supernatant or whole urine (ie, cfDNA, cfRNA, and cfmiR) as potential GU cancer biomarkers.

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