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PERSPECTIVE

Biomarker discovery and beyond for diagnosis of bladder diseases

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Abbreviations used: BPS, bladder pain syndrome; CGM, continuous glucose monitors; DID, diabetes interactive diary; IHC, immunohistochemistry; IC, interstitial cystitis; IoT, internet of things; MAPP, multidisciplinary approach to the study of chronic pelvic pain; NIH, National Institutes of Health; ROC, receiver operator characteristic; SMBG, self-monitoring of blood glucose; UI, urinary incontinence; UTI, urinary tract infection

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ABSTRACT

Molecular biosignatures of altered cellular landscapes and functions have been casually linked with pathological conditions, which imply the promise of biomarkers specific to bladder diseases, such as bladder cancer and other dysfunctions. Urinary biomarkers are particularly attractive due to costs, time, and the minimal and noninvasive efforts acquiring urine. The evolution of omics platforms and bioinformatics for analyzing the genome, epigenome, transcriptome, proteome, lipidome, metabolome, *etc*., have enabled us to develop more sensitive and disease-specific biomarkers. These discoveries broaden our understanding of the complex biology and pathophysiology of bladder diseases, which can ultimately be translated into the clinical setting. In this short review, we will discuss current efforts on identification of promising urinary biomarkers of bladder diseases and their roles in diagnosis and monitoring. With these considerations, we also aim to provide a prospective view of how we can further utilize these bladder biomarkers in developing ideal and smart medical devices that would be applied in the clinic.

Keywords: biomarker; bladder; urine; medical device; biosensor

URINE AND URINARY BIOMARKERS FOR BLADDER DISEASES

Urine is a waste product that is readily produced by all patients and contains a wealth of information. It can be produced in high-volume and procurement of samples is noninvasive. Considering these factors alone, urine is a highly attractive potential resource. However, there are several glaring issues that make urinalysis difficult. Factors such as preanalytical reliability and data analysis can be a major challenge [1,2]. Transport and preservation of urine samples are particularly important. It has been shown that increased time gaps between sampling and analysis, lack of temperature control, and lack of preservatives for samples that cannot be analyzed within two hours after collection can lead to low-quality test results [3]. However, preservatives may also affect the chemical properties and alter the appearance of certain particles [4]. Additionally, urine contains much more complex compounds that can be affected by a wide range of external factors, including diet and environment [5]. A comparative urinary metabolite profiling study of habitual diet discovered that 417 urinary metabolites were correlated with more than one food, beverage, or supplement [6]. Exposure to different environmental toxins and chemicals have been shown to be reflected in urine. A study of pediatric exposure to pyrethroids, an insecticide, found differing concentrations of the chemical in urine based on each child's level of risk [7]. Fortunately, recent advances in technology and standardization have made urinalysis more of a viable option for a number of clinical issues [8]. Because the uroepithelial-associated sensory web may be related to hypersensitive benign urological disorders [9], it is not always necessary that clinopathological status results in a change in urinary components. As the pathology of genitourinary diseases is being better understood, more diagnostic and prognostic biomarkers are also being identified [10]. A recent study reported that 4 urinary biomarkers were associated with kidney injury [11]. By

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integrating newer technologies with increased knowledge of diseases, novel biomarkers can be discovered.

MULTI-OMICS APPLICATION FOR BLADDER BIOMARKER DEVELOPMENT

Omics involves the high-throughput analysis of different domains of biological information, including the genome, transcriptome, proteome, and metabolome [12,13]. Comprehensive omics analysis of urine can be a potentially valuable source of disease biomarkers. For instance, the proteomic profile of healthy urine can be used as a standard to compare disease-state urine to identify proteins of interest [14]. Recent new types of software are being developed to create workflows that involve distinguishing biomarkers *via* integrated comparative and quantitative analysis [15]. Advanced proteomic analysis has led to high-throughput profiling of bladder cancer-related proteins with both high sensitivity and specificity, which has resulted in a wealth of informative biomarkers [16]. A similar strategy was utilized in a recent study that identified 54 potential protein biomarkers of bladder schistosomiasis by quantitatively comparing urinary samples from humans [17]. Other types of omics applications, such as genomics, epigenomics, transcriptomics and metabolomics, were also applied to determine biomarkers of bladder schistosomiasis. Metabolomic profiling using urine and plasma samples revealed that the perturbed glycerophospholipid and sphingolipid metabolisms are associated with schistosomiasis and its associated-bladder cancer pathologies [18]. Epigenetic regulation on RASSF1A and TIMP3 were found using a quantitative methylation-specific PCR assay in urine sediments of patients with schistosomiasis infection. Hypermethylation of both RASSF1A and TIMP3 shows 77.55% of area under the receiver operator characteristic (ROC) curves ($P = 0.023$) [19]. Another study profiled urinary amino acids to identify potential biomarkers for lower urinary tract symptoms in male patients [20]. As non-invasive disease biomarkers, urinary extracellular vesicles such as exosomes have been discovered to contain a variety of molecular and genetic materials including nucleotides, proteins, metabolites, miRNAs, and they function as a cargo and transfer those materials to nearby neighbor cells [21,22]. Progress in these comprehensive tests continues to increase our understanding of the complexity of biomarkers that underlie diseases and, with technology, it is becoming easier to navigate how to utilize them.

MICROBIOME STUDIES IN UROLOGICAL DISEASES

The microbiome is defined as the collective genome of all microorganisms in an environment [23]. Interest in this field has recently boomed as it has been shown that microbiota and alterations in their communities can contribute to the pathogenesis of chronic urological diseases, such as urothelial carcinoma [24]. A preliminary study found an association between urinary dysbiosis and urothelial carcinoma, suggesting that the ratio for microbiota could be used as a potential diagnostic indicator [25]. Another study observed that bacterial richness increased in the urine of patients with cancer compared to controls [26]. However, despite all the promising exploratory data surrounding microbiome's usage in urological diseases, the field is still relatively new and more comprehensive studies are needed [27]. Studies on the influence of microbiota expand beyond the genitourinary tract as well. For instance, *Helicobacter pylori* is, well-documented, increasing the risk of duodenal and gastric ulcer disease and gastric cancer [28]. Bacterial pathogenesis is also noted to be potentially associated with colorectal cancer [29]. Based on the extensive role of microbiomes in many diseases, a better understanding of urinary microbes and their roles in urological diseases may prove to be significant.

Aside from potential utilization of the microbiome in diagnostics and prognostics, identifying present microbiota may be important when it comes to various treatments. For instance, gastrointestinal microbes are known to affect the metabolism and toxicity of various agents [30]. *Mycoplasma hyorhinis* has been shown to metabolize and inactivate gemcitabine, a chemotherapeutic drug, which can result in drug resistance [31]. Additionally, reactivation of the inactive metabolites of irinotecan, a topoisomerase I inhibitor, by gastrointestinal bacteria can lead to adverse toxicities, such as severe diarrhea [32]. For urological diseases, there are also some noted interactions between microbiota and treatment. It has been shown that D-mannose, a simple sugar, can hinder bacterial adhesion to the urothelium, thereby reducing risk of urinary tract infections and aiding in acute cystitis management [33].

The urinary microbiome is believed to play an important role in predicting disease status for many different urogenital diseases. Recently, a pilot study looking into the relationship between the urinary microbiome and bladder cancer uncovered that that bacteria belonging to the genus Fusobacterium were significantly more abundant in urine specimens from cancer patients [34]. Another exploratory study comparatively surveyed the urine microbiota of female patients with interstitial cystitis (IC)/bladder pain syndrome (BPS) and controls who were enrolled in the National Institutes of Health (NIH) Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. It identified potential negative impacts of the presence of *Lactobacillus gasseri* and protective influence of *Corynebacterium* [35]. It should be noted that a different study on urinary incontinence (UI) found a lack of *Lactobacillus* to be associated with urgency UI and resistance to anticholinergic treatments [36]. However, being that these are two different diseases, the conflicting results are not unexpected. Furthermore, there are many species of Lactobacillus and some may contribute to a healthy or disease bladder. On the other hand, another study that collected urinary samples from 21 IC patients and 20 matched controls found no significant differences in urinary microbiota [37]. The conflicting conclusions between these two recent studies highlight controversy surrounding this fairly new field and the need for a more comprehensive longitudinal study.

CHALLENGES AND CONSIDERATION IN URINE BIOMARKER DEVELOPMENT

Despite the promising potential of urinary biomarkers, there are several precautions to consider. One important factor that can affect biomarker outcomes is age. Studies have shown that the maturing kidney can affect biomarker levels and interpretation, suggesting that age-specific biomarker reference ranges may be needed for certain diseases [38]. Furthermore, baseline metabolites have been shown to be different among different age-groups, which may highlight carefully establishing different age groups should be warranted when conducting urinalysis [39]. Gender is another factor to be considered when establishing reference values for urinary biomarkers [14]. Proteomic analysis of female and male urine observed different patterns and variations of proteins

[40]. Given that urine sample can have huge variation in concentration of proteins or metabolites due to the fluid consumption, special care should be taken to data normalization methods to reduce any potential artifacts [41]. Furthermore, external factors that are dependent on individuals can influence the expression of urinary biomarkers. Studies have shown differences in expression of urinary biomarkers in patients who have undergone cisplatin therapy [14]. Certain procedures can also affect urinary levels of metabolites; another study found increased urinary neurotrophin in women with stress urinary incontinence after a midurethral sling procedure [42]. This suggests that in order to effectively utilize urinalysis, there needs to be a comprehensive understanding of the fluctuations in biomarkers that can occur within each individual.

BIOSENSOR FOR THE DETECTION OF URINARY BIOMARKERS

Biosensors are an arising field of great interest when it comes to detecting and monitoring markers in biofluids, such as sweat and urine. Wearable sensors are particularly garnering attention because they can be portable, convenient, non-invasive, and provide real-time evaluation of important biomarkers [43]. In addition to its detection and monitoring benefits, biosensors could also be integrated with therapeutic drugs to monitor for response to treatments [44]. The potential for sensors can extend to many different types of situations. For example, biosensors can be developed into electrochemical sensors or fluid measuring sensors [45]. These biosensors can be constructed to detect various compounds, such as antigens, biomarkers, and bacterial enzymes.

With the advent of smart technologies, there has been exciting developments in utilizing such devices in healthcare as well. In 2015, a team of biomedical engineers at the University of Arizona was able to develop a highly-sensitive and cost-efficient paper-based analytical device (μPad) that could monitor urine for urinary tract infection (UTI) and gonorrhea [46]. A recent study developed a similar device that quantified β-glucuronidase, an enzyme released by 95% of *E. coli*, the bacteria that causes UTI [47]. In addition to these urinalysis-based detection devices, several others have been developed to detect other compounds. A study by the Southern Taiwan University of Science and Technology developed an ultraportable microsensor-lined biosensor that can actually quantify the presence of Gal-1, a protein biomarker indicative of multiple oncological conditions, including bladder cancer [48]. These novel devices only scratch the surface of the great potential for biosensors.

The use of technology can also extend beyond detection. Taking advantage of the fact that most people use a smartphone, a study in the United Kingdom crowdsourced members of the public to grade immunohistochemistry stains of bladder cancer tumor microarrays [49]. Surprisingly, this was found to be a potentially accurate way to screen immunohistochemistry (IHC) data and speed biomarker discovery.

DIGITAL APPLICATIONS OF BIOSENSORS

The rise of digital applications of biosensors is also a rising field of great interest. There are incredible possibilities that comes from being able to use everyday technology to monitor health. Not only would this reduce risks to patients and lower healthcare costs, but it could also lead to an immense wealth of data that can be used to pioneer science even further. The most commonly used interactive app for monitoring has been in diabetes. Currently, there are two major mobile apps that incorporate self-monitoring of blood glucose (SMBG) recording and insulin bolus calculators. These are Diabeo (Voluntis) and Diabetes Interactive Diary (DID) [50]. Studies have shown that monitoring of patients with type 1 diabetes by using Diabeo can lead to substantial improvement in metabolic control in chronic poorly controlled patients without requiring more medical time and at a lower cost than typical standard care [51]. Similar studies with DID show that it can reduce risk of moderate to severe hypoglycemia while also improving quality of life [52]. However, these apps are still a work in progress and have only shown improvements in certain areas of diabetes monitoring. With rapid technological innovation and progress, the focus on making these apps better should be continued.

In addition to real-time monitoring of chronic diseases, digital applications can lead to an enormous wealth of health data that can be used for more comprehensive studies. For instance, adding internet of things (IoT) capabilities to commercially used continuous glucose monitors (CGM) can lead to both the monitoring of patients remotely and crowdsourcing of that data [53]. As personal tech becomes increasingly embedded in the lives of patients, digital phenotypes can be captured to enhance health and wellness [54]. There is one caveat with this integration of technologies with personal health. As information is formed and sourced, careful attention must be paid to decentralizing databases and ensuring that patient health information remains private and protected. With proper cyber security, the promises of digital health monitoring are endless.

CONCLUDING REMARKS

Advances in urine-based molecular profiling technologies, the development of biosensor targeting disease-specific biomarkers and the wirelessly connected medical device would lead to smart diagnosis and monitoring for patients affected by bladder diseases. Thanks to rigorous efforts of scientists and urologists including us to define biomarkers for bladder diseases such as bladder cancer and other types of bladder dysfunction, we have better idea how to manage those bladder diseases. As we discussed in this paper, the current evidence suggests the integration of multi-omics profiling-based characterization of bladder diseases and application of urinary biomarkers into smart medical device could lead future tools for patient care.

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