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Review Article

Development of Non-Invasive Biosensor Devices for the Detection of Bladder Cancer in Urine

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ABSTRACT

Bladder cancer (BC) is the fourth most common malignant tumor in the United States. It is the second most common cancer of the urinary system, accounting for 7% of all new cancer cases. It is also the fifth deadliest cancer, accounting for 4% of all cancer-related deaths in the United States. Our efforts to reduce costs of BC diagnosis and improve patients' quality of life by avoiding unnecessary invasive diagnostic tests resulted in findings of promising urinary biomarkers for the detection of BC. This short review article aims to provide the current status of non-invasive biosensor device development for detection of BC, in particular, in patients' urine samples.

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Bladder Cancer

Bladder cancer (BC) is the fourth most common malignant tumor in the United States. It is the second most common cancer of the urinary system, accounting for 7% of all new cancer cases. It is also the fifth deadliest cancer, accounting for 4% of all cancer-related deaths in the United States. The male to female ratio of morbidity and mortality is about 3:1 [1]. Risk factors are related to age, family history, and genetic factors, environment, diet, and lifestyle, especially smoking and exposure to aromatic amines [2-5]. Cigarette smoking is the single greatest risk factor for BC [6, 7]. Smokers are more than twice as likely to develop BC than non-smokers [8]. In muscle-invasive and metastatic bladder cancer, there is a causal relationship between tobacco exposure and cancer, of which the principal preventable risk factor for the muscle-invasive disease is active and passive smoking [6, 7]. There is a well-established relationship between schistosomiasis and squamous cell carcinoma of the bladder [6]. Exposure to chemotherapy and pelvic radiation is also considered as a risk factor for BC [6]. Other known risk

factors include the ingestion of high levels of arsenic or significant usage of pain relievers containing finazepine [3, 9].

Bladder Cancer Diagnosis

The most common symptom of BC is painless hematuria, which should be followed by physical examination, cystoscopy, urinary cytology, and imaging of the upper urinary tract (UUT). Both computed tomography (CT) and magnetic resonance imaging (MRI) may be used to detect stage T3b or higher BC. Studies suggest that FDG-PET/CT might have potential clinical use for staging metastatic BC [10, 11].

Treatment for Bladder Cancer

For treatments of non-muscle invasive BC (NMIBC, stage Ta-T1 and carcinoma in situ (CIS)), a combination of conventional surgery (transurethral resection of bladder tumor (TURBT)), intravesical chemotherapy, and immunotherapy are applied [12]. For patients with

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high-risk diseases and those whose diseases are difficult to treat, cystectomy may be necessary [12].

For the treatment of muscle-invasive BC (MIBC, stage T2 to T3 and CIS), the two principal treatment choices are radical cystectomy (RC) and TURBT, with concurrent radiation therapy using a radiosensitizer and systemic chemotherapy (multi-modality therapy) [6]. Standard treatment for patients with MIBC is radical cystectomy; however, this treatment only provides 5-year survival in about 50% of patients. The use of chemotherapy is beneficial to improving both survival and patient outcomes [6]. A paper published in 2005 demonstrated a significant 5% absolute survival benefit in favor of neoadjuvant chemotherapy (NAC) [13]. This study also showed that only cisplatin combined chemotherapy could produce significant therapeutic effects [13].

More modern chemotherapeutic regimens, such as gemcitabine/cisplatin, which are effective in metastatic disease, have also shown efficacy in the neoadjuvant setting [14, 15]. Adjuvant chemotherapy (AC) after RC can be used for high-risk M0 patients, such as those with pT3/4 and/or lymph node-positive (N+) disease [16]. A retrospective study from 2009 compared the long-term outcome of preoperative versus no preoperative radiation therapy (RT) in clinical T1-3 tumors [17]. The study showed that preoperative RT could lead to a reduction in order to prolong progression-free survival (PFS) [17]. External beam radiation therapy (EBRT) may be given preoperatively (neoadjuvant), postoperatively (adjuvant), or as radical treatment for patients with muscle-infiltrating cancer with no proven metastases to lymph nodes or distant metastases [6]. For the patients without lymph node metastasis or distant metastasis, external beam radiation therapy (EBRT) can be given before operation (neoadjuvant), after operation (adjuvant) or as radical treatment [6].

Metastatic BC is a serious disease. Before the development of effective chemotherapy, patients with the metastatic disease rarely had a median survival that exceeded 3-6 months [6]. The 5-year survival rate of patients with metastatic bladder cancer was previously estimated to be 5% [18]. The current estimated 5-year survival is 10% [2]. Chemotherapy is still the standard treatment for metastatic bladder cancer; however, patients with ineffective or poorly tolerated chemotherapy still have poor prognosis [6]. The recent discovery of innovative immunotherapies based on the programmed death ligand-1 (PD-L1) inhibitors, such as avelumab, durvalumab, nivolumab and atezolizumab, are promising for patients who are considered unsuitable for chemotherapy [19, 20].

Bladder Cancer Classification

Traditionally, BC has been classified into NMIBC or MIBC, and most of BC belong to transitional cell carcinoma (TCC) [21]. Recent efforts classifying phenotypes based on molecular characteristics reported several BC phenotypes. For example, there are University of North Carolina (UNC), MD Anderson Cancer Center (MDA), The Cancer Genome Atlas (TCGA), Lund University (Lund), and Broad Institute of Massachusetts Institute of Technology and Harvard University (Broad) classification.

Biosensors for Detection of Bladder Cancers Using Urine

Researchers' efforts to reduce costs of BC diagnosis and improve patients' quality of life by avoiding unnecessary invasive diagnostic tests resulted in findings of promising urinary biomarkers for the detection of BC such as telomerase, nuclear matrix protein 22 (NMP22), cytokeratin 19, etc. [22]. Along with the discovery of urinary biomarkers, biosensors for detecting those biomarkers have been developed, which can offer low detection limits, a wide linear response range, good stability and, good reproducibility [23].

The biosensor is a device that transforms a biological response into a quantifiable and processable signal. The key components of biosensors are a bioreceptor and a transducer. The bioreceptor is a molecule that specifically recognizes the analyte, such as enzyme, antibody, and protein, while the transducer is an element that converts the bio-recognition event into a measurable signal, which classifies the types of the biosensors. The biosensors for BC diagnosis with voided urine can be typically categorized into two groups, an optical biosensor and an electrochemical biosensor. The optical biosensor emits an optical signal which is proportional to the concentration of a measure substance (analyte) whereas electrochemical biosensor converts biological event to an electronic signal.

From early research, the optical biosensor has been the most commonly used biosensor to screen target biomarkers in urine [24-27]. Shin *et al.* developed the optical biosensor with silicon microring resonators to detect DNA biomarkers (fibroblast growth factor receptor 3 and Harvey RAS) in urine [24]. They successfully demonstrated linear wavelength shifts pattern in a different concentration range of target biomarkers. Due to the intrinsic advantage of silicon fabrication, a highly sensitive and specific platform was achieved for diagnosis and surveillance of BC. As another prominent urinary biomarker for BC diagnosis, telomerase activity's detection was conducted using a fluorescence method and a colorimetric method on the optical biosensor [25, 27].

Xu *et al.* established the label-free colorimetric optical biosensor based on the merits of hemin-graphene nanomaterial, such as easiness of synthesis, stability, and reliability. The sensor was validated its performance by changing colour from light blue to dark blue as telomerase activity increased due to chromogenic reaction. To detect telomerase activity, not only the optical biosensor but also the electrochemical biosensor can be applied with good reproducibility and selectivity [28]. The electrochemical biosensor was realized using methylene blue (MB) as a G-quadruplex binding on indium tin oxide. The large amount of MB bounded to G-quadruplexes under the activity of telomerase resulted in sharply decreasing diffusion current of MB. Ma *et al.* proved the effectiveness of the electrochemical immunosensor (affinity-biosensor) for detecting NMP22 on graphene oxide-tetraethylene pentaamine and trimetallic AuPdPt nanoparticles [29]. It achieved high accuracy in real urine sample, showing differential pulse voltammetry responses of the immunosensor towards different concentrations of NMP22. Another transducer, a high stability indium gallium zinc oxide field effect transistor (IGZO-FET), was utilized to build the electrochemical biosensor for capturing the NMP22 [30]. The IGZO-FET sensor was tested with real urine samples from BC patients,

and it was able to detect NMP22 with high sensitivity, selectivity, and detection limit.

Point-of-Care Detection Device for Bladder Cancer Using Urine

It is widely known that screening and early diagnosis of cancer are key to improving the likelihood of recovery and 5-year survival. Consequently, many studies have been conducted to develop a point-of-care (POC) device for BC detection, which responds fast and diagnoses non-invasively. The POC device should be low-cost, portable, reliable, and disposable.

To satisfy such requirements, a lab-on-a chip (LOC) technique has been investigated. It is reported that Liang *et al.* developed an integrated double-filtration microfluidic LOC device, which isolates, enriches, and quantifies urinary extracellular vesicles to assist in screening for bladder cancer at the POC [31]. The device detects the multiple biomarkers of BC and transfers diagnostic data in the patient's urine sample to the user through wireless communication using a cell phone. In addition, Geng *et al.* developed a microfluidic chip using polydimethylsiloxane (PDMS) to form a channel using a cast molding method, with a sliding glass coverslip as the substrate to detect BC biomarkers [32]. The chip converts different concentrations of biomarkers in urine to fluorescence intensity of image, which is a quantifiable signal. Chuang *et al.* have reported that an immunosensor chip as POC test device for detection of BC biomarker (Galectin-1 protein) realized with a photolithographically patterned gold microelectrode array [33]. They also designed a portable impedance measurement readout device for this chip, which transferred the data to the computer for improved monitoring.

A few commercial POC diagnosis devices are approved by FDA for BC detection and surveillance, such as UBC rapid, NMP22 BladderChek and BTA-STAT. UBC rapid detects cytokeratin fragments 8 and 18 in the urine to diagnose BC. It takes only 10 minutes to get the result and has the closest sensitivity of cytology in high-grade tumors [34]. NMP22 BladderCheck utilizes the detection of NMP22 as can be guessed from its name, and it takes 30 minutes to diagnose with high specificity. BTA Stat Test detects the presence of bladder tumor associated antigen (BTA) in 5 minutes. Some insists that it has higher potential in detection of early grade BC than cytology [35]. Much of the research has been done to evaluate the commercial test kit by ethnicity, case number, gender and so on [36-41].

As of now, cytology is the only recommended guidelines internationally for diagnosing BC. The aforementioned commercial POC devices have been used in combination with cystoscopy to improve the accuracy. To use the commercial POC as a standalone BC diagnosis devices, there should be more meta-analyses with various conditions such as age, gender, genetic factors, environment, and lifestyle.

Conflicts of Interest

None.

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Author Contributions

Research conception and design: JK, SJ. Data analysis and interpretation: HP, JK. Drafting of the manuscript: HP, JK. Critical revision of the manuscript: JK, SJ. Supervision: JK, SJ. Approval of the final manuscript: all authors.

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