Journal of Cancer Research and Treatment, 2018, Vol. 6, No. 2, 34-38
Available online at http://pubs.sciepub.com/jcrt/6/2/2

DOI:10.12691/jcrt-6-2-2

Biomarkers for Bladder Cancer: Present Challenges and Recent Developments

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Abstract Bladder cancer is the most common carcinoma of the urinary system. Early detection and diagnosis of bladder cancer is a major challenge. Urine cytology has low sensitivity and specificity, especially for low-grade tumors. Cystoscopy is the gold standard for the detection and follow-up of bladder tumors. However, it is an expensive and invasive procedure and can fail to detect many bladder lesions such as carcinoma *in situ* (CIS). It is thus an unmet need to identify a robust set of biomarkers, which can improve the current diagnostic practice. During the last one decade, several bladder tumor markers have been identified for diagnosis, however, none of these diagnostic markers offer sufficient sensitivity and specificity to be routinely used in the clinics. Recent advancement in mass spectrometry (MS)-based quantitative proteomics has emerged as a powerful method to discover wide range of proteins in complex biological samples. In this review, we summarize the status of biomarkers currently identified and used for bladder cancer diagnosis.

Keywords: *bladder cancer, biomarker, diagnosis, non-invasive, mass spectrometry*

Cite This Article: Barnali Deb and Prashant Kumar, "Biomarkers for Bladder Cancer: Present Challenges and Recent Developments." *Journal of Cancer Research and Treatment*, vol. 6, no. 2 (2018): 34-38. doi: 10.12691/jcrt-6-2-2.

1. Introduction

Bladder cancer is the second most common genitourinary tract cancer worldwide and it results in significant morbidity and mortality [1,2]. These tumors have a range of manifestations. The papillary tumors are mostly well differentiated as flat tumors and the higher grade tumors are poorly differentiated and highly aggressive. Bladder carcinoma is divided into two clinicopathological subtypes namely; non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). Majority of the transitional cell carcinomas are NMIBCs (70% – 75%) while approximately 25% – 30% cases are MIBCs at diagnosis [3]. Carcinoma *in situ* (CIS) of the bladder are higher grade non-papillary; non-invasive tumors, which harbor increased risk of progression to muscle-invasive stages as well as recurrence [4,5]. The NMIBCs are treated by Transurethral Resection of Bladder Tumor (TURBT) at diagnosis [6]. However, 50% of these patients have recurrence with a short period of approximately 12 months [7]. Radical cystectomy has been the choice of treatment for MIBCs since decades [8]. The overall 5-year survival of patients is relatively high in superficial bladder carcinoma, however, most of these patients (40% – 80%) will progress to higher stages or recur [9]. In the case of high grade MIBCs, once the cancer progresses to metastatic stages, the overall 5-year survival decreases radically to only 6% [10].

Diagnosis of bladder carcinoma has not evolved considerably in the past decades. Cystoscopy remains to be the major modality for bladder carcinoma diagnosis as well as follow-up [11,12]. However, it is a highly invasive procedure. Thus, owing to high recurrence rate and the frequent need for follow-up implies a very high financial burden on individual and families. Cytological examinations is another additive approach of diagnosis; however its major limitation being poor sensitivity especially for low-grade tumors. Developing cost-effective as well as non-invasive strategies to advance the detection of bladder tumors is the main focus of biomarker discovery. Researchers have long attempted to identify urinary biomarkers for the detection of bladder carcinoma as a potential alternative to cystoscopy [13]. Urinary biomarkers, in particular may be a helpful diagnostic adjunct in bladder cancer, as urine-based diagnostic tests will offer a non-invasive and cost-effective means of detection of carcinoma. With the growth in unbiased profiling techniques (genomics, transcriptomics, proteomics and metabolomics profiling), the data-driven discovery of novel biomarkers has recently gained momentum. The current approaches employed for the early detection of bladder cancer are depicted in Figure 1. In this article, we summarized the challenges and the recent development in the area of bladder biomarker discovery.

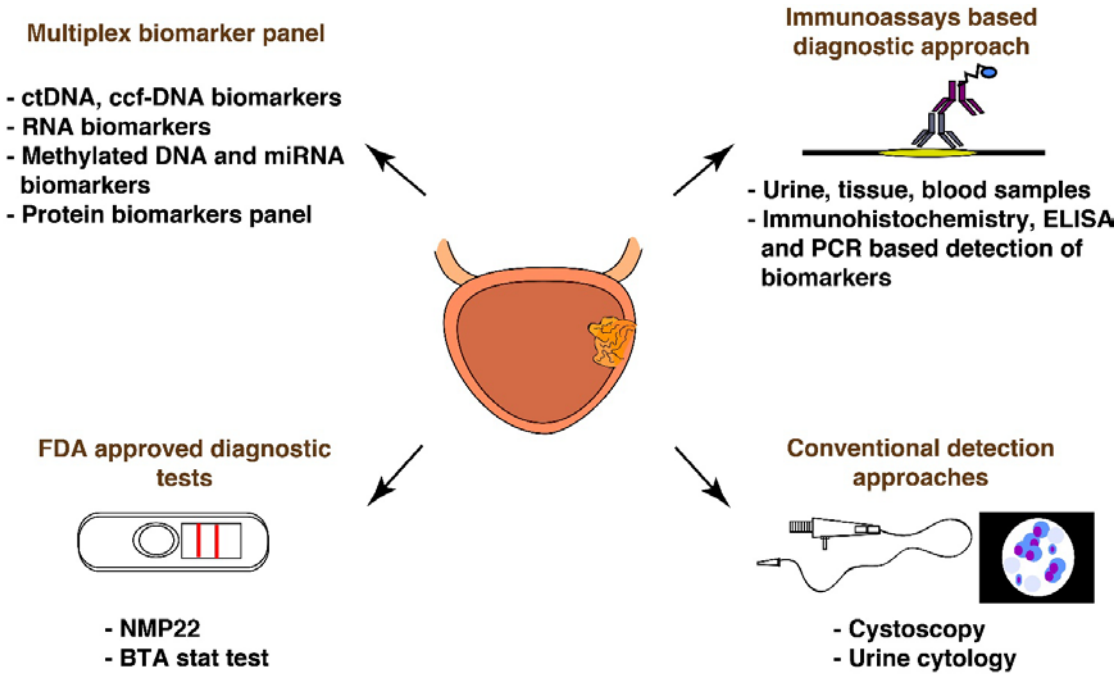

Figure 1. Current approaches for early detection of bladder cancer

2. Proteomics Approaches in Biomarker Discover

In this era of proteomics, the discovery of reliable, sensitive and specific biomarkers is beaming to gain momentum for the better understanding of cancer pathobiology. Mass-spectrometry is a reliable method for the unbiased identification and absolute quantification of proteins, which is thus emerging as an alternative to the immunoassays such as ELISA.

The proteomic profiling of body fluids such as urine and identifying biomarkers with potential clinical utility in non-invasive assays could be an excellent approach for bladder cancer diagnosis. The establishment of a normal urinary proteome has evolved hand-in-hand with the improvement of proteomic techniques. In a study by Thongboonkerd *et al*., studies exploited LC-MS/MS and identified over 100 proteins from unfractionated normal urine [14]. In another multicentre study were the researchers have identified proteins panel such as fibrinogen chains, apolipoprotein A1 (ApoA-1), beta-2-macroglobulin and basement membrane-specific heparan sulfate proteoglycan [15]. The findings from urine proteomics were further verified at the tissue level in several studies [16,17,18]. Based on the urine proteome studies, the secretome of the tumor cell lines were also utilized. Association of TACSTD2 with bladder cancer has been investigated and highlighted the prospective of non-invasive bladder cancer diagnosis using human urinary microparticle [19]. Plasma proteomics studies have also been conducted on bladder cancer patients, however, are comparatively lagging as compared to urine proteomics. Altered expression of S100A4, S100A8, S100A9, carbonic anhydrase I (CA I) and annexin V proteins in pre-operative bladder cancer serum compared to healthy controls were observed. Further it was validated on the follow-up post-operative patients that S100A4, S100A8, S100A9, carbonic anhydrase I (CA I) were significantly reduced while annexin V was progressively increased [20].

3. Biomarkers in Bladder Cancer

Highly specific and sensitive biomarkers for the prediction of clinical course of the patient during and after receiving therapy or treatment are immensely requisite. In previous studies, survivin, fascin, MCT1, MCT4 and CD147 were observed to be associated to bladder carcinoma development and progression [21,22,23]. Recently, in a meta-analysis of twenty-four studies on CD147 expression revealed that its expression in bladder carcinoma patients was significantly higher than in non-cancer tissues. CD147 expression was found to be closely associated with prognostic and clinicopathological characteristics and response to chemotherapy [24]. In a study from urine samples, the prognostic value of matrix metalloproteinase-9 (MMP-9) was evaluated where MMP-9 was measured in urine from bladder carcinoma patients and was suggested as an independent prognostic marker of poor survival [25].

In another study, nicotinamide N-methyltransferase (NNMT) expression was evaluated in urine samples of 55 bladder carcinoma patients. The data revealed that NNMT could be used to diagnose the early NMIBCs through urine-based tests. In another meta-analysis, it was suggested that HA/HAase could be used as a biomarker for the diagnosis of bladder carcinoma [26]. Fast-track diagnosis of bladder carcinoma in a non-invasive approach has utilized urinary markers for affirmative use as they contain proteins of malignant exfoliated cells from the primary tumors.

Several other single protein biomarkers like alpha-1-anti trypsin (SERPINA1), apolipoprotein A1 (APOA1), transforming growth factor-beta-induced protein IG-H3 (BIGH3), keratin, type I cytoskeletal 19 (CYFRA 21-1),

protein/nucleic acid deglycase DJ-1 (DJ1), stathmin-1 (STMN1) and telomerase (TERT) have been proposed for the detection of bladder carcinomas [27-33].

In addition, microRNAs have been implicated as potential biomarkers in tumor initiation, regulation and progression [34,35]. The miR-182/miR-100 ratio has been studied to serve as a biomarker for bladder carcinoma diagnosis and survival prediction [36]. In a panel study of miRNAs, six-miRNAs (miR-152, miR-148b-3p, miR-3187-3p, miR-15b-5p, miR-27a-3p and miR-30a-5p) were considered as a serum signature for bladder carcinoma diagnosis with high sensitivity (sensitivity and specificity of 90%) [37]. miR-126 and miR-96 were also reported to be potential diagnostic markers in bladder carcinoma [38,39].

Among the FDA approved urine based tests, bladder tumor antigens (BTA stat® Test; Bion Diagnostic Sciences, Redmond, Washington, USA) has been reported to be a simple and rapid immune chromatographic test that detects bladder tumors in human urine [40]. Low-specificity of the BTA tests is associated with benign genitourinary conditions as well as intravesicle BCG treatment [41]. Moreover, false-positive results of NMP22 in patients followed-up (for a mean range of approximately 28 months) for bladder carcinoma correlate with future recurrences [42]. UBC tests in combination with bladder washing cytology revealed higher sensitivity in detecting low- and high-grade tumors but at the expense of a lower specificity as compared to urine cytology. Thus, currently cystoscopy cannot be replaced by any of the evaluated methods [43].

4. Urinary Biomarker Panels for Bladder Carcinoma

A widespread approach based on multiplex biomarker panel offers accurate and sensitive detection and diagnosis of bladder cancer patients.

In a study by Kumar *et al*., a robust set of urine biomarkers for bladder cancer detection was identified. The study employed mass spectrometry-based quantitative proteomics approach to identify a panel of potential biomarkers: Coronin-1A, Apolipoprotein A4, Semenogelin-2, Gamma synuclein and DJ-1/PARK7. A multi-analyte assay was established for both non-muscle invasive (Ta/T1) and muscle invasive (T2/T3) bladder cancer detection in urine using MS-based stable isotope labelling approach for the biomarkers discovery. These biomarkers panels were validated in a large cohort of urine samples from 66 healthy subjects, 110 NMIBC (Ta/T1) patients and 63 MIBC (T2/T3) patients using RT-PCR, western blot and ELISA. In the western blot based validation, all the bladder carcinoma patients displayed elevated expression of at least three out of the five biomarkers. This panel achieved an AUC 0.92 and 0.98 respectively using ELISA and western blot data (79.2% and 93.9% sensitivity; 100% and 96.7% specificity). This panel also involves the potential for follow-up of patients and screening asymptomatic subject at high-risk of developing bladder carcinoma. Moreover, these five biomarkers are also able to differentiate between bladder carcinoma patients and patients with different benign conditions such as; inflammation of the bladder, benign prostrate hyperplasia or nephrolithiasis perhaps associated with hematuria [44].

5. Conclusion

Escalating the understanding of the proteomic landscape of bladder carcinoma anticipates the discoveries of new proteomic biomarkers for bladder cancer detection and surveillance. Additionally, it has improved the usefulness and accuracy of diagnostic and prognostic significance in the prediction of cancer progression. However, large cohorts of patient's biological samples and complete clinical annotation are required for validation. This remains a major challenge for the biomarkers discovery and validation. Tumor heterogeneity does not allow the use of single biomarker; hence multiplex biomarker panel will offer high accuracy for the diagnosis of bladder cancer patients. In addition, multiple protein signatures may target tumor heterogeneity and offer a better prospect for non-invasive detection of bladder cancer.

Acknowledgements

PK is a recipient of the Ramanujan Fellowship awarded by Department of Science and Technology (DST), Government of India. BD is a recipient of INSPIRE Fellowship from Department of Science and Technology (DST), Government of India.

Disclosure

All authors declare that no conflict of interest exists.

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