Review Article

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INVESTIGATIVE AND CLINICAL UROLOGY
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Unmasking molecular profiles of bladder cancer

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Precision medicine is designed to tailor treatments for individual patients by factoring in each person's specific biology and mechanism of disease. This paradigm shifted from a "one size fits all" approach to "personalized and precision care" requires multiple layers of molecular profiling of biomarkers for accurate diagnosis and prediction of treatment responses. Intensive studies are also being performed to understand the complex and dynamic molecular profiles of bladder cancer. These efforts involve looking bladder cancer mechanism at the multiple levels of the genome, epigenome, transcriptome, proteome, lipidome, metabolome etc. The aim of this short review is to outline the current technologies being used to investigate molecular profiles and discuss biomarker candidates that have been investigated as possible diagnostic and prognostic indicators of bladder cancer.

Keywords: Biomarkers; Liquid biopsy; Precision medicine; Urinary bladder neoplasms

INTRODUCTION

Molecular profiling is the global analysis of genomic, epigenetic, transcriptomic, proteomic and metabolomics profiles. It represents a critical pre-requisite for the future success in developing tailored treatment strategies for individual patient [1].

Bladder cancer (BC) is the second most common urological malignancy and requires the most expensive care [2]. Since the time of diagnosis directly influences survival rate, early detection and life-long surveillance of BC is very important. Microhematuria testing and urine cytology are currently the most widely used diagnostic tools for BC; however, these methods are limited due to its costliness and invasiveness [3].

Clinico-pathological features classify BC into two distinct groups; non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). MIBC is the main cause of cancer-specific deaths among BC patients [4,5]. Although NMIBC has better survival than MIBC and other malignancies, 30% to 50% of patients will experience recurrence throughout the remainder of their lives. This rate accounts for cases with surgical resection of the primary tumor and adjuvant therapy. About 10% to 20% of these recurrences will progress to MIBC [6,7]. Therefore, the odds of recurrence and progression in BC have been major challenges for patients and physicians. While the introduction of cisplatin-based chemotherapy has increased the chances of recurrence-free survival, there have been no new U.S. Food and Drug Administration (FDA)-approved therapies for those who cannot tolerate or fail to respond to the treatment [8].

BC is also known as a highly immunogenic cancer type that has a higher rate of mutation than other types

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of cancer. In BC, various types of tumor-infiltrating immune cells have been reported. The signaling pathways between the tumor and immune cells have been studied. Immunotherapy has been widely accepted as a treatment option for BC and recent new immunotherapies have been studied in various BC clinical trials. New cancer immunotherapies have been tested and applied using clinically immune checkpoints blockers against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), checkpoint programmed death–1 (PD-1), or programmed death receptor ligand (PD-L1) etc. [9]. A portion of patients with moderate to high-grade NMIBC has been given intravesical immunotherapy with bacillus Calmette-Guérin (BCG) [10-12]. However, this has not been shown to be effective in those with MIBC.

Muscle-invasive disease is managed through cystectomy with or without systemic cisplatin-based chemotherapy. Despite this, it is still not possible to distinguish between patients who will benefit and those who will not from the chemotherapy. It would be tremendously useful and innovative to identify reliable biomarkers that could enable clinicians to distinguish these patients and would provide optimal and personalized treatment plans for each individual case. However, to ensure that the path from discovery to clinical diagnostics continues to be successfully paved, the analytic, diagnostic, and regulatory requirements of a clinical assay need to be understood. Furthermore, active partnerships with industry and effective communication between clinicians and scientists are necessary.

In this short review article, we will provide a general overview of classical and current technologies and molecular findings in BC research. We will also summarize the clinical significance and impacts of these discoveries for future precision medicine in BC patient management and treatment. A simplified diagram shows a series of approaches to precision medicine for BC patients that will be discussed in this short review (Fig. 1).

ESTABLISHED TECHNOLOGY AND RECENT BREAKTHROUGHS IN BLADDER CANCER RESEARCH

Improved understanding of the molecular classification of BC could provide great benefits in the clinical setting. It would serve to bring improved insight and decision-making regarding diagnosis, prognosis, and treatment. The Cancer Genome Atlas (TCGA) includes such comprehensive genomic analyses; such as whole-exome sequencing, mRNA and microRNA (miRNA) sequencing, DNA methylation analysis, and proteomic analysis [13].

Epigenetic modifications include DNA methylation, histone modifications, miRNA, and nucleosome positioning etc. Expression changes and genetic mutations of epigenetic regulatory genes such as DNA methyltransferases, chromatin modifiers and remodelers have been found [14]. Epigenetic alterations contribute to gene expression levels during cancer initiation and progression [14]. As an epigenetic regulator, miRNAs regulate gene expression. For example, miRNAs such as *miR-101, miR-21, miR-148a, miR-126, miR-152,* and *miR-29a/29b/29c* etc. can repress epigenetic regulators like EZH2 (H3K27 methyltransferase) [15], DNMT1 (DNA methyltransferase) [16,17], and DNMT3A/3B [18].

For clinical proteomics, there have been a series of mass spectrometry-based techniques used; including liquid chromatography-mass spectrometry (LC-MS/MS), capillary electrophoresis-mass spectrometry (CE-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, matrix assisted laser de- sorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and nano-liquid chromatography-tandem mass spectrometry (nano-MALDI-MS) [19]. Each of these proteome analytic technologies has their own advantages and disadvantages. LC-MS is sensitive yet expensive, while CE-MS is cheaper than the others. MALDI-TOF MS is relatively cheap and simple; however, nano-MALDI-MS is known more sensitive than MALDI-TOF MS [20-22].

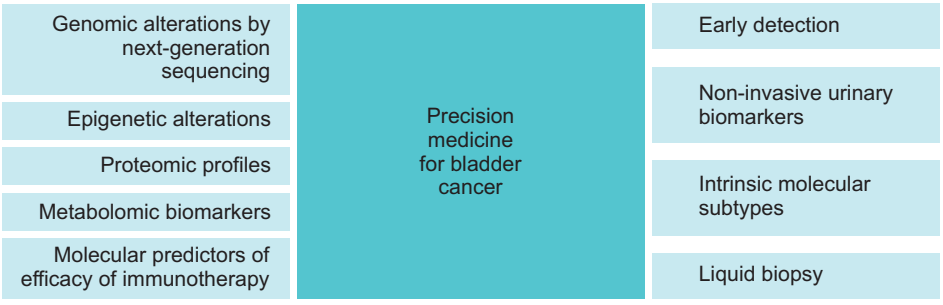

Fig. 1. A diagram showing current translational and clinical approaches to precision medicine for bladder cancer patients.

In order to better understand cancer metabolism, a metabolomics approach has often been utilized. It has since provided information and insight on global chemical fingerprints associated with the physiological and pathological states of cancer [23]. Using various metabolomics technologies, including nuclear magnetic resonance spectroscopy (NMR, also known as, ^{1}H-NMR), gas chromatography mass spectrometry (GC-MS), direct flow injection mass spectrometry, inductively coupled plasma mass spectrometry, and high performance liquid chromatography, possible metabolic fingerprints associated with BC have been tried to be identified.

In addition to analytic technologies, an adequate *ex vivo* BC model, which is currently a major limitation towards identifying predictive biomarkers, is needed to better understand the molecular mechanisms of BC. A series of previous studies have suggested that cancer cells in three dimensional (3D) culture systems respond differently from those in 2D cultures [24,25]. The lack of clinico-physiology of cell line models, and *in vivo* models (e.g., animal and patient-derived xenografts) have greatly limited urological research. However, recent developments of 3D organoids received from patients seem to provide a realistic bladder microenvironment. This pre-clinical BC mimic model has the potential to be used as a method of therapeutic pre-screening for individual patients. Using a rotating wall vessel bioreactor under microgravity conditions, BC organoids have been previously developed from cell line or tissue biopsy samples. Well-constructed 3D organoids play as a functional unit and closely mimic the tissue of origin. 3D organoids are characterized to exhibit 4–6 multiple cell layers, and this amenability allows organoids to be powerful pre-clinical BC models.

Successful construction of BC patient-derived 3D organoids has further broadened our understanding of the molecular mechanisms and drivers that promote BC development. DNA sequencing analysis of patient-derived 3D organoids suggest that these organoids share very similar mutational profiles with those of real tumor samples [26]. Thus, it is speculated that genetic information from patient organoids can be used for personalized drug-prescreening and predicting responses to treatment. While these experimental results need intensive follow-up validation, 3D organoid-based drug response assays seem very promising and will undeniably benefit clinical decisions. If successful, a patient-derived organoid biobank could facilitate personalized medicine in BC research.

APPLICATION TO BLADDER CANCER STUDY

1. Urine biomarkers for early detection of bladder cancer

Molecular profiling methods have been used for phenotyping BC. Recent reports have shown that modern classification of BC into various distinct subtypes is associated with responses to chemotherapy and immune checkpoint inhibitors. There are various commercial BC biomarkers that are currently being used in the clinical setting. They include nuclear-matrix protein 22 (NMP22), UroVysion test, and others. NMP22 levels are shown to be associated with disease recurrence and progression [27]. The UroVysion test is a multicolor fluorescence in situ hybridization (FISH) assay designed to detect aneuploidy of chromosomes 3, 7, or 17, and/or the loss of the 9p21 locus [28]. A series of recent reports showed that utilization of FISH-based assay may be used as an additional tool for sub-classification of patients or determining a treatment option [29-32].

Because it is stored in the bladder before micturition, urine is an attractive non-invasive biomarker resource for BC. As potential urinary BC biomarkers, perturbed levels of urinary miRNAs and DNA methylation have been reported. The most promising urinary BC biomarkers include: *miRNA-96, miRNA-138, miRNA-126, miRNA-182, miRNA-143, miRNA-222, miRNA-21, miRNA-133b, miRNA-518c-5p, miRNA-452, miRNA-129, miRNA-200c, miRNA-99a, miRNA-100*, and *miRNA-29c* [33].

The methylation statuses of *SALL3, CFTR, ABCC6, HPR1, RASSF1A, MT1A, ALX4, CDH13, RPRM, MINT1*, and *BRCA1* in the urine samples of BC patients is also shown to be associated with the disease [34]. In other studies, a specific three-gene panel, consisting of *BCL2, hTERT*, and *DAPK*, was linked with BC in urine [35]. Seven other genes in urinary cell-free DNA (cfDNA) have also been found to be associated with BC. These include *FGFR3, TERT, PIK3CA, TP53, HRAS, RXRA*, and *KDM6A* [36]. This report suggested that one of avenues of biomarker detection includes identifying circulating cell-free tumor DNA.

2. Genomic alterations detected by next-generation sequencing in bladder cancer

BC, and in particular MIBC, has one of the largest mutational burdens of all tumor types studied in the TCGA. The key cause of this is believed to be due to smoking, which leads to the production of reactive oxygen species and resultant DNA damage. TCGA analysis has shown

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that DNA mutation of the *ERCC2* and *APOBEC3B* genes drives BC genomic heterogeneity and disease progression [37,38]. Inactivation of the *TP53* gene is also a well-known mutation in BC. *TP53* gene mutations were observed in approximately 50% of MIBC cases and 20% of NMIBC cases [39]. *TERT* promoter mutations and chromatin-modifying gene mutations are some of the most frequently altered genes, having frequency rates of 73% and 69%, respectively [40,41]. In addition, amplification of *cyclin D1* and *MYC* has been reported in BC [42]. Approximately 20% of NMIBC and MIBC show *cyclin D1* amplification and 13% of MIBC show *MYC* amplification. Activating mutations, fusions, or amplifications of the *EGFR* family have also been reported [43].

DNA alterations of *EGFR*, *ERBB2*, *ERBB3*, and *ERBB4* have been reported in BC [44,45]. *FGFR3* mutations are common in low-grade and low-stage NMIBC. *FGFR3* mutant tumors are known to be associated with higher risk for intravesical recurrence. Both *ERBB2* and *FGFR3* alterations are present in 57% of high-grade NMIBC tumors in a mutually exclusive pattern [46-48].

In BC, oncogenes or tumor suppressor genes, such as those found in the Ras-MEK-ERK pathway and PI3 kinase-AKT-mTOR pathway, are often mutated. PIK3CA and/or P13k/Akt pathway alterations are associated with favorable disease-specific outcomes, independent of tumor and lymph node stage [49]. For better sensitivity and specificity, *FGFR3* mutation levels are sometimes combined with *PIK3CA* or *CDKN2A* alterations [50].

3. Epigenetic alterations in bladder cancer

In this section, we will discuss the epigenetic alterations in BC. Several epigenetic drugs have been used in the clinical and pre-clinical settings. They include: DNMT inhibitors (5-azacytidine and 5-Aza-2'-deoxycytidine), histone deacetylases in-hibitors (SAHA, valproic acid, and romidepsin), and Tazemetostat (an EZH2 inhibitor). These epigenetic drugs are being considered for BC treatment [51].

Modified histone proteins lead to the perturbation of gene expression and other key biological processes [52]. For example, histone modification, such as trimethylation of histone H3 at lysine 4 (H3K4me3), trimethylation on H3 lysine 9 (H3K9me3), lysine 27 (H3K27me3), acetylation on H3 lysine 9 (H3K9Ac), and lysine 27 (H3K27Ac), regulates gene activation [53]. DNA alterations are frequently observed on *histone H3 lysine 27* (*H3K27*) in NMIBC and *histone H3 lysine 4* (*H3K4*) methyltransferase MLL2 in MIBC [54].

DNA hypomethylation of *LINE-1* repetitive element has been found often in BC, and this correlates with activated *MET* oncogene transcription [55]. *RUNX3*-promoter DNA methylation is positively correlated with BC progression and patient survival [56]. It was also reported DNA hypermethylation of *A2BP1, NPTX2, SOX11, PENK, NKX62, DBC1, MYO3A, CA10, POU4F2, HOXA9, MEIS1, GDF15, TMEFF2, VIM, STK11, MSH6, BRCA1, TBX2, TBX3, GATA2, ZIC4, PAX5A, MGMT*, and *IGSF4* [57]. DNA hypermethylation of *CDH1, FHIT, LAMC2, RASSF1A, DAPK, MINT31*, and *SFRP* are also related to BC development and survival [58-60]. Furthermore, DNA methylation signatures of candidate genes were combined and tested to determine if the joint DNA methylation signature shows high sensitivity and specificity in diagnosing BC. The recently identified urinary 3-marker DNA methylation panel (*SOX1, IRAK3*, and *LINE-1-MET*) showed an area under the curve (AUC) of 0.90 (95% confidence interval [CI], 0.86–0.92) with sensitivity of 86% (95% CI, 74%–99%) and specificity of 89% (95% CI, 81%–97%) by the 5-fold cross-validation analysis [61].

4. Molecular predictors of efficacy of BCG therapy

BC is known as one of highly immunogenic cancer types [62,63], and cancer immunotherapies aimed to stimulate the body's immune system (e.g., BCG) have been utilized to treat BC patients [9]. In the last ten years, there have been continued drug developments on new classes of immune checkpoint inhibitors. These include Pembrolizumab, Atezolizumab, Nivolumab, Avelumab, Durvalumab, Ipilimuumab, and Tremelimab etc. The current ongoing clinical trial NCT02324582 was designed to test the efficacy of immune checkpoint inhibitors when combined with BCG in NMIBC. Clinical trials for testing neo-adjuvant and adjuvant immune checkpoint therapy following cystectomy were also designed as well (NCT02451423, NCT02450331).

For over forty years, BCG therapy, the first FDA-approved immunotherapy and the most effective intravesical treatment, has been used to reduce the risk of BC recurrence for high-risk disease. However, approximately 70% of BC patients eventually failed to respond to intravesical BCG therapy and experienced remission after treatment [12]. Interestingly, BC patients who did not respond to BCG therapy exhibit the higher PD-L1 expression than those who responded to it. This suggests that PD-L1 could attenuate responses to BCG therapy by neutralizing T cells and possibly infers a biological role for PD-1/PD-L1 interactions [64].

More recent studies on the genomic alterations correlated with recurrence following BCG therapy suggest a possible association between *ARID1A* mutations and BCG outcomes. When compared to the *ARID1A* wild-type,

ARID1A truncating mutations were significantly associated with an increased risk of recurrence following BCG therapy [65]. Further investigation is needed in determining whether inactivation of *ARID1A*, due to its truncating mutation, can be a reliable predictive biomarker of BCG therapy. *ARID1A* inactivation may also be reversed by epigenetic inhibition, which could benefit patients who fail to respond to BCG treatment [66,67].

5. Proteomics in bladder cancer

The scope of this section is to briefly present on the contribution of proteomics towards BC research. Concerted efforts aimed at discovering biomarkers for BC detection and disease monitoring have led to the discovery of many proteomic biomarkers in the urine, tissue, blood etc. [20,21].

Multiple different approaches have been attempted in order to characterize the BC-specific urine proteome landscape. These include using LC-MS/MS, multiple reaction monitoring, and/or CE-MS. These biomarker candidates were also validated using targeted proteomic techniques such as enzyme linked immunosorbent assay (ELISA).

Global urinary glycoproteomic analysis performed by Kreunin et al. [68] revealed the alpha-1B-glycoprotein as a potential biomarker for BC. A different study found increased levels of urinary fibrinogen, lactate dehydrogenase B, Apo-A1, clusterin, and haptoglobin as being associated with BC [69,70]. Higher levels of histone H2B and nuclear interacting factor 1/Zinc finger 335 were detected in the urine and tumor tissue from BC patients. This was further confirmed through independent ELISA and immunohistochemistry (IHC) analyses. ADAM28, midkine, and hepatocyte growth factor activator inhibitor type 1 (HAI-1) were also found to be significantly elevated in the urine of BC patients, compared to controls [71]. Interleukin 8, matrix metallopeptidase 9, and syndecan-1 are additional metabolites discovered to be heightened in a set of urine samples [72]. Moreover, some secreted proteins from isolated exosomes (e.g., calcium-signal transducer 2) were found in the *in vitro* cell culture as well as in the urine specimens of BC patients.

In addition to urine, the BC-specific proteome has also been obtained from tissue and blood specimens. Dynamin and clusterin were identified as potential biomarkers of BC and were further validated via IHC of tissue arrays. It was found that lowered expression of clusterin is associated with MIBC. Dynamin is negatively correlated with adverse outcomes [71]. From these proteomic analyses, differentially expressed proteins were found when comparing MIBC to NMIBC [73]. Cullin-3 and stathmin-1 were found to have increased expression in BC and are linked with unfavorable outcomes [74]. Differential expression of prelamin-A/C (LMNA), transcription factor AP-1 (JUN), nucleasesensitive element-binding protein 1 (YBOX1), L-selectin (LYAM1), cyclindependent kinase inhibitor 1 (CDN1A), and mothers against decapentaplegic homolog 3 (SMAD3) were reported as tissue-based BC biomarkers. Three proteins, 4F2 cell-surface antigen heavy chain (SLC3A2), stathmin (STMN1) and transgelin-2 (TAGLN2), were revealed as upregulated in BC. The BC-specific blood proteome revealed S100A8 and S100A9 expression as being significantly different between BC and healthy controls (AUC of 0.946) [75].

Evidently, our future research efforts should concentrate on the proper validation of these promising biomarkers through multiple large and independent patient cohorts. Coordinated efforts to utilize existing or developing bio-repositories of clinical samples and perform well-designed proteomic profiling should be maintained. BC molecular subtypes should be considered in the proteomics approach of attributing biological significance to proteomic findings.

6. Metabolomics of bladder cancer

Significant progress has been made from current metabolomic techniques to distinguish BC patients from control subjects. Various techniques such as NMR, GC-MS, and LC-MS have contributed to BC metabolomic research. The intermediates of glucose metabolism, including lactic and citric acids, were found to be significantly different in cancer samples [76]. This phenomenon is widely known as the Warburg effect, which states that cancer cells exhibit increased dependence on the glycolytic pathway for ATP generation, giving rise to enhanced lactic acid production [77]. Hence, measuring lactic acid level in biological samples of BC is useful in BC diagnosis. Increased amino acid levels have been demonstrated in the urine, serum and tissue samples of BC patients. Decreased levels of citric acid and fumarate, which are the metabolic intermediates of aerobic oxidation, were also observed in BC samples [76].

A NMR-derived metabolomics study has also proven to be a potential useful avenue for BC diagnosis [23,78,79]. NMR spectroscopy was found to adequately detect hidden biomarkers for the early detection of BC [78]. In a current study with the urinary metabolomics-based diagnostic approach, both high sensitivity and specificity were found [80]. This approach is non-invasive, needs only a small sample of urine, and the diagnosis can be made relatively quickly and objectively. The study showed that patients with BC had elevated levels of urinary acetyl-CoA and carnitine. It also established several acylcarnitines that were found

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to differentiate between cancer and control groups [80]. Another study using the targeted mass spectrometry found that it is highly sensitive for detecting metabolic alterations. This provides insight into metabolic pathways that are potentially associated with tumorigenesis and tumor progression [77].

CLINICAL IMPLICATIONS OF MOLECULAR PROFILES

1. Intrinsic molecular subtypes of bladder cancer

Intrinsic molecular subtypes of BC were recently established through many studies based on comprehensive genomic data from TCGA. The relationships between subtypes and their clinical implications have been investigated.

Specific genetic alterations have been found in distinct phenotypes, suggesting distinct disease entities. Most of NMIBC primarily show *FGFR3* mutations, *Ras* activation, and wild-type *TP53* [33,81]. Basal/squamous cell carcinoma (SCC)-like MIBC is the most aggressive phenotype. However, it is also the most sensitive to cisplatin chemotherapy [82]. *RB1* and *NFE2L2* mutations are frequently observed in the basal/SCC-like MIBC phenotype. The p53-like MIBC is characterized as being chemo-resistant. Alterations of *FGFR3* and *KDM6A* are associated in the luminal subtype of p53-like MIBC. Luminal cluster I shows lowered expression of CD8+ effector genes and PD-L1 immune or tumor cells. Meanwhile, luminal cluster II subtypes are linked to activated T-effector cells. The Lund classification currently recognizes five subtypes of BC; urobasal A (uroA), urobasal B (uroB), genomically unstable, and infiltrated/SCC-like [83]. Cancerous cells that can switch between the luminal and basal subtypes has also been found. This suggests that longitudinal studies are critical for understanding subtype changes and the associated responses to various chemotherapies. Collectively, based on these genetic alterations of these different phenotypes, research efforts are now moving to consider clinical strategies that can better the management of BC patients.

2. Liquid biopsy

Liquid biopsies are being considered as a potential applicable non-invasive molecular profiling tool. A number of non-invasive multi-marker tests are currently commercially available for BC. In particular, ImmunoCyt™ is able to measure levels of mucin and carcinoembryonic antigens in urine samples for BC diagnosis [84]. Another urine test, Aura Tek FDP Test™, can detect BC recurrence [85]. Circulating factors, including circulating tumor cells (CTCs), cfDNAs, RNAs (miRNAs, long non-coding RNAs [lncRNAs], mRNAs), cell-free proteins, peptides, or exosomes et al., are derived from cells in human body. However, it is still elusive where these circulating molecules are coming from.

CTCs of BC were previously detected in the urine and serum from patients with metastatic BC. Rising levels of CTCs were also positively correlated with aggressiveness [86,87]. CTCs derived from BC can be measured by using CTC-specific proteins, such as c-MET and PD-L1 [88-90]. Increased CTC levels were able to predict clinical outcomes, such as recurrence and survival. CellSearch™, an FDA-approved CTC assay kit, is currently being used in the clinical setting for prognostic purposes.

Tumor-derived DNA is released into the body's circulation. Circulating tumor DNA (ctDNA) may reflect the genetic profile of all tumor sub-clones. In most cases, ctDNA has a very small size, usually between 180–200 base pairs. Quantification of ctDNA levels and the integrity status of ctDNA can be of great potential clinical utility for early diagnosis and prognosis of BC. Like other liquid biopsies, ctDNA testing can also be easily and frequently repeated in order to monitor changes during treatment [91]. In addition, genetic alterations can be detected in ctDNA. In the urine specimens of BC patients, *TERT* promoter mutations correlated with recurrence [92], while *KRAS2* mutations were found in the plasma even before BC diagnosis [93].

Circulating RNA classes, which include mRNAs, miRNAs, and lncRNAs, were also found to be potential non-invasive biomarkers [94]. The urinary *CAIX* splice variant mRNA was reported to have high diagnostic performance and value [95]. Urinary *UBE2C* and *hTERT* mRNA are found to be potential markers for early diagnosis and prognosis of BC [96]. Urinary levels of *miR-126* and *miR-146a-5p* were also discovered to be elevated in BC and are associated with tumor grade and invasiveness [97].

The delivery of circulating molecules employs the use of small vesicles, called exosomes. Exosomes transfer biologically active molecules and can be secreted into the urine, blood, and other body fluids [98]. Hence, exosomes are essential mediators of cell-to-cell communication [98]. There is a strong association between heightened exosome levels and BC [99]. In urinary exosomes, significantly increased levels of active molecules (e.g., *TACSTD2*, lncRNAs–*HOTAIR, HOX-AS-2, ANRIL*, and linc-ROR, et al.) were found to in high-grade MIBC patients [100].

CONCLUDING REMARKS AND PERSPECTIVES

In this short review article, we addressed current concerted efforts on developing molecular profiling focused on BC. Development of high-throughput profiling technologies, including genomics, epigenomics, proteomics, metabolomics, and bioinformatics etc., have accumulated evidence through research in the laboratory and clinical settings. Experimental findings have proposed promising biomarker candidates for clinical application. Subtyping of BC based on molecular signatures associated with clinical outcomes suggest mechanistic clues on how to monitor responses to chemotherapy in patients. However, clinically applicable and personalized biomarkers for early diagnosis and prediction of recurrence, progression, and treatment are unsolved and require more investigation. Focused efforts should continue in order to extract applicable and synergistic benefits from our current findings. This will likely ensure that a clear path from discovery to clinical diagnostics will be successfully paved.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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REFERENCES

1. Diamandis M, White NM, Yousef GM. Personalized medicine: marking a new epoch in cancer patient management. Mol Cancer Res 2010;8:1175-87.
2. Johnson DC, Greene PS, Nielsen ME. Surgical advances in bladder cancer: at what cost? Urol Clin North Am 2015;42:235-52, ix.
3. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234-41.
4. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet 2009;374:239-49.
5. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
6. Messing E. Markers of detection. Urol Oncol 2007;25:344-7.
7. Quan C, Cha EJ, Lee HL, Han KH, Lee KM, Kim WJ. Enhanced expression of peroxiredoxin I and VI correlates with development, recurrence and progression of human bladder cancer. J Urol 2006;175:1512-6.
8. Bellmunt J, Pons F, Orsola A. Molecular determinants of response to cisplatin-based neoadjuvant chemotherapy. Curr Opin Urol 2013;23:466-71.
9. Kim J. Immune checkpoint blockade therapy for bladder cancer treatment. Investig Clin Urol 2016;57 Suppl 1:S98-105.
10. Lenis AT, Chamie K. Bladder cancer in 2014: from the genomic frontier to immunotherapeutics. Nat Rev Urol 2015;12:74-6.
11. Macleod LC, Ngo TC, Gonzalgo ML. Complications of intravesical bacillus calmette-guérin. Can Urol Assoc J 2014;8:E540-4.
12. Steinberg RL, Thomas LJ, Nepple KG. Intravesical and alternative bladder-preservation therapies in the management of non-muscle-invasive bladder cancer unresponsive to bacillus Calmette-Guérin. Urol Oncol 2016;34:279-89.
13. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19:A68-77.
14. Kim WJ, Kim YJ. Epigenetics of bladder cancer. Methods Mol Biol 2012;863:111-8.
15. Martínez-Fernández M, Rubio C, Segovia C, López-Calderón FF, Dueñas M, Paramio JM. EZH2 in bladder cancer, a promising therapeutic target. Int J Mol Sci 2015;16:27107-32.
16. Wu CT, Lin WY, Chang YH, Lin PY, Chen WC, Chen MF. DNMT1-dependent suppression of microRNA424 regulates tumor progression in human bladder cancer. Oncotarget

https://doi.org/10.4111/icu.2018.59.2.72

2015;6:24119-31.
17. Lombard AP, Mooso BA, Libertini SJ, Lim RM, Nakagawa RM, Vidallo KD, et al. miR-148a dependent apoptosis of bladder cancer cells is mediated in part by the epigenetic modifier DNMT1. Mol Carcinog 2016;55:757-67.
18. Palmbos PL, Wang L, Yang H, Wang Y, Leflein J, Ahmet ML, et al. ATDC/TRIM29 drives invasive bladder cancer formation through miRNA-mediated and epigenetic mechanisms. Cancer Res 2015;75:5155-66.
19. Chen Z, Kim J. Urinary proteomics and metabolomics studies to monitor bladder health and urological diseases. BMC Urol 2016;16:11.
20. Di Meo A, Pasic MD, Yousef GM. Proteomics and peptidomics: moving toward precision medicine in urological malignancies. Oncotarget 2016;7:52460-74.
21. Thomas S, Hao L, Ricke WA, Li L. Biomarker discovery in mass spectrometry-based urinary proteomics. Proteomics Clin Appl 2016;10:358-70.
22. Frantzi M, Latosinska A, Flühe L, Hupe MC, Critselis E, Kramer MW, et al. Developing proteomic biomarkers for bladder cancer: towards clinical application. Nat Rev Urol 2015;12:317-30.
23. Chan EC, Pasikanti KK, Hong Y, Ho PC, Mahendran R, Raman Nee Mani L, et al. Metabonomic profiling of bladder cancer. J Proteome Res 2015;14:587-602.
24. Deiss F, Mazzeo A, Hong E, Ingber DE, Derda R, Whitesides GM. Platform for high-throughput testing of the effect of soluble compounds on 3d cell cultures. Anal Chem 2013;85:8085-94.
25. Håkanson M, Textor M, Charnley M. Engineered 3D environments to elucidate the effect of environmental parameters on drug response in cancer. Integr Biol (Camb) 2011;3:31-8.
26. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell 2014;159:176-87.
27. Shariat SF, Savage C, Chromecki TF, Sun M, Scherr DS, Lee RK, et al. Assessing the clinical benefit of nuclear matrix protein 22 in the surveillance of patients with nonmuscle-invasive bladder cancer and negative cytology: a decision-curve analysis. Cancer 2011;117:2892-7.
28. Ho CC, Tan WP, Pathmanathan R, Tan WK, Tan HM. Fluorescence-in-situ-hybridization in the surveillance of urothelial cancers: can use of cystoscopy or ureteroscopy be deferred? Asian Pac J Cancer Prev 2013;14:4057-9.
29. Gomella LG, Mann MJ, Cleary RC, Hubosky SG, Bagley DH, Thumar AB, et al. Fluorescence in situ hybridization (FISH) in the diagnosis of bladder and upper tract urothelial carcinoma: the largest single-institution experience to date. Can J Urol 2017;24:8620-6.
30. Lavery HJ, Zaharieva B, McFaddin A, Heerema N, Pohar KS. A prospective comparison of UroVysion FISH and urine cytology in bladder cancer detection. BMC Cancer 2017;17:247.
31. Liem EIML, Baard J, Cauberg ECC, Bus MTJ, de Bruin DM, Laguna Pes MP, et al. Fluorescence in situ hybridization as prognostic predictor of tumor recurrence during treatment with Bacillus Calmette-Guérin therapy for intermediate- and high-risk non-muscle-invasive bladder cancer. Med Oncol 2017;34:172.
32. Virk RK, Abro S, de Ubago JMM, Pambuccian SE, Quek ML, Wojcik EM, et al. The value of the UroVysion® FISH assay in the risk-stratification of patients with "atypical urothelial cells" in urinary cytology specimens. Diagn Cytopathol 2017;45:481-500.
33. Yoshino H, Seki N, Itesako T, Chiyomaru T, Nakagawa M, Enokida H. Aberrant expression of microRNAs in bladder cancer. Nat Rev Urol 2013;10:396-404.
34. Yu J, Zhu T, Wang Z, Zhang H, Qian Z, Xu H, et al. A novel set of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer. Clin Cancer Res 2007;13:7296-304.
35. Vinci S, Giannarini G, Selli C, Kuncova J, Villari D, Valent F, et al. Quantitative methylation analysis of BCL2, hTERT, and DAPK promoters in urine sediment for the detection of non-muscle-invasive urothelial carcinoma of the bladder: a prospective, two-center validation study. Urol Oncol 2011;29:150-6.
36. Ward DG, Baxter L, Gordon NS, Ott S, Savage RS, Beggs AD, et al. Multiplex PCR and next generation sequencing for the non-invasive detection of bladder cancer. PLoS One 2016;11:e0149756.
37. Kim J, Mouw KW, Polak P, Braunstein LZ, Kamburov A, Kwiatkowski DJ, et al. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. Nat Genet 2016;48:600-6.
38. Burns MB, Temiz NA, Harris RS. Evidence for APOBEC3B mutagenesis in multiple human cancers. Nat Genet 2013;45:977-83.
39. Nagata M, Muto S, Horie S. Molecular biomarkers in bladder cancer: novel potential indicators of prognosis and treatment outcomes. Dis Markers 2016;2016:8205836.
40. Zhang X, Zhang Y. Bladder cancer and genetic mutations. Cell Biochem Biophys 2015;73:65-9.
41. Nickerson ML, Witte N, Im KM, Turan S, Owens C, Misner K, et al. Molecular analysis of urothelial cancer cell lines for modeling tumor biology and drug response. Oncogene 2017;36:35-46.
42. Seiler R, Thalmann GN, Rotzer D, Perren A, Fleischmann A. CCND1/CyclinD1 status in metastasizing bladder cancer: a

prognosticator and predictor of chemotherapeutic response. Mod Pathol 2014;27:87-95.
43. Dadhania V, Zhang M, Zhang L, Bondaruk J, Majewski T, Siefker-Radtke A, et al. Meta-analysis of the luminal and basal subtypes of bladder cancer and the identification of signature immunohistochemical markers for clinical use. EBioMedicine 2016;12:105-17.
44. Ross JS, Wang K, Al-Rohil RN, Nazeer T, Sheehan CE, Otto GA, et al. Advanced urothelial carcinoma: next-generation sequencing reveals diverse genomic alterations and targets of therapy. Mod Pathol 2014;27:271-80.
45. Ross JS, Wang K, Gay LM, Al-Rohil RN, Nazeer T, Sheehan CE, et al. A high frequency of activating extracellular domain ERBB2 (HER2) mutation in micropapillary urothelial carcinoma. Clin Cancer Res 2014;20:68-75.
46. Moss TJ, Qi Y, Xi L, Peng B, Kim TB, Ezzedine NE, et al. Comprehensive genomic characterization of upper tract urothelial carcinoma. Eur Urol 2017;72:641-9.
47. Pietzak EJ, Bagrodia A, Cha EK, Drill EN, Iyer G, Isharwal S, et al. Next-generation sequencing of nonmuscle invasive bladder cancer reveals potential biomarkers and rational therapeutic targets. Eur Urol 2017;72:952-9.
48. Choi W, Ochoa A, McConkey DJ, Aine M, Höglund M, Kim WY, et al. Genetic alterations in the molecular subtypes of bladder cancer: illustration in the cancer genome atlas dataset. Eur Urol 2017;72:354-65.
49. Abbosh PH, McConkey DJ, Plimack ER. Targeting signaling transduction pathways in bladder cancer. Curr Oncol Rep 2015;17:58.
50. Christensen E, Birkenkamp-Demtröder K, Nordentoft I, Høyer S, van der Keur K, van Kessel K, et al. Liquid biopsy analysis of FGFR3 and PIK3CA hotspot mutations for disease surveillance in bladder cancer. Eur Urol 2017;71:961-9.
51. Ding XL, Yang X, Liang G, Wang K. Isoform switching and exon skipping induced by the DNA methylation inhibitor 5-Aza-2'-deoxycytidine. Sci Rep 2016;6:24545.
52. Jenuwein T, Allis CD. Translating the histone code. Science 2001;293:1074-80.
53. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693-705.
54. Gui Y, Guo G, Huang Y, Hu X, Tang A, Gao S, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet 2011;43:875-8.
55. Wolff EM, Byun HM, Han HF, Sharma S, Nichols PW, Siegmund KD, et al. Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. PLoS Genet 2010;6:e1000917.
56. Kim EJ, Kim YJ, Jeong P, Ha YS, Bae SC, Kim WJ. Methylation of the RUNX3 promoter as a potential prognostic marker for bladder tumor. J Urol 2008;180:1141-5.
57. Costa-Pinheiro P, Montezuma D, Henrique R, Jerónimo C. Diagnostic and prognostic epigenetic biomarkers in cancer. Epigenomics 2015;7:1003-15.
58. Maruyama R, Toyooka S, Toyooka KO, Harada K, Virmani AK, Zöchbauer-Müller S, et al. Aberrant promoter methylation profile of bladder cancer and its relationship to clinicopathological features. Cancer Res 2001;61:8659-63.
59. Marsit CJ, Karagas MR, Andrew A, Liu M, Danaee H, Schned AR, et al. Epigenetic inactivation of SFRP genes and TP53 alteration act jointly as markers of invasive bladder cancer. Cancer Res 2005;65:7081-5.
60. Catto JW, Azzouzi AR, Rehman I, Feeley KM, Cross SS, Amira N, et al. Promoter hypermethylation is associated with tumor location, stage, and subsequent progression in transitional cell carcinoma. J Clin Oncol 2005;23:2903-10.
61. Su SF, de Castro Abreu AL, Chihara Y, Tsai Y, Andreu-Vieyra C, Daneshmand S, et al. A panel of three markers hyper- and hypomethylated in urine sediments accurately predicts bladder cancer recurrence. Clin Cancer Res 2014;20:1978-89.
62. Ratliff TL, Gillen D, Catalona WJ. Requirement of a thymus dependent immune response for BCG-mediated antitumor activity. J Urol 1987;137:155-8.
63. Sweis RF, Galsky MD. Emerging role of immunotherapy in urothelial carcinoma-immunobiology/biomarkers. Urol Oncol 2016;34:556-65.
64. Donin NM, Lenis AT, Holden S, Drakaki A, Pantuck A, Belldegrun A, et al. Immunotherapy for the treatment of urothelial carcinoma. J Urol 2017;197:14-22.
65. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkov AV, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. Nat Med 2015;21:231-8.
66. Balbás-Martínez C, Rodríguez-Pinilla M, Casanova A, Domínguez O, Pisano DG, Gómez G, et al. ARID1A alterations are associated with FGFR3-wild type, poor-prognosis, urothelial bladder tumors. PLoS One 2013;8:e62483.
67. Scott SN, Ostrovnaya I, Lin CM, Bouvier N, Bochner BH, Iyer G, et al. Next-generation sequencing of urine specimens: a novel platform for genomic analysis in patients with non-muscle-invasive urothelial carcinoma treated with bacille Calmette-Guérin. Cancer Cytopathol 2017;125:416-26.
68. Kreunin P, Zhao J, Rosser C, Urquidi V, Lubman DM, Goodison S. Bladder cancer associated glycoprotein signatures revealed by urinary proteomic profiling. J Proteome Res 2007;6:2631-9.
69. Li H, Li C, Wu H, Zhang T, Wang J, Wang S, et al. Identification of Apo-A1 as a biomarker for early diagnosis of bladder transitional cell carcinoma. Proteome Sci 2011;9:21.
70. Li F, Chen DN, He CW, Zhou Y, Olkkonen VM, He N, et al.

https://doi.org/10.4111/icu.2018.59.2.72

Identification of urinary Gc-globulin as a novel biomarker for bladder cancer by two-dimensional fluorescent differential gel electrophoresis (2D-DIGE). J Proteomics 2012;77:225-36.
71. Orenes-Piñero E, Barderas R, Rico D, Casal JI, Gonzalez-Pisano D, Navajo J, et al. Serum and tissue profiling in bladder cancer combining protein and tissue arrays. J Proteome Res 2010;9:164-73.
72. Urquidi V, Chang M, Dai Y, Kim J, Wolfson ED, Goodison S, et al. Il-8 as a urinary biomarker for the detection of bladder cancer. BMC Urol 2012;12:12.
73. Srinivasan H, Allory Y, Sill M, Vordos D, Alhamdani MS, Radvanyi F, et al. Prediction of recurrence of non muscle-invasive bladder cancer by means of a protein signature identified by antibody microarray analyses. Proteomics 2014;14:1333-42.
74. Grau L, Luque-Garcia JL, González-Peramato P, Theodorescu D, Palou J, Fernandez-Gomez JM, et al. A quantitative proteomic analysis uncovers the relevance of CUL3 in bladder cancer aggressiveness. PLoS One 2013;8:e53328.
75. Bansal N, Gupta A, Sankhwar SN, Mahdi AA. Low- and high-grade bladder cancer appraisal via serum-based proteomics approach. Clin Chim Acta 2014;436:97-103.
76. Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J, et al. Metabolomics in bladder cancer: a systematic review. Int J Clin Exp Med 2015;8:11052-63.
77. von Rundstedt FC, Rajapakshe K, Ma J, Arnold JM, Gohlke J, Putluri V, et al. Integrative pathway analysis of metabolic signature in bladder cancer: a linkage to the cancer genome atlas project and prediction of survival. J Urol 2016;195:1911-9.
78. Bansal N, Gupta A, Mitash N, Shakya PS, Mandhani A, Mahdi AA, et al. Low- and high-grade bladder cancer determination via human serum-based metabolomics approach. J Proteome Res 2013;12:5839-50.
79. Cao M, Zhao L, Chen H, Xue W, Lin D. NMR-based metabolomic analysis of human bladder cancer. Anal Sci 2012;28:451-6.
80. Jin X, Yun SJ, Jeong P, Kim IY, Kim WJ, Park S. Diagnosis of bladder cancer and prediction of survival by urinary metabolomics. Oncotarget 2014;5:1635-45.
81. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer 2015;15:25-41.
82. Dyrskjøt L, Zieger K, Kruhøffer M, Thykjaer T, Jensen JL, Primdahl H, et al. A molecular signature in superficial bladder carcinoma predicts clinical outcome. Clin Cancer Res 2005;11:4029-36.
83. Sjödahl G, Lauss M, Lövgren K, Chebil G, Gudjonsson S, Veerla S, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res 2012;18:3377-86.
84. Yang M, Zheng Z, Zhuang Z, Zhao X, Xu Z, Lin H. ImmunoCyt™ and cytology for diagnosis of bladder carcinoma: a meta analysis. Chin Med J (Engl) 2014;127:758-64.
85. Siemens DR, Morales A, Johnston B, Emerson L. A comparative analysis of rapid urine tests for the diagnosis of upper urinary tract malignancy. Can J Urol 2003;10:1754-8.
86. Naoe M, Ogawa Y, Morita J, Omori K, Takeshita K, Shichijyo T, et al. Detection of circulating urothelial cancer cells in the blood using the CellSearch System. Cancer 2007;109:1439-45.
87. Flaig TW, Wilson S, van Bokhoven A, Varella-Garcia M, Wolfe P, Maroni P, et al. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. Urology 2011;78:863-7.
88. Qi F, Liu Y, Zhao R, Zou X, Zhang L, Li J, et al. Quantitation of rare circulating tumor cells by folate receptor α ligand-targeted PCR in bladder transitional cell carcinoma and its potential diagnostic significance. Tumour Biol 2014;35:7217-23.
89. Anantharaman A, Friedlander T, Lu D, Krupa R, Premasekharan G, Hough J, et al. Programmed death-ligand 1 (PD-L1) characterization of circulating tumor cells (CTCs) in muscle invasive and metastatic bladder cancer patients. BMC Cancer 2016;16:744.
90. Zhang T, Boominathan R, Foulk B, Rao C, Kemeny G, Strickler JH, et al. Development of a novel c-MET-based CTC detection platform. Mol Cancer Res 2016;14:539-47.
91. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 2013;10:472-84.
92. Kinde I, Munari E, Faraj SF, Hruban RH, Schoenberg M, Bivalacqua T, et al. TERT promoter mutations occur early in urothelial neoplasia and are biomarkers of early disease and disease recurrence in urine. Cancer Res 2013;73:7162-7.
93. Gormally E, Vineis P, Matullo G, Veglia F, Caboux E, Le Roux E, et al. TP53 and KRAS2 mutations in plasma DNA of healthy subjects and subsequent cancer occurrence: a prospective study. Cancer Res 2006;66:6871-6.
94. Fernandez-Mercado M, Manterola L, Larrea E, Goicoechea I, Arestin M, Armesto M, et al. The circulating transcriptome as a source of non-invasive cancer biomarkers: concepts and controversies of non-coding and coding RNA in body fluids. J Cell Mol Med 2015;19:2307-23.
95. Malentacchi F, Vinci S, Melina AD, Kuncova J, Villari D, Nesi G, et al. Urinary carbonic anhydrase IX splicing messenger RNA variants in urogenital cancers. Urol Oncol 2016;34:292.e9-16.
96. Xie XY, Yang X, Zhang JH, Liu ZJ. Analysis of hTERT expression in exfoliated cells from patients with bladder transitional cell carcinomas using SYBR green real-time fluorescence quantitative PCR. Ann Clin Biochem 2007;44:523-8.
97. Sasaki H, Yoshiike M, Nozawa S, Usuba W, Katsuoka Y, Aida K, et al. Expression level of urinary MicroRNA-146a-5p is increased in patients with bladder cancer and decreased in those

after transurethral resection. Clin Genitourin Cancer 2016;14: e493-9.

98. Yu S, Cao H, Shen B, Feng J. Tumor-derived exosomes in cancer progression and treatment failure. Oncotarget 2015;6:37151-68.
99. Nawaz M, Camussi G, Valadi H, Nazarenko I, Ekström K, Wang X, et al. The emerging role of extracellular vesicles as biomarkers for urogenital cancers. Nat Rev Urol 2014;11:688-701.
100. Berrondo C, Flax J, Kucherov V, Siebert A, Osinski T, Rosenberg A, et al. Expression of the long non-coding RNA HOTAIR correlates with disease progression in bladder cancer and is contained in bladder cancer patient urinary exosomes. PLoS One 2016;11:e0147236.

https://doi.org/10.4111/icu.2018.59.2.72