The era of personalized treatments: Updates on immunotherapy within urothelial of bladder cancer

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Abstract

Bladder cancer is a complex disease of the urinary system with high morbidity and mortality. Recently, the introduction of immunotherapies such as immune checkpoint inhibitors (eg, programmed cell death protein 1/programmed death-ligand 1) has proven to be a reliable means of improving survival outcomes, including patients with limited response to conventional treatment. Nevertheless, difficult questions remain in clinical practice, such as how to select appropriate patients for personalized treatment, how to predict and assess therapeutic efficacy in advance, and how to enhance the therapeutic benefits of immunotherapy treatment. These issues require urgent attention. Herein, we describe recent clinical applications of immune checkpoint inhibitors in bladder cancer therapy, examine underlying mechanisms for treatment failure in a subset of patients, and discuss potential approaches to improve their therapeutic effects.

Keywords: Bladder cancer; Combination strategies; Immune checkpoint inhibitors; Molecular subtypes; Predictive biomarkers

1. Introduction

Urothelial bladder cancer (UBC) is the most common carcinoma of the urinary system. According to the tumor, node, metastasis (TNM) classification, UBC is generally divided into nonmuscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), and metastatic UBC (mUBC). Standard treatment approaches differ for these 3 subtypes. Conventionally, management of NMIBC involves transurethral resection with or without adjuvant intravesical agents, such as chemotherapeutic drugs or bacillus Calmette-Guérin (BCG) based on pathological and clinical parameters. For patients with MIBC, radical resection with cisplatin-based chemotherapy is the preferred approach. For patients with disease that has progressed to mUBC, intravenous chemotherapy is considered the best available treatment. Although conventional treatment algorithms are continually updated and optimized, a substantial subset of patients experience treatment failure and eventual death, especially for those with mUBC. Fortunately, the care paradigm of UBC has stepped into the era of precision medicine because of the emergence of 2 profound achievements. First, sequencing technology and omics studies have discovered high heterogeneity within BC tissues. Second, the advent of immunotherapy, such as immune checkpoint inhibitors (ICIs), including programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1), has significantly changed the treatment landscape for BC. Herein, we focus on the current status of immunotherapy for BC and explore the cellular and molecular mechanisms underlying immune response in UBC.

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2. Clinical application of ICIs

Unlike other malignancies, treatment of BC has included immunotherapy for decades. For instance, the therapeutic benefit of BCG in high-risk NMIBC is well established. Because the first ICI drug, atezolizumab, was approved by the US Food and Drug Administration (FDA) in 2016, 4 other ICI drugs, nivolumab, pembrolizumab, avelumab, and durvalumab, have demonstrated robust outcomes in clinical trials for the treatment of mUBC and gained approval from the FDA.[1] Recently, ICIs have been approved as second-line drugs for the treatment of patients with unresectable and metastatic BC.[2–4] Moreover, for platinum-ineligible patients, ICIs can be offered as first-line treatment using different agents in mainstream clinical guidelines.[5,6] In addition, the maintenance role of immunotherapy immediately after first-line chemotherapy in advanced or metastatic BC was also documented in a phase III trial.[7] Currently, the role of adjuvant[8] and neoadjuvant[9,10] immunotherapy in MIBC is undergoing study in clinical trials. While preliminary results are positive, the strength of evidence is low, and more evidence is needed to confirm the application of immunotherapy in these scenarios. In addition, the potential therapeutic effects of ICIs reported in bladder-sparing therapy for MIBC and BCG-refractory NMIBC need further study.[1] Studies investigating other immunotherapies such as adoptive immunotherapy, cytokine therapeutics, costimulatory receptor agonists, and cancer vaccines, but the majority are in early stages focused on preclinical outcomes. We reviewed it in a previously published article.[11] Looking forward, the biggest obstacle for widespread adoption of immunotherapy in the personalized treatment of BC is determining how to choose appropriate patients and how to precisely predict treatment response or prognosis.

3. Mechanisms of treatment failure

Despite the potential promise of immunotherapy, response rates to immunotherapy are still low with associated poor prognosis. Elucidation of the fundamental cellular and molecular mechanisms involved in antitumor immunity may contribute to understanding how to optimize immunotherapeutic strategies. Recent developments

in sequencing technologies, such as next-generation sequencing and single-cell sequencing, have enabled studies confirming that UBC shows high heterogeneity.[12] Differences have been observed in the molecular and genetic features of cancer cells, such as mutations, gene expression patterns, and copy number, which determine tumor aggressiveness and sensitivity to therapeutics. Thus, unraveling the mechanisms by which tumor heterogeneity influence effectiveness of therapies is necessary for improvement of tumor management and surveillance.

Tumor heterogeneity describes the presence of cells with different morphologies, genotypes, or metabolic status, resulting in inconsistent tumor phenotype. Urothelial bladder cancer contains heterogeneity both between different patients (referred to as interpatient heterogeneity) and within tumors (referred to as intratumor heterogeneity).[13] Interpatient heterogeneity could explain why 2 patients with same pathological type respond differently to the same treatment. Indeed, scientists have identified several distinguishable subtypes for both NMIBC and MIBC with distinct clinical outcomes based on comprehensive transcriptional analysis. For example, the European multicenter prospective study of NMIBC (FP7: UROMOL) study found three distinct subtypes of NMIBC via comprehensive transcriptional analysis of 460 patients with early-stage NMIBC and 16 patients with MIBC.[14] Type 1 tumors displayed high levels of expression of early cell cycle genes associated with low-risk NMIBC. Type 2 tumors exhibited high levels of expression of late cell cycle genes closely associated with poor prognosis. Both type 1 and type 2 tumors showed abundant uroplakins expression, which are markers for luminal or umbrella cells. Type 3 tumors demonstrated high levels of expression of KRT5 and KRT15, markers of basal/undifferentiated cells, and upregulated expression of long noncoding RNA. One recent study succeeded in constructing the MIBC molecular classification system with the use of a single sample classifier integrating 6 nonoverlapping patient subtypes, highly conforming to previous classification studies. This classification includes luminal papillary, luminal unstable, luminal nonspecified, basal/squamous, stroma-rich, and neuroendocrine-like subclasses.[15] More and more studies have proven distinct differences exist in terms of epigenetics and metabolic activity between different individuals.[16] These results suggest the need to identify personalized approaches to treatment according to a multiomics data, not just relying on conventional TNM classification.

With regard to intratumor heterogeneity, the concept of the tumor microenvironment (TME) needs to be emphasized, because it is of extreme importance during the processes of cancer progression and immune evasion. The TME consists of malignant cells and nonmalignant compartments. Crosstalk between these cellular compartments influences not only tumor development but also sensitivity to therapy. Recently, research has focused on the role of nonmalignant cells within the TME, in particular immune cells (eg, tumor-infiltrating lymphocytes [TILs], myeloid-derived suppressor cells [MDSCs], tumor-associated macrophages [TAMs], regulatory T [Treg] cells), and stromal cells (eg, cancer-associated fibroblasts [CAFs]). It is noteworthy that many of these cell types have been associated with therapeutic failure and poor prognosis, except for CD8$^+$ T cells, which have been correlated with increased patient survival and improved therapeutic response. Cancer-associated fibroblasts are the major type of stromal cells involved in the TME,[17] with potential ability to remodel the extracellular matrix in normal tissues. In recent years, increasing evidence has suggested that CAFs could serve as a therapeutic target during cancer treatment, because they can modulate crosstalk between immune cells and cancer cells observed in the TME.[17] Compared with normal bladder tissue, an increase in CAFs was detected in primary BC by

immunochemistry assay.[16] The expression level of some CAF-specific biomarkers such as CD90, fibroblast activation protein (FAP), and platelet-derived growth factor receptor-beta, was positively correlated with tumor aggressiveness in UBC.[18] In addition, a lower 5-year survival outcome was found in UBC patients who predominantly expressed FAP protein as compared with those without overexpression in a hierarchical cluster analysis study.[18] These results were further confirmed by an investigation, which suggested that expression of FAP was positively correlated with invasiveness and negatively associated with survival in patients with basal phenotype BC.[19] Regarding immune cells, TILs (such as cytotoxic CD8$^+$ T cells) are responsible for cell-mediated antitumor response. For instance, TILs can promote apoptosis of cancer cells via release of cell-surface expression of death ligands or cytotoxins upon recognition of exogenous antigens.[20] According to the extent of TIL presence, tumors have been generally categorized into 3 subclasses: (1) inflamed tumors with low or high infiltration of TILs, (2) uninflamed tumors with low infiltration of TILs, and (3) tumors with moderate TIL infiltration with the lack of PD-L1$^+$ immune cell. In MIBC, CD8$^+$ T cells have been widely investigated because of their direct antitumor effects, and the number of TILs located within precancerous stroma has been used to analyze immune phenotype, molecular tumor subtype, and patient survival.[21] Myeloid-derived suppressor cells, which belong to the category of immunosuppressive cells, include a wide range of defective dendritic cells and immature myeloid cells that prevent T cells from recognizing tumor antigens.[22] In normal tissues, MDSCs play a vital role in maintaining homeostasis of the immune system through inhibition of excessive inflammation and reduction of autoimmunity.[23] In cancer, MDSCs promote proliferation, foster immune evasion, and induce invasion and metastasis,[24] eventually compromising the effects of immunotherapy. Tumor-associated macrophages refer to macrophages recruited from the bone marrow to intratumor regions of necrosis and hypoxia or those that reside in tumor tissue. Macrophages are often classified into 2 distinctive subtypes, M1 and M2.[25] The M1 subtype initiates acute inflammation and is responsible for the removal of pathogens and tumor cells, whereas the M2 subtype induces chronic inflammation, resulting in immunosuppression and tumorigenesis.[26] In UBC, researchers have succeeded in separating TAMs from tumor tissue, predominantly M2 subtype, and also found a greater quantity of M2 in higher-grade disease than that in low-grade disease.[27] Treg cells hinder activation and proliferation of CD8$^+$ T cells through the secretion of inhibitory cytokines, including granzyme A and granzyme B.[28] In cancer, stromal cells induce recruitment of M2 macrophages to the TME and indirectly stimulate Treg cell infiltration, ultimately hindering immunotherapies.[29]

4. How to improve treatment efficacy

Identification of patients who may be sensitive to immunotherapy is an ongoing problem that needs to be addressed urgently. In 2016, the concept of a "cancer immunogram" was brought forward, including general immune status, tumor foreignness, tumor sensitivity to immune effector mechanisms, absence of inhibitory tumor metabolism, immune cell infiltration capacity, absence of checkpoints, and absence of soluble inhibitors.[30] The immunogram concept was soon thereafter applied to lung cancer.[31] Recently, a study has suggested that this concept could also be extended to BC for the prediction of ICI response.[32] In addition, other parameters including sex, age, commensal microorganisms, and general clinical condition could potentially play important roles influencing ICI

response and antitumor immunity.[33] Based on recent work, we suggest herein that the efficacy of ICIs could be influenced by at least 4 parameters: (1) clinical characteristics (eg, age, sex, and general performance status); (2) blood-based biomarkers (eg, absence of soluble inhibitors, general immune status, and liquid biopsy); (3) tumor tissue-based biomarkers (eg, absence of checkpoints, immune cell infiltration capacity, tumor foreignness, and absence of inhibitory tumor metabolism); and (4) commensal microorganisms. In a previous article, we have discussed in detail the role of these biomarkers in the prediction of ICI efficacy in BC.[11]

Another dilemma regarding ICI treatment is how to enhance therapeutic efficacy. As mentioned previously, a substantial number of patients do not respond to ICI treatment. The underlying mechanisms whereby ICI failure occurs are not well understood. In recent years, it has been suggested that impairment of priming signals, activation of negative signals by recruitment of immunosuppressive cells, dysfunction of antigen presenting cells, and negative stromal interactions are likely mechanisms for immune evasion by tumor cells. A number of studies have demonstrated that combining ICIs with other cancer therapeutics (eg, radiotherapy, chemotherapy, targeted therapy, local therapy, and other immunotherapies) can overcome resistance due to antitumor immune responses, including ongoing clinical trials, which have reported preliminary outcomes.[34] For example, fibroblast growth factor receptor (FGFR), a receptor tyrosine kinase correlated with cell survival, proliferation, and migration, is an established target in UBC treatment, especially for luminal-subtype tumors. Erdafitinib, a pan-FGFR inhibitor, has been studied the most extensively and is currently the only FDA-approved FGFR inhibitor to treat advanced UBC.[35] In addition, antibody-drug conjugates make use of highly expressed tumor proteins as targets for drug delivery. One such agent approved by the FDA, enfortumab vedotin, contains anti–nectin-4 antibody linked to the microtubule-disrupting molecule monomethyl auristatin E.[36] The clinical response rate of patients to this agent seems to be higher in those previously treated with ICIs.[37]

5. Conclusions

Conventional treatment algorithms based on the TNM classification system are unlikely to be successful for all patients experiencing BC, given that BC displays a high degree of interpatient and intratumor heterogeneity. Thus, modern treatment of BC will require a personalized treatment approach, which considers individual patient differences. In recent years, rapid development of immunotherapies has received considerable attention. Among the different types of immunotherapies, ICIs approved by the FDA have been one of the most successful treatment strategies and have changed the treatment landscape for BC. However, in the meantime, clinicians must confront issues such as a lack of sensitive and specific predictors for treatment response and resistance to ICIs in a large proportion of patients, predominantly because of tumor heterogeneity. Fortunately, researchers have devoted efforts toward solving these critical issues through identification of predictive biomarkers from the perspective of multiomics. Although some potential biomarkers have been recommended, such as tumor mutation burden and expression levels of PD-1/PD-L1, consensus has yet to be achieved regarding acceptable biomarkers for predicting the therapeutic efficacy of immunotherapy in BC. Nevertheless, there are probable benefits to be gained from the development of combination approaches with chemotherapy, radiotherapy, targeted therapies, and other immunomodulatory agents.

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