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Seminar Article

Utilizing precision medicine to modulate the prostate tumor microenvironment and enhance immunotherapy

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Abstract

The last two decades of cancer research have seen two major advancements in our ability to treat cancer: precision medicine and immunotherapy. While these approaches have shown striking anticancer efficacy in numerous malignancies, they have not shown similar success and applicability in advanced prostate cancer patients. The fields of precision medicine and immunotherapy have come to realize that targeted therapies are capable of not only inhibiting tumor cell growth, but also promoting antitumor immunity by modulating the tumor microenvironment. Here we examine how personalized medicine can be used to target the tumor immune microenvironment in prostate cancer, with the goal of enhancing clinical responses to immunotherapy.

Keywords: Precision medicine; Immunotherapy; Prostate cancer

Introduction

As technological advances have expanded our ability to identify, isolate, and interrogate unique genetic changes within an individual patient's tumor, so has the effort to develop therapeutic agents that can be used to target these specific alterations. This precision medicine revolution—characterizing the genomics of individual patient tumor samples to develop a personalized treatment regimen targeting aberrations critical to the growth and progression of that unique cancer—has led to a fundamental reconsideration of how we can most effectively treat cancer. In several malignancies with well-defined genomic alterations, utilizing personalized targeted therapies has led to dramatic clinical successes. For example, the use of monoclonal antibodies such as trastuzumab to target human EGF receptor 2 (HER-2) positive breast cancer [1], or small molecule tyrosine kinase inhibitors such as imatinib to target the breakpoint cluster region (BCR)- Abelson (ABL) fusion protein in chronic myelogenous leukemia [2], have elicited clinical responses that have significantly changed the clinical management of these diseases. However, similar advances in personalized medicine have not been seen in the treatment of prostate cancer (CaP), despite continued research into the genomic alterations that occur throughout the development and progression of this disease.

CaP remains one of the most significant health concerns for men, being the most commonly diagnosed and second leading cause of cancer related death in American men [3]. While organ-confined disease can be treated with localized therapies, about a third of patients relapse and develop biochemically-recurrent disease, and are treated with androgen deprivation, which targets the aberrant and critical activity of the androgen receptor (AR) [4]. While most patients experience a period of disease regression, tumors invariably recur as castrate-resistant disease, which often metastasize to the lymph node, bone, and other tissue sites. Metastatic, castrate-resistant CaP (mCRPC) is lethal, despite several new therapeutic options having been approved for patients with this late stage of disease, including second-generation agents targeting the activity of the AR such as abiraterone [5] and enzalutamide [6], taxane-based chemotherapies

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such as docetaxel [7], bone-targeting radiation therapy radium-223 [8], and the immunotherapy sipuleucel-T [9]. However, these agents provide a relatively small median benefit in overall survival, highlighting the urgent need for more effective therapies for patients with advanced disease.

CaP immunotherapy and the tumor immune microenvironment

Like most cancers, the development and progression of CaP occurs within a complex tumor microenvironment composed of tumor cells as well as fibroblasts, endothelial cells, extracellular matrix components, and various immune populations. While this includes immune cells with potential antitumor activity (including T cells [10–13] and NK cells [14]), there are also several suppressive immune populations that are elevated in CaP patients, including regulatory T cells [13], tumor-associated macrophages [15], myeloid-derived suppressor cells [16], and others [17]. In addition to these immune cells, prostate tumor cells produce factors that both prevent the infiltration of effector cells as well as promote their anergy within the tumor microenvironment, including secreted factors such as transforming growth factor beta [18], indoleamine 2,3-dioxygenase [19], as well as surface molecules such Fas ligand [20], checkpoint ligands [21], or decreased expression of major histocompatability complex (MHC) class I to escape detection and lysis by CD8+ T cells [22]. Similarly, other components of the microenvironment can aid in the suppression of antitumor immunity, such as cancer-associated fibroblasts that can not only promote prostate tumor progression but also enhance the activity of suppressive tumor-associated macrophages [23,24]. Working together, the various components of the microenvironment form an immunosuppressive tumor milieu that helps contribute to the progression of CaP while also limiting antitumor immunity.

To overcome this immunosuppressive tumor microenvironment, several immunotherapeutic strategies have been developed that seek to activate a patient's immune system to counteract tumor- and immunemediated suppression with the goal of eliciting clinically significant antitumor immune responses [25]. This includes active vaccination strategies, designed primarily to activate antigen-specific T cells against prostate tumor-associated antigens. Several of these agents have entered clinical evaluation [26–28], including sipuleucel-T, an antigen-presenting cell immunotherapy approved for patients with mCRPC [9]. Immunotherapeutic approaches designed to elicit multiple antigen-specific immune responses through gene-mediated tumor cell lysis [29] or using irradiated tumor cells [30,31] have also been evaluated in clinical trials alone or in combination with other therapeutic approaches. Additionally, therapies designed to modulate the activity of other components of the immune system have been evaluated in patients with mCRPC, such as tasquinimod, which aims to target the suppressive activity of the myeloid immune compartment [32]. The use of immune checkpoint blockade has also been examined in patients with CaP, which utilizes antibodies blocking T cell inhibitory pathways such as CTLA-4 [33] or PD-1/PD-L1 [34] to promote T cell infiltration, expansion, and function within the tumor microenvironment. However, clinical trials evaluating immune checkpoint blockade in CaP have shown clinical responses in a relatively small subset of patients [35] compared to other malignancies, such as melanoma or lung cancer. This may be due to several factors, such as the immunosuppressive prostate tumor microenvironment [36], the low somatic mutation rate and neoepitope generation in CaP compared to other malignancies [37,38], or the high disease burden present in patients with mCRPC [39]. Given this relative lack of single-agent efficacy, combinatorial strategies are now being pursued seeking to pair immunotherapies with other therapeutic modalities, including personalized targeted therapies, with the goal of harnessing both the tumor cell-intrinsic activity of these targeted therapies along with their potential ability to modulate the prostate tumor microenvironment and enhance the antitumor efficacy of immunotherapy.

Using personalized targeted therapies to modulate the prostate tumor immune microenvironment

The advancement of precision medicine has led to an increased understanding of the genomic factors that contribute to the development and progression of CaP. Given the ease of access to primary prostate tumor samples, several studies have provided an in-depth characterization of alterations that occur during the development of localized CaP [40–47]. While these studies have identified the range of alterations that occur throughout the development and progression of this primary malignancy, much of this work has highlighted two of the central challenges that are common throughout personalized cancer medicine—a lack of clinically-actionable alterations, and inter- and intrapatient mutational heterogeneity. However, recent studies aimed at characterizing metastatic prostate tumor samples have identified a set of pathways that are commonly altered in patients with mCRPC [47–53], some of which are also frequently altered in several other metastatic cancers [54]. These pathways include *AR* signaling, the DNA repair pathway, the phosphoinositide 3-kinase (*PI3K*) pathway, the cell cycle pathway, and the Wnt/β-catenin signaling pathway. Alterations to these pathways stand out in that they are shared in a relatively high frequency of mCRPC patients, and can also be targeted using therapeutic agents currently under clinical evaluation. Below, we highlight these pathways, how they have been targeted using personalized medicine approaches in CaP, and how these targeted approaches can potentially be used to enhance the efficacy of immunotherapies.

Androgen receptor

Androgen deprivation therapy (ADT) has been the gold standard for patients with advanced CaP for more than 60 years, eliciting clinical responses in the majority of patients. ADT is also one of the earliest examples of targeted therapy, with the goal of starving prostate tumor cells of androgen and thereby decreasing the activity of the AR, the central driver of CaP development and progression. This was initially achieved through the use of surgical castration, and later through the use of chemical castration using agents designed to target the activity of the AR by preventing androgen production as well as directly binding to and inhibiting the AR. While these approaches provide disease regression in most patients, tumors invariably relapse and begin to grow in a castrate-resistant fashion. While this was initially referred to as "androgen-independent" or "hormone-refractory" disease, evaluation of these recurrent tumors found that the AR is often overexpressed and accumulates alterations that promote its constitutive activity. This occurs through a variety of mechanisms, including *AR* gene amplification, intratumoral androgen biosynthesis pathways, relaxed ligand specificity, and ligand-independent mutations and splice variants [55]. Through these and other mechanisms, the AR remains a target in castrate-resistant prostate cancer (CRPC), leading to the development of next-generation targeted therapies that seek to further inhibit the activity of the AR. This includes agents such as abiraterone [5], enzalutamide [6], and apalutamide [56] which were approved for patients with CRPC and are also currently being investigated in earlier stages of disease.

In addition to its on-target antitumor effects, clinical studies evaluating the effects of androgen deprivation has long been shown to have effects on various aspects of the tumor immune microenvironment. Androgen-deprivation can directly impact the immune phenotype of tumor cells, including increasing expression of chemokines associated with antitumor immune responses in patient tumor tissue compared to benign tissue [57]. Additionally, ADT can induce thymic regrowth and release of naïve T cells, increase immune infiltration into the prostate (both myeloid and lymphocyte populations), decrease Treg infiltration, alter checkpoint expression on immune cells, increase MHC expression, and enhance immune responses to prostate antigens [57–63]. However, ADT has also been implicated in altered infiltration of suppressive immune populations into the prostate tumor microenvironment [64], indicating the importance of evaluating appropriate combinations and sequencing when paired with immunotherapy.

Given the ability of androgen deprivation to target both tumor cell growth via inhibition of the AR, as well as modulate the immune phenotype of tumor cells and the composition of immune cells within the tumor microenvironment, AR-targeted therapies have been combined with immunotherapeutic strategies in a number of preclinical prostate tumor models [65–68] and have also entered clinical evaluation. This includes trials in combination with active vaccination approaches, where androgen deprivation has been combined with either sipuleucel-T or PROSTVAC (a viral vaccine targeting prostate specific antigen) and been shown to augment antigen-specific immune responses, although the timing and sequencing of these agents can impact the clinical response rate (with improved responsiveness observed when immunotherapy is delivered prior to ADT) [69,70]. Additionally, the ability of androgen deprivation to induce the production of naïve T cells and alter T cell infiltration into the microenvironment has led to considerable interest in combination with checkpoint blockade. In either hormone-naïve [71] or castrate-resistant patients [72], patients receiving androgen deprivation along with CTLA-4 blocking antibodies were shown to have enhanced antibody and T cell immune responses. Promising early results have also suggested that enzalutamide can increase PD-L1 expression [62], and demonstrates enhanced responsiveness to PD-1 checkpoint blockade [35]. This report also identified enhanced responsiveness to checkpoint blockade in a patient with microsatellite unstable (MSI) CaP, suggesting alterations to the DNA repair pathway may also result in alterations to the tumor microenvironment to promote antitumor immunity. These data have provided compelling evidence that harnessing the antitumor and immunostimulatory effects of ADT can enhance the efficacy of immunotherapy in CaP.

DNA repair pathways

One of the common aberrations identified in tumors from mCRPC patients was alterations to components of the homologous recombination DNA repair pathway, which was found to be disrupted in up to 30% of patients [48,73], including nearly 12% of patients with germline mutations [74]. Commonly altered members of this pathway include *BRCA1/2, CHEK2, CDK12, RAD51, and ATM*, which create a homologous-recombination deficiency (HRD) state, that can contribute to CaP progression. However, alterations to these pathways also result in the potential for synthetic lethality with agents such as poly-(ADP-ribose) polymerase (PARP) inhibitors [75], that can block an additional DNA repair mechanism, leading to catastrophic cell death. Several PARP inhibitors have entered clinical evaluation and have elicited promising results in patients with HRD alterations. In particular, the TOPARP-A trial, where mCRPC patients were treated with the PARP inhibitor olaparib, identified clinical responses in 16/48 patients, with 14/16 of these responding patients having HRD alterations [73], illustrating the potential of this therapeutic strategy in a genomically-identified patient population.

Agents targeting the DNA repair pathway have also been shown to have a direct effect on the tumor immune microenvironment, leading to emerging interest in combining these agents with immunotherapy. The potential to enhance neoantigen burden with PARP inhibitors, has led to

considerable interest in evaluating PARP inhibitors in combination with checkpoint blockade in mCRPC patients (NCT03572478, NCT02484404, NCT02861573, and NCT03338790), some of which have described preliminary results that have suggested this combination has encouraging clinical activity [76]. This agrees with results seen evaluating this combination in other malignancies, including preliminary results from the TOPACIO/Keynote-162 trial in breast and ovarian cancer (NCT02657889), in which PARPi in combination with checkpoint blockade led to durable clinical responses (including higher response rates in patients with alterations to the DNA repair pathway) [77,78].

PI3K *signaling*

The PI3K/Akt/mTOR signaling pathway plays a critical role in the development and progression of CaP, where it serves to mediate tumor cell proliferation and survival, as well as resistance to a variety of therapeutic approaches [79]. Approximately 50% of mCRPC patients have alterations that lead to hyperactivation of the PI3K pathway, either through deletion of *PTEN* (which negatively regulates the PI3K pathway) or amplification or activating mutations in *PIK3CA* or *PIK3CB* [48,80]. Given their critical role in tumor progression, inhibitors of this pathway (blocking the activity of PI3K, Akt, and/or mTOR) have been developed to treat the broad range of malignancies with alterations to this signaling pathway and are also under clinical evaluation in CaP (NCT01884285 [81]). However, limited success has been achieved with these monotherapies, including in mCRPC [82]. This has led to an influx of clinical trials looking at combinatorial approaches with other targeted therapies, including combining second-generation AR targeted therapies with PI3K inhibition (NCT02215096, NCT02407054) or Akt inhibition (NCT01485861, NCT02525068), the latter of which have reported interim analyses that have suggested potential clinical responses [81,83].

In addition to combination approaches with other targeted therapies, agents targeting the PI3K pathway present numerous opportunities for combination strategies with immunotherapy. Given that PI3K signaling mediates a variety of cellular functions in disparate tumor and immune cell types, it is not surprising that its inhibition alters the activity of a variety of components of the tumor immune microenvironment [84]. In preclinical models of CaP, pan-PI3K/mTOR inhibition led to altered myeloid-derived suppressor cells infiltration leading to enhanced responsiveness to checkpoint blockade [85]. Clinical trials are currently underway in other malignancies combining PI3K inhibition with checkpoint blockade (including NCT02637531, NCT02646748, NCT03257722, and NCT02535286), and clinical trials combining PI3K targeted agents in combination with checkpoint blockade are currently being planned in patients with mCRPC.

Cell cycle pathway

Alterations to key mediators of the cell cycle pathway, including loss of *RB1, CKDN2A, CDKN2B,* as well as amplification and overexpression of *CCND1*, have been observed in at least 20% of patients with mCRPC [48,86]. These proteins help regulate the tightly controlled process of cell division, wherein Rb is normally hypophosphorylated, but becomes phosphorylated by cyclin-dependent kinases (including CDK4/6), leading to expression of genes that drive cell proliferation. Given their critical role in regulating cell cycle progression, *RB1* and *CDKN2A/B* (inhibitors of CDKs that serve to maintain Rb in a hypophosphorylated state) are two of the most common alterations across all metastatic malignancies [54], leading to a significant interest in developing effective inhibitors of this pathway. In particular, CDK4/6 inhibitors have been developed and approved in breast cancer, and are currently being evaluated as single agents in CaP (NCT02555189, NCT02059213). However, as these agents are designed to inhibit CDK4/6 (and thus allow hypophosphorylated Rb to appropriately regulate entry into the cell cycle), they require maintained Rb expression; as such, these agents would be predicted to have limited efficacy in *RB1*-deficient disease as opposed to malignancies lacking *CDKN2A* or those with wild-type Rb expression [87].

In addition to targeting entry into the cell cycle, the use of CDK inhibitors has also been shown to have effects on the tumor immune microenvironment to promote antitumor responses in preclinical models [88–90]. However, given that CDK agents are critical not only to tumor cell proliferation but also other cell types, there are several on-target off-tumor effects that can impact the effectiveness of tumor immunotherapy. For example, prolonged exposure to CDK inhibition can also have detrimental effects on the tumor microenvironment, driving senescence in nontransformed fibroblasts that promote tumor cell growth [91]. Combinatorial clinical trials evaluating CDK inhibitors along with checkpoint blockade are currently being conducted in breast and lung cancer (NCT02791334, NCT02079636, NCT02779751), and would be a rational approach for the treatment of advanced, Rb-proficient, or CDKN2A-deficient CaP.

Wnt/β-catenin signaling

In samples from mCRPC patients, alterations in the Wnt/β-catenin signaling pathway are identified in nearly 20% of patients [48,92]. In addition to its role in promoting CaP progression, Wnt signaling has also been shown to play an important role in prostate stromal cells, helping to promote tumor cell proliferation and therapeutic resistance [93]. However, targeting alterations to this pathway presents significant challenges, as this pathway is involved in a broad range of critical cellular functions that make the development of effective therapeutics challenging due to on-target,

off-tumor toxicities. Therapeutic approaches being evaluated include agents targeting Wnt secretion, blockade of Frizzled receptor interactions, as well as therapeutics targeting the activity of the β-catenin transcription factor downstream of Wnt activation [94], some of which have moved into clinical trials. This includes a Wnt mimetic peptide Foxy-5, which has been shown to have activity in Wnt-high CaP in preclinical models, and is being evaluated in a Phase I clinical trial in CaP patients (NCT02020291).

The activity of Wnt/β-catenin signaling has been implicated in a broad range of cellular functions, including modulating the tumor immune microenvironment. In multiple malignancies, including bladder cancer, tumors with active Wnt/β-catenin signaling are associated with immune exclusion and an immunologically cold tumor microenvironment [95,96]. However, Wnt signaling is also important in the maintenance of T cell memory and function [97], illustrating the challenge of properly combining agents blocking this pathway with immunotherapeutic approaches as these strategies enter preclinical evaluation.

Future directions

One of the central challenges in evaluating how targeted therapies and immunotherapies modulate the mCRPC tumor microenvironment is the difficulty in obtaining series biopsies of metastatic lesions. As CaP most commonly metastasizes to the bone, obtaining viable tumor material from pre- and post-treatment biopsy samples presents logistical, economic, and technical hurdles that have hindered their widespread incorporation into mCRPC clinical trials. Additionally, when tissue can be procured, issues remain regarding heterogeneity between lesions in patients with disease, and ensuring that the targeted lesion is representative of those that are responding to therapy. This complexity highlights the critical unmet need of companion longitudinal imaging to guide in the selection of biopsy targets. While methods evaluating circulating tumor cells or circulating tumor DNA can provide insight into alterations to genetic alterations within tumor cells (including evaluation of clonal evolution of tumor escape mutations), they preclude the evaluation of alterations to immune and other stromal components of the microenvironment. Additionally, as peripheral measures of immunity are a poor surrogate to changes in the tumor immune microenvironment, evaluating the response within the tumor microenvironment is a critical research area that needs to be addressed as combinatorial trials move into the future. The possibility of evaluating these therapeutic approaches in the neoadjuvant space provides access to additional tissue−however, the lower frequency of targetable alterations in primary lesions compared to metastatic disease increases the number of patients needed to evaluate these combinations.

The second major challenge in the development of novel immune-oncology combination strategies in advanced CaP is the identification of predictive biomarkers of response and resistance. In a variety of malignancies in which PD-1 therapies have been approved, PD-L1 expression in the tumor microenvironment (whether on tumor or immune cells) has been validated as a biomarker of responsiveness to PD-1 checkpoint blockade in some malignancies [98]. However, the relatively low expression of PD-L1 in CaP patients requires further evaluation of PD-L1 or other checkpoint molecule expression (such as PD-L2, as recent data has suggested) as potential prospective biomarkers in CaP patients [21]. In addition, patients with mismatch repair deficiency, or MSI tumors, have also been shown to have enhanced responsiveness to checkpoint blockade [99]. For example, alterations to genes such as *MLH1*, *MSH2*, *MSH6,* and *POLE* involved in the mismatch repair process lead to increased mutational rates, neoantigen presentation, and T cell infiltration in a variety of malignancies, potentially contributing to the enhanced efficacy of checkpoint blockade [99−101]. In CaP, approximately 3% to 5% of patients have MSI-high alterations [102] and recent studies have suggested these patients may have elevated response rates to checkpoint blockade [103]. This further highlights the importance of further examining these responses in CaP patients receiving immunotherapy, and identifying potential mechanisms of activity by which MSI may correlate with enhanced responsiveness. Conducting these studies in CaP will be critical toward not only potentially validating this biomarker in these patients, but can also be used to develop rational combinations with precision medicine as well as immunotherapeutic strategies outside of checkpoint blockade. The widespread utilization of next-generation sequencing in most major cancer centers, along with preclinical interrogation of tumor-intrinsic genomic drivers of immune-responsiveness/resistance, will lead to robust personalized biomarker development for immuno-oncology combination therapies in CaP.

Conflicts of interest

The authors declare no relevant potential conflicts of interest.

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