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Review Article

Prostate cancer autoantibodies - applications in diagnosis, prognosis, monitoring disease progression and immunotherapy

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Abstract: Although PSA testing is widely used in prostate cancer diagnosis, it remains an imperfect assay due to its lack of accuracy. While several urine or tissue-based gene expression assays are available to identify patients with higher risk of adverse disease and to aid in deciding treatment options, there is still a critical need for reliable biomarkers to monitor disease progression and treatment response. Autoantibodies (AAbs) produced by the humoral immune response against tumor associated antigens offer an attractive alternative, as they target a wide variety of prostate cancer specific antigens and can be collected by using clinically non-invasive methods. Herein, we review the transition from traditional methods that identify individual AAbs to high throughput approaches that detect multiple targets simultaneously in patient sera. We also discuss how these approaches improved the sensitivity and specificity of AAb detection and enhanced prostate cancer diagnosis and prognosis. Cancer vaccines offer potential as a novel therapeutic strategy in their ability to stimulate both cell-mediated and antibody-mediated cytotoxic responses. Ongoing efforts aim to identify immunotherapy targets that also stimulate a strong antibody response, since antibodies activated by the anti-cancer humoral response can eliminate cancer cells effectively via several distinct mechanisms. Autoantibodies are useful not only for the diagnosis of prostate cancer, predicting disease progression, and tracking response to treatment, but can also be harnessed as therapeutic agents for prostate cancer treatment.

Keywords: Prostate cancer, autoantibodies, humoral response, tumor associated antigens

Introduction

Diagnosis, prognosis, and treatment of prostate cancer

Prostate cancer is the most prevalent cancer among US men in 2022 with 268,490 diagnoses and 34,500 deaths projected. This approximates to one in four of all diagnosed cancers and one in nine cancer deaths among men [1]. Diagnosis of earlier stage disease, through prostate-specific antigen (PSA) testing and advances in treatment, decreased prostate cancer death rate by about 4% per year during the late 1990s and the 2000s [2]. Fluctuations of PSA levels in patients with benign prostatic hyperplasia (BPH), however, can confound the accuracy of PSA tests. Due to concerns about overtreatment, arising from the low specificity and high false positive rate of the assay, the US Preventive Services Task Force (USPSTF) recommended against PSA screening for men over 75 in 2008 and for men of all ages in 2012 [3]. This was later revised due to an apparent rise in higher grade, stage, and risk upon diagnosis [4]. Overtreatment of prostate cancer patients remains a concern in the U.S., as about 30-40% of men who have undergone surgery or other treatments likely had indolent tumors [5].

Beyond the initial prostate cancer diagnosis, patients are faced with the need to assess the

risk of disease progression. For better results, risk predictive models used in the clinical setting usually incorporate PSA as a molecular marker together with digital rectal examination (DRE), trans-rectal ultrasonography (TRUS), or multiparametric magnetic resonance imaging (MRI) [6]. The integration of prostate cancer susceptibility associated genetic polymorphisms and inherited variants identified from genome-wide association studies in polygenic models provided only modest improvements [7, 8]. Applications of urine-based RNA assays such as Mi-Prostate Score, SelectMDx, and ExoDx have improved the prediction of indolent and aggressive disease and helped to identify patients who may benefit from prostate biopsy [9]. In men with previous negative biopsies, the Progensa Prostate Cancer Antigen 3 (PCA3), a post-DRE urine test, and ConfirmMDx, a tissue-based methylation marker test, are predictive of follow-up biopsy outcomes and could help to decide on whether a rebiopsy is necessary [10]. Several commercially available tissue-based assays such as Decipher, Oncotype DX, and Prolaris, which measure the mRNA expression of multi-gene panels were shown to successfully identify men at the highest risk of adverse outcome and helped to improve prostate cancer risk stratification [11-13].

Among patients with advanced disease, most of whom receive androgen deprivation therapy (ADT) treatment and are likely to progress to castration-resistant cancer, it is critical to be able to predict disease progression and monitor response to treatment. Positive detection of AR-V7 expression, for example, could predict resistance to abiraterone or enzalutamide [14]. Meanwhile, metastatic prostate cancer patients harboring mutations in DNA damage repair genes could benefit from poly (ADP-ribose) polymerase-1 inhibitors and platinum-based chemotherapy [15, 16].

In both pre-treatment active surveillance and follow-up monitoring for treatment response, the PSA test continues to be the cornerstone assay. While most solid tumors can be evaluated reliably using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria as a measure of objective response, this guideline is impractical for prostate cancer as metastatic lesions are often smaller and considered "unmeasurable". Bone scans to confirm metastasis are recommended in symptomatic men or in asymptomatic men with a PSA of > 20 ng/ml. The sensitivity of 18F-flouro-2-deoxy-2-D-glucose (FDG)-positron emission tomography (PET) can be unpredictable because prostate cancer has a low metabolic glucose activity and high bladder activity due to urinary FDG excretion can occlude tumors [17]. Consequently, there remains a critical need for dependable biomarkers that can help monitor disease progression and response to treatments.

Cancer autoantibodies and their applications in prostate cancer

Circulating AAbs are attractive candidate biomarkers because of their association with tumor proliferation and because they target a diverse range of prostate cancer specific antigens.

During carcinogenesis, TAAs are introduced by mutations in malignant cells that create new epitopes, or neoepitopes that alter protein structures, which become exposed to the host when cells undergo apoptosis (**Figure 1**). The host perceives these self-antigens as foreign and mounts a humoral immune response against them by generating AAbs [18]. Antigen presenting cells (APCs), such as macrophages and dendritic cells, engulf, lyse, and present the TAAs on their cell surface to T and B cells. CD4+ helper T cells that recognize the TAAs are activated to release cytokines and chemokines, which enhances B cell production, proliferation, and clonal expansion [19]. TAAs presented on APCs also activate B cells, particularly a subset of tumor-infiltrating B lymphocytes that include B-1 or $CD5^+$ cells, that produce AAbs directed against self-antigens [20, 21]. These B cells further activate the proliferation of helper T cells that, in turn, may attach to and increase the production of TAA-bound B cells [22]. Thus, a multitude of B cells are primed against the same antigen. Of these, many remain as memory B cells, while others differentiate into antibody-producing plasma cells that bring about systemic release of the specific antibody, which can facilitate the destruction of tumor cells [23].

During the neoplastic process, this immune dysregulation may be further enhanced by the loss of self-tolerance through clonal deletion,

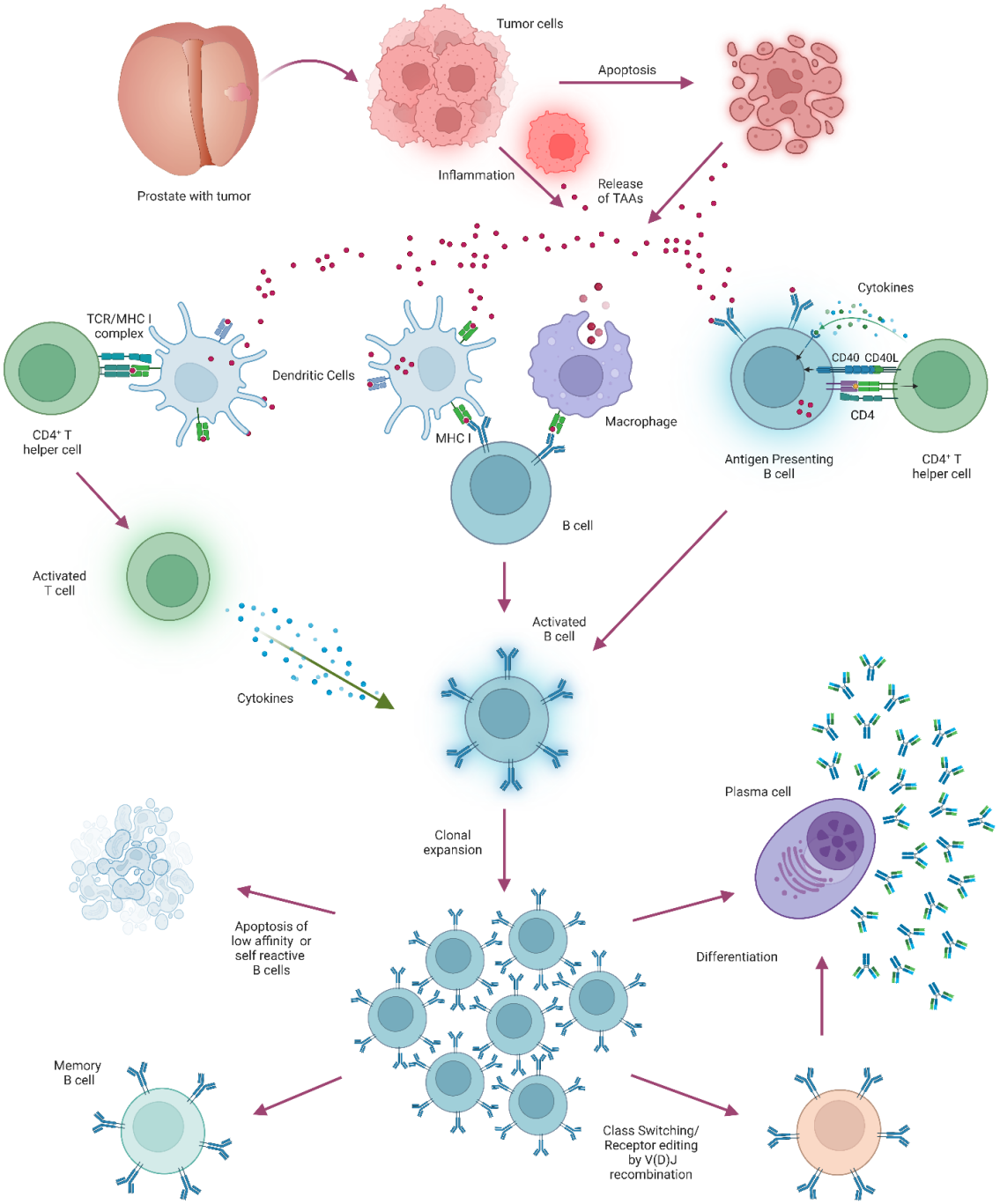

Figure 1. Humoral immune response against tumor associated antigens in prostate cancer. Tumor cells that are inflamed or undergo apoptosis release TAAs that are ingested by APCs in the form of macrophages and dendritic cells. TAAs presented on the cell surface of these APCs are recognized by $CD4^+$ helper T and B cells. $CD4^+$ helper T cells that recognize TAAs are activated to release cytokines and chemokines that stimulate B cells to proliferate by clonal expansion. A subset of B cells that bind to TAAs on APCs are similarly activated to produce AAbs. B cells that lose self-tolerance or have low affinity against TAAs undergo clonal deletion and are removed by apoptosis. Some B cells remain as memory B cells, others undergo antigen receptor editing by V(D)J recombination, still others differentiate into antibody-producing plasma cells that bring about systemic release of TAA-specific AAbs, which can recognize and destroy tumor cells (Figure is created by authors using Biorender.com).

whereby self-reactive B lymphocytes are removed by apoptosis [24], or clonal anergy, when lymphocytes are silenced into a non-reactive state [23, 25, 26]. Conversely, lymphocytes

may be preserved when antigen receptors are edited or revised by VDJ recombination and class switching [27]. High AAb titers could be maintained by downregulation of regulatory T (Treg) cells and increased effector T helper cells [28, 29], or by an inflamed tumor microenvironment that facilitates the release and exposure of more TAAs to the immune system [30].

Autoantibodies against TAAs in patients across multiple malignancies that include melanoma [31], colorectal [32], gastrointestinal [33], breast [34], and bladder [35] cancers could be detected by various high-throughput approaches. Besides products of mutated genes, tumor antigens that elicit immune responses include differentiation antigens, or proteins that are over-expressed in cancer [36]. Tumor suppressor p53 protein, for example, has consistently elicited a humoral response between 12% to 40% of affected patients with prostate, breast, colon, oral, or gastric cancers [37, 38]. In contrast to low levels of their corresponding antigens, AAbs often remain stable at high levels in serum, persist in circulation [39], and are detectable months or years before clinical symptoms appear. The onset of AAb production may also reveal molecular processes in disease etiology that allow us to predict its course [40]. Additionally, testing AAbs, much like PSA, involve a relatively simple, non-invasive procedure, making its use invaluable to clinical practice [41-46]. In this review, we discuss the feasibility of different approaches used in AAb detection for prostate cancer diagnosis and prognosis, the use of AAbs in monitoring treatment response, and their potential role as agents of prostate cancer immunotherapy.

Methods of autoantibody detection

Historically, prostate cancer AAbs were detected using traditional methods that apply enzyme-linked immunosorbent assays (ELISA) (**Figure 2A**), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and western blot analysis (**Figure 2B**) in the serum of prostate cancer patients. These methods, however, could only detect the most abundant AAbs against specific cognate TAAs. ELISA, for example, was used to show that AAbs against cancer testis antigen 1B (NY-ESO-1), were present at higher titers in sera of hormone refractory prostate cancer than those of localized cancer, which were correlated with poor survival [47]. Meanwhile, high throughput methods that employ panels of TAAs, which can yield higher diagnostic value over a single TAA, have been used to identify multiple prostate cancer specific AAbs [48]. In addition to serological proteome analysis (SERPA) and serological identification of antigens by recombinant expression cloning (SEREX), we discuss high throughput techniques that use protein arrays, electrochemistry, microfluidics, and other advances in proteomic methods, which have facilitated the discovery of an increasing number of prostate cancer AAbs with specificity against novel tumor antigens.

Serological proteome analysis (SERPA)

SERPA requires the separation of a complex mixture of proteins extracted from tumor or cell cultures in two-dimensional (2D) gel electrophoresis by their isoelectric points (pI) and molecular weights followed by identification by mass spectrometry (**Figure 2C**). SERPA has the advantage of enabling both the antibody response and the identity of the immunogenic tumor proteins, including post-translational modifications, to be determined based on their reactivity with autologous patient sera [49]. Unfortunately, SERPA requires a large amount of tumor proteins, and is limited by the low resolution of 2D electrophoresis and poor reproducibility. A comparison of pooled serum samples of prostate cancer patients and healthy controls using SERPA by Ummanni et al. [50], detected 18 antigens immunoreactive to serum from cancer patients. Further validation using recombinant antigens and an independent set of cancer sera confirmed an increased abundance of peroxiredoxin-6 (PRDX6) and annexin A11 (ANXA11) antibodies in prostate cancer patients. A comparative screen of sera from European-American (EA) and African American (AA) men with prostate cancer using SERPA detected higher levels of AAbs against nucleophosmin 1 (NPM1) in prostate cancer patients, especially in AA men, than in BPH patients and healthy individuals [51]. These findings suggest the possibility of race-associated differences in the AAb response of prostate cancer that may define novel biological determinants of prostate cancer health disparities.

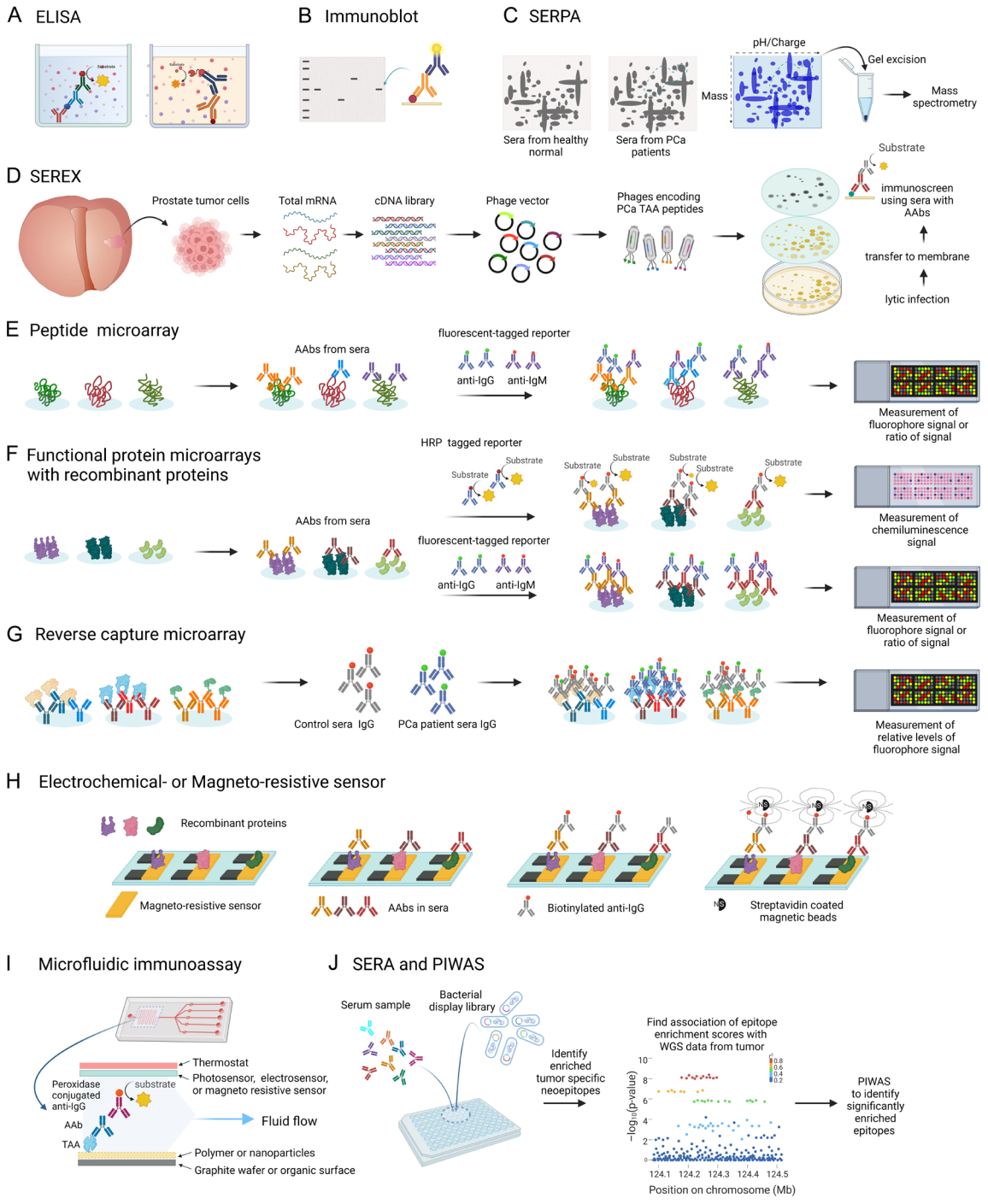

Figure 2. The development of methods of autoantibody detection. AAbs against cancer TAAs were initially identified using traditional methods such as ELISA (A) and immunoblot assay (B). SERPA (C) and SEREX (D) allowed the detection of multiple prostate cancer specific AAbs. Further immobilization of peptides (E) or functional recombinant proteins (F) on glass slides in microarray format followed by detection using chemiluminescence- or fluorescence-tagged secondary antibodies elevated the high-throughput screening of AAb targets to the scale of several hundreds of thousands. In reverse capture microarray (G), high-affinity antibody microarrays were used to capture native antigens from cell extracts of tumors or cell lines before control and cancer AAbs labelled with different fluorophores were applied to allow the relative abundance of the AAbs in a serum sample to be determined from the ratio of fluorescence signals. AAb detection by electrochemical or magneto-resistive sensor (H) use immobilized antigens to selectively capture target antibodies, which were detected using secondary antibodies tagged with electroactive molecules or magnetic nanoparticles that emit measurable electrochemical or magnetic signals for the quantifica-

tion of AAbs. Microfluidic devices containing nanostructures with automated fluidic handling allow AAbs to be detected with high sensitivity from femtoliter volumes of serum samples with minimal sample handling and processing (I). In the SERA and PIWAS approach (J), serum epitope enrichment scores that match somatic mutation-specific epitopes identified by genomic sequencing of tumor specimens were used to find enriched epitopes and prevalent antibodies in serum samples of prostate cancer patients [90] (Figure is created by authors using Biorender.com. The figure in H is adapted from Xu et al., under the terms of the Creative Commons Attribution License [63]).

Serological identification of antigens by recombinant expression cloning (SEREX)

In SEREX, a cDNA expression library constructed from prostate tumor specimens is cloned into lambda phage expression vectors and transduced into *Escherichia coli*. Thousands of recombinant peptides, expressed during lytic infection of bacteria, are transferred onto nitrocellulose membranes, which are then incubated with diluted serum samples (**Figure 2D**). Immunoreactive clones identified by anti-human IgG secondary antibodies are then sequenced to identify the autoantigen [52]. The multitude of peptides presented using SEREX enables the simultaneous assay of a large number of antibody and recombinant tumor protein interactions. SEREX, however, may bias the detection of AAbs against proteins with higher mRNA transcripts, those that could be expressed in bacteria, or the detection antibodies against non-TAAs, while missing AAbs against TAAs of low abundance. The time-consuming and labor-intensive protocol involved as well as the poor reproducibility of this method remains a great disadvantage. SEREX based on phage-display random-peptide libraries was used to screen serum samples of prostate cancer patients for peptides that bind to AAbs against heat shock protein 70 family protein 5 (HSP70/HSPA5/GRP78) [36], and fetuin-A or alpha 2-HS glycoprotein (AHSG) [46]. A screening of 18,000 clones from a phage display prostate tumor cDNA library using autologous patient antibodies identified several antigen-AAb interactions that included NY-ESO-1, X antigen family member 1 (XAGE-1), DJ-1/parkinsonism associated deglycase (PARK7), and transcription factor 25 (TCF25) [53].

Serological analysis by protein or antibody microarrays

The integration of protein microarrays in SEREX or SERPA has enabled the analysis of multiple targets in a single step and increased the number of assays that can be performed within a single serum sample. Microarrays immobilized with hundreds to thousands of known antigens can be probed with serum samples from patients and healthy controls to identify antigens that specifically elicit an immune response to cancer (**Figure 2E** and **2F**). Protein microarrays immobilized with 8,000 to 80,000 recombinant antigens, such as ProtoArray protein chips, have been developed and used to screen AAbs in patients with other cancers [54, 55]. The small reaction surface that allows uniform sera distribution across the surface of microarrays enables the detection of a high dynamic range of signal intensities and collection of large amounts of data in a reproducible and high-throughput manner within a single experiment [56]. The use of recombinant proteins in protein microarrays, however, may miss the detection of post-translationally modified targets. Other disadvantages include higher costs in addition to the need for sophisticated data analysis software to analyze and interpret the large volume data collected.

By using a 22-phage peptide microarray panel to analyze serum samples from 119 prostate cancer patients and 138 controls, Wang et al. [45], were able to discriminate between prostate cancer and control groups at 88.2% specificity and 81.6% sensitivity, an improvement over the PSA test. Four of the 22 targets that encoded known proteins that include bromodomain containing 2 (BRD2), eukaryotic translation initiation factor 4 gamma 1 (eIF4G1), ribosomal protein L13a (RPL13A), and ribosomal protein L22 (RPL22) were later confirmed to be deregulated in prostate tumors by immunoblot of tissue extracts and by meta-analysis of gene expression data.

Working on the premise that aberrant post-translationally modified cancer-associated proteins are likely to be AAb targets, Wandall et al. [57] developed N-hydroxy succinimide (NHS)-activated hydrogel microarray slides printed with O-glycopeptides. When used to screen sera from newly diagnosed breast, ovarian, and prostate cancer patients, specific IgG antibodies against three distinct aberrant mucin 1

(MUC1) O-glycopeptide epitopes, Tn-MUC1, STn-MUC1, and truncated core 3 O-glycopeptides were detected. These results showed that aberrantly glycosylated O-glycopeptides are potential AAb-inducing TAAs, and AAbs against these epitopes may represent sensitive biomarkers for early detection of prostate cancer.

Reverse capture microarray

Reverse capture microarray requires the initial immobilization of well-characterized, highly specific, and high-affinity antibodies on glass slides. Cell extracts from tumors or cell lines are applied to these slides to capture native antigens before adding control and cancer AAbs labelled with different fluorophores, from which the ratio of fluorescence signals could be used to determine the relative abundance of the AAbs in a serum sample [58] (**Figure 2G**). When the numerous immobilized antibodies are exposed to a single protein lysate, multiple TAAs can be captured, which allows for the detection and quantitation of multiple TAA-AAb interactions, but this advantage is offset by the requirement for highly specific capture antibodies and large amounts of protein lysate [59].

An evaluation by reverse capture, using serum samples from ten biopsy-positive prostate cancer patients and five BPH subjects, successfully identified 28 antigen-AAb reactivities with the potential to discriminate prostate cancer from BPH [60]. Autoantibodies against five antigens - nuclear factor of activated T-cells, cytoplasmic 2 (NFATC2/NFAT1), heat shock factor protein 4 (HSF4), tumor suppressor p53, caspase-8 (CASP8), and transcription factor PU.1 (SPI1) - were able to distinguish prostate carcinoma from normal sera in 83% of cases examined. Further refinement of this approach was applied in a study using serum samples from 41 prostate cancer patients and 39 BPH patients that identified a panel of AAb signatures comprising TAR DNA binding protein (TARDBP), talin 1 (TLN1), PARK7, PC4 and SFRS1 interacting protein 1 (LEDGF/PSIP1), and caldesmon 1 (CALD1), which outperformed the PSA test in discriminating prostate cancer from BPH [61]. While the immobilization of antigens in their native configuration and post-translationally modified state allows the immediate identification of antigens, the increased cost of generating high-quality, specific antibodies or properly folded proteins limit the adoption of reverse capture microarrays.

Detection by electrochemical or magneto-resistive nanosensor signals

In electrochemical detection, target antibodies are first selectively captured by antigens and then detected using a secondary antibody tagged with electroactive molecules generating measurable signals that allow the number of AAbs to be quantified [62, 63] (**Figure 2H**). A readout proportional to levels of the AAb can then be obtained by measuring the electrical impedance signal, or current, while the potential is either fixed, or varied.

In Magneto-resistive nanosensors (MNS), recombinant proteins are immobilized onto arrays on a chip to capture target AAbs when serum samples are applied [62]. Biotinylated anti-human IgG antibodies are later added, followed by streptavidin-coated magnetic nanoparticles. The bound magnetic nanoparticles disturb local magnetic fields and induce changes in the resistance of the MNS at a given spot, signaling the capture of a specific AAb. An MNS assay that used a panel of four AAbs against TARDBP, TLN1, CALD1, and PARK7, together with total and free PSA, was able to successfully distinguish between prostate cancer and non-cancer samples [63]. While electrochemical or magneto-resistive nanosensors can be initially costly and require time to set up, the ease of miniaturization can assist in developing assays that are highly sensitive and specific, which respond quickly to changes in analyte concentrations [64].

Microfluidic immunoassay

The rapid development of advanced nanofabrication techniques has enabled the manufacture of various nanostructures that include plasmonic gold-on-gold (Au/Au) films [65], nanoparticles [66], nanopillars [67], nanorods [68], and nanowells [69] that have dramatically improved the sensitivity of immunoassays. Microfluidics allow researchers to reduce reagent and sample volumes to femtoliter levels while integrating automated fluid transfer steps and multiplexed detection of antibodies with minimal sample handling and processing (**Figure 2I**). The high surface-area-to-volume ratios and smaller distance scales of this plat-

form can enhance sensitivity and selectivity of detection, enabling faster and more efficient analysis [70]. A microfluidic chip that combined micropumps, micromixers, and microvalves was able to detect p53 antibody at a detection limit of 4 ng/mL and distinguish relative levels of p53 AAbs from saliva of oral cancer patients [71]. Although microfluidics is highly developed, to date, most devices are designed to immobilize a limited number of antibodies for capturing specific cancer protein biomarkers such as PSA, prostate specific-membrane antigen (PSMA), carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5/CEA), platelet factor 4 (PF-4), interleukin 6 (IL-6), and alpha fetoprotein (AFP) [72]. This promising technology has yet to be used for the detection of AAbs in prostate cancer patients.

Autoantibodies as diagnostic biomarkers of prostate cancer

Prostate cancer patients have been shown to develop immune responses against both universal TAAs and prostate cancer specific TAAs. In the clinical setting, AAb detection by low-throughput methods was used at the outset to confirm cancer diagnosis in previously identified prostate cancer patients. Initial identification of AAbs, such as those against alpha-methylacyl-coenzyme A racemase (AMACR) [73], Anoctamin 7 (ANO7/NGEP) [74], huntingtin-interacting protein 1 (HIP1) [75], and ETS transcription factor (ERG) [76] were performed using a combination of immunoblots and ELISA. AMACR is a prostate cancer-enriched TAA in prostate tumor epithelia [77-79] that was found to elicit an AAb with 72% sensitivity and 62% specificity in detecting prostate cancer [73]. HIP-1 is a cellular survival factor that is upregulated in prostate cancer compared to benign prostatic tissue [80]. Bradley et al., was able to detect HIP1 AAbs in sera from 97 prostate cancer patients and 211 controls by ELISA or immunoblot at 56% sensitivity and 69% specificity [75]. For comparison, a similar evaluation of 68 prostate cancer patients with PSA > 4.0 ng/mL and 29 age-matched controls achieved 88% sensitivity and 64% specificity ($P \leq 0.001$). The combined detection of both HIP1 and AMACR AAbs increased the specificity to 97%. These findings support the functional role of HIP1 in prostate cancer tumorigenesis and the importance of HIP1 AAbs as a serum biomarker.

Mohsenzadegan et al., used ELISA to show that AAbs against the prostate-specific NGEP could discriminate between prostate cancer patients and healthy controls with an area under the curve (AUC) for receiver operating characteristic (ROC) of 0.7 and 0.68, respectively [74]. Univariate analysis revealed a statistically significant inverse correlation between seropositivity against NGEP with higher Gleason scores, which concurs with reduced NGEP expression in prostate tumor tissues [81, 82], suggesting a possible utility for detecting early stages of prostate cancer.

Considering the higher prevalence of *TMPRSS2-ERG* gene fusion and ERG protein expression among Caucasian American (CA) prostate cancer patients [83], Rastogi et al., hypothesized that ERG AAbs may be induced among patients that harbor ERG fusions [76]. To resolve this, they evaluated sera from 37 healthy controls and 93 age-matched CA prostate cancer patients from an equal access miliary treatment facility by using ELISA. Higher levels of anti-ERG AAbs were detected in the prostate cancer patients, compared to the healthy individuals ($P = 0.0001$; AUC = 0.715). Further AAb screening using a triplex antigen panel, comprising ERG and AMACR recombinant proteins and a GAG-HERV-K peptide, showed discrimination of prostate cancer patient sera from healthy controls at an AUC = 0.792. In addition to detecting the presence of anti-ERG AAbs in the sera of prostate cancer patients, these findings suggest that AAbs against ERG together with AMACR and GAG-HERV-K may be a useful panel for the for diagnosis and prognosis of prostate cancer.

An evaluation for AAbs to cyclin B1 (CCNB1) and 14 other TAAs by using ELISA in sera from 174 patients with prostate cancer, 21 with BPH, and 89 healthy controls, detected cyclin B1 AAbs in 31% of prostate cancer patients versus 4.8% of sera from those with BPH [84]. On further analysis of sera from early-stage prostate cancer patients and patients who had normal PSA, cyclin B1 AAbs were detected at a specificity of 31.4% and 29.4%, respectively. The positive detection of AAbs against a panel of seven selected TAAs that included cyclin B1, survivin (BIRC5), p53, DFS70/LEDGFp75 (PSIP1), Ras-related protein Ral-A (RALA), MDM2, and nucleophosmin (NPM1) in prostate

cancer patients reached a sensitivity of 80.5%. The findings suggest that AAbs against cyclin B1 might be useful for the diagnosis of early stage prostate cancer, especially in patients with normal PSA levels.

Higher throughput detection methods that employ novel technologies have increased the identification of multiple prostate cancer AAbs. Schipper et al. [85] used a previously developed phage library [45], to screen 48 biopsy-positive and 48 clinically negative serum samples iteratively on a high-throughput Luminex platform to identify 18 biomarkers. Further logistic regression modeling of results from a training set of serum samples from 268 prostate cancer and 251 controls refined the selection to eight protein markers, which included casein kinase 2 alpha 2 (CSNK2A), centrosomal protein 164 (CEP164), NK3 homeobox 1 (NKX3-1), aurora kinase A interacting protein 1 (AURKAIP1), BMI1 polycomb ring finger protein (BMI1), ADP ribosylation factor 6 (ARF6), and desmocollin 3 (DSC3). When applied to the training set and the validation set, the panel excelled in discriminating between the cancer and control samples at an AUC of 0.74 and 0.69, respectively. The scores from the algorithm developed in this assay could potentially be used to indicate the risk of prostate cancer, especially for patients with intermediate PSA levels of between 4 and 10 ng/ml.

In another high-throughput approach that employed a microarray of 37,000 recombinant human proteins to profile serum samples from 20 prostate cancer patients and 20 healthy controls, Klocker and colleagues detected AAbs against 174 antigens that were found exclusively in prostate cancer patients [86]. Further validation of these AAbs against an independent patient cohort confirmed the utility of the panel to discriminate between prostate cancer, benign disease, and healthy patient sera. ROC curve analysis of the top 15 AAbs showed that AAbs against tubulin tyrosine ligase like 12 (TTLL12), could distinguish prostate cancer from benign disease patients with an AUC of 0.71. Further screening using a low-density protein array of 4,012 recombinant proteins on serum samples from 70 radical prostatectomy patients with localized disease, and selected based on levels of infiltrating lymphocytes as an indication of inflammation (38 high inflammation and 32 low inflammation) [87], identified 165 AAbs that were significantly more abundant in the serum of high inflammation patients. The top three AAbs included those against spastin (SPAST), syntaxin 18 (STX18), and speckle-type POZ protein (SPOP), which were significantly different by *p*-value and fold change in high inflammation patients. Further examination in prostate cancer tissue specimens detected significantly increased gene and protein expression of SPAST in samples from the high inflammation patients compared to those from the low inflammation group. Assessment in an inflammation-independent tissue microarray by IHC detected increased SPAST and STX18 expression in most tumor samples. Further cross-validation of the inflammation AAb profile on an independent sample set using a Luminex-bead protein array retrieved 51 of the 165 significantly discriminating AAbs. The AAbs against methylmalonyl-CoA mutase (MUT), Ras-related protein Rab-11B (RAB11B), and cysteine and glycine rich protein 2 (CSRP2) were significantly upregulated in both screens, while AAbs against SPOP and zinc finger protein 671 (ZNF671) approached statistical significance. These findings provide evidence for a prostate cancer inflammation-specific AAb profile and support the evaluation of AAbs as non-invasive biomarkers for prostate inflammation.

The diversity of AAbs capable of discriminating prostate cancer patients from healthy controls highlight the extent of humoral response against prostate cancer TAAs in patients. The importance of these AAbs as a diagnostic biomarker could perhaps be concluded from their reported AUC values, which reflected the discriminatory power to distinguish between prostate cancer patients and heathy controls: AAbs against fetuin-A [46], TARDBP, and TLN1 [61] were shown to have outstanding discriminative power (AUC > 0.9), while NPM1 [51], and PARK7 [61] AAbs had excellent discriminative power (AUC = 0.8-0.9), whereas AAbs against NGEP [81], ERG [76], AMACR [73], TTL12 [86], PSP1, and CALD1 [61] demonstrated acceptable discriminative power (AUC = 0.7-0.8) (**Table 1**). Furthermore, the detection of AAbs against AMACR [73, 75, 76, 88], GAG-HERV-K [76, 89, 90], and NY-ESO-1 [53, 88, 90, 91] in serum samples of independent studies, their adoption as a benchmark for the detection of

Table 1. Methods used for the detection of prostate cancer autoantibodies

AAbs and methods of detection	Sensitivity (%)	Specificity (%)	AUC or *P* value	Notes	Study
Immunoblot and ELISA					
HIP1	88%^{HIP1}	64%^{HIP1} 97%$^{HIP1+AMACR}$	P ≤ 0.001^{HIP1}	Positive score by either ELISA or immunoblot. Diagnostic.	[75]
ANO7/NGEP	-	-	P < 0.001, AUC = 0.71, 95% CI (0.63-0.74)	Significantly higher AAbs against ANO7 protein in PCa patient sera vs. healthy controls. Diagnostic.	[74]
GAG-HERVK	-	-	Association with overall survival: P = 0.006$^{Gehan-Breslow-Wilcoxon}$ P = 0.053$^{Log-rank}$ HR = 1.98 (1.23-11.85)	Detected higher GAG-HERV-K AAb levels in PCa patients that increased with tumor stage. Seropositivity in RP patients is associated with worse survival. Diagnostic and prognostic.	[89]
ERG, AMACR, GAG-HERV-K			P = 0.0001, AUC = 0.715^{ERG} AUC = 0.792$^{ERG, AMACR, GAG-HERV-K}$	Combined ERG, AMACR and GAG-HERV-K improved discrimination of PCa from control sera. Diagnostic.	[76]
CCNB1, BIRC5, p53, PSIP1, RALA, MDM2, and NPM1	80.5%$^{7-TAAs}$	91%$^{7-TAAs}$	P = 0.013, AUC = 0.942, 95% CI (0.916-0.968)	Potential use of Cyclin B1 AAbs for the diagnosis of early stage PCa, especially in patients with normal PSA level. Diagnostic.	[84]
SERPA					
PRDX6, ANXA11	70%^{PRDX6} 80%^{ANXA11} 90%$^{PRDX6 \& ANXA11}$	-	-	Detection of AAbs in serum discriminated between prostate tumor and healthy. Controls. Diagnostic.	[50]
NPM1	75.9%	75.9%	AUC = 0.86^{Test} AUC = 0.82^{Validation}	Significantly higher AUC values for AA PCa patients. Diagnostic.	[51]
SEREX					
HSP70/HSPA5/GRP78	-	-	Log-rank P = 0.07	Reactivity against GRP78 associated with more aggressive disease. Prognostic.	[36]
Fetuin A/AHSG	-	-	AUC = -0.91, 95% CI (0.830-0.992)	Strongest AAb reactivity to Fetuin-A in sera from mCRPC patients; AUC of 0.91 in distinguishing mCRPC and normal controls. Prognostic.	[46]
Phage display library & protein microarray					
BRD2, eIF4G1, RPL13A, RPL22	81.6% 95% CI (0.70-0.90)	88.2% 95% CI (0.78-0.95)	-	Four peptides of the 22-phage peptide panel encoded known proteins. Diagnostic.	[45]
CSNK2A, CEP164, NKX3.1, AURKIAP, ARF6, BMI1, and DSC3	65%	65%	AUC = 0.74$^{Training set}$ AUC = 0.69$^{Validation set}$	Scores from the developed algorithm could be used to indicate relatively higher or lower PCa risk, particularly for patients with 4.0 to 10 ng/ml PSA. Diagnostic.	[85]
Protein, peptide, or antibody microarray					
AMACR	-	-	AUC = 0.789$^{AMACR (Immunoblot)}$ (95% CI = 0.705-0.872; P < 0.001) AUC = 0.492^{PSA} (95% CI = 0.381-0.603). P = 0.009$^{Protein microarray}$ P = 0.011^{ELISA}	Initial screen with protein array of 12 proteins, validated by immunoblot and ELISA. More sensitive in distinguishing sera of PCa patients vs. controls. Diagnostic.	[73]
TTL12	-	-	AUC = 0.71	Protein microarray with 37,000 recombinant proteins discriminated between PCa patients and benign disease patients. Diagnostic.	[86]

Glycosylated MUC1	-	-	-	Four out of 10 prostate cancer patients showed induction of AAbs against mucSTn, T, and core 3 MUC1 glycopeptides. Diagnostic.	[57]
Detected significant changes in AAb responses against PCAT-14 (lncRNA), ribosomal proteins (BRD2, RPL13a, RPL22 and LAMR1), ACPP, VCP and PRDX6	-	-	-	Association of AAb detection with clinical stage of disease, especially between patients with castration-sensitive and castration-resistant disease. Prognostic.	[40]
Serum Epitope Repertoire Analysis (SERA) and Protein-Based Immunome Wide Association Studies (PIWAS)					
Identified 11 mCRPC immunogenic proteins, including NY-ESO-1, NY-ESO-2, GLV1-47, HERVK-113, HERVK-24, SLC2A5, RIPK3, ST8SIA5, TRBV25-1, and SART3	-	-	-	Detected cancer-specific enrichment of AAbs to mutant peptides in select genes and to nonmutant peptides in the NYESO-1 and HERVK-113 proteins in mCRPC. Prognostic.	[90]
Protein microarray and Luminex beads					
SPAST, SPOP, and STX18 identified in initial screen SPOP, MUT, ZNF671, RAB11B and CSRP2 identified by cross validation using Luminex platform	80%	67%	Screening: P = 0.001^{SPAST}; FC = 14.3 P = 0.003^{SPOP}; FC = 4.3 P = 0.014^{STX18}; FC = 7.8 Validation: P = 0.051^{SPOP}; FC = 1.14 P = 0.003^{MUT}; FC = 1.65 P = 0.051^{ZNF671}; FC = 1.12 P = 0.003^{RAB11B}; FC = 1.37 P = 0.051^{CSRP2}; FC = 1.29 AUC = 0.85	AAbs against five antigen panel were upregulated significantly in the high inflammation group of both screening and validation cohort. Diagnostic.	[87]
Reverse capture					
28 unique Ag-AAb reactivities, including CHD3, NFAT1, EGFR, and p53	-	83%$^{(NFAT, HSF4, p53, CASP8 & SPI1)}$	P = 0.0001^{CHD3} P = 0.001^{NFAT} P = 0.004^{EGFR}	Identified 28 unique Ag-AAb reactivities from 500 specific antibody-antigens on microarray with potential to discriminate PCa from BPH (p-values < 0.01). Diagnostic.	[60]
TARDBP, TLN1, PARK7, PSIP1/LEDGF, CALD1	95%$^{combined\ panel}$	80%$^{combined\ panel}$	AUC = 0.93^{TARDBP} AUC = 0.91^{TLN1} AUC = 0.89^{PARK7} AUC = 0.79^{PSIP1} AUC = 0.77^{CALD1} AUC = 0.95$^{combined\ panel}$	AAbs against five-antigen panel could distinguish between PCa and BPH in patients with higher serum PSA vs. PSA alone more accurately (AUC of 0.95 vs. 0.5) and sensitivity (95% vs. 12%). Diagnostic.	[61]
Electrochemical/Magneto-resistive sensor					
TARDBP, TLN1, CALD1, PARK7, total PSA, free PSA	-	-	Discrimination of PCa from BPH samples: AUC = 0.500^{PARK7} AUC = 0.793^{TARDBP} AUC = 0.625^{TLN1} AUC = 0.820^{CALD1} AUC = 0.693$^{free/total\ PSA\ ratio}$ AUC = 0.916$^{AAb\ panel\ +\ PSA\ ratio}$	AAb panel together with PSA and free PSA can potentially distinguish between PCa and non-cancer patients with higher sensitivity and specificity than PSA alone. Diagnostic.	[63]

PCa, Prostate Cancer; BPH, Benign Prostatic Hyperplasia; mCRPC, metastatic castrate resistant prostate cancer; NHS, Normal Human Sera; AA, African-American; CA, Caucasian-American; FC, Fold change.

other AAbs, and their ability to enhance the sensitivity of AAb panels, highlight their importance as diagnostic biomarkers.

Among multi-TAA panels, the combined panel detecting TARDBP, TLN1, PARK7, TLN1 and PSP1 AAbs using reverse capture microarray platform showed the highest sensitivity and specificity and best discriminative power (AUC = 0.95) [61]. The panel of AAbs identified in this assay (TARDBP, TLN1, CALD1, and PARK7) was used in combination with total and free PSA to develop an MNS multiplex assay. In an evaluation on serum samples from 49 prostate cancer patients and 50 patients without cancer, the panel was able to distinguish between prostate cancer and BPH in patients with increased accuracy, compared to PSA ratio alone (AUC of 0.916 vs. 0.693) [63]. These results showed that AAb detection could overcome the limitations of the PSA test to detect prostate cancer in BPH patients exhibiting increased serum PSA. Meanwhile, the panel of AAbs comprising SPOP, MUT, ZNF671, RAB11B and CSRP2 was able to distinguish prostate cancer patients with low inflammation from those with high inflammation at an AUC of 0.85. Lastly, an eight-AAb biomarker panel against CSNK2A, CEP164, NKX3.1, AURKIAP, ARF6, BMI1, RhoEGF, and DSC3 was shown to be useful for determining prostate cancer risk among patients with intermediate PSA levels.

Autoantibodies as prognostic biomarkers of prostate cancer

The ease by which serum samples can be collected and the potential to detect AAbs at early stages of disease have both enhanced the utility and improved the value of AAbs as prognostic markers. Higher levels of AAbs against GRP78 [36], fetuin-A [46], and GAG-HERV-K [89] were demonstrated to be predictive for progression to more aggressive disease and underscore their importance as prognostic biomarkers of prostate cancer. Both GRP78 and fetuin-A AAbs were identified by screening combinatorial peptide phage libraries using SEREX. Increased reactivity of GRP78 AAb was observed using ELISA in sera of locally advanced, androgen-dependent metastatic, and androgen-independent metastatic prostate cancer patients compared to those from patents with organ confined disease. Kaplan-Meier survival analysis showed an association between GRP78 reactivity with a trend towards a shorter overall survival (log-rank test, P = 0.07) [36]. Using sequential serum samples from an index patient, reactivity to fetuin-A was shown to increase during progression of disease, and strong reactivity was detected in a large cohort of metastatic prostate cancer patients [46]. Reactivity to fetuin-A AAb could distinguish between both castrate-sensitive metastatic or castrate-resistant metastatic prostate cancers and control samples with AUC of 0.91. Reis et al. [57], used ELISA to screen sera from 1,367 patients with different cancer types, including 483 prostate cancer patients and 148 healthy donors for reactivity to the endogenous retrovirus group K member 7 gag polyprotein (GAG-HERV-K). Autoantibodies against GAG-HERV-K were detected most frequently in prostate cancer patients compared to healthy men (6.8% vs. 1.8%) and more frequently in advanced prostate cancer than in those with early disease (21.0% vs. 2.1%). Furthermore, the detection of GAG-HERV-K AAbs was associated with worse survival of prostate cancer patients, and a trend towards faster biochemical recurrence [57].

Multi-AAb panels with prognostic value or that reflect treatment-associated changes were also identified using high throughput methods. Potluri et al. [40] used a prostate cancer-specific microarray represented by 177,604 16-mer peptides of 1,611 cancer-associated proteins to probe samples from healthy volunteers and a prostate cancer patient cohort that ranged from organ confined, castration-sensitive and castration-resistant non-metastatic, to castration-resistant metastatic disease. Although the overall count of AAbs was unaffected by disease burden, AAb composition was found to be associated with clinical stage, especially between patients with castration-sensitive and those with castration-resistant disease. Interestingly, anti-tumor vaccination resulted in a noticeable increase in antibody response over time when compared to the ADT treatment group. These findings support the detection of AAbs to monitor disease progression and response to immunomodulatory therapies in patients from the outset of their diagnosis.

To discover enriched epitopes and potentially prevalent antibodies in the serum samples, Chen et al. [90] used the Serum Epitope Repertoire Analysis (SERA) approach to com-

pare the landscape of AAbs against tumor-specific neoepitopes in serum samples from a subset of metastatic castration resistant prostate cancer (mCRPC) patients and healthy controls. Serum epitope enrichment scores obtained were compared to somatic mutation-specific epitopes identified by whole genome sequencing of metastatic tumor biopsies and germline blood samples, followed by a protein-based immunome-wide association study (PIWAS) [92] (**Figure 2J**). They observed a 0.44% association between somatic mutations and antibody response specific to the mutated peptide. Specifically, enriched motifs in 11 proteins, including NY-ESO-1 and the human endogenous retroviruses HERV_K113 Gag antigen, were immunogenic in patients with mCRPC. Follow-up studies on a separate cohort of 106 patients with melanoma, using PIWAS, next-generation sequencing, and ELISA, also detected enriched cancer-specific antibody responses to NY-ESO-1.

The role of autoantibodies in cancer immunotherapy

The discovery of inhibitory immune checkpoint receptors modulating anti-tumor immunity, and their inhibition that can unleash the immune system to attack cancer, has revolutionized cancer treatment. Immune checkpoint inhibitors (ICI) targeting these receptors, particularly against the cytotoxic T lymphocyte antigen 4 (CTLA-4), the receptor cell death protein 1 (PD-1) and its ligand (PD-L1), have been approved by the US Food and Drug Administration (FDA) to treat a variety of cancers including melanoma, lung, liver, kidney, and bladder cancers. A recent study of melanoma patients treated with adjuvant immunotherapy using nivolumab, ipilimumab, or ipilimumab plus nivolumab found that high baseline serum AAb signatures were predictive of recurrence and severe toxicity [93]. Compared to many other cancers, prostate cancer has a relatively low tumor mutation burden (TMB) and diminished neoantigen diversity [94, 95], which can lead to a lower attraction of immune cells to the tumor site, fewer tumor-specific epitope - class I major histocompatibility complex (MHC I) interactions, and reduced priming of tumor infiltrating lymphocytes (TILs) by APCs [96]. These factors are likely to contribute to the evolution of a non-inflamed, or cold, prostate cancer tumor immune microenvironment (TIME) and affect the response to ICI therapy [97, 98]. An increase in genetic aberrations that enhance the diversity of AAbs has been shown to improve the response to ICI therapy [99]. Deficiency in DNA mismatch repair [100-102] and an increase in DNA damage repair gene mutations [103], are found to have a higher TMB and a stronger response to ICI therapy. Similarly, advanced prostate cancer patients with increased gene fusions that arise due to biallelic *CDK12* inactivation have reportedly elevated neoantigen burden and may benefit from immunotherapy [104]. Furthermore, observations of primary and metastatic prostate cancers with low expression and even complete loss of MHC I, which is essential for neoantigen presentation on the tumor cell surface for recognition by $CD8^+$ cytotoxic T cells, are correlated with poor prostate cancer prognosis and resistance to therapy [105-107].

Considering the increasing number of mAb-based immunotherapy drugs approved for cancer treatment, the importance of humoral anti-tumor response is often overlooked. Most therapeutic cancer vaccines are designed to generate cancer specific cytotoxic $CD8^+$ T lymphocytes (CTL) that can recognize and kill cancer cells upon recognition of specific TAAs. This recognition, which is mediated by the binding of T cell receptors of CTLs to TAA epitopes mounted on the MHC I molecule on the surface of cancer cells, induces cancer cell death via multiple pathways that includes degranulation and apoptosis [108]. Cancer vaccination can also harness antibody-mediated cytotoxic mechanisms to effectively prevent tumor growth. Antibodies activated by anti-cancer humoral immune responses can specifically bind to cancer cells and trigger their elimination by antibody-mediated cellular cytotoxicity (ADCC), antibody-mediated cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC) (**Figure 3**) [109]. Antibodies bound to epitopes of TAAs exposed on the cancer cell surface can be recognized via their Fc receptors, by innate immune cells, including natural killer (NK) cells, macrophages, and neutrophils, which induce cell lysis via ADCC, or phagocytosis via ADCP. The induction of ADCP by macrophages is mediated mainly by the binding of FcγRIIa/CD32a receptors to antibodies on tumor cells [110]. Meanwhile, ADCC by NK cells is highly dependent on FcγRIIIa/CD16a receptors [111, 112]. In CDC, antibodies directly kill

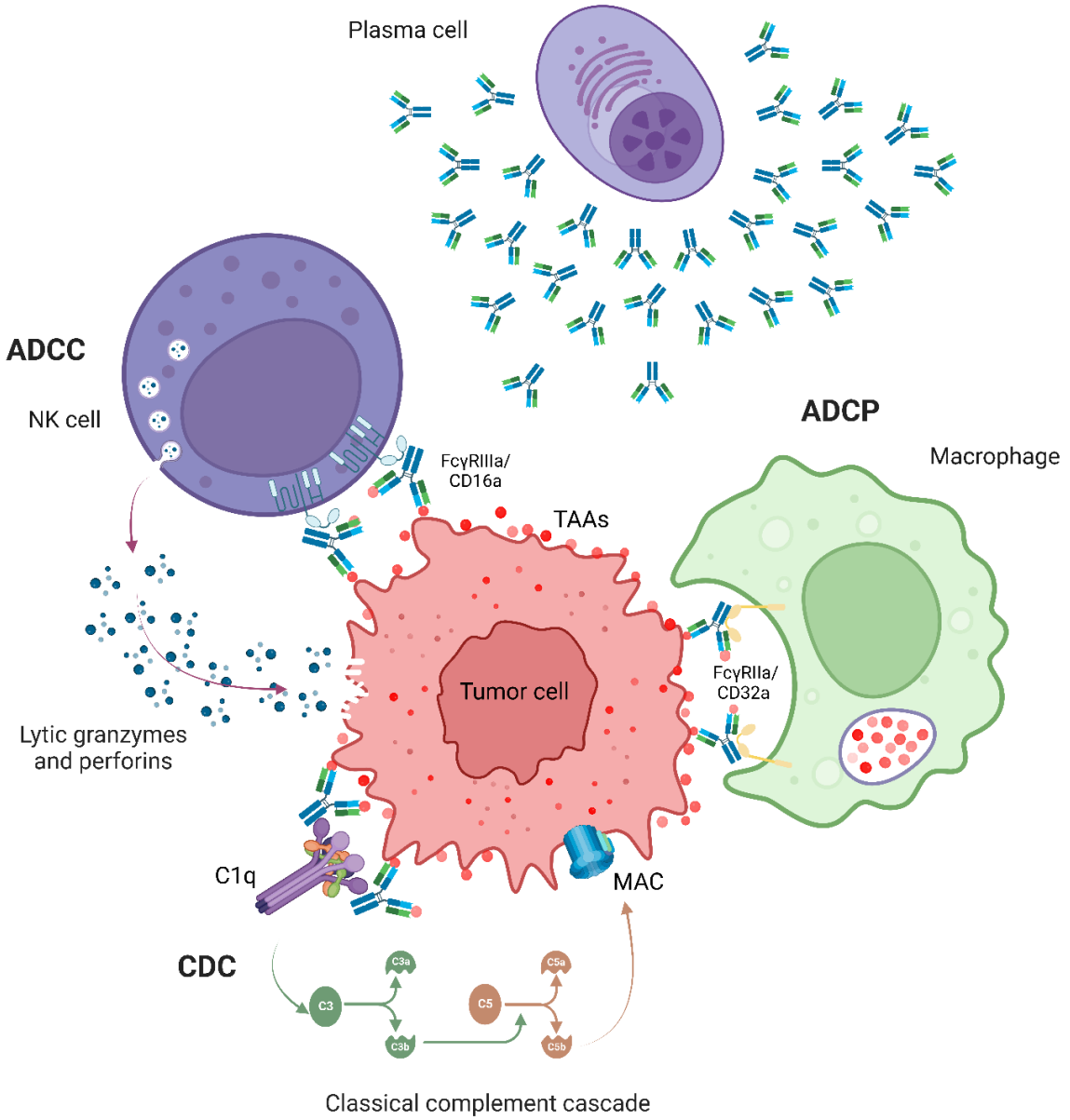

Figure 3. Antibody-mediated cytotoxic mechanisms against tumor cells. AAbs bound to TAAs on tumor cells signal via Fc receptors (FcR) on innate immune cells, to induce antibody-dependent cell phagocytosis (ADCP) of tumor cells by macrophages and is mediated mainly by the binding to FcγRIIa/CD32a receptor, or antibody-dependent cell cytotoxicity (ADCC) by the binding to FcγRIIIa/CD16a receptors on NK cells. In complement-dependent cytotoxicity (CDC), antibodies induce the direct destruction of tumor cells by activating the complement cascade, which results in the formation of membrane attack complexes (MAC) that perforate the tumor cell membrane (Figure is created by authors using Biorender.com).

the cancer cell by activating the complement cascade, which leads to the formation of membrane attack complexes (MAC) that perforate membranes of cancer cells with cytolytic pores, inducing their death. Such vaccine induced antibody mediated anti-tumor responses, like the cellular response, are antigen-specific and provide durable long-term adaptive immune memory [113]. Vaccination also primes the immune system to induce antigen spreading or antigen cascade, a phenomenon where vaccine mediated tumor cell lysis exposes the immune system to additional TAAs, leading to immune responses against TAAs not targeted

by the vaccine [114]. Therapeutic prostate cancer vaccines are also particularly promising treatment options because prostate cancers grow slower than most other cancers, which allows the patient to generate a prolonged targeted cellular and humoral immune response, long after treatment has been discontinued [115, 116].

Prostate cancer TAAs commonly targeted by therapeutic vaccines include PSA, PSMA, and prostatic acid phosphatase (PAP). Presently, sipuleucel-T is the sole therapeutic cancer vaccine approved by the FDA in 2010 based on results of a phase III trial [117]. The vaccine employs autologous peripheral-blood mononuclear cells (PBMCs), including APCs, that have been activated *ex vivo* with a PAP-granulocyte-macrophage colony-stimulating (GMCSF) recombinant fusion protein. PROSTVAC-VF vaccine is a recombinant vaccinia and fowl pox virus vaccine designed to target PSA and a triad of T-cell co-stimulatory molecules. A Phase III trial of the vaccine as a single agent in asymptomatic or minimally symptomatic mCRPC patients [118], however, was halted when it failed to meet the primary Overall Survival end point [118]. Likewise, PSMA-VRP, a PSMA targeting vaccine based on an attenuated Venezuelan Equine Encephalitis alphavirus was shown to be well tolerated in a phase I clinical trial, but it elicited no cellular response and only a weak humoral response in patients [119].

The limited efficacy of these vaccines has encouraged the ongoing development of vaccines against novel immunogenic TAAs. The MVA-brachyury-TRICOM, for example, is a Modified Vaccinia Ankara (MVA) vector-based vaccine designed to target brachyury, a transcription factor known to mediate epithelial mesenchymal transition [120]. In another approach, highly expressed neoantigens with strong predicted binding affinity to MHC Class I, identified from the genomic sequencing of patient tumor samples, were selected as targets of a personalized genomic therapeutic peptide vaccine (PGV-001) [121]. Several of these prostate cancer vaccines are being assessed in clinical trials, either as a single agent or in combination with other treatments, such as androgen deprivation therapy, docetaxel chemotherapy, radiotherapy, and immunotherapy [122-124].

The search for novel immunotherapy targets that also stimulate a strong antibody response have taken on different approaches. One strategy involves probing TAAs identified in prostate cancer for immunogenic peptide sequences that are predicted to bind the MHC I molecule. Using microarray analysis of prostate cancer and normal prostate tissues followed by RT-PCR validation, Arredouani et al. [125], identified single-minded homologue 2 (SIM2) among the top genes with TAAs that have differentially elevated expression in prostate cancer. Significantly higher levels of SIM2 AAbs were detected by ELISA in sera of prostate cancer patients compared to controls, suggesting immune responsiveness to the TAAs from SIM2. Potential HLA-A2.1 (MHC class I)-restricted epitopes within SIM2 protein, predicted by using multiple algorithms, were further shown to bind to and stabilize human HLA-A2.1 using T2 cell line and induced SIM2-specific CTL responses when used to immunize transgenic HLA-A2.1 mice [125]. Results showing SIM2 overexpression in malignant prostate tissue, detection of SIM2 AAbs in sera of prostate cancer patients, and the induction of MHC I restricted cellular immune responses by SIM2-derived peptides in humanized A2.1 transgenic mice, support a strategy for identifying novel prostate cancer TAAs for immunotherapy through the detection of AAbs.

One therapeutic use of AAbs is demonstrated by the action of AAbs against Complement factor H (CFH) in non-small cell lung cancer (NSCLC). CFH protects host cells from destruction by binding to Complement C3b, preventing its deposition on the cell surface to form cell-lytic MAC [126]. Working on the basis that CFH antibodies may enhance anti-tumor activity because significantly higher levels of AAbs against CFH were detected in patients with early-stage than those with late-stage NSCLC, Bushey et al., isolated B cells from patients expressing high affinity CFH AAbs. They then amplified the cDNA encoding variable regions of the heavy and light chains of the CFH-specific antibodies by RT-PCR to produce recombinant antibodies [127]. One of the recombinant CFH antibodies was shown to cause complement activation, stimulate the release of anaphylatoxins, promote CDC, and inhibit tumor growth *in vivo* [127]. In prostate cancer, higher Complement C1q expression in biopsy tissues of BPH

patients was significantly associated with subsequent development of prostate cancer [128]. The use of AAbs to enhance mAb-based immunotherapy against tumor cells through activation of CDC or to stimulate an enduring adaptive anti-tumor immune response in prostate cancer remains to be explored [129]. Promising studies with ICI therapy in cancer have also revealed the association of AAb development following ICI treatment with better survival and improved therapeutic response [130]. In some cases, higher circulating levels of AAbs following ICI are associated with fewer organ-specific immune-related adverse events [131, 132], further suggesting that AAbs may serve as unique biomarkers of disease management and immune response.

Another therapeutic use of AAbs is demonstrated by the co-opting of AAbs against Inhibitor of apoptosis (IAP) proteins for cancer treatment [133]. IAP proteins are essential through their inhibition of caspases in helping cancer cells evade apoptosis, escape immune surveillance, and survive cytotoxic therapies [133]. IAP proteins that include survivin, cellular inhibitor of apoptosis protein 1 (CIAP1/BIRC2), cellular inhibitor of apoptosis protein 2 (CIAP2/BIRC3), and X chromosome-linked IAP (XIAP), are often upregulated in multiple malignancies, including prostate cancer [134]. AAbs against several IAP proteins, including melanoma inhibitor of apoptosis protein (ML-IAP) and survivin, are frequently detected in the serum of cancer patients, including melanoma and colorectal cancer, suggesting that IAPs [133-136] function as TAAs and could be potential targets for cancer immunotherapy through antigen-based vaccination [137, 138]. Phase I trials that vaccinated urothelial [139] and oral [140] cancer patients using survivin derived antigen peptides showed increased peptide specific CTL levels without adverse side effects and even reduced tumor volume in individual patients. In another approach, *Salmonella typhimurium* (SL7207) was used to deliver an oral DNA vaccine encoding survivin TAAs in a syngeneic neuroblastoma mouse model. Delivery of the vaccine as a prophylaxis induced a cytotoxic $CD8^+$ T cell-mediated anti-tumor immune response that resulted in a 48-52% reduction in tumor volume, weight, and metastatic progression. Therapeutic vaccination with the DNA vaccine eliminated neuroblastoma in more than half of the immunized mice and decreased tumor growth by 80% in the remaining mice [141]. The feasibility of whether AAbs against TAAs of prostate tumor-enriched IAPs could be identified and developed for cancer immunotherapy through antigen-based vaccination remains to be explored.

Summary

Prostate cancer poses a unique challenge for clinicians in its difficulty to both detect the disease as well as to provide prognosis without clinical examination, laboratory testing, and invasive imaging. While PSA remains an excellent diagnostic marker for screening over time, it's diagnostic and prognostic capabilities remain limited. Where PSA falls short, however, AAbs directed against prostate cancer enriched TAAs may hold the key to improving clinical outcomes. The detection AAbs, especially prostate cancer specific AAbs, either individually or as a panel, offer improved methods to diagnose prostate cancer, aid in the prognosis of disease progression, and assist in the design of novel treatment modalities. Further research is required to elucidate the true potential for clinical application of a vast majority of these antibodies, but the prospect for improving prostate cancer outcomes is incredibly promising.

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Disclosure of conflict of interest

None.

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