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Review

REVIEW

Prostate cancer: updates on current strategies for screening, diagnosis and clinical implications of treatment modalities

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Abstract

Prostate cancer is the most common cancer in men by way of diagnosis and a leading cause of cancer-related deaths. Early detection and intervention remains key to its optimum clinical management. This review provides the most updated information on the recent methods of prostate cancer screening, imaging and treatment modalities. Wherever possible, clinical trial data has been supplemented to provide a comprehensive overview of current prostate cancer research and development. Considering the recent success of immunotherapy in prostate cancer, we discuss cell, DNA and viruses based, as well as combinatorial immunotherapeutic strategies in detail. Furthermore, the potential of nanotechnology is increasingly being realized, especially in prostate cancer research, and we provide an overview of nanotechnology-based strategies, with special emphasis on nanotheranostics and multifunctional nanoconstructs. Understanding these recent developments is critical to the design of future therapeutic strategies to counter prostate cancer.

Introduction

Second only to skin cancer, prostate cancer is a common cancer type diagnosed in men and a major cause of cancer-related deaths. The prostate tumors incidences have been increasing worldwide and differ between the countries (1,2). According to one report, in the year 2016, about 180 000 new prostate cancer cases have been diagnosed in the United States (3,4). As far as the worldwide occurrence of prostate cancer is concerned, it has greater incidence in western countries, partly due to lifestyle and environmental risk factors. Detection and treatment modalities for prostate cancer have advanced in recent years. Furthermore, the biochemical mechanism of occurrence of prostate cancer has also been investigated. Prostate-specific antigen (PSA)-based screening is one of the most common method of prostate cancer diagnosis (5,6). Expression of PSA has also been reported in other tissues such as normal epithelium of prostate, small intestine, kidney cells and salivary organs; however, the expression level in these parts is ~100–1000 folds lesser than prostate cancer (7,8). However, PSA-based prostate cancer detection remains controversial, and, therefore, new tests are imperative to be a part of the primary screening. Several novel and effective drugs have been approved for the treatment, and concomitant improved survival of patients suffering from advanced prostate cancer.

The current trend shows that about 15% of diagnosed prostate cancer patients are at very high risk of the disease (9). One of the major reasons of this high-risk disease is heterogeneity in the patient's pool with a range of prognosis. In this context, it has been seen that some of the prostate cancer patients develop

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Abbreviations

CTLA4	CTL-associated antigen 4
GM-CSF	granulocyte/macrophage colony-stimulating factor
NIR	near infra-red
PAP	prostatic acid phosphatise
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen
SPIONs	superparamagnetic iron oxide magnetic nanoparticles.

a lethal phenotype, which can be mortal, while others may be cured during the primary tumor stage treatment. The best treatment and management of latter patient group is continually evolving. However, the former group of patients needs more effective treatment strategies to enable the early-stage disease identification, thus optimal management. In this review, we comprehensively and critically review the existing literature on the current strategies of screening, diagnosis and clinical implications on treatment modalities the prostate cancer and future directions to optimize patient care.

Screening and diagnosis

Early detection and precise staging of prostate cancer are vital because the survival rate decreases dramatically when cancer has migrated beyond the prostate organ (10). Due to tissue heterogeneity in prostate cancer and the lack of specificity of conventional imaging techniques, no universally approved imaging methods exist for the early detection of prostate cancer. However, some of the most researched strategies are discussed below.

Prostate-specific membrane antigen-based screening

Prostate-specific membrane antigen (PSMA) is an excellent target for molecular imaging of prostate cancer because it is overexpressed in ~90–100% of local prostate cancer lesions. Additionally, few reports suggest PSMA expression levels are further enhanced in high-grade, metastatic and castration-resistant prostate cancer cells (11–14). It is also reported that PSMA is expressed in other cells of the body such as normal prostate epithelium, small intestine, renal tubular cells and salivary glands; however, the level of expression is about 100–1 000 fold lesser than prostate cancer tissues (15). PSMA-targeted optical imaging modality is one of the common methods to screen the prostate cancer cells under *in vitro* and *in vivo* experimental models. Probes emitting near infra-red (NIR) light are currently being used for *in vivo* optical imaging, as it is extremely sensitive and offers non-invasive, inexpensive, image-guided therapy and real-time monitoring of the progress of the disease (16,17).

Humblet *et al.* (18) showed a PSMA-based high-affinity (9 nM), single nucleophile-containing, small molecule, which could target the active site of PSMA enzyme. Authors demonstrated the production of a tetra-sulfonated heptamethine indocyanine NIR fluorescent derivative of this molecule using a high-yield liquid chromatography/ mass spectrometry (LC/MS) purification strategy. They also reported the sensitive and specific *in vitro* imaging of endogenous and ectopically expressed PSMA in human cells and *in vivo* imaging of xenograft tumors. This method paved a way to the nearly complete preclinical development of an optically based small-molecule contrast agent for image-guided surgery of prostate cancer (18). There have been recent efforts to develop chemical inhibitor scaffolds as carriers for diagnostic and therapeutic payloads delivered to PSMA-expressing prostate cells. A schematic representation has been shown in Figure 1. However, limited success have been achieved due to the fewer efforts devoted to determining the optimal length of linker molecule between PSMA inhibitor and payload agents. In order to address this limitation, Liu *et al.* (19) have developed three spacer-length varied fluorescent inhibitors (FAM-CTT-54, FAM-X-CTT-54 and FAM-PEG_8-CTT-54). The enzymatic inhibition studies were found to be linker length-dependent with variations in inhibitory potency (IC_{50} = 0.41 nM, 0.35 nM, 1.93 nM) and modes of binding (reversible, slowly reversible, irreversible), respectively. Furthermore, cell labeling imaging revealed the spacer length-related change of fluorescence intensity (FAM-X-CTT-54 > FAM-PEG_8-CTT-54 > FAM-CTT-54). Based on these results, authors concluded that the choice of linkers and their lengths are the essential considerations when developing next-generation prostate tumor-targeted imaging probes and therapeutic agents, which are specifically guided to home in PSMA-expressing tumor cells/tissues. Additionally, Banerjee *et al.* have reported the synthesis of a urea-based fluorescence compound ReL2; however, they could not image the targeted tissues because this compound showed insufficient depth penetration in tissues under *in vivo* experimental conditions (20). Chen *et al.* (21) extended this work and reported the synthesis of a compound (YC-27), which could be used to visualize the PSMA-expressing cells in xenografted tumors in mice. Authors

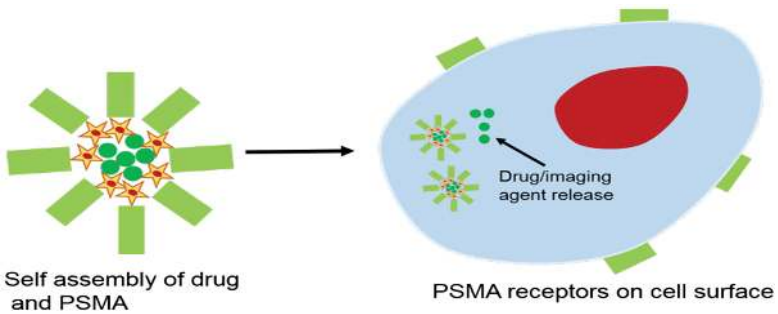

Figure 1. Exploiting PSMA receptors for delivery of diagnostic/therapeutic payloads. Schematic diagram showing the targeting of PSMA receptors. First, the drug molecules and/or imaging agents are allowed to self-assemble with PSMA. PSMA is used to specifically target the cancer cells that express its receptor. Binding of PSMA to its receptor is exploited for the internalization of cargo that contains drug and/or the imaging agents. Internalized imaging agent(s) can be used for diagnostic purposes while the internalized drug can specifically kill cancer cells.

further extended this work and synthesized a series of compounds by simply varying the fluorophores and linkers. Their imaging results established that it is indeed critical to have a linker of suitable length between the PSMA-binding urea and the bulky fluorophore, for enhanced targeting of PSMA under *in vivo* experimental conditions (22). Recently, Neuman *et al.* (23) have reported the development of a laparoscopic imaging system (including an optimized light source, LumiNIR), which could image small tumor burdens with high signal to noise ratio of fluorescence in real-time laparoscopic extirpative surgery of small prostate tumor xenografts in murine and porcine models. The strategy was based on the use of YC-27 compound, which is a low-molecular-weight, urea-based agent that enables NIR imaging of PSMA under *in vivo* conditions. Additionally, Kularatne (24), Kelderhouse (25) and Wang (26) have developed a series of optical agents using Glu-Glu-urea scaffold with varying linkers. They have shown that these compounds can also bind specifically to prostate cancer cells through PSMA. Several other preclinical agents are also reported, which use an optical component such as hetero-bivalent compounds targeting both PSMA and integrin αvβ3 (27) and dual modality agents, which enable sequential single-photon emission computed tomography (SPECT) and optical (28) and bionized nanoferrite nanoparticles for optical and SPECT imaging (29).

Magnetic resonance-based imaging and detection of PSMA

Magnetic resonance-based molecular imaging has the potential to combine the ubiquity of its established clinical modality and high spatial resolution along with molecular profiling under *in vivo* experimental conditions. However, due to the low sensitivity of agents exhibiting magnetic resonance, magnetic and other nanoparticles are often used to improve the sensitivity and contrast. Furthermore, for imaging cell surface receptors there is a need to conjugate with the nanomaterial-based contrast enhancing agents, and indeed, nanomaterials provide an exceptional platform to achieve this. The utilization of PSMA-decorated iron oxide nanoparticles as T_2-contrast enhancer has been a recent trend, and worldwide several research groups are working towards it. The most common method is to employ aptamers or antibody to target PSMA expressed on the cell surface (30–35).

Abdolahi *et al.* have studied the J591-conjugated superparamagnetic iron oxide magnetic nanoparticles (SPIONs) displaying enhanced specific T_2-magnetic resonance contrast of LNCaP cells (expressing PSMA); however, not in DU145 cells (do not express PSMA) (33). Authors observed that SPIONs decorated with PSMA antibody (J591) generated PSMA-specific magnetic resonance contrast enhancement in a preclinical model of orthotopic prostate cancer (34). Furthermore, administration of J591-conjugated SPIONs resulted in significant darkening in the T_2-weighted magnetic resonance images of the prostate cancer region within 2 or 24 h of injection. The generated contrast enhancement was many folds higher than untreated and untargeted SPIONs in prostate region. Polypeptides have also been studied in greater depth for the targeting prostate cancer and imaging. In a recent effort by Jhu *et al.*, a PSMA-conjugated polypeptide (CQKHHNYLC, C1–C9 disulfide) was decorated on the surface of SPIONs and used for the PSMA-specific prostate cancer imaging (35). *In vitro* studies revealed that the polypeptide-conjugated SPIONs were actively internalized in PSMA-expressing cells. Under *in vivo* magnetic resonance experiment, it was found that contrast signals in PSMA-expressing tumors were clearly enhanced, which was further complemented with Prussian blue staining. This staining revealed that the distribution and deposition of SPIONs were heterogeneous in the tumor tissues. Banerjee *et al.* have reported methods based on T_1-contrast enhancement by using Gd (III)-based low-molecular-weight compounds (36). Authors synthesized three novel high-affinity, low-molecular-weight Gd(III)-based PSMA-targeted contrast agents containing one to three Gd(III) chelates per molecule, employing a PSMA-targeted Glu-Lys-urea-linker construct. The rexometric characteristics of synthesized contrast agents revealed that T_1-weighted, PSMA-based magnetic resonance contrast enhancement could be achieved in *in vivo* experimental model. Interestingly, when injected into mice bearing xenograft tumors of PC3 cells (expressing PSMA) and PC3 cells (not expressing PSMA), it was observed that ~36% enhancement of image contrast in the former case within 30 min post injection and remained high till 3 h. However, tumors without PSMA expression showed rapid decay in contrast signal within 30 mins of post injection. Additionally, when other animals injected a trimeric Gd (III) probe without a targeting moiety, did not show any enhancement in tumor contrast at similar time points (37). Therefore, it could be concluded that PSMA is expressed on the prostate tumor cells in sufficient quantity, and significant signal enhancement could be achieved if a potent magnetic resonance agent, conjugated with a suitable target, is used. Such strategies are successful in targeting and imaging of prostate cancer under *in vitro* and *in vivo* experimental models with almost similar efficiency.

New triage and screening markers for prostate cancer

With the recent research and development in the area of molecular biology, it is expected that new methods and markers would be developed, which could efficiently differentiate the low-risk cancers from aggressive. These methods and markers could also be used for efficient targeting and imaging of the much larger proportion of cancer found. Theoretically, this would also be translated into non-invasive and relatively cheaper methods of disease detection, and possible multiple serum or urinary markers may also be detected. The multi-parametric magnetic resonance system or assay could also be generated for potential use in needle biopsies. Such strategies could also be useful for avoiding radical prostatectomy and radiotherapy in patients, thus circumvent the morbidity associated with these treatments.

Serum and urine markers

In the context of non-invasive detection of prostate cancer, several potential improvements in the traditional PSA assay have been investigated. The prostate health index (PHI) is a measure of a molecular isoform of free PSA in the biological fluid (38). PHI is the most recently developed concept and has greater relevance than using total PSA level or percentage of free PSA. The addition of Kallikrein proteins, the serum marker for prostate cancer, to PSA-based markers has found advantage in PSA assays (39). However, PSA and Kallikrein needs to be investigated in detail in the context of screening, giving special emphasis as to how they must be integrated and compared against the current calculation of risk. The current detection methods for urinary markers are complicated and require trained laboratory personnel to run the assay. Two methods of detection have received the attention. PCA3 marker, more specific than PSA, measures the mRNA selectively (Figure 2), produced from prostate tissue and markedly overexpressed on prostate cancer cells

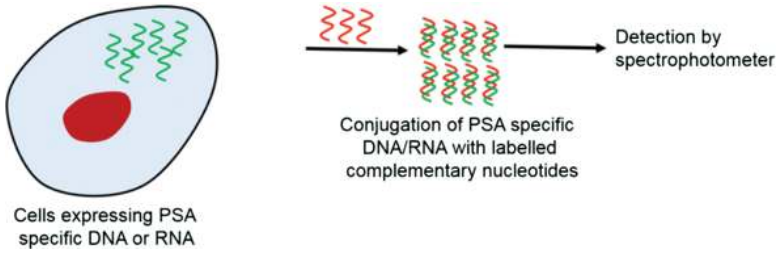

Figure 2. Detection via complementary nucleotides. Schematic diagram showing the use of complementary nucleotides for detection of prostate cancer cells. Prostate cancer cells express PSA whose mRNA transcripts or the gene itself can be recognized by complementary nucleotides. The nucleotides are labeled for quick, easy and efficient detection.

(40). Additionally, unlike PSA, PCA3 also provides the information about the total prostate volume. Recent reports suggest that PCA3 identifies cancer effectively; however, it does not differentiate between low-risk and aggressive form of prostate cancer (41). Another urinary marker that spots the fusion of TMPRSS2 with ERG (ETS, erythroblast transformation specific,-related gene) is under development but may be able to efficiently differentiate between aggressive and low-risk early-stage prostate cancer forms. It has been well established that PHI, the four marker Kallikrein panel and PCA3 provide more accurate information than traditionally used PSA detection method, probably due to the better specificity (42,43). DNA methylation markers are another class which could be useful for the easy diagnosis and prognosis of prostate cancer; however, the research is still infancy. Furthermore, research is also required to establish that how the use of needle biopsy specimens is better than markers identified from serum or urine samples.

Additionally, age factors must also be considered to accurately predict the onset of prostate cancer. It is established that the detection of PSA level at ages between 45 and 65 years can provide indication of the risk of development of prostate cancer several years later. Such strategies could also help in identifying the stage of cancer, i.e. likely to become metastatic or lead to death (39,44).

Markers in needle biopsies

The needle biopsy is done at the advanced stage of the disease progression. Sometimes the identified markers help in avoiding the unnecessary radiation treatment or other painful management of the disease. The well-known Ki-67 detection by immunohistochemistry is the most recognized marker and can differentiate between early-stage and aggressive stage prostate cancer type (45). Immunohistochemistry and FISH (fluorescent *in situ* hybridization) assays are developed for the detection of PTEN and have shown some success (46,47). Similarly, overexpression of MYC and p53 in prostate cancer and their detection by FISH and Immunohistochemistry, respectively, have shown some prognostic potential (48,49). Additionally, a four protein (PTEN, SMAD4, cyclin D1 and SPP1) signature assessment by Immunohistochemistry has also been developed, which offers a potential method to predict the biochemical recurrence of prostate cancer (50).

Men with elevated PSA but negative biopsies

Certain patients show negative signature in biopsy but have a high level of PSA. Such cases present another important question and need to be looked differently than regular prostate cancer patients. It has been shown that after negative biopsies, certain men show a high incidence of development of prostate cancer after subsequent few years of following up (51–53). Pinsky *et al.* (54) have studied the patterns of repeat prostate biopsy in a cohort of men undergoing prostate cancer screening with negative initial biopsy. They performed Prostate, Colorectal, Lung and Ovarian (PLCO) cancer screening trial, which consists of six annual screens with measurements of PSA level and a digital rectal examination. Out of 1736 men, the probability of having a repeat biopsy within 3 years of initial biopsy was 43% with suspicious PSA levels and 13% (out of 1025 men) with suspicious digital rectal examination results. Percentages of third and fourth biopsy (previous negative biopsy) were similar to the initial repeat biopsy rate in PSA-positive patients. Most patients had a repeat biopsy only after having an additional round of screening. Therefore, the need of additional markers like Kallikrein panels for triage of such patients is essential and need further investigation (42).

Surgery versus observation for men with localized prostate cancer

Similar to the above-discussed issue, the management of men with low grade (e.g. Gleason score 6) of prostate cancer need to be observed closely. It is well known that a Gleason score of 6 is the poorly defined entity, and further, its history and appropriate active surveillance methods are unclear; therefore, these protocols must be redefined and validated clinically. In this context, Wilt *et al.* (55) studied the effectiveness of surgery versus observation for men with localized prostate cancer detected by means of PSA testing. In their study, they included randomly assigned 731 men with localized prostate cancer (mean age, 67 years; median PSA value, 7.8 ng/ml) to radical prostatectomy or observation and followed them for 8 years. The primary outcome was all-cause mortality, whereas, the secondary outcome was prostate-cancer mortality. The results indicated that in men with localized prostate cancer detected during the early era of PSA testing, radical prostatectomy did not significantly reduce all-cause or prostate-cancer mortality, as compared with observation, through at least 12 years of follow-up. Therefore, it is concluded that not only treatment-related morbidity and mortality, observation alone may also help to avoid biopsy-related morbidity when under active surveillance. However, the major challenge remains to identify the subgroup of patients who can be managed through this way. It is also imperative to discover and validate new markers of aggression, especially in men with prostate cancer of Gleason score 6 and PSA level < 10 ng/ml.

Prostate cancer treatment modalities

Immunotherapy

Currently, immunotherapy is considered as a viable strategy for prostate cancer treatment. Although there are several ways to target the immune system, signalling pathway inhibitors

and therapeutic cancer vaccines are the most successful candidates in advanced stages of clinical trials. In prostate cancer, PSA, prostatic acid phosphatase (PAP) and PSMA serve as targets for activated immune cells (11,56,57). Prostate cancer is predominantly well suited for immunotherapeutic approaches because it is a non-essential organ and elimination of residual normal prostate tissue as a result of the immune response or immunotherapy will have no clinical consequences. There are few immunotherapeutic strategies for prostate cancer, as discussed below (Table 1).

Cell-based vaccines

Sipuleucel-T:
It is an autologous dendritic cell vaccine designed to target PAP approved for the treatment of certain patients with metastatic castration-resistant prostate cancer (58). It is one of the first personalized immunotreatment for the prostate cancer patients. Very frequently, patients undergo leukapheresis and reinfusion procedures after the treatment of this vaccine; therefore, extreme care is recommended to the patients. Sipuleucel-T is expected to stimulate the dendritic cells, which in turn activate effector T cells leading to enhanced antitumor effect (59). The antitumor effect depends on the number of CD54+ dendritic cells. The quality of an immunotherapy product is also ensured by testing of CD54+ dendritic cells prior to the administration. The minimum established threshold for CD54+ dendritic cells in a product is ≥40 million (60). It has also been reported that patients administered products containing higher CD54+ cells show prolonged overall survival than those receiving lower CD54+ cells containing the product. This observation suggests that Sipuleucel-T's main antitumor effect is due to the infused dendritic cells, which could activate tumor-specific effector T cells.

GM-CSF–transduced tumor cell vaccine (Gvax):
It is another example of cell-based immune vaccine in Phase III of clinical testing. It consists of two immortalized cell lines of prostate cancer (PC-3 and LNCaP), which are transfected to express granulocyte/macrophage colony-stimulating factor (GM-CSF) (61,62). Considering that several of the antigens present on the surface of normal prostate cell epithelium are also found on cancerous cells, immune tolerance is expected to happen. Therefore, by transducing the PC-3 and LNCaP cell lines to express GM-CSF, GVAX was prepared as an early treatment model. The intention behind this was to break the immune tolerance and induce a potential antitumor immune response (63). Although the initial clinical trials showed exceptional GVAX activity by enhancing the immunogenic response in advanced prostate cancer patients, its activity was compromised to show an overall long-term survival advantage in metastatic stage (62,64). The lack of survival response could be argued that immunotherapies are generally successful during minimal metastatic conditions.

Virus-based vaccines

Viral vaccines are constructed by involving the insertion of a plasmid encoding for tumor proteins into a viral vector (65). These vaccines, upon administration, infect the host's epithelial cells, which upon lysis, release the encoded antigens that are taken up by antigen-presenting cells and finally activate CD4+ and CD8+ T cells. It has been suggested that the advanced viral vaccines not only use plasmids for encoding tumor-associated antigens but also co-stimulate the required molecules (61,65). One of the major drawbacks of viral vaccines is that the antibody response to vector antigen is hyperactive than the effect of plasmid-encoded tumor antigens, especially during multiple administration of viral vaccines (66,67). It is considered that boosting the immune system is required before such immunological treatment strategies. For example, the heterologous vaccine ProstVac-VF uses two viral vectors, one (vaccinia virus) and fowlpox virus (68). This viral vaccine is designed to express PSA as well as the triad of T-cell co-stimulatory molecules (TRICOM): B7.1, ICAM-1, and LFA-3.

DNA-based vaccines

Naked plasmid DNA has also been used for immunotherapy. Generally, the DNA-based vaccines are administered either subcutaneously or intramuscularly; subsequently, the DNA is taken up by host cells and proteins are expressed (69). The DNA vaccines are easy to formulate; therefore, it is an attractive mean for engineering a vaccine. Furthermore, DNA vaccines are also less immunogenic than some of the other vaccination methods; however, the repetitive immunization strategy has been suggested as an effective mean, which could circumvent the weak immune response (70). In the context of prostate cancer, the PAP-encoding plasmid (pTVG-HP) has been extensively studied in clinical settings. During Phase I of clinical study, a PAP-specific T-cell response in men population with recurrent prostate cancer was observed (70,71).

Passive immunotherapy

Passive immunotherapy is an attractive and most advanced strategy in immune-based cancer therapeutics as it involves general monoclonal antibody for the desired therapeutic efficacy. Rituximab, targeting CD20+, was the pioneering antibody approved for the treatment of any cancer as an immunotherapeutic strategy. Later, several other monoclonal antibodies such as trastuzumab, bevacizumab and cetuximab have also been approved for use (72). Although these antibodies were being used for any cancer, their mechanism of action was different. For example, cetuximab inhibits the signals for cellular growth, whereas bevacizumab inhibits the signals for pro-angiogenesis (73,74). Additionally, antibodies also show a direct cytotoxic effect when used in high quantity. Rituximab and trastuzumab were expected to induce their antitumor effect by antibody-dependent cell-mediated cytotoxicity, which involves the interaction between antibody's Fc region with the Fc receptor

Table 1. List of immunotherapy options against prostate cancer

	Type	Target	Mechanism	Treatment option for
Gvax	Cell-based vaccine		Immune stimulation through GM-CSF	Recurrent prostate cancer
J591	Monoclonal antibody	PSMA	Attacks PSMA-expressing cells	Metastatic prostate cancer
ProstVac-VF	Virus-based vaccine	PSA	Educates immune system to recognize and attack cells expressing PSA	Metastatic castration-resistant prostate cancer
Sipuleucel-T	Cell-based vaccine	PAP	Stimulates dendritic cells	Metastatic castration-resistant prostate cancer

present on the surface of natural killer cells, neutrophils and macrophages. It further leads to the death of antibody-coated cell by involving several mechanisms (75). While some antibodies undergo activation of the complement cascade, others conjugated to toxins act more as a mean to localize the cytotoxic agent to cancer deposits (76).

In the context of passive immunotherapy in prostate cancer, there has been no major success so far, suggesting the need for better strategies. Several monoclonal antibodies have gone to clinical testing but the majority has failed; however, the J591 antibody has perhaps gone through the most of the stages of clinical testing (77). The initial efficacy study of J591 antibody used an unlabelled antibody and showed dose-dependent antibody-dependent cell-mediated cytotoxicity effect; however, it did not result in enhanced antitumor effect in patients. Therefore, the current focus is on developing more effective J591 antibody by radioimmunotherapy. More agents are additionally conjugated with this radio-antibody to focus on enhanced cancer treatment efficacy. In a recent study, J591 antibody labelled with 177-Lutetium has demonstrated a robust antitumor activity in stage I and II studies of clinical trials (78). It is also expected that this radio-antibody can also be combined with other drugs and anticancer agents to exert better treatment consequences.

Combination approaches

In the context of development of resistance in cancer cells against the treatment agents, it becomes necessary to look for alternatives, which could significantly overcome this issue. Recent research on combination therapeutic approach has given some indication that it could be a reality in the near future. Furthermore, the combination therapy could also be selective to have maximum effect on cancer cells, if the key cancer survival signalling pathways are considered while designing the combination of anticancer agents. The combinations could use a variety of anticancer agents. Some of the recent strategies are discussed below.

The combination of multiple immunotherapies

As discussed above, the discovered immunotherapies can be combined to achieve the goal of effective anticancer immune response. Since the mechanism of action of several immunotherapies is unveiled, several murine models have shown that combined immunotherapies (Figure 3) could lead to the effective cancer treatment (79–82). It has been shown that CTL-associated antigen 4 (CTLA4) molecules are expressed on activated T cells and provides an inhibitory signal to associated T cells. Therefore, inhibition of CTLA4 with antibody has been found to boost the antitumor immunity in several *in vitro* cell culture and *in vivo* animal models (83,84). Due to its success, this strategy is being developed as a potential treatment option for cancer patients. In prostate cancer treatment, Fong *et al.* (85) have reported the combination of combination immunotherapy with CTLA4 blockade and GM-CSF. Patients (24) having metastatic, castration-resistant prostate cancer in a Phase I trial were administered increasing doses of ipilimumab, a fully human anti-CTLA4 antibody. Patients were also given s.c. injections of a fixed dose of GM-CSF. Out of six patients, given highest dose, three patients confirmed the decrease in the levels (>50%) of PSA. Authors also screened the sera with protein array and found that this treatment can induce antibody responses to NY-ESO-1. Therefore, from this study, it was concluded that combination of immunotherapy can induce the expansion of activated effector CD8 T cells as well as the levels of T cells, which are specifically tumor-associated antigens from endogenous immune stock. Additionally, ipilimumab has also been combined with other immunotherapies, such as GVAX, and have shown positive results (86). Several other combinations of antibodies are currently in clinical trials, e.g. Prostvac-VF with ipilimumab, interleukin 21 (IL-21) with an anti-PD1 (programme death-1) antibody, Sipuleucel-T with an anti-PD1 antibody and cyclophosphamide and J591 with IL-2.

The combination of radiotherapy with immunotherapy

Use of radiation with immunotherapy has been the common trend currently for effective treatment of cancer. Radiation exposure exerts local cytotoxicity and therefore enhances the patient's anticancer immune response, which in turn results in the "immunogenic cell death" (87). The abscopal effect has been most discussed with respect to the radiotherapy. This effect describes a phenomenon where local radiation leads to the regression of metastatic tumors outside the radiation field, which was first reported in year 1950s (88). Although, it was postulated as an immune system-mediated phenomenon until 2004 when Demaria *et al.* confirmed that it is an immunological phenomenon (89). Radiotherapy can activate the major histocompatibility complex overexpression along with the cancer cell surface antigens. Additionally, it can also induce the generation of reactive oxygen species and DNA damage, which could further complement the cell death from the tumor. It is well documented that radiation can also alter the production of several interleukins and growth factors such as IL-1, IL-2, IL-6, TGFs and TNFs (90,91). The combination of radiotherapy and immunotherapy has been in clinical

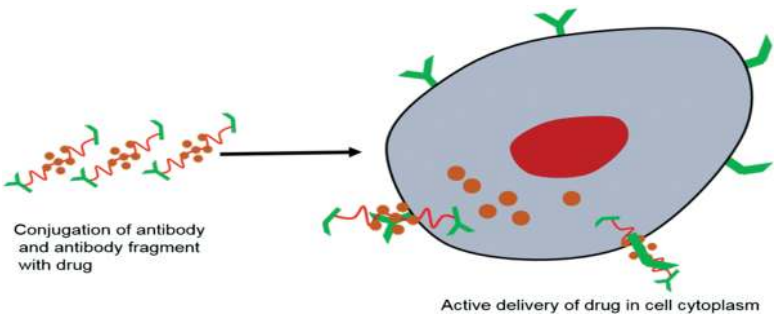

Figure 3. Co-delivery of drug with antibody and antibody fragment. Conjugation with antibody and antibody fragment represents an attractive strategy for targeted delivery of therapeutic agents. The strategy uses specificity of antibody to deliver the drug to prostate cancer cells for efficient killing.

trials, in which ipilimumab is combined with radiation therapy in patients with metastatic castration-resistant prostate cancer (92). Recently, external beam radiation therapy when combined with PSA-TRICOM vaccine has resulted in significant increase in immune responses against tumor-linked antigens, then concomitant immune-based treatments alone (93). Thus, in the context of above-discussed points about the combination of radio and immunotherapy, it seems that immune-based treatment strategies can enhance the radiation-based local tumor responses, and concomitantly, radiation could also potentially enhance the immunomodulatory effects for cancer treatment (87,94).

The combination of chemotherapy with immunotherapy

Chemotherapies are considered as immunosuppressive because their higher doses can kill the effector cells. Contrary to it, the recent research suggests that effect of chemotherapy on the immune system is complicated and have produced mixed observations. It is found that certain chemotherapy could activate the immune system by several mechanisms, such as inhibiting the cells mediating immune tolerance (e.g. Tregs- and myeloid-derived suppressor cells), activation of immune effector cells (e.g. NK cells, cytotoxic T cells, B cells) and exerting cytotoxicity, which results in the uptake and cross-presentation of tumor antigens by antigen-presenting cells (95,96). It is well documented that dose and mechanism of action of chemotherapeutic agent direct the immune response; therefore, the immune responses could be significantly different with certain agents but at varying concentrations (97). Such observation suggests that a conceptual framework must be designed for rationale-based identification and selection of combination of a chemotherapeutic drug and immunotherapeutic agents for clinical applications. The immunomodulatory effects produced by chemotherapeutic agents act to boost the effects of immunotherapeutic-based anticancer effect. A recent neoadjuvant trial in men consisted of a combination of low-dose cyclophosphamide with GVAX vaccination. This treatment was followed by androgen deprivation therapy prior to prostatectomy. In this context, a preclinical work has shown that low dose of cyclophosphamide may revoke the immune tolerance by enhancing the production of CD8+ T-cell infiltration in prostate (96,98). The main goal of this trail is to quantitatively estimate the CD8+ T-cell infiltration in patients receiving GVAX and cyclophosphamide compared with ones receiving only hormonal treatment. Although the initial phase study reveals that the combination of chemotherapy with immunotherapy can enhance the cancer treatment efficacy significantly; however, additional human studies are required to comprehensively understand the implications of this strategy.

Nanotechnology in prostate cancer treatment

Nanotechnology research has given significant impetus to the biomedical applications, especially point-of-care devices and personalized cancer medicines. Nanotechnology is expected to revolutionize the diagnosis, imaging and targeted treatment of several diseases, including prostate cancer (99–101). Use of nanocarriers has shown improvement in pharmacokinetics and pharmacodynamics of the chemopreventive agents, which has resulted in tremendous success to the therapeutic index (102,103). In case of prostate cancer, derivatives of green tea have shown protective effect, wherein green tea catechins, mainly epigallocatechin 3-gallate (EGCG), has shown potent anticancer activity (104,105). Mukhtar *et al.* have reported that EGCG successfully induces apoptosis and promotes cell death in multiple prostate cancer cell culture models, however, with no effect on non-cancerous cells. Furthermore, when administered in prostate animal models, EGCG exhibited delayed progression of prostate cancer. Also, this study was extended to clinical trials in prostate cancer patients, with the observation that patients treated with green tea showed good prognosis (105). Nanoparticles made from a polylactic acid-polyethyleneglycol (PLA-PEG) encapsulating EGCG were also used in preclinical testing against prostate cancer (106). A list of nanoparticle-based formulations used for the treatment of prostate cancer (107–120) is summarized in Table 2.

Langer and Farokhzad have also devised several nanotechnology-based approaches for controlled release of docetaxel drug for enhanced efficacy of prostate cancer. They used safe or US Food Drug Administration (FDA)–approved biocompatible materials to synthesize nanocarriers such as biocompatible polymers, such as PLGA and PEG (121–123). Several types of nanoparticle-based drug delivery agents such as nanoemulsions, liposomes and polymeric NPs can be used as drug delivery system for a variety of hydrophobic and hydrophilic anticancer agents. Nanocarriers exhibit enhanced permeability and retention (EPR) effect, which results in enhanced retention of drugs at the tumor site through a passive targeting strategy (124). Nanocarriers also offer several binding sites to conjugate active molecules to provide active targeting of cancer cells. Additionally, diagnosis of prostate cancer by detecting the level of PSA in the blood has been one of the well-known strategies. In this quest, gold nanoparticles (AuNPs) decorated with PSA recognize DNA probes and antibodies. This ultrasensitive technology could detect PSA present at extremely low concentrations in the blood sample (125). When compared with the existing PSA testing method available commercially, the reported method was found ~300 times more sensitive. Strategies for selective and sensitive imaging of prostate cancer cells have also been developed utilizing the unique properties of nanomaterials. In this context, SPIONs have been used for non-invasive imaging of prostate cancer cells. This method was further taken into a clinical investigation of institutional hyperthermia in patients. Using biopsy, the local recurrence of prostate cancer following radiation exposure was obtained (125).

A magnetic nanoparticle-based fluorescent polymeric nanoparticle was prepared by Lee *et al.* for prostate cancer imaging under *in vivo* experimental conditions. They used bombesin-conjugated *N*-acetyl histidine-glycol chitosan construct to target gastric-releasing peptide receptors, overexpressed on prostate cancer cells. When used, this nanoconstruct resulted in higher binding with prostate cancer cells than untargeted nanoparticles with bombesin. Furthermore, SPIONs were encapsulated in this nanoconstruct as an MRI contrast generating agent, which showed better accumulation at the tumor site in tumor-bearing mice. These results suggest that bombesin-based targeting could be successfully used for targeted drug delivery and imaging of cancer tissues (126).

Another important aspect of using nanomaterials for cancer treatment and imaging is that they can be engineered to perform multiple functions simultaneously. In this context, several theranostic nanosystems have been developed, which could simultaneously perform multimodal imaging, diagnosis and therapy with similar efficacy (126,127). Integration of such capabilities into one nanosystem could be used in diverse scenarios ranging from improving diagnosis, therapy to real-time monitoring of treatment efficacy. Kim *et al.* (128) have reported the synthesis of a multifunctional theranostic nanoparticle system for targeted computed tomography imaging and therapy of

Table 2. A list summarizing nanoparticle-based formulations used for the treatment of prostate cancer

Nanoparticle type	Drug used	Cell culture/animal model used	Result	Reference
Poly(lactic-co-glycolic acid)-CUR nanoparticles (PLGA-Cur NPs)	Curcumin	LNCaP, C4-2 DU-145 and PC-3/C4-2-xenograft	PLGA-Cur NPs inhibited nuclear β-catenin expression in pancreatic cells and in tumor xenograft tissues. Suppression of STAT3 and AKT phosphorylation was also observed, which lead to the apoptosis by inhibiting key Mcl-1, Bcl-xL and induced poly ADP ribose polymerase (PARP) cleavage.	(107,108)
Peptide-based NPs	Co-delivery of docetaxel and curcumin, and imaging	Prostate cancer cells and xenografts	Docetaxel and curcumin could be simultaneously delivered into prostate tumor cells using epidermal growth factor receptor (EGFR) peptide targeting and the enhanced permeability and retention (EPR) effect of NPs. Peptidee-based NPS can also be used for imaging.	(109,110)
PLGA NPs	Cell penetrating peptide and 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (a radio-sensitizer), encapsulate bicalutamide	PC-3	Formulations showed a bi-phasic release of encapsulated radio sensitizer and were actively taken up by PC-3 cells in a dose- and magnetic field-dependent manner. The NPs were effective radiation sensitizers of prostate cancer cell lines *in vitro*. PLGA-NPs have also been used to encapsulate bicalutamide	(111–113)
Superparamagnetic iron oxide nanoparticle	Prostate-specific membrane antigen and docetaxel	C4-2 and PC-3	NPs were efficiently internalized in prostate cancer cells and exhibited potent anticancer efficacy by induction of the expression of apoptosis-associated proteins, downregulation of antiapoptotic proteins and inhibition of chemo-resistance associated protein in cells.	(114,115)
Solid lipid nanoparticles	Retinoic acid	LNCap cells	NPs showed reduced cell viability with increased drug concentrations. The cellular uptake of NPs showed localization within the cytoplasm of cells, and flow cytometry analysis indicated an increase in the fraction of cells expressing early apoptotic markers.	(116–118)
Poly(ε-caprolactone) nanoparticles	Celastrol, a triterpenoid extracted from the Chinese herb Tripterygium wilfordii, paclitaxel	LNCaP, DU-145 and PC3	It was found that NPs inhibited the proliferation of tested cells, dose dependently, in all prostate cancer cell culture models (MIC < 2 μM). NPs significantly increased the cytotoxicity at lower/medium dose (0.5 and 1.0 μM) on DU145 and PC3 cell lines with respect to free drug, with modulation of apoptotic and cell cycle machinery proteins.	(119,120)

prostate cancer. They achieved this by modifying the surface of AuNPs with Aptamer to bind with PSMA, which showed a selective computed tomography signal and antiproliferative activity in prostate cancer cells when loaded with an anticancerous drug, doxorubicin. Agemy *et al.* also reported the synthesis of nanoparticle composed of a pentapeptide, Cys-Arg-Glu-Lys-Ala, which preferably accumulates in tumor cells through binding with fibrin-associated clotted plasma proteins (129). Optical and MRI modalities of imaging confirmed tumor-specific targeting of this nanoparticle, and ultrasound imaging showed lower blood flow in tumor vessels. Furthermore, the treatment to mice bearing prostate cancer with multiple doses induced significant tumor necrosis and reduction in tumor proliferation.

Although nanotechnology has introduced several exciting materials; however, the lack of fundamental understanding of the interaction of nanomaterials with living mammalian cells, especially at the nano-bio interface, hinders the full potential use of nanotechnology in biomedical sciences. This becomes furthermore essential as the interaction of nanomaterials with living cells depends on the particle size, shape, composition, charge and surface capping molecules. These pieces of information are essentially needed for the successful outcomes of clinical trials of any disease including prostate cancer.

Conclusion and future perspectives

Understanding the underlying causes of prostate cancer, including genetics and pathogenesis, has improved substantially in last several years. Several new drugs with improved therapeutic efficacy have been investigated, including those with proposed action against metastatic and castration-resistant prostate disease. Several immunotherapies are also FDA approved for use in prostate cancer. Nanotechnology-based efficient delivery nanovehicles have shown initial success in prostate cancer disease diagnosis, imaging and treatment. Despite these advancements, controversies about the screening of prostate cancer and localized treatment modalities still remain a big challenge. Furthermore, many of the above-mentioned strategies showed better results in *in vitro* and *in vivo* experimental models of prostate cancer, only to fail in clinical phase studies. PSA-based testing of prostate cancer is very common but remains controversial. Therefore, more genetic testing–based detection strategies are needed to identify the individuals at high prostate cancer risk. Novel drugs need to be evaluated to substantially improve the clinical care of patients suffering with prostate cancer. Clinical and translational research must continue, which could be key to improvements in prostate cancer imaging and diagnosis leading to personalized treatment and management of prostate cancer.

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