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Review Article

Functionalized nanoparticles targeting biomarkers for prostate cancer imaging and therapy

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Abstract: Nanomedicine is an evolving field of scientific research with unique advantages and challenges for the detection and treatment of medical diseases. Since 1995, the FDA has approved the administration of nanoparticle-based therapies. The initial generation of nanoparticles relied on an enhanced permeability and retention effect, associated with an increased penetrability of tumor related blood vessels. With increasing knowledge of biomarkers and molecular targets, active targeting of circulating tumor cells by nanoparticles provides an exciting area for application. The selective targeting of prostate cancer cells using a nanotechnology-based mechanism has the potential to optimize the delivery of therapeutic payloads directly to prostate cancer cells while minimizing systemic toxicities. The molecular targets that have been studied include prostate specific membrane antigen, gastrin-releasing peptide protein, glucose related protein, CD44, claudin, C-X-C chemokine receptor type 4 (CXCR-4), and adenosine. The clinical potential for nanoparticle-based therapies is supported by several studies that have progressed past the preclinical stage into clinical trials. In this review, we present the molecular biomarkers that have been targeted by ligands conjugated to the surface of nanoparticles for prostate cancer imaging and therapy.

Keywords: Prostate cancer, nanomedicine, biomarkers, tumor targeting, prostate specific membrane antigen

Introduction

Nanomedicine represents the developing field of medical research that involves the use of materials at the nanoscale level for the diagnosis and treatment of medical diseases [1]. Nanomaterials are being fabricated to serve as sensors for bioelectric devices, probes for diagnostic modalities, and vehicles for drug-delivery mechanisms [2-4]. In the area of cancer research, the past decade of advancements in the field of nanotechnology has provided unique solutions to the current challenges of treatment paradigms. The proposed benefits of nanomedicine include its ability to detect malignancies at an early stage, to minimize systemic toxicities of therapeutics with targeted delivery, and with the use of certain applications, to directly treat disease entities [4-8]. Solutions based on nanotechnology have been introduced into current management strategies of diseases, and since 1995 several nanomedicine-based therapies have been approved for utilization by the Food and Drug Administration [9].

Advances in nanomedicine have been coupled with an increased understanding of tumor biology and the discovery of novel biomarkers for cancer targeting. The first generation of nanoparticles was designed with the intent of delivery via an enhanced permeability and retention (EPR) effect. In this passive form of delivery, the EPR effect relies on the increased penetrability of the tumor vasculature and limited lymphatic drainage, which allow for increased delivery to, and retention of nanoparticles and macromolecules at, the tumor site [10]. More recently, the field of nanomedicine has focused on active targeting, where researchers have modified the surface of nanoparticles to include targeting moieties that directly bind to tumor cells, *in vivo* [11]. The premise of tumor-targeting nanomedicine is to identify specific receptors and antigens that are overexpressed on tumor cells, but remain minimally expressed on non-malig-

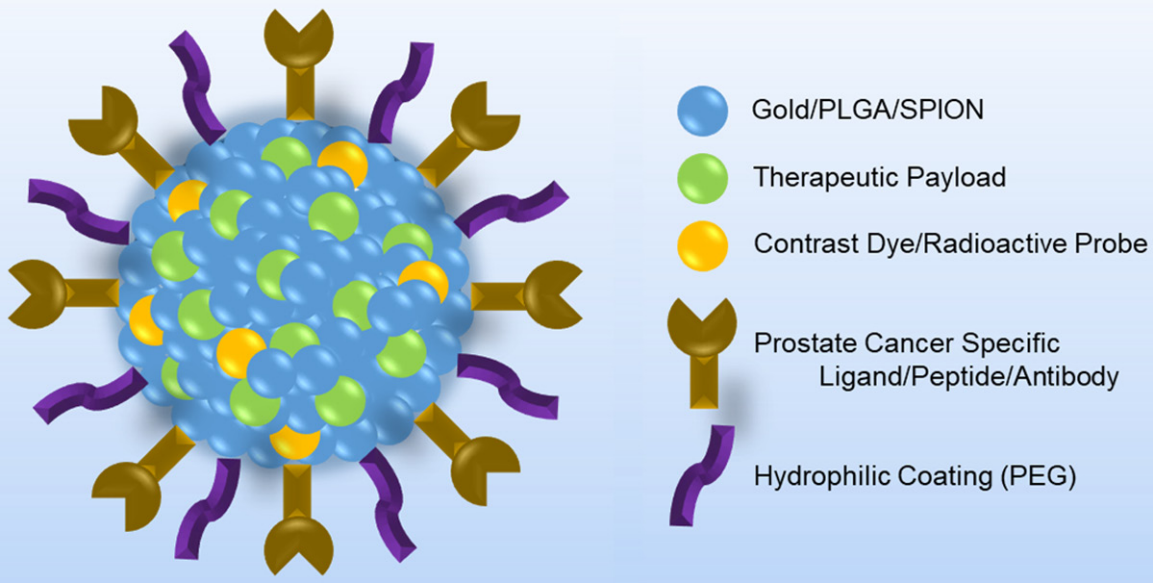

Figure 1. Schematic showing the design of a multi-functionalized theranostic nanoparticle with associated moieties including prostate cancer specific targeting ligands, a therapeutic payload, an imaging probe, and hydrophilic side-chains.

nant cells. Multi-functionalized nanoparticles are then designed with a receptor-specific ligand and a chemotherapeutic and/or imaging enhancing payload (**Figure 1**). As the understanding of tumor biology and molecular targets evolves, the development of multi-functionalized nanoparticles may improve the site-specific delivery of chemotherapeutic payloads to tumor cells [12].

One of the solid tumor malignancies that would benefit from selective targeting is prostate cancer. Prostate cancer is one of the leading cancer-related causes of death in males, with approximately one in nine males being diagnosed during their lifetime [13]. While locally advanced prostate cancer has been successfully treated with surgical resection, patients diagnosed with metastatic disease must rely on medical management with hormone therapy, chemotherapy, and immunotherapy, which are often associated with systemic side effects that negatively affect quality of life [14]. The selective targeting of prostate cancer cells using nanotechnology-based delivery vehicles containing chemotherapeutic payloads has the potential to optimize the transport of therapies to the prostate cancer while minimizing systemic toxicities. In this review, we present an overview of the potential antigens and receptors studied by investigators in the development of novel, functionalized nanoparticles for targeting prostate cancer.

Pre-clinical targets identified for prostate cancer

Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA) is a type II cell surface membrane glycoprotein consisting of an intracellular, transmembrane, and extracellular domain. PSMA is predominantly expressed by the prostate cells and also is found in the central nervous system, kidney, proximal small intestine, and salivary glands [15]. PSMA has been found to be overexpressed on prostate carcinoma and metastatic prostatic cancer cells, as compared to benign prostate cells, with the up-regulated expression of PSMA being associated with more aggressive disease [16, 17]. PSMA has been a widely targeted moiety due to its large extracellular domain, its overexpression in malignant cells, and its demonstrated potential for internalization of bound substrates, supporting its potential role in targeted drug delivery.

Ligands that can decorate the surfaces of biocompatible nanoparticles have been designed to target the extracellular domain of PSMA. Hrkach *et al*. functionalized a docetaxel-encap-

sulated polymeric nanoparticle with a small molecule ligand, ACUPA (2-(3-((S)-5-amino-1-carboxypentyl) ureido) pentanedioic acid), to target PSMA [18]. *In vitro* binding studies demonstrated a higher affinity of organic nanoparticles functionalized with ACUPA for PSMA as compared to non-functionalized polymeric nanoparticles. In a mouse xenograft model, studies validated the enhanced delivery and accumulation of docetaxel to PSMA-expressing solid tumors with the use of targeted polymeric nanoparticles as compared to solvent based docetaxel formulations. Early clinical trials with these docetaxel-loaded ACUPA functionalized nanoparticles, now referred to as BIND-014, revealed a potential for clinical translation. A Phase II, single-arm trial was performed with the administration of BIND-014 to 42 chemotherapy naïve patients with metastatic, castration-resistant prostate cancer (mCRPR) [19]. Overall, patients with mCRPR who were treated with BIND-014 exhibited a median radiographic progression-free survival of 9.9 months. Even though the authors interpreted the trials as being favorable, this was a single-armed trial and was not compared directly with a docetaxel-free arm. The most common side effects reported by participants in the trial were fatigue, nausea, and neuropathy, with the latter event reported at a rate similar to that of non-targeted docetaxel formulations, which is concerning for off-site toxicity. In preclinical studies in xenograft models the nanoparticles exhibited an increased tumor accumulation of docetaxel, a lower volume of distribution, and a slower clearance compared to conventionally administered docetaxel [18].

A theranostic agent with both imaging and therapeutic capabilities was developed by Mangadlao *et al*. in which a PSMA-1 ligand was used to target the PSMA receptor on prostate cancer cells [20]. The gold nanoparticles designed by this group also conjugate Pc4, a fluorescent photodynamic therapy drug, to assist in intra-operative detection of PSMA positive prostate cancer cells. Using spectroscopic techniques, *in vitro* experiments demonstrated increased uptake of the gold nanoparticles by PSMA-positive PC3pip cells compared to PSMA-negative cell lines, suggesting active targeting of the PSMA-1 ligand for the PSMA receptor.

A monoclonal antibody, J591 (mAb HuJ591), also has been designed to target the extracellular domain of the PSMA receptor, and investigators have designed nanoparticles that express J591 on its surface. Nagesh *et al*., designed superparamagnetic iron oxide nanoparticles (SPIONs) encapsulating docetaxel that target PSMA via the J591 antibody [21]. The targeting efficacy of the J591-SPION-docetaxel nanoparticles was evaluated by *in vitro* studies, which demonstrated increased uptake of the nanoparticles in PSMA - positive cell lines. Western blot analysis demonstrated an upregulation of pro-apoptotic proteins in prostate cancer cells after treatment with the J591-SPION-docetaxel nanoparticles, showing both the delivery of docetaxel and its anti-tumor properties. The preclinical effectiveness of J591-SPION nanoparticles was evaluated by Tse *et al.* in a study in which J591-SPIONs were assessed as a potential tracer for magnetic resonance imaging [22]. *In vivo* experiments revealed increased magnetic resonance contrast uptake of J591-SPIONs by prostate cancer cells, which was not seen with non-targeted SPIONs.

In addition to iron oxide nanoparticles, researchers have developed bovine serum albumin (BSA) - polyethylenimine biocompatible nanoparticles that target the PSMA receptor via an antibody approach. Pang *et al.* have designed a BSA nanoparticle that co-delivers docetaxel and p44/42 mitogen-activated protein kinase siRNA directly to prostate cancer cells, and have demonstrated a two-fold decrease in the required dose to achieve a 50% inhibition of cellular growth as compared to nanoparticles loaded with docetaxel alone [23]. For prostate cancer (CWR22R) xenograft mice treated with the docetaxel-p44/42 siRNA conjugate BSA nanoparticle, median survival improved to 45 months as compared to 18 months for mice treated with docetaxel alone. Another polymeric nanoparticle to take advantage of the J591 antibody - PSMA receptor complex, is the poly (lactic-co-glycolic acid)-curcumin nanoparticle (PLGA-CUR-NP) developed by Yallapu *et al.* [24]. Curcumin is a natural phytochemical, which may have potential in anti-cancer therapeutics via the induction of apoptosis in malignant cells [25]. By radiolabeling the J591 antibody with ^{131}Iodine, the investigators were able to visualize the targeted delivery of PLGA-CUR-NPs to prostate cancers, and PCR analysis demonstrated an upregulation of apoptotic proteins and a downregulation of oncogenic mRNA [24]. Hence, the targeting of the external do-

main of PSMA via the mAb HuJ591 is a potential tool to aid in the localization of the prostate cancer. Investigators have demonstrated the ability to synthesize both metallic and polymeric nanoparticle compositions with the mAb HuJ591 antibody functionalized to its surface.

Aptamers are small, single-stranded DNA or RNA oligonucleotides that can selectively bind to a specific target with high affinity. The small size and facile synthesis of aptamers has made them a promising oligonucleotide for targeting PSMA. The A9 and A10 aptamers are common sequence oligonucleotides that have been used to target prostate cancer secondary to its three-dimensional tertiary structure and its binding to PMSA. Relative to antibodies, the PSMA aptamers have the advantage of smaller size and molecular weight and decreased immunogenicity [26]. The facile synthesis of nanoparticles functionalized with targeting aptamers has been well described for prostate cancer imaging and therapy [27].

Functionalizing the surface of polymeric PLGA nanoparticles with the A10 PSMA aptamer, Dhar *et al.* delivered cisplatin to prostate cancer cells via the endocytosis of the nanoparticle carrier [28]. Nanoparticles functionalized with the A10 aptamer had increased internalization of the nanoparticle as compared to non-targeted nanoparticles. The specific binding of A10 to PSMA was further confirmed by the increased internalization of nanoparticles in experiments involving PSMA-positive prostate cancer cell lines as compared to PSMA-negative cell lines. Similar A10 aptamer functionalized PLGA - PEG polymeric nanoparticles were designed by Cheng *et al.* with tunable size characteristics [29]. Bio-distribution studies performed on a LNCaP xenograft mouse model revealed a four-fold increase in nanoparticle delivery using the aptamer targeting nanoparticle (0.83% ± 0.21% injected dose/gram tissue) as compared to non-targeting nanoparticles (0.22% ± 0.07%). The same group of investigators designed a novel quantum dot, nano-scaled semiconductor with optical properties, functionalized with the A10 aptamer and doxorubicin for prostate cancer targeting and drug delivery [30]. Doxorubicin, an anthracycline with fluorescent properties, intercalates into the double strands of the aptamer. When doxorubicin is bound to the aptamer, doxorubicin absorbs and quenches the fluorescence of the quantum dot. Upon targeted delivery of the quantum dot vehicle to the prostate cancer cell, doxorubicin is released from the complex, resulting in activation of the fluorescence of the quantum dot, which can be visualized using fluorescent microscopy.

Metallic nanoparticles also have been functionalized to exhibit the PSMA aptamer on its surface. Kim *et al.* developed a gold nanoparticle functionalized with the A9 PSMA aptamer for use as a computed tomography contrast agent [31]. Using A9 aptamer functionalized gold nanoparticles, *in vivo* studies demonstrated a four-fold increase in CT intensity of LNCaP PSMA-expressing prostate cancer cells (Hounsfield units 130 ± 17) as compared to PC3 PSMA non-expressing prostate cancer cells (Hounsfield units 28 ± 3). The investigators were able to load doxorubicin to the A9 aptamer functionalized gold nanoparticle and using an MTT assay showed equipotent cytotoxicity of the doxorubicin loaded nanoparticle with free doxorubicin in LNCaP prostate cancer cells.

Hence, PSMA, when it is expressed, is a suitable target for prostate cancer cells as revealed by established pre-clinical and clinical nanoparticles. Nanoparticles have been functionalized with ligands, antibodies, and aptamers that target the extra-cellular domain of PSMA. Binding to the PSMA receptor results in endocytosis of the nanoparticle complex, allowing for a potent delivery method of antineoplastic agents. Selection of patients with prostate cancer cells that express PSMA will likely optimize future treatments with PSMA targeting therapies.

Gastrin-releasing peptide receptor

The gastrin-releasing peptide receptor (GRPR) is a G-protein coupled receptor that activates the phospholipase C signaling pathway, regulating the release of gastrointestinal hormones and smooth muscle contractions [32]. The GRPR is aberrantly expressed in several malignancies including prostate cancer and is involved in epithelial cell proliferation and the promotion of mitosis. Bombesin is a neuropeptide that contains a homologous set of amino acids with the mammalian gastrin-releasing peptide, allowing for its high-affinity binding to the GRPR [33]. Several bombesin analogs have been designed for targeting the GRPR to target prostate cancer.

A preclinical magnetic resonance imaging probe based on a SPION formulation functionalized with bombesin was developed by Martin *et al.* [34]. Bombesin and rhodamine, a fluorescent dye, were conjugated to the surface of the SPION through click chemistry, allowing for facile synthesis [34]. Selective uptake of the bombesin functionalized SPIONs as compared to non-targeting SPIONs was demonstrated in PC3 prostate cancer cells, with *in vitro* studies demonstrating internalization of the SPION.

Radiolabeled metallic nanoparticles functionalized with bombesin have been developed to target the GRPR. Zambre *et al.* synthesized gallium radiolabeled gold nanoparticles functionalized with bombesin [35]. Biodistribution studies demonstrated an approximate six-fold greater uptake of the bombesin functionalized gold nanoparticles in PC3 tumors as compared to non-targeted gold nanoparticles [35]. Technetium radio-labeled gold nanoparticles functionalized with bombesin and hydrazinonicotinamide (HYNIC) were developed by Mendoza-Sanchez *et al.* for *in vivo* imaging of the GRPR [36]. *In vitro* analysis demonstrated that the bombesin functionalized to the surface of the gold nanoparticles exhibited a high affinity for GRPR positive, PC3 prostate cancer cells. Micro-SPECT imaging in a PC3 prostate tumor-bearing mouse model demonstrated tumor uptake of the radiolabeled gold nanoparticles within 1 hour of administration. In addition to gold nanoparticles, radiolabeled copper sulfide nanoparticles have been designed by Cai and colleagues [37]. After the surface modification of the copper sulfide nanoparticle with Poly (ethylene glycol) (PEG), bombesin was linked to PEG through an amino-carboxyl condensation reaction. In a PC3 orthotopic prostate tumor mouse model, bombesin functionalized nanoparticles demonstrated an enhanced PET/CT signal approximately 6 hours post-injection [37].

Lee *et al.* synthesized chitosan nanoparticles conjugated with bombesin to target the GRPR [38]. The chitosan nanoparticle conjugates were labeled with Cy5.5, a near-infrared fluorophore [38]. Relative to non-targeting nanoparticles, bombesin-targeting chitosan nanoparticles had increased binding to the PC3 cell surface and tumor accumulation. SPION were further conjugated to the chitosan complex for use as a potential MRI tracer.

Glucose-regulated protein (78-kDA)

Glucose-regulated proteins are stress - inducible chaperone molecules that belong to the heat shock protein family and are involved in protein folding and the transport of misfolded proteins for degradation [39]. They are found in the endoplasmic reticulum and mitochondria, and are integral in the proper functioning of these organelles. Glucose-regulated proteins are classified by their molecular weight, with GRP78 established as an ubiquitous protein involved in the homeostatic balance of the endoplasmic reticulum. In the setting of endoplasmic reticulum stress, the nucleus transcribes unfolded protein response - associated genes, following which GRP78 is induced to regulate protein folding and to maintain cellular homeostasis. Neoplastic cells induce endoplasmic reticulum stress and hence upregulate GRP78 to maintain protein folding. In mouse models, GRP78 has been shown to be overexpressed in several cancer types, including prostatic adenocarcinomas, and is involved in tumorigenesis, cell proliferation, downregulation of apoptotic proteins, and the adhesion between metastatic prostate cancer cells and the bone microenvironment [40]. Furthermore, malignant cells have an increased localization of GRP78 to its cell membrane, an effect not seen in benign cells. Several peptides and antibodies have been designed to target and inhibit GRP78 on both the cell surface and the endoplasmic reticulum [41].

Zhang *et al.* designed a preclinical calcium phosphate - polymeric conjugate nanoparticle that targets GRP78 with the Arg-Gly-Asp (RGD) ligand to co-deliver docetaxel and GRP78 siRNA for an anti-prostate cancer effect [42]. The delivery of GRP78 siRNA is hypothesized to silence GRP78, and to sensitize prostate cancer cells to chemotherapy [43]. *In vitro* studies of PC3 prostate cancer cells demonstrated a synergistic induction of apoptosis upon treatment with nanoparticles co-encapsulating both GRP78 siRNA and docetaxel when compared to the simultaneous administration of a combination of free docetaxel and free GRP78 siRNA or either alone [42].

The KDEL amino acid sequence can bind to the carboxy-terminal domain of GRP78, and anti-KDEL antibodies have been employed by Delie *et al.* to functionalize the surface of polymeric

nanoparticles loaded with Paclitaxel [44]. PC3 and DU145 prostate cancer cell lines were tested for GRP78 targeting, with the non-cancerous PNT1B cell line serving as a negative control. Using these functionalized nanoparticles *in vitro* cell viability studies demonstrated increased cell sensitivity to Paclitaxel in the DU145 prostate cancer cell line which highly expresses GRP78 on its cell membrane compared to PC3 and PNT1B cells which either have a low expression or no expression of GRP78 on its cell membrane.

CD44 antigen

The CD44 antigen is a multi-structural cell surface and transmembrane glycoprotein that is involved in cell differentiation, proliferation, migration, and in the presentation of chemokines and cytokines to appropriate receptors [45]. CD44 is a single chain molecule composed of an extracellular domain that has a ligand-binding site, a membrane-proximal region, a transmembrane domain, and a cytoplasmic tail [46]. CD44 serves as a receptor for hyaluronan, a component of extracellular matrices. The CD44 antigen has been shown to be overexpressed in several cancer types, including prostate cancer [47].

In order to target CD44, investigators have functionalized the surface of polymeric nanoparticles with hyaluronic acid (HA). Huang *et al.* synthesized HA - functionalized polymeric PEG - gelatin nanoparticles to deliver epigallocatechin-3-gallate (EGCG), the natural constituent of green tea extracts [48]. The HA-PEG-gelatin EGCG nanoparticle demonstrated increased cellular uptake in PC3 (CD44+) prostate cancer cells as compared to CD44 knock out PC3 cells. Western blot experiments revealed an increase in pro-apoptotic proteins following treatment with the functionalized nanoparticle. Alternatively, Wei *et al.* designed salinomycin - encapsulated lipid PLGA nanoparticles, which were functionalized with the rat anti-human CD44 Alexa Fluor® 488 antibody [49]. Initial studies demonstrated anti-cancer activity of salinomycin against prostate cancer - initiating cells. Magnetic sorting techniques were used to differentiate the DU145 and 22RV1 prostate cell lines by their expression of CD44. For CD44+ cells, the antibody conjugated PLGA nanoparticles demonstrated increased delivery of salinomycin compared to salinomycin alone or non-targeted nanoparticles.

Sanfilippo *et al*. synthesized gold nanospheres functionalized with hyaluronic acid (HA) for targeting CD44 receptors [50]. The targeting capability of the HA-gold nanospheres was evaluated in CD44+ PC3 prostate cancer cells. Compared to non-functionalized gold nanoparticles, a higher cellular uptake was seen for the HA-functionalized gold nanoparticles in PC3 prostate cancer cells.

Claudin

Claudins are a family of tight junction proteins that reside within the cell membrane. Histochemical analysis has demonstrated the upregulation of claudin-3 and claudin-4 in neoplastic prostatic cells as compared to benign tissue [51]. Martin et al. demonstrated a higher expression of claudin-3 and claudin-4 in higher grade human prostate cancer biopsy specimens as compared to lower grade specimens [52].

The Clostridium perfringens enterotoxin (CPE) is well established to bind to the claudin-3 and claudin-4 receptors with high affinity, resulting in increased porosity of the cellular membrane of prostate cancer cells. The increased porosity of the cellular membrane creates an osmotic disequilibrium and the eventual lysis of the cell [53, 54]. Rajapaksa *et al.* have synthesized PLGA polymeric nanoparticles that target claudin-4, however their work focused on targeting microfold (M) gut-associated cells [55]. The investigators utilized the carboxyl-terminal 30 sequence of amino acids of CPE as a targeting ligand for claudin-4.

Martin *et al.* targeted claudin-3 and claudin-4 receptors by functionalizing the surface of PLGA polymeric nanoparticles with a non-toxic truncated CPE peptide fragment [52]. The functionalized nanoparticles co-encapsulated SPION and rhodamine, for potential use in fluorescent-based and MRI studies. In a PC3 prostate tumor xenograft mouse model, CPE functionalized nanoparticles had an increased uptake by prostate cancer cells. Initial MRI studies in a mouse model correlated with increased uptake in prostate tumors following administration of CPE functionalized nanoparticles. Hence, utilizing the affinity for claudin-3 and claudin-4 by

the CPE peptide represents a potential means by which prostate cancer can be targeted [52].

CXCR-4

CXCR-4 is a chemokine receptor specific for the C-X-X motif chemokine 12 (CXCL12), which has a role in lymphocytic chemotactic activity, in the function of CD4+ T cells in HIV infections, and in directing hematopoietic stem cells to the bone marrow [56]. CXCR-4 has been shown to be upregulated in several malignancies including prostate cancer and is involved in tumor migration and metastatic spread [57, 58]. In addition to the CXCR-4/CXCL12 axis, synthetic ligands such as AMD3100 and FC131 have been developed to target and inhibit the CXCR-4 pathway. The synthesis of AMD3100 functionalized gold nanoparticles doped with radioactive copper has been described by Zhao and colleagues [59]. The AMD3100 functionalized gold nanoparticles showed high specificity for CXCR-4 in a breast tumor mouse model, with potential applications in positron emission tomography and drug delivery.

Although a CXCR-4 targeted nanoparticle platform has not been well tested in prostate cancer cell lines, the effect of free AMD3100 on prostate cancer chemosensitivity has provided promising results. Domanska and colleagues have shown that when PC3 prostate cancer cells were treated with AMD3100, there was increased anti-cancer sensitivity to docetaxel [60]. The investigators also noted that the expression of CXCR-4 was increased in bony metastases as compared to primary tumor sites, suggesting a potential application for targeting metastatic prostate cancer.

Adenosine

Adenosine receptors are over-expressed in prostate cancer cell lines, and their activation increases cellular proliferation [61, 62]. Swami et al. targeted the adenosine receptor in DU145 prostate cancer cells using adenosine receptor ligand functionalized nanoparticles encapsulating docetaxel [63]. The functionalized nanoparticles enhanced endocytosis, mediated uptake by the DU145 prostate cancer cells, and permitted the use of lower doses of docetaxel without decreasing the therapeutic effect [63]. Currently, there are two active clinical trials in progress that are targeting the Adenosine A2A receptor in metastatic castration-resistant prostate cancer patients (**Table 2**).

Discussion

In this review, we have described multiple prostate cancer targeting moieties, in various stages of development, employing nanoparticle vehicles (**Table 1**). The selective targeting of neoplastic cells using nanoparticle - based carriers has promising potential in cancer imaging and for the delivery of chemotherapeutics, while minimizing systemic toxicities. The targets described in this review, which include PSMA, GRPR, GRP78, claudin, CD44 antigen, CXCR-4, and adenosine are advantageous due to their upregulation in malignant cells, increasing the stochastic likelihood of targeted delivery of imaging or therapeutic agents to tumor cells as compared to benign cells. From studies that have been performed to date, it is evident that tumor biology and the expression of specific biomarkers within individual patient tumor microenvironments play a significant role in further optimizing treatment strategies.

The utilization of a nanoparticle vehicle for cancer targeting and drug delivery continues to remain prospective. There are several national clinical trials evaluating the use of these nanoparticles in solid tumors. National clinical trials related to prostate cancer are listed in **Table 2**. Of the studies included, the planned clinical indications for use of these nanoparticles include directed anti-tumor actions on metastatic and non-metastatic disease, *in vivo* enhancement of radiation therapy, *in situ* photo thermal ablation, and their use as diagnostic tracers in MRI/PET imaging modalities. Many of the current nanoparticles that are being tested rely on the EPR effect for the delivery of payload to the cancer site, a passive form for payload delivery. A nano-particulate form of paclitaxel, NanoPac®, was clinically evaluated in a single institution phase 2a trial, NCT03077659, and demonstrated reductions in mean tumor volume, PSA-density, and percent adenocarcinoma in biopsy specimens. Only three studies have addressed nanoparticles that actively target biomarkers on prostate cancer cells *in vivo;* NCT04167969 (PSMA) and NCT04089553 and NCT04495179 (Adenosine A2a receptor). The payloads involved in these studies include camptothecin, a natural alkaloid with anti-topoisomerase

Table 1. Summary of receptors and targeting agents employed to target prostate cancer using a nanoparticle carrier

Receptor	Targeting Agent	Nanoparticle Formulation	Therapeutic Payload	Prostate Cancer Cell/Mouse Model	Reference
Prostate specific membrane antigen	ACUPA	PLA[a], PLGA[b]	Docetaxel	LNCaP	[18, 19]
	PSMA-1	Gold	Pc4 fluorescent dye	PC3pip	[20]
	mAb HuJ591	SPION	Docetaxel	LNCaP, C4-2	[21, 22]
		BSA-polyethylenimine	Docetaxel, p44/42 siRNA	CWR22R	[23]
		PLGA	Curcumin, ^{131}Iodine	LNCaP, C4-2, DU145, PC-3	[24]
	A10 aptamer	PLGA, PEG	Cisplatin, Doxorubicin	LNCaP	[28-30]
	A9 aptamer	Gold	Docetaxel	LNCaP	[31]
Gastrin-releasing peptide receptor	Bombesin	SPION	Rhodamine dye	PC3	[34]
		Gold	^{67}Gallium, ^{99}Technicium, HYNIC	PC3	[35, 36]
		Copper Sulfide	^{64}Copper	PC3	[37]
		Chitosan, SPION	Cy5.5 fluorophore	PC3	[38]
Glucose-regulated protein (78-kDA)	RGD ligand	Calcium Phosphate-DSPE[c]-PEG	Docetaxel, GRP78 siRNA	PC3	[42]
	Anti-KDEL antibody	PLA	Paclitaxel	PC3, DU145	[44]
CD44 antigen	Hyaluronic acid	PEG-gelatin	EGCG[d]	PC3	[48]
	anti-human CD44 Alexa Fluor® 488 antibody	PLGA	Salinomycin	DU145, 22RV1	[49]
	Hyaluronic acid	Gold	N/A	PC3	[50]
Claudin	CPE peptide	PLGA	SPION, Rhodamine dye	PC3	[52]
CXCR4	AMD3100/FC131	Gold	^{64}Cu, Docetaxel	PC3	[59, 60]
Adenosine	Adenosine receptor ligand	SLN[e]	Docetaxel	DU145	[63]

[a]PLA = poly (D, L-lactide), [b]PLGA = poly (D, L-lactide-co-glycolide), [c]DSPE = distearoyl phosphatidylethanolamine, [d]EGCG = epigallocatechin-3-gallate, [e]SLN = solid lipid nanoparticles (Glyceryl monostearate, soya lecithin, and stearic acid).

Table 2. Ongoing clinical trials employing a nanoparticle carrier for the detection or treatment of prostate cancer

NCT number	Receptor/Target	Nanoparticle Formulation	Therapeutic Payload	Clinical Use	Estimated Completion Date
04167969	PSMA	NOTA-PSMAi-PEG-Cy5.5-C'	^{64}Cu radiolabel	MRI/PET tracer	11/2022
03531827	N/A[a]	cyclodextrin-PEG	Camptothecin, Enzalutamide[b]	Anti-tumor activity in mCRPC	12/2021
04240639, 02680535	N/A[a]	Gold nanoshell-silica core-PEG	N/A	Photothermal ablation	06/2023
02805894	N/A[a]	Hafnium-oxide	NBTXR3 radio-enhancer	External beam radiation therapy	11/2022
04221828, 03077659	N/A[a]	nanoparticulate paclitaxel	paclitaxel	Anti-tumor activity in prostate cancer	01/2022
04261777	N/A[a]	Dextran-coated superparamagnetic iron oxide	Ferumoxtran-10	MRI detection of metastatic prostate cancer	03/2023
04089553, 04495179	Adenosine A2a receptor	AZD4635	AZD4635, Oleclumab[b], Durvalumab[b]	Anti-tumor activity in mCRPC	12/2021

[a]Targeting based on enhanced permeability and retention (EPR) effect; [b]Co-administered with nanoparticle therapy.

activity, paclitaxel, and NBTXR3, a radioenhancer for external beam radiation therapy. The limited presence of nanoparticles with prostate cancer targeting capabilities in national clinical trials suggests the need for ongoing laboratory research focused on novel targeting approaches.

Despite the discussed advantages of nanoparticle - based cancer targeting strategies, there are several limitations that need to be overcome prior to the translation of these novel agents to the clinic. Starting from the synthesis and production of nanoparticles, precise experimental conditions need to be maintained, which depending on the type of nanoparticle being developed, can differ significantly from physiologic conditions. After production, nanoparticles need to undergo rigorous toxicity screening and evaluations. This requires evaluation of the physiochemical profile along with *in vitro* and *in vivo* cytotoxicity studies [64]. Once the production, stability, and toxicity of the nanoparticles have been addressed, the nanoparticles need to achieve optimal pharmacokinetic performance. The surface of the nanoparticles is generally coated with a polyethylene glycol layer to render hydrophilicity, in order to prolong the blood circulation time and to avoid the monocyte phagocytotic system and eventual excretion by the liver. However, by increasing the hydrophilicity of the nanoparticle conjugate, the potential for cellular internalization and tumor delivery is decreased. While specific targeting generally affords enhanced delivery of the nanoparticle and therapeutic payload to the tumor site, a study that reviewed 117 nanoparticles applied for cancer theranostics revealed a median nanoparticle delivery efficiency of only 0.7% of the administered nanoparticle dose to the targeted solid tumor [65]. When examining the preclinical studies associated with BIND-014, which utilized a PSMA-targeting docetaxel encapsulated nanoparticle for prostate cancer targeting, the targeting nanoparticle demonstrated a seven-fold increase in delivery of docetaxel, relative to free docetaxel, to circulating tumor cells (21.2 ± 5.2 vs. 3.30 ± 0.76 ng of docetaxel/mg of tumor). While BIND-014 was successful in targeting prostate cancer with tolerable adverse events, the study eventually failed when it was expanded to target cervical and head and neck cancers. Likely reasons for the failure of BIND-014 may be linked to tumor size and tumor density, which were not taken into consideration [66]. In addition, the histologic evaluation of the tumor and circulating tumor cells, from this trial, demonstrated the heterogeneous expression of PSMA, which suggests the need to identify patients that would be optimal candidates for the BIND-014 targeted nanoparticles. Moving forward, there needs to be an appropriate selection of targeting nanoparticles based on the expressed biomarkers on the patient's tumor cells. It becomes imperative that these limitations are addressed in the preclinical phase of nanoparticle synthesis and characterization prior to clinical translation.

Conclusion

This review presents several prostate cancer molecular biomarkers that have been targeted by ligands conjugated to the surface of nanoparticles. While PSMA has been the most studied target, other targets such as GRPR, GRP78, CD44, claudin, CXCR-4, and adenosine have substantial supportive preclinical data to suggest clinical translation. The development of nanoparticle-based targeting strategies are within various phases of development, with a majority of the current clinical trials focusing on the enhanced permeability and retention effect. As the knowledge of biomarkers and molecular targets evolves, the active targeting of prostate cancer cells using nanoparticle-based carriers is an exciting field of research and precision medicine.

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Disclosure of conflict of interest

None.

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