EXPERT REVIEWS

Multifunctional nanoparticles for prostate cancer therapy

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Multifunctional nanoparticles promise significantly better treatment for prostate cancer. This review begins with a molecular and physiological overview of prostate cancer, including current treatments in various stages of disease development. Emerging nanoparticle technology in chemotherapy, hyperthermia therapy and gene therapy will be discussed. We highlight novel advances in nanoparticle technology for prostate cancer and indicate future challenges in the rational design of multifunctional nanoparticles, such as understanding tumor characteristics and the activation of the complement system.

KEYWORDS: chemotherapy • gene therapy • hyperthermia • multifunctional • nanoparticle • prostate cancer

Prostate cancer (PCa) is the most prevalent non-skin malignancy in the USA [1]. It is estimated that PCa alone accounts for approximately 29% of cancer cases in men, with one in six men expected to develop invasive PCa in his lifetime. The prevalence of PCa varies widely across different geographical areas; incidence is higher in the USA and Europe relative to Asia. Advanced age, diet, genetics (race and family clinical history) and social factors (sexual behavior and alcohol consumption) are risk factors for PCa.

The prostate gland is mainly composed of stromal cells (smooth muscle cells, endothelial cells and fibroblasts) and epithelial cells (basal cells, intermediate cells, neuroendocrine cells and secretory luminal cells). Androgen receptors (ARs) on the prostate regulate the growth and differentiation of the prostatic tissue by binding to cognate receptors. ARs are expressed in the cells of the secretory epithelium and the smooth muscle cells of the stroma. Intermediate cells express very few or no ARs [2]. Research suggests that PCa may occur when prostate epithelial cells acquire an autocrine mechanism for androgen-stimulated proliferation. Molecular changes to epithelial cells allow AR proteins to regulate a new series of genes that affect cell proliferation and survival [3]. Ligand-independent activation of ARs by growth factors may also cause PCa [4]. Her2/neu, which is overexpressed in androgen-independent cell lines, increases ligand-independent activation of ARs [5].

Damber *et al.* have recently reported that familial aggregation occurs in PCa [6]. They attribute this aggregation to the inheritance of genes that cause PCa; some show high penetrance, while others show polymorphism and low penetrance [7]. The first gene locus was identified in 1996 and named hereditary PCa locus-1 [6]. Other genes have also been associated with familial PCa, including *EPAC2*, *RNASEL*, *MSR1*, *CHEK2*, *CAPZB*, vitamin D receptor and *PON1* [8–10]. For example, a germline mutation in *BRCA2* can increase the risk of PCa and causes approximately 5% of cases in men younger than 55 years [11]. In 2005, Tomlins *et al.* discovered a new pathogenic mechanism in PCa when they demonstrated the existence of recurring gene fusions between a prostate-specific gene, *TMPRSS2* on chromosome 21, and members of the ETS transcription factor family [12]. It is still unclear which cells are responsible for oncogenic change, especially tumor-initiating cells within the prostate and preferential sites of metastatic disease in the bone [13].

In clinical settings, PCa can roughly be classified as localized, expansively localized or in metastatic stages [14]. The consensus is that localized cancer is potentially curable, but the metastatic stage leads to androgen-independent progression and death within a few years [15]. The risk groups of PCa are defined on the basis of three well-established pretreatment prognostic factors: pretreatment prostate-specific antigen (PSA), clinical T stage (tumor, nodes and metastases classification) on the basis of digital rectal examination and biopsy Gleason score (a measure of the extent of glandular differentiation).

Treatment decisions after the diagnosis of PCa depend on several factors, such as tumor characteristics and patient life expectancy [6].

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Local therapies include retropubic radical prostatectomy [16], laparoscopic prostatectomy [17], cryosurgery [18], radiation with conformal external beam radiation [19] and brachytherapy [20]. Treatment options for locally recurrent PCa include androgen deprivation [21], salvage radical prostatectomy [16,22], salvage brachytherapy [23] and cryotherapy [24]. Treatment for advanced PCa begins with androgen deprivation (AD; also known as hormone therapy), which consists of withdrawal of androgen either surgically or pharmacologically. The latter includes several luteinizing hormone-releasing hormone agonists (goserelin, leuprolide and buserelin) and AR antagonists (steroidal, cyproterone acetate and megesterol acetate) [2]. On the molecular level, AD has been demonstrated to promote apoptosis (programmed cell death) and may act synergistically with radiation to kill PCa cells [21]. However, after AD, PCa cells become progressively hormone refractory (HR) and insensitive to antiandrogen therapy. This patient population is a candidate for chemotherapeutic options (docetaxel may confer survival benefit in this setting).

Novel therapeutic approaches

Current chemotherapeutic agents used to treat cancer have many disadvantages. Drugs, such as the widely used docetaxel and paclitaxel, cannot cure tumors within a tolerable dosage [25]. Although cancer cells are inherently more vulnerable than normal cells to the effect of chemotherapy, drugs are also nonselective and cause injury to normal tissues. Toxicity to normal cells remains the main constraint to dose and frequency; both important factors that modulate the persistence of cancer cells after completing therapy. Other disadvantages include increased drug resistance, limited solubility and low therapeutic index. In general, traditional treatment can only reach a median survival of 18 months after AD fails [26]. There are no good second-line options. Consequently, novel therapeutic approaches for PCa treatment are urgently needed.

Multifunctional nanoparticles (NPs) capable of performing several simultaneous functions may signal a paradigm shift in PCa treatment. The main components of a typical multifunctional nanocarrier are: a nanocarrier, a target ligand conjugated to the nanocarrier and the payload. Hence, targeting, imaging, sensing and the delivery of therapeutic payloads are the most distinctive functions of such nanodevices (Figure 1). These novel drug carriers carry chemotherapeutics to inhibit or kill PCa cells, but also control drug release, confer immune clearance (most commonly by polyethylene glycol [PEG] functionalizing to confer 'stealth' properties) and target delivery (by further functionalization with homing molecules to allow selective binding to PCa cells). These NPs can be engineered further to increase drug solubility in aqueous media. In addition, the longer the plasma half-life of a drug, then the more the required dose can be reduced. These characteristics reduce drug toxicity and increase antitumor efficacy.

In this article, we review the most recent research advances in developing multifunctional NPs, which will enable a better PCa diagnosis and treatment.

Chemotherapy based on multifunctional nanoparticles for PCa

Nanoparticles can be delivered into the tumor via a passive or active process. In the former, NPs pass through leaky tumor capillary fenestrations into the tumor interstitium and cells by passive diffusion or convection [27]. The latter involves drug delivery to a specific site, based on molecular recognition [27]. The most common approach conjugates targeting ligands to NPs. The target ligands enhance the interaction between NPs and receptors at the target cell site, increasing local drug concentration. Many ligands have been successfully conjugated to the NPs including antibodies [28,29], transferin receptor [25], aptamers (RNA and DNA) [30], folate receptors [31] and a wide range of biomolecules [32].

Polymers that are biodegradable, biocompatible, have large engineering flexibility and sustained-release characteristics are the most promising drug delivery carriers. Over the last decade, research groups have developed a vast arsenal of NPs designed to better treat cancers such as PCa [33]. Polymeric NPs, particularly poly(D,L-lactide glycol; PLGA)-based NPs [34,35], have been used extensively. To our knowledge, multifunctional NPs based on PLGA conjugated with PEG are the only system that has been applied in PCa (Figure 1A). Besides the aforementioned advantages, the PLGA conjugated NP system is attractive owing to PLGA having been approved by the US FDA, which may facilitate and accelerate the process of reaching clinical trials.

Gu *et al.* reported a recent advance in the development of a new multifunctional NP design to treat PCa [36]. An end-to-end linkage of PLGA, PEG and A10 aptamer assembled an amphiphilic tri-block copolymer. The tri-block approach has a comparative advantage over the conventional di-block approach because it makes it possible to precisely engineer NPs to balance molecular targeting and immune invasion by adjusting target ligand density [37]. The tri-block approach can also be very useful for exploring the targeting ability of other aptamers relevant to PCa research. However, this approach may be limited by the low tolerance of some ligands, such as aptamers, in the harsh reaction environment needed for conjugation.

In general, aptamers are stable at a wide range of pH levels (~4–9), physiological conditions and solvents. Aptamers are known to be less immunogenic than antibodies and can penetrate a tumor more easily owing to their size. The shape of aptamer binding sites, which includes grooves and clefts, provide highly specific characteristics and drug-like capabilities [38]. Active targeting, however, does face challenges. For example, researchers must find RNA aptamers that discriminate cancer cells from normal cells.

Besides A10 RNA, there are other aptamers that are potential candidates to target proteins overexpressed in PCa cells. For example, pegaptanib is a pegylated anti-VEGF aptamer, a single-stranded nucleic acid that binds with high specificity to a particular target [39]. Although the pegaptanib aptamer was originally approved by the FDA in 2004 to treat age-related macular degeneration disease, it has the potential to treat PCa because it binds specifically to VEGF165, a protein recognized as the key inducer of tumor angiogenesis [40]. Several studies have reported the overexpression

of VEGF in prostate tumors [41]. Also, Latil *et al.* have suggested that VEGF expression could be used as a prognostic marker in early-stage tumors [42].

Chu *et al.* have reported the use of a single A9 RNA aptamer conjugated with a gelonin protein as an efficient method to target and destroy prostate-specific membrane antigen (PSMA)-positive PCa cells [43]. Gelonin is a small *N*-glycosidase protein that causes cell death by cleaving a specific glycosidic bond in rRNA, disrupting protein synthesis. The authors report that this aptamer system not only promotes uptake into target cells, but also decreases the toxicity of gelonin in nontargeted cells.

Zhang *et al.* have also reported a novel multifunctional NP design for PCa treatment: polymer–lipid hybrid NPs [44]. Polymer–lipid hybrid NPs combine characteristics of both liposomes and polymeric NPs since they are composed of a hydrophobic core and a hydrophilic shell. Docetaxel can be encapsulated in the core of these NPs. Polymer–lipid hybrid NPs have a higher drug encapsulation yield of docetaxel (60%) than PLGA–PEG (20%) and PLGA (35%) NPs [44]. The presence of the lipid monolayer at the interface of the PLGA core and the PEG shell might act as a molecular fence to retain the drugs inside the NPs. In addition, A10 RNA aptamer-targeting polymer–lipid NPs were selectively delivered to cells from the human prostate adenocarcinoma cell line (LNCaP) that overexpress PSMA, demonstrating the potential use of the hybrid NPs to treat PCa. Polymer–lipid hybrid NPs may offer another way to increase chemotherapeutic efficacy in PCa treatment if they combine hydrophobic drugs, such as docetaxel, encapsulated in the NP core, with the intercalation of hydrophilic chemotherapeutics, including goserelin and leuprolide, within the A10 PSMA aptamer [45].

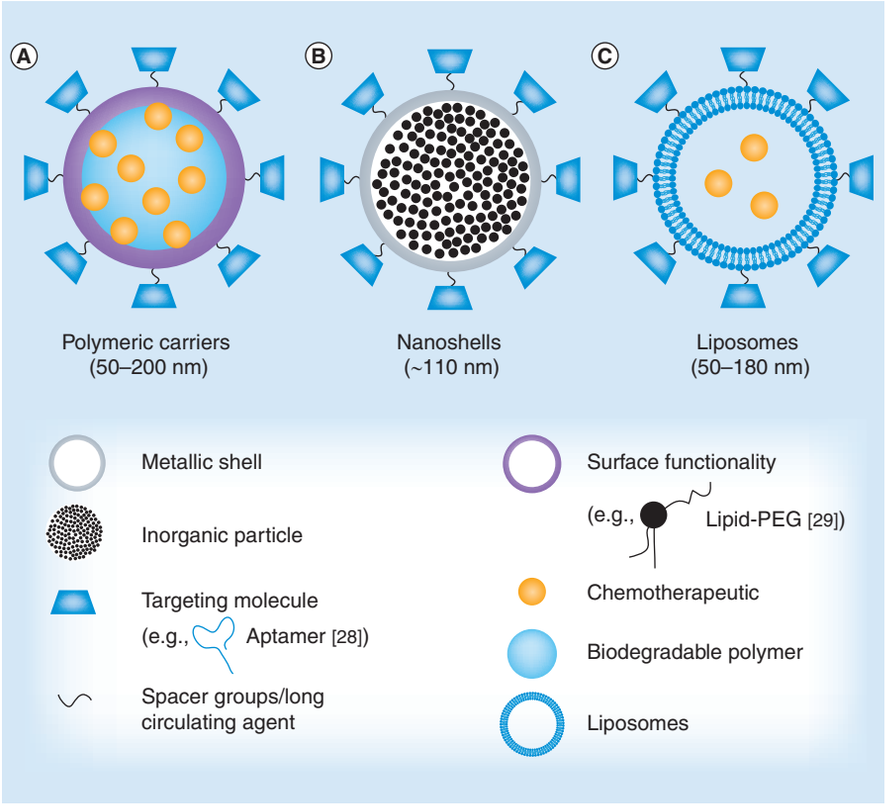

Figure 1. Examples of multifunctional nanocarriers for the treatment of prostate cancer. The main components of a typical multifunctional nanocarrier include a nanocarrier, a target ligand conjugated to the nanocarrier and payload. **(A)** Biodegradable polymer nanocarriers capable of encapsulating hydrophobic or hydrophilic drugs in their matrix and releasing them upon degradation. To target prostate cancer cells, the surface of polymeric nanoparticles [36] and lipid-polymeric hybrid nanoparticles [44] have been functionalized with A10RNA aptamer, which recognizes the prostate-specific membrane antigen (PSMA) expressed in prostate cancer cells. **(B)** Metal nanoshells can work as a dual platform, for both imaging and therapy. In particular, the performance of gold nanoshells has been investigated for prostate cancer therapy using a murine tumor model [47]. **(C)** Liposomes have been the most extensively utilized nanoparticle-based carriers for delivering anticancer drugs. Recently, researchers have reported the use of rhodamine-labeled supermagnetic liposomes to target solid human prostate adenocarcinoma tumors implanted in mice [48].
Adapted with permission from [33].

It is important to note that multifunctional nanocarriers must overcome or diminish the tumor resistance phenomenon observed after delivering several doses of chemotherapeutic drugs into tumor cells. Therapeutic resistance results from multiple, stepwise changes in DNA structure and gene expression. One cause of this phenomenon is uneven intratumoral distribution of the chemotherapeutic agent. This distribution may be due to the binding of extracellular matrix proteins to the drug [46]. The extracellular matrix of a solid tumor is composed of macromolecules, such as fibrous proteins including collagen and elastic. They represent a source of physical resistance to drug transport. To overcome this problem, NPs must be bioengineered to minimize binding to the extracellular matrix.

Nonpolymeric multifunctional carriers for PCa treatment

There is a wide range of papers that report on the proof-of-concept of nonpolymeric multifunctional nanodelivery systems that are able to efficiently target PCa cells. They represent the first step towards the discovery and improvement of therapies for cancers including PCa.

Nanoshells

Nanoshells can be used as multifunctional nanodevices. In general, nanoshells are NPs often consisting of a dielectric silica core covered by a thin gold shell (Figure 1B). To provide optically guided hyperthermic ablation, nanoshells can be designed to significantly absorb near-infrared light that is converted to heat energy when excited electrons in the gold shell relax to the ground state [47]. A delicate balance must be struck between accurate tumor targeting,

energy delivery and preservation of surrounding vital structures. The nanoshell particles offer minimally invasive targeted ablation for PCA [47]. In a recent *in vivo* study by Stern *et al.*, approximately 110-nm gold nanoshells were administered by intravenous injection, followed by treatment with a near-infrared laser in an ectopic murine tumor model [47]. These studies showed 93% tumor necrosis and regression and a sharply limited ablation zone in 14 out of 15 tumors in 21 days.

However, it is not clear in these studies why gold nanoshells were not found at the tumor site after laser ablation and tumor involution. It is hypothesized that the reticuloendothelial system clears the area of nanoshells in 21 days. Therefore, there is no effective means by which to quantify nanoshell delivery to a tumor bed that will subsequently be ablated [47].

Liposomes

Liposomes are self-assembling vesicles with an inner aqueous compartment surrounded by a lipid bilayer that commonly consists of phospholipids and cholesterol. Hydrophilic drugs can be encapsulated into the interior aqueous compartment, whereas lipophilic and amphiphilic drugs can be embedded in the liposome bilayers (Figure 1C).

Recently, reports show magnetic targeting of rhodamine-labeled superparamagnetic liposomes to solid human prostate adenocarcinoma tumors implanted in mice [48].

These liposomes are composed of superparamagnetic nanocrystals of maghemite (γ-Fe_2O_3) encapsulated within pegylated unilamellar phospholipid vesicles referred to as magnetic fluid-loaded liposomes (MFLs).Their size is approximately 180 nm ± 25 nm. MFLs can circulate in blood for approximately 8 h as intact vesicles, without loss of magnetic fluid. They were unclear in the tumor owing to a dense and heterogeneous vascular network within the tumoral tissue and regular blood flow. However, the use of rhodamine as a fluorescent label and maghemite nanocrystals, which act as efficient magnetic resonance contrast agents allowed the accumulation of MFLs at a tumor site to be detected. This multifunctional NP design constitutes a powerful tool to treat PCa, not only for simple diagnosis but also for active targeting of drugs.

Theodossiou *et al.* described a novel multifunctional liposome design to enhance doxorubicin retention and cytotoxicity in DU145 human prostate cells [49]. In this model, doxorubicin is encapsulated in the hydrophilic core of the liposomes, whereas amiodarone, a multidrug resistance inhibitor, is incorporated in the lipid bilayer of the liposomes. These were further functionalized with guanidinium groups in order to achieve better binding to the cell plasma membrane. This model offers the possibility of better drug resistance reversal.

Kawai *et al.* have reported the use of cationic liposomes as carriers for magnetite NPs, referred to as magnetite cationic liposomes (MCLs), to induce intracellular hyperthermia with exposure to an alternating magnetic field [50]. MCLs were injected into 7-mm diameter tumor male rats and exposed to repeated alternating magnetic field. Tumor proliferation of PCa cells was suppressed in the bone environment.

Hyperthermia treatment for PCa by magnetic NPs

Hyperthermia, using iron oxide NPs, has demonstrated potential as an alternative for patients with locally recurrent PCa. Low-temperature hyperthermia treatment usually involves temperatures between 39 and 41°C for 1–72 h. Moderate temperature hyperthermia involves temperatures between 41 and 45°C for 30–60 min [51]. Elevated temperature causes tumor cell apoptosis or necrosis.

Moderate hyperthermia appears to be more successful for the treatment of PCa. A novel approach in moderate hyperthermia uses the synthesis of a magnetic fluid. This contains magnetic NPs, particularly iron oxide NPs, which are injected into the prostate gland. The NPs locally generate heat when the tumor region is immersed in an external 100 kHz magnetic field [51]. The results of a recent Phase I trial of iron oxide NP technology developed by German company Magneforce have been published [52]. In this report, iron oxide NPs were administered via intraprostatic injection. The magnetic NPs were able to remain in the prostate for more than a year [52]. The results from Phase I clinical trials also show that patients who received the thermotherapy treatment did not experience obvious toxicity or significant deterioration of quality of life at any of the previous time points observed [52].

In comparison with other cancer treatments (e.g., chemotherapy and radiotherapy), hyperthermia is a technique that does not induce tumor resistance. However, it faces issues that are related to the physical and anatomical position of the prostate (e.g., due to the anatomical position of the prostate, external application of a thermal treatment is problematic). This might be overcome by the use of monoclonal antibodies linked to the iron oxide magnetic NPs [53]. The use of the monoclonal antibodies renders the NP system tumor-specific and thereby increases the efficacy of the hyperthermia. Lehmann *et al.* report that this technique causes the complete death of human PCa cells (DU145) in the presence of external beam radiation therapy [53].

Hyperthermia may be more effective if magnetic NPs not only destroy the malignant tumor, but also penetrate cancer cells selectively to generate lethal heating from inside the cell [54]. An example of this is maghemite anionic NPs that are efficiently captured by human prostatic tumor cells (PC3) and concentrate within intracellular vesicles [55]. In a subsequent publication, Wilhelm's group reported the use of maghemite and cobalt ferrite anionic NPs as magnetic mediators to cause death in prostate tumor cells. Both NP types entered tumor cells (PC3) with similar efficiency and followed the same intracellular pathway.

Maghemite NPs with a size of 14 nm are high efficiency nanoheaters because their heating efficiency improves more than tenfold [55], thereby reducing the number of NPs required intracellularly by the same factor. The reason for the dramatic heating efficiency improvement is still unclear. Maghemite and cobalt ferrite NPs may be better candidates for PCa hyperthermia than iron oxide NPs owing to their higher heat capacity and lower dose in the tumor site. The challenge of injecting tumor-specific NPs intravenously has not been addressed. The biocompatibility and toxicity of novel multifunctional NPs intended for

intravenous administration will be discussed in the section 'The role of the complement system in the rational design of NPs to treat PCa'.

The combination of antibodies and different magnetic NPs for hyperthermia treatment promises an effective multifunctional NP design for future PCa treatment.

Gene therapy using NPs for PCa

Gene delivery has increasingly become an important strategy for treating a variety of human diseases, including cancer [56]. For example, it offers the opportunity to tackle PCa using different therapies, including tumor-suppressor therapy, suicide gene therapy, immunomodulatory gene therapy and anti-oncogene therapy [57]. In tumor-suppressor gene therapy, the goal is to cause the reversal of a malignant phenotype by introducing a normal tumor suppressor. In PCa, wild-type (*P53*), retinoblastoma (*RB*) and *p21* have been studied [58]. However, in the case of *P53*, the therapy has not reached clinical applications because not all PCa cells express a *P53* gene abnormality [59]. Suicide gene therapy activates a prodrug in transfected cells, while immunomodulatory gene therapy aims to provoke the induction and enhancement of a tumor-specific immune response. Anti-oncogene therapy uses antisense oligonucleotides (ASOs) to downregulate gene expression, such as *c-myc* and *ras*. ASOs are single-stranded chemically modified DNA-like molecules that are 17–22 nucleotides in length. They are designed to be complementary to the mRNA of selected genes and thereby specifically inhibit expression of that gene [60]. Currently, there are several ASO drugs in clinical development against HR PCa, including OGX-011, GTI-2040 and OGX-427. OGX-011 targets human clusterin mRNA. Increased levels of clusterin in PCa are correlated with the Gleason score [60]. Preclinical studies have suggested that clusterin suppresses the apoptotic cell death in response to androgen withdrawal, chemotherapy and radiation [61]. Results from Phase I indicate that the weekly use of OGX-011 has the potential to suppress clusterin expression in PCa tissues [62]. GTI-2040 is an antisense molecule that inhibits the expresson of the R2 subunit of ribonucleotide reductase [63]. Ribonucleotide reductase is often expressed in tumors to increase their malignant potential and promote drug resistance. Nine patients out of 22 bearing HR PCa showed a PSA response when they were treated with GTI-2040 in combination with docetaxel [64]. OGX-427 targets heat-shock protein (HSP)27, which is found in high levels in HR PCa. HSP27 has been associated with treatment of resistance and apoptosis [65]. Although ASOs produce a therapeutic effect in PCa, a vehicle to protect them from nuclease degradation and alleviate chemistry-related side effects is needed. Schwab *et al.* reported in 1994 that a small amount of antisense oligonucleotide can inhibit the neoplastic growth of HBL100 ras1 cells in a very effective manner when they are adsorbed in polyalkylcyanoacrylate NPs [66].

Transcriptional targeting is another gene therapy method, which refers to the use of particular cell-specific regulatory elements (promoters or promoter/enhancers) to restrict gene expression to a particular tissue or cell type. Nonviral vectors such as NPs have demonstrated success of *in vivo* gene delivery. For example, Sawicki *et al.* have conjugated polymeric C32 NPs with toxic genes, taken advantage of PSA promoter and have attempted to regulate the expression of toxic genes specifically in PCa cells, preserving the healthy tissue in close proximity to targeted cells [67]. Chumakova *et al.* have attempted to use ultrasound with NPs to enhance drug and gene delivery in PCa cells *in vivo* because ultrasound alters the properties of tumor vasculature and the cell membrane [68]. Multifunctional NPs reported for gene delivery in PCa include multivalent cations, such as polyamines, polyethylenimine (PEI) and peptides, such as poly-L-lysine, which help condense DNA during gene delivery [69]. Depending on their molecular weights, PEI is known to help disrupt the endosomal membrane and enhance gene delivery [70]. Hattori *et al.* have also tried to combine PEI with cationic cholesterol derivatives to increase transfection efficiency [71].

Folate-linked lipid-based NPs in combination with the herpes simplex virus thymidine kinase gene/ganciclovir (prodrug) has the potential to deliver DNA with high transfection efficiency and selectivity. This combination leads to the inhibition of tumor growth in the prostate, as reported by Hattori *et al.* [31]. This technique has not been used *in vivo* and might have some difficulties. For example, the deficiency of gap junctions found in human prostate tumors may hamper the extent of the bystander effect in suicide gene therapy [72].

The delivery of siRNA by a new biomaterial called atelocollagen has been show to successfully inhibit the secretion and expression of VEGF in PC3 human prostate carcinoma cells *in vitro* and *in vivo* [73]. This inhibition results in potent tumor growth suppression. Before siRNA can reach clinical trials, there are issues with atelocollagen that must be cleared up: delivery, distribution and clearance. Cationic cardiolipin liposomes have also been used as drug delivery carriers for siRNA to silence *Raf-1*, a gene found in PCa [74]. These novel nanocarriers were able to inhibit tumor growth in a xenograft model of human PCa.

Recently, Li *et al.* have used anisamide-targeted stealth liposomes to silence the EGF receptor (EGFR) [75], which seems especially relevant in hormone-resistant stage of PCa [76]. They used a mouse xenograft model and found that three daily injections (1.2 mg/kg) of siRNA formulated in the targeted NPs silenced the EGFR in the tumor and induced approximately 15% tumor cell apoptosis.

Challenges for nonviral vector gene delivery include improving the efficiency of the expression vector intracellular uptake, the escape from the degradative environment inside the endo/lysosomal compartment, the dissociation of DNA from the vector and the localization of DNA into the nucleus [77]. For example, even though some polymeric NPs are successful in encapsulating and delivering hydrophobic drugs, the encapsulation efficiency of siRNA is low compared with the other nanocarriers, such as atelocollagen. This is due to the negative charge of siRNA, which gives it a hydrophilic nature incompatible with the hydrophobic core in polymeric nanoparticles. Moreover, these polymeric NPs cannot readily escape the endosomal compartment. However, they still represent a new platform for siRNA delivery to silence several genes expressed at different stages of the development of PCa including *EZH2* and *Raf-1*.

Challenges in the rational design of multifunctional NPs for PCa

The efficient use of novel multifunctional NPs to treat PCa depends on understanding the anatomy and physiology of the PCa, and the physicochemical properties of the drug. The tumor vasculature, extracellular components, interstitial fluid pressure, tumor cell density, tissue structure and composition phenomena are important characteristics to consider [46].

Tumor vasculature & NP size

Tumor vasculature refers to the characteristics and functionality of tumor blood vessels. It plays a pivotal role in the delivery of therapeutic agents to the solid tumor. The tumor blood vessels are generally more heterogeneous in distribution, density, length and diameter; they are larger in size and more permeable than normal ones [78]. Some of these characteristics may be important based on the intended therapy. For example, for hyperthermia treatment, the anatomical position and the high perfusion characteristics of the prostate must be considered to overcome practical difficulties while providing treatment (as discussed in the section 'Hyperthermia treatment for PCa by magnetic NPs').

One of the unique features of tumor microvessels is their leakiness as a result of endothelium discontinuity [79]. This allows NPs to escape the blood vessels and deliver drugs in solid tumors via diffusion and/or convection through the discontinuous endothelial junctions [80]. In the prostate, the endothelium gap in the blood vessels ranges from 300 to 780 nm. As a result, NPs within this range will penetrate the prostate tumor. The defective lymphatic flow in solid tumors also decreases the clearance of high-molecular-weight compounds from the tumor interstitium. Defective lymphatic flow, together with leaky tumor blood vessels, results in enhanced accumulation and retention of high-molecular-weight compounds in solid tumors, which is known to cause the enhanced permeability and retention (EPR) effect explained in the section 'Chemotherapy based on multifunctional NPs for prostate cancer'. The vasculature of the tumor becomes particularly relevant for the design of polymer NPs planned as drug-delivery vehicles.

NP transport in the tumor environment

An important factor in deciding which drug to encapsulate in a nanocarrier for solid tumors is tumor blood flow. Slow flow affects drug transport through vascular space in the tumor. The presence of tumor cells and large molecules (e.g., proteins and collagen) perturbs blood flow, affecting the viscosity of the tumor blood [81]. In this regard, poorly soluble chemotherapeutic drugs might diffuse less efficiently than those with good dispersibility properties in aqueous solution. A good example is docetaxel, which needs polysorbate 80 as a water-soluble molecule to enable it to be administered. Complement system reactions generated by the use of this chemotherapeutic agent also need to be considered and will be discussed later. Drug selection influences the design of nanocarriers since the chemical features of the drug must balance specificity, circulation time and low toxicity characteristics.

The presence of extracellular matrix proteins in a tumor is also a source of physical resistance to drug transport. One of these proteins is glycosaminoglycan, which reduces the hydraulic conductivity and convective flow in the interstitium [82]. Collagen is another substance that causes drug transport resistance in the interstitium. Indeed, collagen is considered to be a major determinant of resistance of drug transport in solid tumors. It is unknown, however, whether the reduction of collagen assures the even distribution of the drug throughout a tumor.

Remodeling leaky chaotic neoplastic vasculature agents to reduce tumor interstitial fluid pressure may reduce drug resistance. This procedure may improve tumor blood flow, drug delivery and efficacy [46].

The challenge of cancer nanotechnology does not end with a nanovehicle that specifically delivers a drug into tumor cells, but rather in finding ways to kill the cancer cells in a homogenous manner. This is challenging owing to new vasculature as the tumor grows. Time-dependent changes in cell density also generate as the result of drug-induced cell death [26]. In addition, when a drug is not distributed homogeneously along the tumor, tumor cells become more resistant to the drug. An important area that has received relatively little attention is the spatial drug distribution within a tumor. Work in this area is urgently needed to make better use of the technologies currently available.

In summary, chemotherapy based on NPs faces many challenges that are closely related to the chemical nature of the chemotherapeutic agent and the challenges encountered in the vasculature environment. Those challenges may be overcome with the use of alternative therapies, such as hyperthermia and gene therapy. However, each technique has its own technical barriers and unforeseen disputes. In the section 'Hyperthermia treatment for PCa by magnetic nanoparticles' we mentioned techniques, such as hyperthermia, which do not induce tumor drug resistance. More importantly, when this technique is used with monoclonal antibodies linked to iron oxide NPs, the efficacy of hyperthermia to kill cancer cells improves enormously. Gene therapy, compared with targeted-NPs and hyperthermia, may overcome the inhomogeneous killing of cancer cells since it prevents PCa development on the genetic level.

Role of the complement system in the rational design of NPs to treat PCa

Multifunctional NPs for PCa treatment usually require minimum immune evasion. The advancement of nanotechnology has enabled the delicate fabrication of NPs. Advances in technology also allow for a better study of the underlying mechanisms of NPs in the activation of the complement system. Different physicochemical characteristics of NPs, such as surface properties and nanocarrier shape, may confer different immunological properties. Ideally, NPs could remain in the blood stream for a long period of time, while effectively resisting uptake by tissue macrophages and nontargeted cells, thereby increasing their residence time at the site of administration.

The administration route of novel drug delivery carriers dictates the type of biocompatibility and toxicity studies that must be conducted to validate their use in clinical settings. For example,

if NPs are administered intravenously, then the study of the complement and coagulation system is essential. Abraxane® is a clear example that illustrates the need for studies that tackles the activation of the complement system. Acute hypersensitivity reactions have been observed when Abraxane, the only example of an FDA regulatory-approved NP formulation for cancer therapy, is intravenously administered to cancer patients [83]. Paclitaxel, a well known chemotherapeutic agent for PCa, generates hypersensitive reactions related to the activation of the complement system. The acute hypersensitivity reactions are characterized by dyspnea, flushing, rash, chest pain, tachycardia, hypotension, angioedema and generalized urticaria [84]. The mechanism by which paclitaxel activates complement is still obscure. However, it is clear that the presence of Chemophor EL contributes to complement activation [84]. Docetaxel, a drug widely used in PCa treatment, is known to activate the complement system due to several factors, including the presence of its vehicle polysorbate 80. Although some measurements can be taken to reduce the risk of hypersensitivity reactions to paclitaxel, including a slow intravenous infusion of this substance to the patients together with high-dose steroid and antihistamine premedication, there is no guarantee against occasional severe reactions [84].

The surface chemistry of the nanocarrier can also cause hypersensitive reactions from complement activation. For example, although the consensus holds that the use of methoxy-PEG should dramatically suppress blood opsonization, Moghimi *et al.* have demonstrated that liposomes bearing phospholipid–methoxy-PEG conjugates in their bilayer unexpectedly activate the complement system and fix complement proteins [85]. This observation is worthwhile since this approach has been used in polymeric NPs and other drug delivery systems. The behavior of the key complement proteins in the presence of the surface chemistry associated to drug delivery carriers needs to be carefully understood.

Overview of *in vivo* studies of multifunctional NPs for PCa

Although there are currently quite a few research articles describing the use of multifunctional NPs for the treatment of different types of cancers, only a few report *in vivo* studies using a PCa model. To date, the multimodality of novel multifunctional NPs seems to be focused on targeting, imaging and therapeutic payload. New designs that could be used to treat PCa would involve the use of a wide range of targeting moieties, including A10RNA–siRNA conjugate [86] and anti-HER2 scFv [87]. The aptamer–siRNA chimera is a multifunctional system that uses an A10 RNA aptamer to bind PMSA and siRNA to silencing *PLK1* and *BCL2* genes that are overexpressed in most human tumors [86]. Notably, this siRNA delivery approach effectively mediated tumor regression in a xenograft model of PCa [86]. Another recent novel multifunctional platform tested *in vivo* is the quantum dot-conjugated immunoliposome-based NP system, which is functionalized with anti-HER2 scFv [87]. The aforementioned experiments proved the efficacy of targeting moieties and therapeutic payloads of multifunctional NPs to specifically bind and kill tumor cells. However, biodistribution and pharmacokinetic studies of multifunctional NPs for PCa have not been addressed in depth. If multifunctional NPs are to be used in clinical settings, we must take into account the fact that the biodistribution and pharmacokinetic profiles of the nanocarrier will be influenced by the route of administration, particularly with intravenous administration. Hemolysis, thrombogenicity and complement system activation are the three main possible consequences of an adverse response of the immune system to multifunctional NPs. These immunological responses could affect the biodistribution of the NPs. It is well-accepted that the surface charge of the NPs triggers hemolysis, thrombogenicity and the activation of the complement system [88]. In the latter, the surface charge influences NP uptake and clearance by macrophages. There are still unsolved questions regarding the fate of the nonbiodegradable elements of multifunctional NPs and their stability, both in blood and at the tumor site.

A key aspect to bioengineering multifunctional NPs with a long circulating life is minimizing the activation of the complement system. One strategy is sequestration of factor H by the nanocarrier. Factor H is the main downregulator of the complement system via the alternative pathway. Salvador-Morales *et al.* have demonstrated that the sequestration of factor H by chemically modified carbon nanotubes can greatly diminish the activation of the complement system [89]. Similar approaches could be used to reduce complement system activation induced by multifunctional NPs.

Expert commentary

We believe that with continued research and development, biodegradable and low immunogenic NPs will have a great impact on the fight against PCa as drug carriers and molecular probes/diagnostic devices. It is important to note that the nonhomogeneous delivery of therapeutic agents to tumors via NP delivery systems may lead to ineffective drug efficacy for drugs which intracellular delivery is required for bioactivity (e.g., siRNA); additionally non-homogeneous drug delivery may enhance the emergence of chemotherapeutic drug resistance [46]. There are currently an increasing number of NP designs being developed for combination-drug therapy, enhanced tumor penetration and effective intracellular delivery (example: targeted lipid-polymer hybrid NPs) that might maximize the therapeutic effectiveness of drugs which need intracellular delivery, while minimizing drug resistance problems common to anticancer agents.

Which anticancer technique available today effectively addresses the PCa problem? While we won't know the answer to these questions for many years to come, several technologies have already reached clinical evaluation, including for example, the induction of therapeutic hyperthermia using iron oxide NPs. The wide range of magnetic NPs intensively investigated by scientists has opened new doors for the treatment of PCa. The combination of antibodies with iron oxide NPs offers the possibility of overcoming some difficulties observed in hyperthermia therapy. The use of other magnetic NPs, such as maghemite and cobalt ferrite, can potentially improve hyperthermia for the treatment for PCa.

Gene therapy using NPs for PCa is still in its infancy, although significant advances have been made in this area in the last couple of years. Gene therapy offers the most comprehensive treatment for PCa but faces many challenges. The hybridization of all three leading therapies may help to combat PCa. A wide range of questions remain on the table: should we develop NPs that delay the progression to androgen independence? This may involve targeting antiapoptotic factors, use of chemotherapy at the time of androgen ablation or the blockage/downregulation of AR activity [90]. In addition to these novel options, there are a number of promising strategies, such as targeting signal transduction pathways, cell cycle regulations, and differentiation and angiogenesis.

Five-year view

Many of the technical problems that NP systems currently face will be overcome within a few years. Polymeric NPs are not only capable of delivering chemotherapeutic agents but also siRNA, which may silence the most important PCa genes. Treating cancer on a genetic level in most effective since secondary effects induced by treatment are either reduced or eliminated. New methods to tackle PCa will be developed; Cordon-Cardos' group managed to quantitatively assess the concentration and localization of relevant proteins expressed in PCa [91]. Nanotechnology applications are rapidly developing for tumor imaging, predictive oncology and targeted therapy. Although other nanosystems, such as quantum dots, are not biodegradable, they are potential nanodevices detecting molecular targets *in vivo*.

Understanding the fundamentals of tumor biology will help nanotechnologists to design more effective nanosystems. Identifying mechanisms that cause the tumor angiogenic switch may lead to novel diagnostic and therapeutic approaches as indicated by Marsha Moses (Children's Hospital of Boston, MA, USA) in the last American Association for Cancer Research meeting [15]. The toxicity and biocompatibility issues associated with different architectural designs of drug delivery carriers can be overcome by diminishing complement activation and inactivating the coagulation cascade. Engineers of NPs must also deal with the heterogeneity in tumor vasculature that contributes to uneven drug distribution within solid tumors. Nanotechnology is going through a period of intense development and discovery, overcoming the challenges of synthesizing novel biocompatible materials to fashion powerful nanomaterial, biological devices.

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Key issues

- The use of multifunctional, biodegradable and low immunogenic nanoparticles can be effective as imaging devices and as chemotherapeutic carriers for prostate cancer diagnostic and therapeutic applications. Although it is true that the encapsulation of the chemotherapeutic drugs in nanodevices, such as polymeric nanoparticles, diminishes the harmful effects associated with chemotherapeutic agents without any carrier, chemotherapy delivery may face issues such as drug resistance and complement activation if nanocarriers are not optimally engineered.
- Currently, the use of multifunctional nanoparticles in combination with hyperthermia is an emerging technique for prostate cancer treatment.
- Gene therapy using nanoparticles to deliver siRNA and antisense oligonucleotides into prostate cancer cells is, perhaps, the most promising technique to combat this disease since this tackles the problem on a genetic level. However, several technical barriers must be overcome to make this technique efficacious.
- The ideal design of multifunctional nanoparticles to treat prostate cancer depends on an understanding of the anatomy and physiology of the prostate glands and tumors, and the physicochemical properties of the drug.

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