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Nanoformulation of natural products for prevention and therapy of prostate cancer

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Abstract

There is a need for developing improved therapeutic options for the management of prostate cancer, able to inhibit proliferation of precancerous and malignant lesions and/or to improve the effectiveness of conventional chemopreventive and chemotherapeutic agents. In this perspective, application of nanotechnology based strategies for the delivery of natural compounds for effective management of the disease is being actively researched. Here, after highlighting the most promising natural compounds for chemoprevention and chemotherapy of prostate cancer, the state of the art nanotherapeutics and the recent proof-of-concept of “nanochemoprevention”, as well as the clinical development of promising targeted nanoprotoypes for use in the prostate cancer treatment are being discussed.

Keywords

Prostate cancer; Natural products; Nanoparticles; PSMA; Nanochemoprevention; Targeted nanoparticles

1. Introduction: prostate cancer as a social emergency

Cancer of the prostate gland is one of the most frequently diagnosed cancers amongst men in Western and most of the developing countries [1]. According to the figures available from American Cancer Society, prostate cancer (PCa) has surpassed heart disease as the top killer of men over the age of 85 years [1,2]. In the United States alone, the number of new cases projected to be diagnosed with PCa in 2012 is likely to be more than 240,000, with over 28,000 deaths probable from the disease [1,2]. Racial and ethnic differences is an integral part of PCa incidence and mortality, with African-American men being at the greatest risk for diagnosis (incidence rate, 271.3; death rate, 70.4), followed by Caucasian (incidence rate, 167.4; death rate, 28.8) and Hispanic (incidence rate, 140.0; death rate, 23.5) with Asian Americans carrying the lowest threat for PCa (incidence rate, 100.7; death rate, 13.0)

[3,4]. One primary reason considered important for low incidence of PCa in the Asian continent and people of this origin is the fact that their normal diet is heavily comprised of vegetables, fruits and other naturally occurring dietary food components [5,6]. This low incidence of PCa in Asian population is in fact supported by the concept of “chemoprevention”, where bioactive food components are utilized to block, reverse or delay the process of carcinogenesis [7]. In fact PCa exemplifies an ideal candidate disease for chemoprevention since, (i) it is a unique malignancy which is slow progressing, likely for decades, before symptoms arise and a diagnosis is finally established and (ii) is generally detected in elderly men. Thus even a slight delay in the neoplastic development, achieved through pharmacological or therapeutical intervention could result in substantial reduction in the incidence of the clinically detectable disease [6–8].

In recent years, significant progress has been made, which in fact has resulted in identification of novel PCa chemopreventive agents [7–11]. Despite promising results in preclinical settings, applicability of chemoprevention to human for any cancer including PCa has met with limited success largely due to inefficient systemic delivery and bioavailability of promising chemopreventive agents [7,12]. We envisioned that nanoparticle-mediated delivery could be useful to limit the perceived toxicity and enhance the bioavailability of the chemopreventive agents and introduced the concept of ‘*nanochemoprevention*’, where nanotechnology was incorporated for the enhancement of chemopreventive efficacy of agents [12]. Our proof-of-principal study was well accepted and subsequently exploited by several laboratories and has now being considered as an advancing field of chemoprevention research.

Here we will highlight some of the naturally occurring dietary agents that have been shown to exhibit efficacy in prevention and treatment of PCa in cell culture, animal models and limited clinical trials. We will then describe the recent developments in natural product-based nanotechnology for management of PCa. The development of the first targeted nanoprotoypes in clinical use for the PCa treatment, are also detailed and discussed.

2. Natural product for PCa chemoprevention

There are several naturally occurring dietary food components that are under investigation for their efficacy against PCa and are demonstrating potential usefulness against the disease (reviewed in [3,8,10,13]). Only the agents that have shown considerable efficacy against PCa in nanotechnology based chemoprevention settings (see Table 1) are being discussed here.

Green tea, obtained from the plant *Camellia Sinensis* Theaceae, with its high polyphenolic content, is a well-known and effective chemopreventive agent against various cancers including PCa [14–17]. The polyphenols present in green tea, more commonly known as catechins, are epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate of which the epigallocatechin-3-gallate (EGCG, Fig. 1a) is the most well studied and understood. Our laboratory first initiated a program to assess whether tea consumption could afford chemopreventive effects against PCa development [18]. Since then, research has come a long way in demonstrating that green tea and its individual polyphenols modulate

variety of pathways which collectively afford chemoprevention of PCa [3–5,15,17]. Studies have also shown that EGCG results in an induction of apoptosis in PCa cells [19–22]. Our disease specific preclinical trials have also shown that green tea and its polyphenols are effective against PCa [23–25]. The readers are recommended to review articles [14–16,26–29] on the topic for more comprehensive details about the efficacy of green tea polyphenols against PCa.

Resveratrol (3,5,4'-trihydroxystilbene, Fig. 1b), a phytoalexin antioxidant found in grapes, red wines, berries, and peanuts, has been shown to afford protection against several diseases, including cancer [30–32]. This agent has been studied intensely for cancer prevention and treatment including PCa and the mechanistic and preclinical studies clearly indicate that resveratrol is capable of preventing and delaying malignant growth both *in vitro* and *in vivo* [13,33–39]. The pharmacokinetic, pharmacodynamic and safety properties of resveratrol are currently being investigated in early clinical phase I trials [40]. The efficacy of resveratrol are well understood and widely studied (reviewed in [8,40–43]).

Curcumin, or diferuloylmethane, is another major bioactive polyphenol extracted from the rhizome of turmeric (*Curcuma longa*) (Fig. 1c) [44]. A significant number of studies indicated that curcumin produces chemopreventive and chemotherapeutic effects against PCa [45,46]. Curcumin has been shown to induce antiproliferative, anti-invasive, antiangiogenic and apoptotic activities in PCa cell lines *in vitro* and *in vivo* [47–52]. So far only few clinical studies with curcumin have been performed despite the large amount of preclinical studies. The pharmacokinetics properties and efficacious doses of curcumin are not well established. In a pilot study of a standardized oral Curcuma, doses up to 180 mg per day for up to 4 months were without any toxicity or detectable systemic bioavailability [53]. A subsequent study suggested that doses even up to 8 gm could be administered daily for 3 months without overt toxicity [54].

2.1. Natural products for PCa therapy

Taxol or Paclitaxel (Fig. 1d) is a potent anti-cancer agent and has stimulated an intense research effort over recent years. Paclitaxel is among the first clinically and FDA approved chemotherapy drug that originated from natural sources, and demonstrated activity against leukaemias and a number of solid tumors including breast, lung, ovary, gastric, brain, and PCa [55–58]. Docetaxel, a taxol related derivative, is a semisynthetic compound produced from 10-deacetylbaccatin-III, which is found in the needles of the European yew tree (*Taxus baccata*) [59]. Docetaxel is approved to be used alone or with other drugs to treat advanced and recurrent hormone-refractory PCa (HRPCa) [60,61]. In particular, Docetaxel and Paclitaxel have been proposed in single or combination therapy in HRPCa, and weekly Paclitaxel/extramustine phosphate (EMP) and Docetaxel given every 3 weeks or by weekly infusion with EMP are useful treatment options for patients with progressive HRPCa [62,63].

Despite overcoming the initial difficulties surrounding a limited drug supply, both products (Paclitaxel and Docetaxel) also suffers from their water insolubility. For this purpose Cremophor EL (CrEL) and polysorbate 80 have long comprised the standard solvent system for the commercial formulation of Paclitaxel and Docetaxel, respectively [64]. Since the use

of these adjuvants is generally associated with their own side effects, several efforts have been made to explore novel formulation options [65]. Paclitaxel protein (albumin)-bound NPs (Abraxane) is a clinically validated formulation of Paclitaxel that does not employ the CrEL solvent system [65,66]. Therefore, since problems related to solubility of both agents remain challenging, pharmacokinetic and pharmacodynamic improvement may play a key role in the development of future taxane-based therapies for PCa.

3. Nanotechnology as a strategy for cancer control

3.1. General

Since cancers originate from alterations in biologic processes at the molecular or nanoscale level, nanotechnology based strategies are an emerging and promising approach with a great potential to facilitate the diagnosis and treatment of cancer [67–75]. Nanoparticle (NP) therapeutics can offer solutions to the current obstacles in cancer therapies, because of unique characteristics such as the high surface area to volume ratio, and controllable optical, electronic, magnetic, and biologic properties. By involving nanotechnology, we can achieve several objectives including, (a) aid the transport of therapeutic or diagnostic agents through biological barriers, (b) improve pharmacokinetic profile, (c) obtain targeted delivery of drugs, (d) develop innovative diagnostic tools, and (e) combine therapeutic agents and diagnostic probes into theranostic platforms. With the advances of nanomedicine and the understanding of properties and physical characteristics of materials in the nano-range, several distinct therapeutic systems have been approved or entered clinical development for medical applications [69,76]. Meanwhile, many nanomaterials are being studied in clinical trials or have been approved by the Food and Drug Administration (FDA) for use in humans, and several are in the proof-of-concept stage in research laboratories [77–79]. Depending upon the method of preparation, many types of nanoparticles (NPs) such as polymeric nanospheres and nanocapsules can be obtained. The micelles consist of an inner core of assembled hydrophobic segments capable of solubilizing lipophilic substances and an outer hydrophilic corona serving as a stabilizing interface between the hydrophobic core and the external aqueous environment, whereas dendrimeric nanosystems are branched nanostructures where each terminus contains reactive functional groups that allow the addition of more chemical units to increase the size of the scaffold. Furthermore, nanoliposomes contain amphiphilic molecules, which have hydrophobic and hydrophilic groups that self-assemble in water (Fig. 2). However, despite significant advances on nanoformulation over the last years, only few examples of nanosystems for the clinical management of PCa have been reported [79–81].

3.2. “Passive” and “active” targeting

Conventionally the drugs are distributed nonspecifically in the body where they affect both cancerous and normal cells resulting in suboptimal outcome. Molecularly targeted therapy has emerged as one approach to overcome the lack of specificity of chemotherapeutic agents and, in this context, nanotechnology represents a powerful strategy [67,82–85]. For example, the infusion of antineoplastic agents with nanomaterials as carriers results in an increased payload of drugs to the tumor. Moreover, NPs circulating into the bloodstream can accumulate in tumors owing to the enhanced permeability and retention effect (EPR), when

the vasculature of immature tumors has pores smaller than about 400 nm, thus permitting extravasation of nanodevices from blood and selective accumulation in the tumor interstitium by a mechanism known as ‘passive’ targeting (Fig. 3a) [86–88]. Another contributor to passive targeting is the unique microenvironment surrounding tumor cells, which is different from that of normal cells. In fact, hyperproliferative cancer cells show a high metabolic rate, which uses glycolysis to obtain extra energy, resulting in an acidic environment. Thus, pH-sensitive nanoprotoypes can be designed to be stable at a physiologic pH, but degraded to release active drug in target tissues in which the pH is less than physiologic values, such as in the acidic environment of tumor cells [69,89].

‘Active’ targeting *via* the modification of NP surface by inclusion of affinity ligands with specificity to disease tissues and cells is envisioned to provide an effective strategy (Fig. 3b) [71,75,82,84,90]. Taking advantage of this array of targeting moieties, many recently active targeting nanodevices have been developed to constitute a ternary structure composed of (a) a polymer or lipid as a carrier, (b) antibodies, aptamers, peptides, sugars, and small organic molecules as a targeting moiety, and (c) a chemopreventive/therapeutic bioactive molecule (Fig. 3c). When constructing ternary structure for NPs, some factors, such as antigen or receptor expression, internalization of targeted conjugates, and potential of nanomaterials to overcome drug resistance, must be considered to create more efficient delivery systems. Ideally, cell-surface antigens and receptors should have several properties that render them particularly suitable tumor-specific targets [91]. First, they should be expressed exclusively and homogeneously on tumor cells and not expressed on normal cells. Second, cell-surface antigens and receptors should not be shed into the blood circulation.

On this basis, nanodevices, by using both passive and active targeting strategies, can dramatically increase the intracellular concentration of drugs in cancer cells, thus enhancing their effectiveness while avoiding toxicity in normal cells.

3.3. PSMA as a valid target for PCa

To achieve selectivity for cancer cells, tumor-associated antigens appeared to be suitable biological targets for therapeutic intervention. To this end, functionalization of NPs with ligands that bind the extracellular domain of these receptors, to selectively target drugs to diseased cells, can represent a suitable therapeutic strategy. Prostate specific membrane antigen (PSMA), a 750-residue type II transmembrane glycoprotein that is highly expressed in PCa cells and nonprostatic solid tumor neovasculature has demonstrated a promising potential as target for anti-cancer imaging and therapeutic agents, in addition to its role as a tumor marker [92,93]. PSMA (also termed as *N*-acetyl- α -linked acidic dipeptidase I, NAALADase I), a member of a superfamily of zinc-dependent exopeptidases, acts as a glutamate carboxypeptidase II (GCPII) on small molecule substrates, such as folate and the neuropeptide *N*-acetyl-L-aspartyl-L-glutamate (NAAG) [94]. Recently, a solved crystal structure of the PSMA ectodomain, and several other high-resolution X-ray crystal structures of PSMA with glutamate-containing PSMA inhibitors, have been reported [95–98]. The active site structure of PSMA and its implications for substrate binding and catalysis provide insights into the architecture and function of this important enzyme. These structural and mechanistic features are useful on designing reagents for detection and

treatment of cancer and/or neurological disorders, particularly on the management of PCa therapy.

4. A new proof-of-concept: “Nanochemoprevention”

Our team recently introduced the concept of ‘*nanochemoprevention*’, which uses nanotechnology for enhancing the outcome of chemopreventive intervention [12,99,100]. This approach was first followed to assess the effectiveness of delivery of EGCG loaded in polylactic acid-polyethylene glycol (PLA-PEG) NPs against human PCa cells, under *in vitro* and *in vivo* conditions [12]. In this study, we observed that encapsulated EGCG retains its biological effectiveness with over 10-fold dose advantage over nonencapsulated EGCG for exerting its proapoptotic and angiogenesis inhibitory effects [12]. Such evidences constituted important determinants for the chemopreventive properties of EGCG in both *in vitro* and *in vivo* systems, thus establishing that the effective concentration of EGCG could be lowered by nanoformulation, thus being able to enhance the therapeutic effectiveness of EGCG. Moreover, nanoencapsulated EGCG was found to retain its mechanistic identity upon nanoformulation. Thus, results support the statement that nanoformulation can enhance the stability of EGCG as well as of those of other natural related chemopreventive agents *in vivo*, providing a target-specific enhanced bioavailability and limiting any unwanted toxicity, thus leading to a significantly better clinical outcome.

Since PSMA appeared to be suitable biological target for therapeutic intervention, in another study [101] we developed novel targeted EGCG encapsulated NPs, densely decorated by low molecular weight organic molecule as targeting ligands in their polymeric [poly(lactide-co-glycolide)-PEG, PLGA-PEG] shell surface, to selectively bind to PSMA. PLGA-PEG(-COOH) was used as a polymer system because of its well-established safety profile in clinical use and by considering its biocompatible properties. We selected the pseudomimetic dipeptide N-[N-[(S)-1,3-dicarboxypropyl] carbamoyl]-(S)-lysine (DCL) as PSMA targeting ligand, previously reported [102], capable of targeting PSMA with a similar high affinity and specificity to antibodies and aptamers. These PLGA-PEG-DCL NPs significantly enhanced the binding to PSMA, and exhibited an increased antiproliferative activity in *in vitro* assays against PSMA-expressing LNCaP cells with respect to the non-functionalized ones, without affecting normal cell viability.

Other nanosystems, such as polysaccharide NPs, have been studied in the nanotechnology setting as drug delivery vehicles for chemopreventive agents. EGCG was incorporated into a carbohydrate matrix of gum arabic and maltodextrin with improved biological profile in *in vitro* assays on reducing the cell viability and inducing apoptosis of PCa cells [103]. Another strategy in treatment of solid tumor, such as PCa, focuses on the therapeutic use of prostate tumor specific EGCG functionalized radioactive gold NPs (AuNPs) [104]. These prototypes demonstrated remarkable *in vitro* stability in various buffers as well as significant affinity and cellular internalization toward prostate and breast cancer cells. Therapeutic biocompatible AuNPs derived from the Au-198 isotope were developed to provide cross-fire effects of a radiation dose delivered intratumorally to cells within the prostate gland. Pharmacokinetic studies in PC-3 xenograft SCID mice showed significant retention of

¹⁹⁸AuNP-EGCG in tumors after intratumoral administration along with 80% reduction of tumor volumes [105].

Despite its immense potential in the PCa chemoprevention armamentarium, not much progress has been made yet on the nanoformulation of resveratrol ([43] and unpublished studies from our laboratory). Currently, most of nanotechnology-based approaches are pursued to improve the bioavailability of resveratrol. Narayanan et al. used liposome-encapsulated resveratrol and curcumin individually and in combination [106]. *In vitro* assays using PTEN-CaP8 cancer cells were done to investigate the combined effects of curcumin with resveratrol. Combination of liposomal forms containing co-encapsulated agents revealed that curcumin plus resveratrol effectively inhibited cell growth and induced apoptosis in *in vitro* studies and significantly decreased prostatic adenocarcinoma *in vivo* in PTEN mice.

As far as nanoformulation of other natural chemopreventive agents for PCa is concerned, curcumin has been extensively investigated. To overcome the low systemic bioavailability when administered orally, due to its poor aqueous solubility, a set of PLGA-curcumin nanospheres were prepared using solid/oil/water emulsion solvent evaporation method, and investigated for its efficiency [107]. Cell viability studies revealed that these curcumin-loaded nanoprototypes were able to exert a more pronounced effect on the cancer cells as compared to free curcumin, thus indicating that NP-based formulation of curcumin has high potential as an adjuvant therapy of PCa.

In another study by Thangapazham et al. [108], curcumin was incorporated into the liposomes coated with PSMA specific antibodies, to enhance targeted delivery of for PCa treatment. The conjugates resulted in a dramatic inhibition of cellular proliferation without affecting their viability. On the other hand, free curcumin exhibited similar inhibition only at 10-fold higher doses. Several other curcumin nanoformulations have recently been reported to investigate the comparative cellular uptake and cytotoxicity evaluation of materials such as β -cyclodextrin (CD), hydroxypropyl methylcellulose (cellulose), PLGA, magnetic NPs, and dendrimer-based nanoformulations in PCa cells [109]. Curcumin loaded cellulose NPs formulation exhibited the highest cellular uptake and caused maximum ultrastructural changes related to apoptosis in PCa cells. Secondly, the anti-cancer potential of the cellulose-based curcumin formulation was evaluated in cell culture models and this formulation showed improved anti-cancer efficacy compared to free curcumin.

Again, curcumin loaded fibrinogen NPs have been designed and prepared by a two-step coacervation method using calcium chloride as the cross-linker [110]. The cytotoxicity studies suggested that curcumin-fibrinogen NPs were comparatively toxic to PCa cells with significant internalization and retention of NPs inside the cells. Besides EGCG, resveratrol and curcumin, other natural products are being actively considered to have a role in nanochemoprevention. For example, in a recent study silibinin has been incorporated in nanosuspension and tested for its *in vitro* antiproliferative activity in PC-3 cells [111]. MTT assay, observation of morphological changes and apoptotic body showed that silibinin nanosuspension could significantly enhance the cytotoxicity against PC-3 cells compared to the silibinin solution. Overall these studies aggressively suggest that nanotechnology based

delivery of natural products can enhance the effectiveness of currently employed chemopreventive and chemotherapeutics for PCa [12,100,109,112,113].

5. Nanoproductions for PCa therapy

5.1. Nanotherapeutics

The development of “nanochemotherapeutics” that include bioactive molecules, a major effort has been done by Langer and Farokhzad, who designed customized controlled-release NPs, loaded with Docetaxel [114–117]. These nanoprototypes are constituted by several safe or FDA approved materials as biocompatible polymers, each one performing a specific function in the final NP prototype [118]. Docetaxel chemotherapy is the optional treatment in patients with HRPc, and Docetaxel-loaded polymeric NPs have the potential to improve clinical responses. Concerning the structural features of the nanoprototypes, hydrophilic spacers (i.e., PEG) were incorporated into the NP’s outer shell, in order to (a) have a minimal self-self and self–nonself interaction, (b) prevent non-specific binding of NP’ surface, and (c) escape mononuclear phagocytic system capture, enabling “stealth” properties for immune evasion. This team obtained NPs tumor-targeting capabilities by “decorating” the shell surface with RNA A10-Aptamer (Apt), to bind PSMA [114,118]. The results indicated that the encapsulated drug efficiently interacted to the tumor and selectively delivered Docetaxel to PCa cells and tissues, both in *in vitro* and *in vivo* experimental models [67,82,116,119]

As modification of this strategy, Chandran et al. used a combined approach to obtain novel polymeric targeted Docetaxel-loaded NPs [120]. The targeted nanosystems were formulated using a mixture of PEG–poly-caprolactone (PEG–PCL) conjugated with the urea-based PSMA substrate analog (namely DCL, see on Section 4) as a targeting moiety. These NPs displayed selective *in vitro* cytotoxicity against PSMA-expressing LNCaP cells along with an enhanced affinity for the targeted NPs to PSMA-overexpressed cells.

More recently, we also investigated two novel biodegradable block-copolymers, PLA–PCL and PLGA–PCL for the formulation of Docetaxel-loaded NPs [121]. *In vitro* cytotoxicity on human PCa cell line demonstrated advantages of the Docetaxel-loaded PLGA–PCL NPs over pure Docetaxel in both time- and concentration-dependent manner.

In another study [122], nanoparticulate Paclitaxel was evaluated for antiproliferative activity in a human PCa cell line PC-3, and for its effect on tumor inhibition in a murine model of PCa. It was observed that, under *in vitro* conditions, the NPs exhibited sustained release of the encapsulated Paclitaxel. Cytotoxicity of the drug with transferrin (Tf)-conjugated NPs (Ptx-Tf-NPs) was about 5-fold lower than with unconjugated NPs or drug in solution. Animals studies, in which mice received a single-dose intratumoral injection of Ptx-Tf-NPs demonstrated complete tumor regression and greater survival rate than those mice that received either Ptx-NPs or Ptx-CrEL formulation.

A novel method for combining Paclitaxel chemotherapy and Herceptin (a monoclonal antibody) to generate a treatment for advanced PCa has been developed by the Goldstein’s group [123]. This research team showed that oil droplets in nanoemulsion, with Herceptin

molecules attached to their surface, are able to target to HER2-overexpressing cells. Formulation of Trastuzumab-coupled emulsions containing the drug Paclitaxel-palmitate was tested on cancerous PCa cells and on mice with induced PCa. These studies showed that the preparation did not cause any hypersensitive reaction with respect to the additive CrEL, and even yielded better results than known drug treatments in inhibiting tumor growth.

Currently, several other studies on the nanoparticulate delivery of taxanes toward PCa models have also been reported [124–129].

5.2. The development of BIND-014

The use of targeted NPs for PCa therapy has been recently validated by the Phase-I clinical development of the prototype BIND-014, a PSMA-targeted polymeric NPs containing the antitumor agent Docetaxel, as a successful programmable nanomedicine that combines a targeting ligand and therapeutic NPs [130,131]. This is the result of research work coordinated by Langer and Farokhzad, and developed by BIND Biosciences, where library of targeted self-assembled polymeric NPs were screened resulting in the first targeted and controlled release polymeric nanoprototype for cancer chemotherapy to reach clinical development [82]. They followed a modular self-assembly approach, using pre-functionalized polymeric materials to generate a combinatorial library of targeted NPs (containing more than 100 distinct NP compositions) by introducing physicochemical diversity in the NP design [130]. More specifically, NPs were obtained by systematically varying critical parameters such as size, surface hydrophilicity, polymer composition and concentration, targeting ligand density, drug loading, and drug release, and processing method, in order to optimize the bio-physicochemical properties of the encapsulated bioactive molecule [130]. These targeted Docetaxel-loaded NPs were constituted by the above mentioned ternary structure composed of (a) a hydrophobic biodegradable block copolymeric core (PLA or PLGA and PEG), (a) a small molecules as PSMA-targeting ligand, and (c) Docetaxel as drug. As far as the targeting ligand is concerned, the pseudomimetic dipeptide DCL (namely ACUPA), already used by us on EGCG-loaded PSMA-targeted NPs [101], and by the Denmeade's group, for Docetaxel-PLA/PCL-based targeted NPs [120], has been incorporated on the NP' surface.

In preclinical cancer animal models and mouse xenograft studies, BIND-014 was shown to deliver up to 10 times more Docetaxel to tumors than an equivalent dose of Docetaxel. The increased accumulation of Docetaxel at the site of disease translated to marked improvements in antitumor activity and tolerability. First clinical data in patients with advanced solid tumors, conducted to evaluate the tolerability and pharmacokinetic properties of BIND-014, and to obtain a preliminary assessment of its efficacy in patients with advanced and metastatic solid tumors, indicated different pharmacological profile for such nanoprototype. The data suggested more favorable pharmacokinetic properties of BIND-014 with prolonged circulation half-life and retention of Docetaxel in the vascular compartments, as well as multiple cases of tumor shrinking at doses up to about 5-fold lower than the Docetaxel dose clinically administered.

6. Conclusions and future perspectives

As the adult male population ages, PCa is increasingly becoming a considerable health and social problem. This has raised particular attention in potential chemoprevention of this disease, and a major interest is addressed in developing a localized therapeutic option for treatment of early stage PCa that is associated with reduced toxicity. Chemoprevention, possibly through the use of naturally occurring products, such as dietary nontoxic phytochemicals, has emerged as one important approach to fight this disease, which may be appropriated for high-risk patients, especially those with isolated HG-PIN, elevated PSA, and negative biopsy.

Since its conceptualization in 1976 by Dr. Michael Sporn, chemoprevention has been extensively used as a successful strategy in a variety of preclinical studies however its applicability to humans has met with only 'limited success' [7]. There are several clinical trials performed with EGCG alone [132–137] and some positive outcome has been noted in these studies. However, the outcome of these studies is not strong enough to recommend the use of green tea for general population to replace PCa risk. One reasoning for this is that all these intervention studies, understandably so, were conducted in patients with established disease and none were performed in a typical chemopreventive setting. One general belief for the lack of success of chemoprevention in humans relates to inefficient systemic delivery and bioavailability of promising chemopreventive agents. A recent editorial summarized whether efficient delivery system have any leverage leading to potential benefits [138]. The author suggested that “an appropriate dose aided by suitable delivery system can make better medicines by mitigating the risks”.

Other major factor responsible for any natural agent development for cancer chemoprevention is the lack of interest shown by the pharmaceutical industry who are in the business of making money. Despite positive indications from several clinical trials [134,136,139–141] neither of these agents were followed up by the pharmaceutical industry. Such agent development at the moment is at the mercy of only small scale industries that formulate and markets the agents as food supplements. For the naturally occurring botanicals as chemopreventive agents to be able to receive a first line status, a rigorous promotional campaign has to be undertaken and both care givers and public has to be educated regarding the benefits associated with cancer chemoprevention. That means that the scientific community must make efforts to fully consider limitations and avoid unnecessary claims for cancer prevention associated with the use of natural agents [7].

Also, increased efforts should be devoted on enhancing the bioavailability of natural products, by improving their pharmacokinetic and biodistribution features as well as the ability to selectively kill tumor cells without affecting normal cells by a controlled release mechanism. These basic strategies (i.e., to simultaneously increase the intracellular concentration of agents and to reduce dose-limiting toxicities) are directly related with improvement in patient survival and quality of life. In this scenario, nanotechnology based approaches are demonstrating significant impact on the delivery of natural products, and engineered NPs have shown great potential as suitable candidates to be used in the anti-cancer armamentarium. We recently introduced a novel idea in which nanotechnology was

used for enhancing the outcome of chemoprevention, by demonstrating the usefulness of nanoprotypes on improving the therapeutic effectiveness of natural agents, such as EGCG. This concept, termed by us “nanochemoprevention”, was subsequently exploited by several other laboratories and is becoming an advancing and convincing field in chemoprevention research. However, further prospective animal and clinical studies are required to definitely validate the benefits of chemopreventive agents for PCa, and combinations of chemopreventive agents for PCa treatment should be carefully investigated because of potential additive or synergistic mechanisms of action. Also, the lack of a careful understanding of the interaction that occurs between NPs and many cellular processes constitutes one of the major problems, which should be kept into account during the clinical development process.

As far as the combination of nanoparticulate advanced technology with natural bioactive compounds is concerned, a pioneering work led to proof-of-concept and the development of the first targeted nanoprotypotype (BIND-014, Accurin™) for PCa chemotherapy (as well as for an array of other solid tumors) to be translated from the lab to clinical practice [80,82,131]. In summary, Paul Erlich’s “magic bullet” vision [81,142], which also inspired the nanoparticle based targeting approach paradigm, could be validated for PCa chemoprevention/chemotherapy.

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Fig. 1. Structures of the effective natural products for prevention and treatment of prostate cancer (PCa) currently studied for nanochemoprevention: (a) EGCG; (b) resveratrol; (c) curcumin; (d) taxanes discussed in this mini-review.

Fig. 2. Schematic representation of the structure of various types of nanoparticles used for drug delivery, which are being studied in clinical trials or have been approved by the Food and Drug Administration for use in humans.

Fig. 3.

(a) Passive tissue targeting in blood vessels by nanoparticles that selectively extravasate through the leaky vasculature surrounding the solid tumor, and preferentially accumulate reaching the tumor cells (EPR effect). (b) Active drug delivery by targeted NPs, where the presence of ligands grafted at their surface allow active targeting by binding to receptors overexpressed on tumor cells, thus resulting in enhanced accumulation and cell uptake through receptor-mediated endocytosis. (c) Schematic features of a targeted nanoparticle prototype.

Table 1

Natural agents encapsulated in nanoparticles that have demonstrated significant efficacy against prostate cancer.

Agent	Type of nanoformulation	Efficacy shown in prostate cancer	Reference
EGCG	Encapsulation in polylactic acid-poly ethylene glycol NPs	Ten-fold dose advantage over native EGCG for exerting proapoptotic effects and angiogenesis inhibition	[12]
	EGCG:bovine serum albumin NPs	Better uptake by the cells and enhanced cytotoxicity	[143]
	Incorporation in carbohydrate matrix of gum arabic and maltodextrin	Retention of biological activity, reduction in cell viability and induction of apoptosis	[103]
	EGCG functionalized radioactive gold NPs	<i>In vitro</i> stability, significant affinity and cellular internalization	[104]
	Encapsulation in di-block-copolymer PLGA-PEG-COOH functionalized with PSMA inhibitor	Controlled release of EGCG, targeted delivery to PSMA positive cells, PSMA specific cell growth inhibition	[101]
	Biocompatible gold NPs derived from AU-198 isotope	Significant tumor retention and tumor growth inhibition	[105]
Curcumin	Curcumin loaded PLGA nanospheres	Robust intracellular uptake, more pronounced effects on cell viability	[107]
	Incorporation in liposomes coated with PSMA antibody	Enhanced targeted delivery, robust inhibition of cell proliferation, 10-fold dose advantage	[108]
	Hydroxypropyl methylcellulose NPs	Higher cellular uptake, ultrastructural changes related to apoptosis, improved anticancer efficacy	[109]
	Fibrinogen NPs with CaCl ₂ as linker	Toxicity to cancer cells with internalization and retention	[110]
Resveratrol Taxanes	Liposome encapsulated; co-encapsulation with Curcumin	Significant increased levels in serum and prostate, cell growth inhibition and induction of apoptosis, decreased prostatic adenocarcinoma	[106]
	Transferrin conjugated	Sustained drug release and greater antitumor activity	[122]
	Polymeric NPs with RNA A10-aptamer	Targeted delivery to prostate <i>in vitro</i> and <i>in vivo</i>	[114,118]
	Polymeric targeted NPs	Selective <i>in vitro</i> toxicity	[120]
	Poly(lactide-co-caprolactone) and Poly(lactide-coglycolide-co-caprolactone) NPs	<i>in vitro</i> cytotoxicity in time and dose dependent manner	[121]
	Transferrin conjugated NPs	Enhanced cytotoxicity, complete tumor regression, greater mice survival	[122]