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PERSPECTIVE

# Nanochemoprevention by Bioactive Food Components: A Perspective

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**ABSTRACT** Chemoprevention through the use of bioactive food components is a practical approach for cancer control. Despite abundant efficacy data under preclinical settings, this strategy has resulted in limited success for human cancer control. Amongst many reasons, inefficient systemic delivery and bioavailability of promising chemopreventive agents are considered to significantly contribute to such a disconnect. We recently introduced a novel concept in which we utilized *nanotechnology* for enhancing the outcome of *chemoprevention* (*Cancer Res. 2009; 69:1712–6*) and termed it *nanochemoprevention*. To establish the proof-of-principle of nanotechnology for cancer management, we determined the efficacy of a well-known chemopreventive agent epigallocatechin-3-gallate (EGCG) encapsulated in polylactic acid (PLA)-polyethylene glycol (PEG) nanoparticles in preclinical settings and observed that, compared to non-encapsulated EGCG, nano-EGCG retained its biological efficacy with over 10-fold dose advantage both in cell culture system and *in vivo* settings in athymic nude mice implanted with human prostate cancer cells. This study laid the foundation of nanochemoprevention by bioactive food components. Since oral consumption is the most desirable and acceptable form of delivery of bioactive food components, it will be important to develop nanoparticles containing bioactive food components that are suitable for oral consumption for which experiments are underway in this laboratory.

**KEY WORDS** bioactive food components · chemoprevention · nanochemoprevention · nanotechnology

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## INTRODUCTION

Chemoprevention is the use of one or more synthetic or dietary chemopreventive entities to prevent the initiation of premalignant lesions or their progression to cancer or recurrence of cancer. From a practical point of view, dietary substances are more likely to be useful for chemopreventive agents for human consumption. Consistent with this notion, the search for potentially useful cancer chemopreventive agents in the diet and beverages human consume has become an intense area of investigation. Studies from this and other laboratories worldwide have established the efficacy of many such agents for cancer prevention in many sites in a wide variety of preclinical animal tumor model systems (1–4). This preclinical data in some cases is also supported by the epidemiological and geographical studies of cancer occurrence (5–7). Since most bioactive food components are multi-targeted agents, they hold great promise for cancer risk reduction in human population (3,6,8,9). Some of the well-studied bioactive food agents, in addition to possessing preventive effects, also demonstrate therapeutic potential or enhance the therapeutic efficacy of agents when given in combination with established chemotherapeutic regimen. One such class of bioactive food components that has received considerable importance in experimental models of cancer is the polyphenolic group of compounds. Amongst all the polyphenolic compounds, green tea and its individual polyphenols have received considerable attention because of their demonstrated efficacy against variety of cancers in preclinical settings and in human intervention trials (2,3,8,10).

Chemoprevention is often considered as a tool to attain complete prevention of cancer, which, unfortunately, is an unachievable goal. Since the process of cancer development is *carcinogenesis*, we believe that our aim should be to prevent

or slow down the process of carcinogenesis, which in turn will lead to lower cancer burden. We, therefore, define chemoprevention as "slowing the process of carcinogenesis by chemopreventive agents," a goal that is likely attainable.

Despite remarkable success of chemoprevention by bioactive food components in preclinical settings in a wide variety of animal models, the applicability of chemoprevention from "bench to bedside" for human use has met with very limited success. Some of the reasons that may be responsible for the disappointment of chemoprevention in clinical trials are i) diverse genetic background of individuals at risk, ii) varied food habits amongst the people, and, more importantly, iii) inefficient systemic delivery and poor bioavailability of active agents. Thus, in order to achieve maximum response of bioactive food components as chemopreventive agents for human use, strategies that can bypass these limitations are required. Strategies that could lead to sustained release of the active agents could critically improve their bioavailability and in turn reduce the perceived toxicity associated with high doses that are typically required for optimum response with an agent.

## NANOCHEMOPREVENTION

Nanotechnology is an emerging multidisciplinary field that frequently includes biology, engineering, chemistry and medicine. Nanotechnology (nanotech), is the study of the control of matter on an atomic and molecular scale. Generally, nanotechnology deals with structures of the size 100 nanometers or smaller in at least one dimension and involves developing materials or devices within that size. It is noteworthy that, in recent years, nanotechnology has been assessed and implemented in different areas of cancer therapeutics and cancer management and is expected to lead to major advances in cancer diagnosis, detection and treatment (11–15). *Cancer Nanotechnology* as it is being referred to, is already being applied to cancer in two broad areas: the development of nanovectors, such as nanoparticles, which can be loaded with drugs or imaging agents and then targeted to tumors, and high throughput nanosensor devices for detecting the biological signatures of cancer. Cancer nanotechnology seeks to describe the relations of devices that are in nanoscale with cellular and molecular components specifically related to cancer diagnosis and therapy. Cancer nanotechnology has great potential because of the ability to engineer devices with unique therapeutic properties that, because of their small size, can infiltrate tumors deeply with a high level of specificity. The National Cancer Institute has also recognized that nanotechnology offers an extraordinary, paradigm-shifting opportunity to make significant advances in cancer diagnosis and treatment. (14). The basic rationale is that metals, semiconductors, and polymeric particles have novel optical, electronic, magnetic and structural properties that are often not available from individual molecules and bulk solids (13,16). Because most biological processes, including those that are cancer-related, occur at nanoscale, nanoparticulate technology has been appreciated as a potential tool to diagnose and treat cancer. We recently employed the use of nanotechnology to improve the outcome of chemopreventive intervention and coined the term *nanochemoprevention* (17).

Currently, nanotechnology is being utilized in regards to cancer in various ways, including molecular imaging, early detection, targeted therapy, and cancer bioinformatics. Cancer-related nano-devices include, but are not limited to, injectable nanovectors, such as liposomes; biologically targeted, nanosized magnetic resonance imaging contrast agents; and novel, nanoparticles-based methods (12). Over the last two decades, a variety of nanoscale vehicles, including gelatin (18,19), ceramic (20), liposomes (21), and micelles (22), have been under development for therapeutic use. For delivery of a drug, the core of the nanoparticles can contain one or several payload drugs, as well as permeation and visibility enhancers based on intended applications. The surface could also be bare or conjugated to targeting ligands like polyethylene glycol, aptamer or antibody to prevent macrophage uptake of the nanoparticles (Fig. 1) (23). Utilization of nanotechnology for the development of efficient drug delivery system is one of the most recent developments of medical science. The structure and tunable surface functionality of nanoparticulate system allows it to encapsulate/conjugate single or multiple entities either in the core or on the surface, rendering them ideal carriers for various anticancer drugs (Fig. 1). Further, most drugs have poor solubility and low bioavailability and are formulated with undesirable solvents. With the employment of nanotechnology and use of nanocarriers, preparation of low water-soluble cancer medications as solid or liquid formulations could be easily achieved. Nanoparticles made up of the biodegradable and biocompatible polymers, like polylactic acid (PLA), poly (DL-lactide-co-glycolide acid) (PLGA), starch and chitosan, etc., have been extensively employed for the delivery of various drugs (23,24). In particular, homo- and copolymers of lactic acid and polylactic glycolic acid have been extensively used for numerous drug deliveries (13,23,25). Significant advantages of these biodegradable polymers include their history of safe use, proven biocompatibility, and ability to control the time and rate of polymer degradation and the release of the incorporated entity. However, there are a few limitations with the use of nanoparticles, such as when PLA/PLGA nanoparticles are injected systemically for drug delivery, they are rapidly

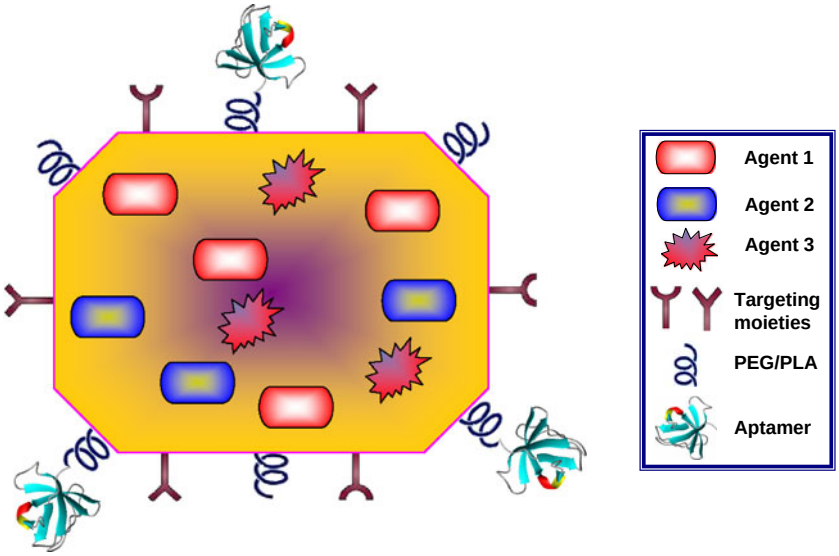


**Fig. 1** Multifunctional nanoparticles. The nanoparticles could be developed with an ability to carry one or more therapeutic agents. The surface of the nanoparticles could be conjugated to one or more targeting moieties, like antibodies or other recognition agents, and polyethylene glycol (PEG)/ polylactic acid (PLA) could be added for the avoidance of uptake by macrophages. The surface of the nanoparticles could also contain aptamers conjugated for target organ delivery. The nanoparticles could be developed fwith one or more of these characteristic based on the requirements. Any such developed nanoparticles could be employed for nanochemoprevention.

cleared from the blood-stream by the mononuclear phagocyte system, thus restraining their potential as controlled drug delivery vehicles (13). This shortcoming could be easily overcome with the presence of a hydrophilic polymer, like polyethylene glycol (PEG), which increases the circulation time of the PLA/PLGA nanoparticles by sterically stabilizing them against opsonization (23). This property of PEG thus improves pharmacokinetic and pharmacodynamic properties of the drugs that have been encapsulated in PLA/PLGA nanoparticles. PEGylation (i.e., the attachment of PEG to proteins and drugs) is an upcoming methodology for drug development, and it has potential to revolutionize medicine by drastically improving the pharmacokinetic and pharmacodynamic properties of administered drugs (26). Several investigators have demonstrated that PEGylated PLA/PLGA nanoparticles exhibit significantly increased blood circulation time and relatively lowered accumulation in different organs compared to non-PEGylated counterparts (27–29). Also, *in vivo* experiments show a significantly high accumulation of the PEGylated nanoformulation in the tumor tissues due to the enhanced permeation and retention effect (EPR). This EPR is mainly due to the difference in the vasculature between tumor tissue and normal tissue. Normal tissue vasculatures are lined by tight endothelial cells which prevent the nanoparticles from escaping into the tissue, whereas tumor tissue vasculatures are porous with leaky endothelium, which easily allows the nanoparticles to permeate in the tissue. Once the nanoparticles carrying the drugs enter the blood-flow, they move freely until they reach the tumor tissue, where, due to the leaky environment, they allow the encapsulated drugs to be released and get accumulated in the tissue. The nanoparticles could also be conjugated with targeting moieties which facilitate the nanoparticles to be delivered only to the tumor cells.

We recently employed nanoparticle-mediated delivery for sustained release of potentially useful chemopreventive agent. This sustained release and lower dose requirement of the agent could be a valuable tool to limit the perceived toxicity associated with their repeated use and at the same time is likely to enhance its bioavailability, a must for human use. To establish the proof-of-the principle, we assessed the effectiveness of delivery of a well-known chemopreventive agent, epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, encapsulated in PLA-PEG nanoparticles against human prostate cancer (PCa) under *in-vitro* and *in-vivo* situations (17). Our choice for the selection of EGCG and PCa in this study was based on several facts: including i) PCa is the most prevalent cancer among men, accounting for an estimated 192,280 new cases and 27,360 deaths in the year 2009 in USA alone (30), ii) because of its long latency period, PCa is an ideal candidate disease for chemoprevention, iii) EGCG demonstrates remarkable chemopreventive potential in a wide range of cell culture studies (31,32), iv) we have extensive experience with EGCG use in PCa, and v) there are remarkable preclinical and clinical efficacy data of green tea and PCa (32–34). Although several nanoparticles made up of biodegradable and biocompatible polymers have been studied for the delivery of various drugs, we used PLA nanoparticles because when these are injected systemically for drug delivery, they are rapidly cleared by endocytosis, thereby minimizing carrier-induced undesirable cytotoxicity.

## ROLE OF NANO-EGCG ON CELL PROLIFERATION AND APOPTOSIS

We and others have demonstrated that EGCG has apoptotic potential in a variety of cancer cells both *in vitro*

and *in vivo*, including cancer of the prostate gland (31,35–38). Though encouraging data has emerged from a recent clinical trial (34), overall effectiveness is not as encouraging as expected. This may be related to inefficient systemic delivery and bioavailability. Limited available data on the bioavailability of flavanols from green tea or green tea extract suggested poor bioavalibility of EGCG and other flavanoids (39,40). Lambert *et al.* in two different studies suggested that EGCG is absorbed but extensively glucuronidated following oral administration (41,42). We thus recently employed nanotechnology as a basis for the use of nanoparticle-mediated delivery to enhance bioavailability and limit any unwanted toxicity of EGCG (17). To establish the proof-of-the-principle of nanochemoprevention, we tested the efficacy of EGCG encapsulated in PLA-PEG nanoparticles, hereafter referred to as nano-EGCG, *versus* non-encapsulated EGCG on proliferative ability of PCa cells. Our data demonstrated that treatment of the cells with nano-EGCG produced remarkably superior effects at 24 h post-treatment with over 10-fold dose advantage compared to non-encapsulated EGCG. This effect was seen with an $IC_{50}$ value of 3.74 μM compared to 43.6 μM of non-encapsulated EGCG. Similar effects of nano-EGCG were observed in 22Rν1 prostate carcinoma cell line (Siddiqui *et al.*, unpublished data), indicating that the effects we observed are general and not cell-type specific. We also observed that nano-encapsulation removes the penetration barriers at the cell surface, and nano-EGCG was readily available to the cells, along with a lowering of the effective concentration of EGCG with 2.74 μM nano-EGCG resulting in similar extent of apoptosis as 40 μM of non-encapsulated EGCG, thereby providing a remarkable dose advantage (17). Similar effects were also observed in 22Rν1 cells treated with nano-EGCG, where we observed that 5.48 μM nano-EGCG induced 81% apoptosis in 22Rν1 cells, whereas to achieve a similar extent of apoptosis, over 40 μM of non-encapsulated EGCG was required; thereby providing a remarkable dose advantage (Siddiqui *et al.* unpublished data). Additionally, nano-EGCG provided similar extent of dose advantage for inhibiting colony formation of human prostate carcinoma cells.

In our published study (17), we also determined that EGCG retained its mechanistic identity even when encapsulated in nanoparticles. The efficacies of nano-EGCG on several molecules which have been shown to be modulated by EGCG were determined, and we observed that the key regulators of apoptosis, Bcl-2 family of proteins, were significantly modulated by nano-EGCG. Like non-encapsulated EGCG, nano-EGCG treatment resulted in a significant increase in pro-apoptotic Bax levels with a concomitant decrease in the levels of anti-apoptotic Bcl-2, thereby shifting the Bax/Bcl-2 ratio in favor of apoptosis. Further, an appreciable increase in PARP cleavage was also observed. Importantly, these responses were observed at a very low concentration of nano-EGCG, further confirming a remarkable dose advantage when EGCG was delivered encapsulated in nanoparticles. This study also evaluated the effects of nano-EGCG on induction of the cyclin-dependent kinase inhibitors WAF1/p21 and CIP1/p27 and observed a marked induction of p21 and p27 in dose-dependent manner with significant dose advantage over EGCG. Collectively, these data confirm that nano-EGCG retained its mechanistic signature and behaved exactly as EGCG, albeit with over 10-fold lower dose.

## ROLE OF NANO-EGCG ON ANGIOGENESIS

Earlier studies have demonstrated that EGCG is an efficient inhibitor of angiogenesis (43–47). We thus tested the efficacy of nano-EGCG on inhibition of angiogenesis. We employed *ex ovo* chick chorioallantoic membrane (CAM) assay for evaluating the effects of nano-EGCG on angiogenesis and observed a 57% inhibition of FGF-induced angiogenesis with nano-EGCG containing only 3 μg EGCG compared to only 35% inhibition with 30 μg non-encapsulated EGCG. Further, this study also observed a significant inhibition of mean branch formation as well as tumor weight of neuroblastoma-induced angiogenesis in CAM by nano-EGCG, with a clear dose advantage over non-encapsulated EGCG (17). These data clearly indicate that while EGCG suppressed angiogenesis, the concentration required to achieve this inhibition was significantly reduced by nanoformulation.

## *IN-VIVO* EFFICACY OF NANO-EGCG

To establish the *in vivo* relevance of our *in vitro* findings, we employed a tumor xenograft mouse model. We compared the effect of nano-EGCG (100 μg/mouse; intraperitoneal administration) and non-encapsulated EGCG (1 mg/mouse, intraperitoneal administration) on the growth of human prostate tumors implanted in athymic nude mice. We observed a significant decrease in the tumor volume at 45 days post-inoculation in both treatment groups as compared to control mice, with nano-EGCG demonstrating better efficacy than non-encapsulated EGCG (17). This data demonstrated that to achieve a similar extent of tumor growth inhibition, 10-fold lower dose of nano-EGCG was required compared to non-encapsulated EGCG. Further, these data also demonstrated a significant inhibition in the serum PSA of treated mice, with nano-EGCG showing better efficacy even at 10-fold lower doses (17). The observed decrease of serum PSA by nano-EGCG at such a low concentration, is an important observation because serum PSA is arguably regarded as the best marker in the diagnosis and prognosis of PCa in human (48).

We further evaluated the rate of degradation of the EGCG formulations and observed that non-encapsulated EGCG was rapidly degraded, with a complete degradation within four hours, whereas nano-EGCG had a significantly longer half-life. These data suggest nanoformulated EGCG given to mice is released slowly, which in turn may be responsible for its superior efficacy. Since we have earlier demonstrated that EGCG acts in synergy with other therapeutic approaches (47,49), we believe that nano-EGCG may offer a much better response than nonencapsulated EGCG when used in combination with other therapeutic approaches.

The only inadequacy, we think, with our approach is that with the use of PLA-PEG nanoparticles, oral delivery is not possible, as these nanoparticles are very unstable in acidic environment and thus are not suitable for oral delivery. Despite extraordinary advancement in other routes of drug administration, oral drug delivery remains the preferred delivery route because of several factors, including the ease of administration in non-hospital settings. To overcome this obstacle, it will be important to formulate bioactive food components employing nanomaterials suitable for oral delivery.

## CONCLUSIONS

Our proof-of-principle study (17) demonstrated the usefulness of nanoparticulate technology to enhance the therapeutic effectiveness of EGCG *in vitro* as well as *in vivo*, though in an athymic nude mouse model not truly reflective of chemoprevention protocol. Using PCa cells, our data clearly demonstrated that nano-EGCG exhibits over 10-fold dose advantage over non-encapsulated EGCG and that EGCG delivered in nanoparticles retains its mechanistic identity. We utilized PLA-PEG nanoparticles to provide EGCG to cancer cells, and the therapeutic/clinical significance of PLA-PEG nanoparticles remains in the fact that they rarely pose any toxicity against cells (50). Moreover, being biodegradable, these nanoparticles are considered to be safe (23). It remains to be seen if such a protocol could be translated to other tumor types. Such a protocol could be very effective in treating visible tumors like melanoma and non-melanoma skin cancers.

Since oral consumption is the most desired and acceptable form of delivery of chemopreventive agents, it will be important to resolve the problem of oral consumption of nano-encapsulated EGCG and other bioactive food components by incorporating biodegradable polymers, such as chitosan, as the starting material, which will be more stable in the acidic environment of the gut to slowly release the agent for absorption by the body.

In addition to our study, a limited number of studies from other laboratories have also evaluated the usefulness of nanotechnology for delivery of natural products mostly in culture system. In a recent study, poly(lactide-co-epsilon-caprolactone) (PLCL), was successfully developed as EGCG-eluting polymeric stent, which could be utilized for preventing thrombosis, inflammation and in-stent restenosis (51). In another study, Italia *et al.* also suggested the potential of biodegradable nanoparticles in improving the therapeutic efficacy of EGCG (52). Other studies have also demonstrated the efficacy of delivery of other natural products through incorporation of nanotechnology. Sahu *et al.* and Thangapazham *et al.*, in two separate studies (53,54), have demonstrated that curcumin could also be delivered via nanotechnology-based carriers for prevention and therapy of cancer. In a recent study, curcumin was nanoformulated with three biocompatible polymers—alginate (ALG), chitosan (CS), and pluronic—by ionotropic pre-gelation followed by polycationic cross-linking. Pluronic F127 was used to enhance the solubility of curcumin in the ALG-CS NPs. This study demonstrated cellular internalization of curcumin-loaded composite NPs (55). A study also demonstrated that curcumin-loaded poly (caprolactone) nanofiber matrix is bioactive and has potential as a wound dressing with reduced inflammatory induction and increased rate of wound closure (56). In a different study, PEGylated curcumin conjugate was demonstrated to have much more potent effects on pancreatic cancer cell growth inhibition than free curcumin (57).

Resveratrol-loaded nanoparticles at lower concentration were recently observed to lead to significantly higher cell death compared to equivalent dose of free Resveratrol, and this difference of cytotoxicity was not found to be abrogated by the antioxidant Vitamin E (58). In a separate study (59), 12-h pre-incubation of resveratrol-loaded nanoparticles was found to protect cells from beta-amyloid peptide (Abeta)-induced damage in a dose-dependent manner by attenuating intracellular oxidative stress and caspase-3 activity. Narayanan *et al.* (60), in a recent study, used the liposome-encapsulated curcumin and resveratrol individually and in combination in male B6C3F1/J and prostate-specific PTEN knockout mice. *In vitro* assays using PTEN-CaP8 cancer cells were also performed to investigate the combined effects of curcumin with resveratrol. In this study, HPLC analysis of serum and prostate tissues showed a significant increase in curcumin level when liposome-encapsulated curcumin was co-administered with liposomal resveratrol. Combination of liposomal forms of curcumin and resveratrol significantly decreased prostatic adenocarcinoma *in vivo* in PTEN mice, and the *in vitro* studies revealed that curcumin plus resveratrol effectively inhibited cell growth and induced apoptosis. Findings from this study for the first time provide evidence on phytochemicals in combination to enhance chemopreventive efficacy in prostate cancer.

These and other studies on the subject recommend that nanochemoprevention (i.e. delivery of bioactive food components via nanotechnology-based carriers) may be utilized

with considerable advantage over currently employed chemopreventive approaches for cancer. We suggest that the concept of *nanochemoprevention* for cancer prevention should be explored further for its potential use in preclinical phytochemical-based chemopreventive studies. This could also be developed as an inexpensive, tolerable and readily applicable approach for cancer control and management. Supportive data from these studies could pave the way for effective chemoprevention of cancer in human population. This advancement might also help us achieve the higher concentrations of the phytochemicals which are unattainable when the agents are provided as part of diet. With the current studies in mind, our ultimate goal could be a once-a-day or once-a-week capsule containing one or more nanoencapsulated bioactive food component(s) for effective chemoprevention and chemotherapy of cancers.

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