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Perspectives on the recent developments with green tea polyphenols in drug discovery

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Abstract

Introduction: Increasing evidence has expanded the role of green tea from a traditional beverage to a source of pharmacologically active molecules with diverse health benefits. However, conclusive clinical results are needed to better elucidate the cancer-preventive and therapeutic effects of green tea polyphenols (GTPs).

Areas covered: The authors describe GTPs' chemical compositions and metabolic biotransformations, and their recent developments in drug discovery, focusing on their cancer chemopreventive and therapeutic effects. They then review the recent development of GTP-loaded nanoparticles and GTP prodrugs.

Expert opinion: GTPs possess potent anticarcinogenic activities through interfering with the initiation, development and progression phases of cancer. There are several challenges (*e.g.*, poor bioavailability) in developing GTPs as therapeutic agents. Use of nanoparticle-based delivery systems has provided unique advantages over purified GTPs. However, there is still a need to determine the actual magnitude and pharmacological mechanisms of GTPs encapsulated in

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Declaration of interest

QP Dou and TH Chan are inventors of the issued patent claiming the use of pro-EGCG for proteasome inhibition and the treatment of cancer. TH Chan is also the co-inventor of the patent for use of Pro-EGCG in the treatment of endometriosis (US9,713,603). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

nanoparticles, in order to address newly emerging safety issues associated with the potential “local overdose” effect. The use of Pro- epigallocatechin gallate (Pro-EGCG) as a prodrug appears to offer improved *in vitro* stability as well as better *in vivo* bioavailability and efficacies in a number of animal studies, suggesting its potential as a therapeutic agent for further study and development.

Keywords

Bioavailability; biotransformation; cancer; catechin; epigenetic target; EGCG; nanoparticles; pharmacology; pro-EGCG; target delivery

1 Introduction

Tea, made from the leaves of the *Camellia senenisis* plant in the family Theaceae, is now the second most consumed beverage worldwide after water, with more than two-thirds of the world population being consuming this beverage. Consumption of tea is believed to date back to ancient China and India, where it was mostly used as a medicinal plant to help rid the body of excess fluid, control bleeding, heal wounds, and improve heart health. In past decades, interest in tea consumption is growing due to accumulating evidence from cellular, animal, and epidemiological studies that have linked tea consumption to various health benefits, such as chemoprevention of various cancers (prostate, breast, lung, skin, pancreatic, colon and head and neck cancers), chronic inflammation, heart disease, diabetes, and neurodegenerative diseases [1–7]. Although all these health benefits have not been consistently achieved in reported intervention trials, positive results from clinical trials have provided direct evidence supporting the protective effect of tea against human cancer [8,9]. In addition, multiple mechanisms of action have been discovered that could explain how the green tea polyphenols (GTPs) exert their chemopreventive effects [10, 11]. Recently, we did a literature search in PubMed database with the keywords “tea polyphenol and cancer”, and obtained 1272 publications (1976–2018), suggesting that tea polyphenol has been a promising candidate for chemoprevention and therapeutic application of human cancers.

In this review article, after a brief description of GTPs, we will focus on their recent developments in drug discovery, with a special emphasis on potential molecular mechanisms involved in therapeutic implication of GTPs in various cancers and other diseases.

2 Chemical compositions and structures of GTPs

To date, more than three hundred varieties of tea are produced by different manufacturing processes, of which green, black, oolong, white, yellow and post fermented teas are six major commercial forms depending on the extent of fermentation. Green tea (unfermented, 20% of total tea consumption) is produced by steaming and drying the fresh tea leaves to prevent fermentation *via* inactivating the polyphenol oxidase activity. Black tea (fermented) is the most consumed type in Europe and United States, accounting for 78% of global tea production and consumption according to a recent estimation of the United Nations [12]. It is produced by allowing the oxidation and polymerization of tea catechins by polyphenol oxidases during fermentation to form oligomeric polyphenols (theaflavins) and polymeric derivatives (thearubigins), which contribute to the characteristic aroma and color of black tea. Oolong tea (semi-fermented, 2% of total tea consumption) is especially popular in south

China and Southeast Asia, and it is produced through a process including withering the plant under strong sun and oxidation before curling and twisting. White tea and yellow tea are considered as semi-fermented tea. White tea is produced similarly to oolong tea, but minor differences between them are that white tea does not require bruising, oxidation, and curing steps, while yellow tea's production is very close to that of green tea but needs an extra yellowing process.

In general, tea polyphenols are the major component of these teas, accounting for up to 30% of the dry weight of tea leaves. These polyphenols are composed of flavanols, flavandiols and phenolic acids. The major monomeric flavanols are referred to as catechins, including: (+)-catechin (C), (−)-catechin gallate (CG), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), (−)-gallocatechin gallate (GCG) and (−)-epigallocatechin gallate (EGCG). Their chemical structures are illustrated in Figure 1. These catechins contain the same basic structural features, which are two aromatic rings (rings A and B) joined by three carbons and an oxygen forming a cyclic pyran ring (ring C). The catechins differ in the number and distribution of hydroxyl groups on the B-ring, the type of configuration (*cis*- or *trans*-isomers), and the presence or absence of a galloyl moiety (ring D). EGCG is the most abundant in these catechins, representing 50~80% of total catechins in green tea and about 16.5% wt in the water extraction fraction of green tea. It is also considered to be the major contributor to the various health benefits of green tea in cell culture and animal studies, as well as in clinical studies [13–16]. Commercial products derived from green tea are mainly in a powdered form (pill or capsule). In general, these green tea extract supplements contain about 90% total polyphenols with EGCG as primary active ingredient, which provide more catechins than the daily intake from a typical green tea beverage in order to achieve the proclaimed health benefits. Besides these, green tea contains some other constituents including gallic acid, chlorogenic acid, caffeic acid and flavonols such as kaempferol, myricetin and quercetin.

3 Metabolism, biotransformation, bioavailability and toxicology of GTPs

Encouraging data from epidemiological, cellular, animal and clinical studies support numerous health benefits of GTPs and green tea major active ingredient EGCG. However, intervention trials have generally not yet achieved a consistent conclusion in terms of the efficiency and the dose for all the illnesses and diseases of interest [17]. For example, reports from U.S. Food and Drug Administration (FDA) revealed that "there is no credible evidence to support qualified health claims for green tea or green tea extract reducing the risk of heart disease," and "it is highly unlikely that green tea reduces the risk of breast cancer or prostate cancer." [18, 19]. This discrepancy might be attributed to a variety of factors, such as the composition of tea extract, treatment dose and times, individual and local variables, environmental conditions, and even lifestyles of the patients. Especially, metabolic biotransformation of GTPs under physiological conditions is a critical factor influencing their bioavailability and therapeutic application *in vivo* [20].

The metabolic biotransformation of green tea catechins affects their absorption, distribution, metabolism, excretion, and toxicity, as well as overall efficiency in disease prevention and therapy. Metabolic fate of green tea catechins has been extensively investigated in *in vitro*

systems (including human liver microsomes, human placental cytosol, human jejunal cytosol, human Caco-2 cytosol and human saliva), animals (mice, rats and rabbits), and some human models [21–23], and their metabolic pathways including methylation, glucuronidation, sulfation and ring-fission in the liver, kidney and gastrointestinal tract [24, 25] have been reported (Figure 2). Moreover, it is evident that these biotransformation reactions are species- or tissue-dependent, and the type of finished metabolic products vary depending on the types of parental catechins and the tissues where metabolic transformation takes place. For instance, the glucuronidation of EGCG was much greater than that of EGC. The greatest catalytic efficiency for glucuronidation is observed in mouse small intestinal microsomes, followed by mouse liver, human liver, rat liver, and rat small intestine for EGCG-4”-O-glucuronide. In the case of EGC-3’-O-glucuronide, the catalytic efficiency in microsomes was decreased in the order of: mouse liver > human liver > rat liver > rat or mouse small intestine [26]. EGCG is also sulfated depending on time and concentration in human, mouse, and rat liver cytosol, with the rat having the strongest catalytic activity. The concentration of the parental catechins also affects the type of the metabolic products. A good example is the methylation of EGCG by rat liver cytosol: the demethylated form is predominate at low EGCG concentrations (< 1.0 μM) while the monomethylated species could prevail at high EGCG concentrations (> 3.0 μM) [27].

To better understand the mechanism of actions behind various pharmacological activities of GTPs, bioactivities of some tea polyphenol metabolites have been examined. In general, these metabolites formed by the glucuronidation, methylation, and sulfation of green tea polyphenols exhibited decreased bioactivities (i.e. antioxidant, anti-inflammatory and cancer-preventive activities) [28]. Studies on structure-activity relationship of some analogs of green tea catechins suggest that the addition of a methyl group on the B- or D-ring of (−)-EGCG or (−)-ECG led to decreased proteasome-inhibitory activity and, as the number of methyl groups increased, the inhibitory potencies further decreased [29]. Taken together, these results imply that the metabolic transformation of GTPs is an important issue leading to a poor bioavailability and restricting their clinical applications as therapeutic agents.

The oxidation of EGCG is another important aspect associated to pharmacological activities of GTPs. The chemical structures of EGCG endows it highly reactive property, making them very susceptible to oxidation in air under neutral or alkaline conditions [30]. EGCG oxidation involves many pathways including auto-oxidation, transition metal ion or oxidant-promoted oxidation and enzyme-catalyzed oxidation [31]. The auto-oxidation of EGCG occurs not only in cell culture conditions, but also in different tissues and circulating bloods. However, compared with *in vitro* conditions, the auto-oxidation degree of EGCG *in vivo* is believed lower due to more complicated environmental conditions [32], such as the lower oxygen partial pressure (< normal oxygen partial pressure of 160 mmHg), and the presence of antioxidant enzymes in tissues. However, studies showed that some *in vivo* factors promoted the EGCG oxidation degree, including cytochrome P450 peroxidase, myeloperoxidase and tyrosinase, oxidants released from neutrophils and endogenous copper [33, 34]. It is evident that the pro-oxidant effects of EGCG or its oxidation was affected by the dose levels and the environment. Numerous EGCG oxidation products have been characterized and their biological activities have been evaluated using *in vitro* and *in vivo* models. Compared to the parental catechin, some enzyme-catalyzed catechin polymers

exhibited higher antioxidant and xanthine oxidase-inhibitory activity and anti-hyperglycemic effect [35, 36]. Interestingly, some EGCG auto-oxidation products have equivalent cytotoxic activities to EGCG, but enhanced capacity for cysteine depletion, suggesting that anti-cancer activity of EGCG involves a joint contribution of EGCG and vast numbers of bioactive EGCG oxidation products formed extracellularly [31]. The generation of ROS as a result of the auto-oxidation of EGCG is shown to induce ERK1/2 activation in Jurkat cells [37]. Although the *in vitro* mechanisms report cannot explain the effect of EGCG *in vivo*, the bio-activity of EGCG is likely independent of its oxidation.

It is noteworthy that studies from experimental animals and epidemiological surveys have shown that GTPs have a dose-dependent toxicology [38]. When the mice were administrated with diets containing 0.01% and 0.1% GTPs, a hepato-protective activity was observed. By contrast, the treatment with a high dose of GTPs (1%) exhibited symptoms of nephrotoxicity, such as the increased serum creatinine level. Moreover, this treatment deteriorated oxidative damage and decreased the expression of antioxidant enzymes and heat shock proteins (HSPs) in colitis and normal mice. Some case reports also indicated that excessive intake of tea extracts induce liver toxicity [39], which is probably due to the pro-oxidant property of tea polyphenols [40]. It is proposed that low and moderate doses of tea polyphenols produced lower levels of ROS which activate Nrf2 to attenuate oxidative stress whilst high dose of tea polyphenols produced high levels of ROS to induce toxicity [1]. These findings suggest that dose-dependent functionality and toxicology of GTPs should be considered when GTPs or related supplements are used to obtain beneficial effects.

4 GTPs and cancer prevention and therapy

4.1. GTPs and prostate cancer

Prostate cancer (PC) appears to be a suitable model for chemoprevention by means of diet intervention, considering that there is a relatively longer intervention period before the symptoms accompanied with prostate cancer arise and a diagnosis is finally established [41]. It is evident that the redox homeostasis imbalance resulting from reactive oxygen species (ROS) and cellular antioxidant defense is one of the important mechanisms in initiation and progression of PC, and interactions between multiple genetic and ambient factors are significant determinants in PC development [42].

GTPs and EGCG have shown chemopreventive and anti-carcinogenic effects on prostate cells, as supported by the data from cellular, animal experiments, and human clinical trials. For example, the relationship between the prostate cancer risk and habitual green tea intake among Chinese men was investigated in Hong Kong, and results showed an inverse correlation between PC risk and green tea consumption and EGCG intake [43]. The typical chemical characteristics of tea polyphenols allow them to affect cellular redox homeostasis by exerting antioxidant or pro-oxidant activity. The antioxidant/pro-oxidant behavior of green tea polyphenols varies with different experimental conditions, such as concentration, temperature, pH, presence of metal ions, and differences in culture medium composition. For example, the influences of EGCG concentrations and pH on the net anti-oxidant and pro-oxidant activity of EGCG in flaxseed oil-in-water (o/w) emulsions are examined [44]. In low pH (pH 2–4) emulsions, EGCG (1–100 μM) was observed to yield pro-oxidant effects.

However, with the increase of EGCG concentration (500 μM), a competition between antioxidant and pro-oxidant activities of EGCG was observed, leading to lower TBARS concentrations than those observed at 100 μM EGCG. In high pH (pH 5–7) emulsions, high EGCG concentrations showed the best antioxidant effects with 500 μM EGCG resulting in the lowest TBARS concentrations in emulsions. The presence of oxygen and temperature significantly affected the stability [32]. In addition, the presence of some transition metals Cu(II) plays important roles in affecting antioxidant or pro-oxidant activity of EGCG. Co-treatment of HL-60 human leukemia cells with tea catechins and Cu(II) led to the formation of 8-oxo-guanosine [45]. But co-treated with tea catechins and the catalase or the copper chelator, bathocuproine, catechin-mediated oxidative damage was decreased.

GTPs have shown the effects on intracellular ROS production and antioxidant enzymes activity [46]. Besides the anti-oxidant and pro-oxidant activity, some other specific mechanisms are involved in the chemopreventive actions of GTPs, including regulation of the activities of cell surface receptors and their signaling pathways [*i.e.* Receptor tyrosine kinases (RTKs), insulin-like growth factor receptor 1 (IGF-R1)], modulation of gene expression through either direct effect on transcription factors (such as Sp1, NF-kB, AP-1, STAT1, STAT3 and Nrf2) [47, 48], or indirect epigenetic mechanisms [DNA methylation, histone modifications, and expression of noncoding regulatory micro RNA (miRNAs)] [49]. In addition, GTPs are found to interfere with intracellular proteostasis at various levels, such as to target HSPs and proteasome functions, and to interfere with the autophagic flux [50, 51]. Moreover, some EGCG analogues have been identified as novel heat shock protein 90 (Hsp90) inhibitors [52]. Table 1 summarizes the reported effects of GTPs against prostate cancer in experimental systems.

4.2. GTPs and lung cancer

Lung cancer is the leading cause of cancer-related deaths worldwide in both men and women, which could be categorized into two histological groups: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). NSCLCs include adenocarcinoma, squamous cell carcinoma (SqCC), and large cell carcinoma [70]. SCLC accounts for approximately 15% of all lung cancers and has a poor prognosis, with the 5-year survival at diagnosis rarely exceeding 15%. EGCG shows similar inhibitory effects on drug-sensitive (H69) and drug-resistant (H69VP) SCLC cells, which is attributed to their abilities in reducing telomerase, inducing caspases 3/9 activities and blocking the cell-cycle in S phase [71]. NSCLC represents 85% of lung cancers, and patients of stage IIIB & IV disease have the five-year survival less than 10%. Treatment with 10–50 μM of EGCG for 3 days led to promoter demethylation and restoration of WIF-1, an antagonist of Wnt proto-oncogene in NSCLC cells (H460 and A549) [72]. EGCG is a potent inhibitor of cell proliferation, independent of EGRF inhibition, in several NSCLC cell lines including those resistant to EGFR kinase inhibitors and those overexpressing c-Met (H2122, H358, H460, H1975 and H1993 cells). EGCG is able to completely inhibit ligand-induced c-Met phosphorylation and partially inhibit EGFR phosphorylation. When EGCG is used in combination with erlotinib, a synergistic inhibition of cell proliferation and colony formation was observed [73]. Co-treatment of NSCLC cells (*i.e.* PC-9, A549 and ChaGo K-1 cells) with EGCG and celecoxib also shows synergistic induction of apoptosis by upregulation of GADD153 genes, which

might be attributed to the activation of the mitogen-activated protein kinases (MAPKs), such as ERK1/2 and p38 MAPK pathways [74]. EGCG is also effective in attenuating cardiopulmonary bypass-related lung injury by reducing lung edema, pulmonary neutrophil infiltration, and presumably initiating poly (ADP-ribose) polymerase (PARP) dependent cell death signaling [75].

Studies on lung cancer-related micro RNA (miRNA) is a research focus recently due to the potential roles of miRNAs in lung tumorgenesis. The identified miRNAs include miR34-c, miR145, miR142–5p and miR210. EGCG upregulates the expression of microRNA (*i.e.* miR-210) by binding HIF-1α in mouse (CL13) and human NSCLC cells (H1299, H460 and A549), resulting in reduced cell proliferation and anchorage-independent growth [76]. The overall miRNA levels in the oncogenic K-ras-induced mouse lung cancer is decreased [77], which is consistent with the findings that that lung-specific knockout of Dicer results in the abnormality of lung development and function [78]. Furthermore, the genomic and bioinformatics approaches were employed to analyze the role of microRNA in the EGCG inhibition of tobacco carcinogen-induced lung tumors in A/J mice [79]. Results showed that the expression levels of 21 miRNAs had modest changes and 26 potential targeted genes of the 21 microRNAs were identified by the computation method. Further pathway analysis revealed that the most impacted pathways of EGCG treatment are the regulatory networks associated to AKT, NF-κB, MAPKs, and cell cycle, and the identified miRNA targets are involved in the networks of AKT, MAPKs and cell cycle regulation.

4.3. GTPs and breast cancer

Breast cancer is the leading cause of cancer death among females worldwide, with an estimated 14 million new cases and roughly 8 million deaths each year [70]. Breast cancer is categorized into three main subtypes according to their molecular profiles: hormone receptor-positive (Estrogen receptor-positive/ ER+ve), HER2-positive (ErbB2-positive, a member of EGFR family) and triple-negative tumors. The heterogeneity of breast cancer, with the distinct morphological and phenotypic profiles through gene expression, proliferation, and metastatic potential, make it difficult to treat with chemotherapy.

It is found that GTPs, particularly EGCG, inhibit proliferation, migration, angiogenesis, and tumor growth in breast cancer, as well as inducing apoptosis and cell cycle arrest in the cancerous cells [80]. EGCG has been shown to interfere with estrogen receptor function, inhibit estrogen-induced breast cancer cell proliferation, and sensitize hormone responsive tumors to drugs that target steroid receptors (e.g. tamoxifen) [81]. For example, treatment with 10 μM EGCG for 72 h led to reactivation of estrogen receptor (ER)-α expression in low basal ER-α MDA-MB-231 breast cancer cells. Moreover, this effect is enhanced when combined with a histone deacetylase (HDAC) inhibitor [82]. The combination of EGCG and curcumin was efficacious in both *in vitro* and *in vivo* models of ER-α-positive breast cancer. EGCG possesses potent anti-invasive activity by activating the FOXO3a/ERα/MTA3/E-cadherin pathway and overcomes trastuzumab drug resistance of HER2 positive breast cancer cells [83]. In addition, EGCG treatment reduced cell proliferation, ATP production, and Akt activity, and a concomitant elevated level of nuclear FOXO3a and $p27^{Kip1}$ levels in trastuzumab-resistant breast cancer cells, suggesting that the combination of EGCG with

trastuzumab may be an effective approach to treat HER2-overexpressing breast cancers [84]. Moreover, GTPs are able to target breast cancer stem cells (CSCs) and their signaling pathways, by blocking Wnt signaling through a transcriptional repressor. Considering that CSCs are responsible for the initiation of tumor growth, cancer recurrence, anticancer drug resistance, and metastasis, they are believed to be promising therapeutic targets for breast cancer in the future [85].

GTPs and EGCG are able to modulate epigenetic events to breast cancer prevention and therapy, and are thus used as epi-drugs for prevention, prognosis, and perhaps treatment, which might aid in overcoming the drawback of standard therapy for estrogen-dependent breast cancer by using the selective ER modulators [86]. EGCG reduced DNA methyltransferase 1 (DNMT1) activity by either reducing S-adenosyl-L-methionine (SAM) and increasing S-adenosyl homocysteine (SAH), or blocking the entry of the key nucleotide cytosine into its active site by hydrogen bonding [87, 88]. Also, EGCG specifically inhibited histone acetyl transferase (HAT) but not histone deacetylases (HDAC) in B lymphocytes [89]. When used in combination with trichostatin A, EGCG reactivated ERα expression *via* remodeling of the chromatin structure of the ERα promoter by altering the status of histone acetylation and methylation [82]. In addition, EGCG affected miRNAs to cause subtle changes in multiple molecular targets and pathways. For example, treatment with EGCG led to upregulation of MiR-16 in tumor cells, which down-regulates IκB kinase α and subsequently induces IκB accumulation in tumor-associated macrophages and inhibits M2 polarization [90].

Taken together, there are several proposed mechanisms of action of green tea polyphenols against breast cancer, including anti-angiogenesis, interaction with target proteins [PI3K, 67-kDa laminin receptor, Ras-GTPase activating protein (GAP) SH3 domain-binding protein 1 (G3BP1), Bcl-xL and Bcl-2, vimentin, Fyn, GRP78, and insulin like growth factor 1 receptor (IGF-1R)], inhibition of cell signaling pathways [EGFR, Wnt, hepatocyte growth factor signaling pathway, Met phosphorylation and ERK-1/2, as well as Akt/protein kinase B (PKB)], inhibition of enzyme activities (CDK 2/CDK4, proteasomes), induction of cell cycle arrest and apoptosis, and effects on microRNAs [80].

4.4. GTPs and neurodegenerative diseases

Excessive ROS production causes oxidative damages to normal neuronal biomolecules and leads to accumulation of iron in specific brain area, which is believed to contribute to the major pathological aspects of Parkinson's disease (PD) and Alzheimer's disease (AD) [91]. Green tea extract and EGCG are found to exert neuroprotective/neurorescue activities in a wide array of cellular and animal models of neurological disorders.

Accumulation of phosphorylated tau protein (P-tau) aggregates and the 42 amino acid form of β-amyloid ($A\beta_{42}$) is a pathological hallmark of AD. EGCG has been implicated as a drug candidate that is able to modulate aggregation of proteins, such as Huntingtin, β-amyloid and α-synuclein in neurodegenerative diseases [92]. For instance, treatment with 10 μM EGCG provides a protective effect on cultured rat primary prefrontal cortical neurons against iAβ-induced cytotoxicity *via* the activation of the AKT pathway [93] or by increasing the levels of acetylcholine [94]. Moreover, EGCG is effective in suppressing Al-

induced $A\beta_{42}$ fibrillation by preventing further conversion of Aβ42 monomers into a folded conformation [95]. EGCG also inhibits the aggregation of tau protein into toxic oligomers at ten- to hundred-fold substoichiometric concentrations to rescue neuronal cells from tau-induced neurotoxicity [96]. Results from Chesser *et al.* (2016) revealed that EGCG treatment enhances the clearance of three AD-relevant phosphorylated tau epitopes in primary neurons [97]. These clearance are likely through increasing adaptor protein expression in a highly specific manner, which differs from the previously reported mechanisms such as those independent of both Nrf2 activation and enhanced autophagy [98].

PD is neuropathologically characterized by the misfolding and aggregation of α-synuclein protein, damage and loss of dopamine (DA) neurons in the substantia nigra pars compacta (SN), and mitochondrial dysfunction caused by oxidative stress [99]. EGCG treatment inhibits the α-synuclein protein aggregation in a time- and concentration-dependent manner with the ED_{50} of 250 nM [100]. Similar results were obtained in *in vivo* human brain tissues. It is suggested that the inhibitory effect of EGCG on α-synuclein aggregation is attributed to intermolecular hydrophobic interactions. Studies on animal experiments show that oral administration of EGCG is able to significantly reduce dopamine neuron loss in the substantia nigra pars compacta and can also prevent striatal dopamine and tyrosine hydroxylase protein level depletion [101]. Some proposed molecular mechanisms behind therapeutic actions of catechins includes scavenging of ROS and iron chelating, inhibition of lipid peroxidation, induction of endogenous detoxifying enzymes, modulation of cell signaling pathways (activation of PKC and PI3K/Akt signaling pathways and inhibition of MAPK signaling pathway), and regulation of cell survival and apoptotic gene expression [102, 103]. By interaction with these signaling cascade, EGCG further strengthens the response of the cell to environment or the stressor, ultimately leading to responses such as cell proliferation, apoptosis, synthesis of inflammatory mediators, and neurite growth.

GTPs and diabetes

According to the WHO report, about 347 million people worldwide have diabetes mellitus (DM), from which total deaths are projected to rise by more than 50% in the next 10 years. Type 2 DM (T2DM) accounts for approximately 90% of all diabetes in almost all countries [104]. There are some discrepancies regarding the effect of tea consumption on diabetes based on human epidemiological studies. Results from some cohort studies in Taiwan and Japan show that the consumption of green tea is effective against type 2 diabetes [105, 106]. However, some randomized trials showed contrary results that daily consumption of green tea by patients with diabetes for several months failed to ameliorate diabetes-related parameters [107].

Some mechanisms of actions of GTPs against diabetes have been proposed. First, oxidative stress has been implicated in the progression of diabetic complications. The health beneficial effects of tea polyphenols on type 2 diabetes are partly attributed to their antioxidant activity, as well as their modulatory action towards some oxidative stress-related signals [108]. Secondly, EGCG is capable of inhibiting cellular glucose uptake either by blockade of insulin signaling or by direct competition with glucose for GLUTs. Thirdly, tea catechins also affect the insulin pathway either by inhibiting the carbohydrate digestive enzymes α-

amylase, α-glucosidase, and intestinal sucrase in the intestine of the rats, or by enhancing the insulin sensitivity and insulinotropic activity [109]. In addition, GTPs are also effective in improving endothelial dysfunction and modulation of inflammation (cytokine expression).

4.6. GTPs and endometriosis

Endometriosis is a disorder due to the implantation of endometrial glands and stroma outside the uterine cavity. It is generally accepted that endometriosis is an angiogenesis-dependent disorder [110]. New blood vessel formation to deliver the oxygen and nutrient supply is essential to the development and progression of endometriosis [111]. Animal models have confirmed that angiogenesis occurs in endometriosis [112,113]. Anti-angiogenesis therapy therefore offers an opportunity for the treatment of endometriosis [114]. The anti-angiogenic activity of EGCG has been previously demonstrated *in vitro* and *in vivo* [115]. Furthermore, EGCG suppressed the angiogenesis signaling pathway and inhibited the growth of experimental endometriosis in mice [113,116]. According to one study, EGCG inhibited the E2-stimulated activation, proliferation and VEGF expression of endometrial cells *in vitro* [117]. EGCG also selectively suppressed angiogenesis and blood perfusion of endometriotic lesions *in vivo*.

4.7. GTPs and angiogenesis

Accumulating data based on *in vitro* and *in vivo* tumor models has confirmed the antiangiogenic properties of GTPs. Various molecular mechanisms have been proposed, such as the suppression of cell proliferation, induction of apoptosis, inhibition the expression of angiogenic factors, suppression the phosphorylation of receptors and inhibition of binding of VEGF to its receptor. For example, green tea extract significantly reduced the expression of Flt-1 and KDR/Flk-1 in HUVEC, suggesting that they may have preventive effects on tumor angiogenesis and metastasis through reduction of expression of VEGF receptors [118]. EGCG is recognized as the only catechin that inhibits VEGF binding to its receptor. It was able to reduce the expression of VEGF and therefore regulates VEGF expression through blocking ERK and Akt phosphorylation [109]. In addition, EGCG significantly decreased the expression of HIF-1α, a strong activator of VEGF expression [120]. It also was able to block the signal transduction events of VEGF, such as phosphorylation of ERK1/2, autophosphorylation of VEGF-R1 and VEGF-R2 and the expression of early growth response-1 (Egr-1) transcription factor in human umbilical arterial endothelial cells [121]. Recently, GTPs, especially EGCG, exhibited influence on some miRNAs and activation/inhibition of various cellular/molecular pathways which are involved in angiogenesis in various cancer types [122]. For example, the incubation of MCF-7 cells with low concentration of Polyphenon-60 (green tea extract) for 48 h lead to significant changes of 23 miRNAs [123]. With more and more studies being carried forward in the future, some new miRNAs and novel roles of miRNAs involved in anti-angiogenesis effect of GTPs may be discovered. Figure 3 summarizes the biological activities and physiological mechanisms of green tea catechins as reviewed above. Please also see Section 7 for anti-angiogenesis effects of pro-EGCG.

5 Challenges for use of GTPs as therapeutic agents

Diverse health-promoting activities of green tea and its major constituent EGCG have been supported by the evidence from cellular, animal and clinical studies. Case-control and cohort studies have reported beneficial effects of green tea on patients with various cancers, such as breast, colon and rectum, pancreas, stomach, ovary, and lung, as well as prevention of recurrence in Stage I-II breast cancer patients. However, some studies did not show an association between the intake of tea and/or polyphenols and the decreased risk of human cancers. For instance, a phase II clinical trial was conducted in Italy to investigate the effect of green tea catechins in patients with high-grade prostatic intraepithelial neoplasia (HG-PIN). After sixty volunteers with HG-PIN took daily 600 mg of green tea catechins (Categ Plus®) for 6 and 12 months, no any statistical difference in PCa incidence was observed between the experimental groups through prostatic biopsy and measurements of prostate-specific antigen (PSA) [124]. Results from a meta-analysis of prospective cohort studies in Asian populations revealed that cumulative tea consumption in the highest consumption range (> 5 cups per day) led to a 22% reduction of the relative risk of liver cancer, but significantly only for women, not in men [125]. This limited effect in a subpopulation of Asian women does not endow a general recommendation for green tea as a preventive agent of hepatocellular carcinoma. Furthermore, meta-analysis including two studies and breast cancer recurrence and seven studies of breast cancer incidence did not find a significant effect of green tea on breast cancer prevention [80]. Many published reviews have discussed the possible reasons between the *in vitro* and *in vivo* studies as wells as between the epidemiological and clinical trials [1,10,126,127]. Taken together, the offered explanations include: a) higher quantities of GTPs used in *in vitro* and in animal studies as compared to the doses used for human clinical trials. The concentrations of GTPs used for *in vitro* studies usually range from 20 μM to 100 μM or even higher. By contrast, these levels cannot be achieved *in vivo,* especially inside or surrounding cancer cells [10]. Even after 7 to 9 cups of tea, the physiological level of tea in blood is less than 1 μM. Furthermore, the concentration of catechins in the tissues is affected by the duration of tea intake; b) Experimental systems are generally simple and homogenous in terms of genetic, host and lifestyle factors, and experimental conditions are easily controlled. However, clinical trials faced more complicated experimental conditions, such as the additional variables of host and lifestyle factors, exposure complexity and metabolic competence; c) Time of exposure to green tea might be different. For example, for the chemopreventive effect of GTPs, exposure is normally before, during and after carcinogen treatment in experimental models when cells/tissue/processes are norm while the administration of green tea in clinical trials is after damage/disease, high-risk individuals/cancer patients. These inconsistent results suggest that there is still a long way to go before the clinical use of GTPs as therapeutic agents.

The low bioavailability of GTPs is still a major hurdle that hinders their clinical application as therapeutic agents. The rates of absorption and bioavailability are extremely low in orally administered EGCG (perhaps < 3.5% of the dose) [128]. Many EGCG bioactivities observed in *vitro* are not reproduced *in vivo*, suggesting that the irrelevance of *in vitro* results associated with tea polyphenols. Molecular and cellular mechanisms associated with low bioavailability of GTPs have been proposed [20], including loss or inactivation owing to

metabolic transformation or degradation, low cellular uptake due to poor hydrophobicity to cross the cell membrane, and active efflux of many polyphenolic compounds by the multidrug resistance-associated protein 2.

Many strategies aiming improving bioavailability and therapeutic efficiency of GTPs have been put into practice. First, it might be applicable to synthesize more GTPs-based analogs or prodrugs to find more potent, stable and specific active agents. Second, design and development of effective delivery systems, such as nanoparticles and liposome, might assist in improving the bioavailability of GTPs. Various nanoparticles are believed to be promising candidates for the encapsulation of chemically labile bioactive substances since they manifested some unique advantages over enhancing performance of the loaded drugs, including higher stability and bioavailability, improved water solubility, sustainable and controlled release, and higher absorption property [129]. Encapsulation of catechins in carbohydrate nanoparticles and liposomes significantly improved their therapeutic efficacy [130]. Third, the combined use of GTPs with other drugs and inhibitors as therapeutic agents might be a promising approach. This combination generates more effective therapeutic activity, such as a synergistic effect, than that of individual drug alone. For example, combination of EGCG and NS-398 significantly resulted in enhanced cell growth inhibition, apoptosis induction, proapoptotic proteins expression, as well as inhibition of nuclear factor-κB compared to the additive effects of the two agents alone [66]. The combination of EGCG and erlotinib induces apoptosis of SCCHN cells by regulating Bim and Bcl-2 at the posttranscriptional level [131]. Some combinations of EGCG and anticancer compounds induced similar synergistic anticancer effects for both *in vitro* and *in vivo* experiments, with an average reduction in tumor volume reaching by 70.3% in xenograft mouse models [132].

6 Development of nanoparticle-based delivery system of GTPs

Various attempts have been made in the past decades to overcome the above-mentioned limitations of GTPs, among which, nanoparticle-based delivery system appears to be a promising alternative for improving the bioavailability of GTPs due to their unique advantages over traditional delivery methods, including improved stability, enhanced anticancer performance, higher bioavailability, and sustainable and controlled release and targeted delivery to the site of action. A summary of the listed references on the development of nanoparticle-based delivery systems of GTPs is shown in Table 2.

6.1. Protecting GTPs against harsh conditions

GTPs are very unstable and reactive owing to their chemical structure, and thus highly susceptible to chemical, physical and biological stress factors such as light, oxygen, thermal processing, pH changes, metal ions, and other harsh conditions. Nanoparticle-based delivery systems are considered as plausible options to overcome these limitations. For example, encapsulation in chitosan-tripolyphosphate nanoparticles is effective in stabilizing tea catechins in alkaline solution [157]. It took 8 and 24 hours for the non-encapsulated and encapsulated (+)-catechin, respectively, to be degraded to 50% of their initial levels, and the corresponding values for the nonencapsulated and encapsulated EGCG were 10 and 40 minutes, respectively. Shpigelman *et al.* (2010) used thermally modified β-lactoglobulin to

form co-assembled nanovehicles for delivery of EGCG, and found that these complexes conferred considerable protection to EGCG against oxidative degradation. A 33-fold lower initial degradation rate and a 3.2-fold slower degradation over 8 days were observed for nano-entrapped compared to unprotected EGCG [158].

6.2. Sustainable and control release of GTPs

The half-life for EGCG is about 5 hours, and for EGC and EC the half-lives vary between 2.4 and 3.4 hours [159]. Such a short half-life greatly hinders its clinical development. Extensive studies have been conducted on the sustainable release of GTPs by various nanoparticle delivery systems. Colloidal complexes from association of methylcellulose and EGCG presented a sustained EGCG release from the complex with around 60% release in intestinal pH at the end of 2 hours [160]. Electrospray-aided nanoencapsulation process improved the release and permeability properties of catechins, especially at lower core-to-wall ratios (1:50 or 1:10) compared to higher core-to-wall ratio (1:05) or as catechins in free form [161]. In addition, some pH-responsive and magnetic-responsive nanoparticle systems have been developed by incorporating pH/magnetic-responsive polymers in nanoparticles [162]. Self-assembled nanoparticles composed of chitosan (CS) and an edible polypeptide, poly(γ-glutamic acid) (γ-PGA) for the delivery of tea catechins, were pH-responsive and demonstrated different tea catechin release profiles in simulated gastrointestinal tract (GI tract) media [147].

In vitro release studies show that the release of GTPs from nanoparticles is affected by pH values, temperature of the release medium, and structure of the nanoparticles, whilst the loading concentrations of drugs seem to be independent to the cumulative percentage of the drug release [163]. In GTPs-loaded liposomal nanoparticles, when the pH value of release medium changed from 3.0 to 9.0, the cumulative release percentages of GTPs in 48 h were about 50% and 74%, respectively. The cumulative release ratio of the drug increased with the temperature. Under the same conditions, about 53%, 60% and 79% of GTPs were released within 48 h at room temperature, 37°C and 45°C, respectively. These differences might be explained that the pH and temperature were able to affect the structure of the liposomal nanoparticles or the binding between liposomes and GTPs. Release kinetics analysis shows that the drug release for nanoparticulate dosage forms or sustained release formulations fitted with Zero order kinetics with anomalous mode of transport. In chitosan-based nanoparticles, GTPs release *in vitro* was divided into two phases: a very rapid initial burst (about 6 h) and a slow release (up to 48 h). The composition and structure of chitosan nanoparticles play important roles in the release behavior of GTPs from chitosan-based nanoparticles delivery system [150].

6.3. Improved bioavailability of GTPs

Results from the study on EGCG-loaded solid lipid nanoparticles (EGCG-SLN) showed that the lipid core of nanoparticles not only provides an additional structural reinforcement to the nanoparticle assembly, but also makes it biologically compatible. The cytotoxicity of EGCG-SLN was found to be 8.1 times higher against MDA-MB 231 human breast cancer cells and 3.8 times higher against DU-145 human prostate cancer cells than that of the pure EGCG [164]. Compared with free EGCG, EGCG encapsulated in polylactic acid-

polyethylene glycol nanoparticles retained its biological effectiveness with over 10-fold dose advantage for exerting its proapoptotic and angiogenesis inhibitory effects in cell assays, and also showed anti-tumor effect in a tumor xenograft mouse model [165]. Similarly improved bioavailability of EGCG was also observed by Hu *et al.*, who synthesized a novel type of nanochemoprevention by encapsulation of EGCG with caseinophosphopeptides and chitosan nanoparticles, and found an enhanced penetration of EGCG through Caco-2 cell monolayers [166].

6.4. Targeted delivery of GTPs

Targeted treatments are aimed at blocking specific biologic transduction pathways or cancer proteins that are involved in tumor growth and progression, *i.e.* molecular targets (receptors, growth factors, kinase cascades or molecules related with apoptosis and angiogenesis) that are present in normal tissues, but are found overexpressed or mutated in cancer. Modification of the surface of the coating material not only provides active targeting characteristics to the particles, but also improves therapeutic efficacy of encapsulated drugs and overcomes the multidrug resistance (MDR) [167]. Various ligands are extensively used to functionalize the surface of nanoparticles for encapsulation of EGCG, such as albumin, hyaluronic acid, biotin, folate, transferrin, and monoclonal antibodies [168]. In order to selectively deliver EGCG to cancer cells, EGCG-loaded nanoparticles consisting of PLGA-PEG functionalized with small molecules like the prostate specific membrane antigen (PSMA) were found to exhibit a selective *in vitro* efficacy against PSMA-expressing prostate cancer cells [156].

7. Pro-drug of GTPs

Our laboratory successfully introduced chemical modifications to GTPs to form prodrugs and synthesized a series of analogs based on structure-activity relationship [169, 170]. Pro-EGCG (Figure 1), the octaacetate of EGCG, was found to be 6 times more stable than (−)-EGCG in a culture medium (RMPI) which mimics the body fluid with a pH value around 8. Therefore, peracetate protection of the phenol groups of (−)-EGCG aids in stabilizing in culture (presumably physiological) conditions [169]. When pro-EGCG was incubated with an extract of leukemia Jurkat T cells, it was degraded with the formation of EGCG, suggesting that pro-EGCG can be hydrolyzed presumably by cellular esterases to give EGCG. (−)-EGCG has been found to potently inhibit the chymotrypsin-like activity of proteasomes *in vitro* and *in vivo* [171]. In contrast, Pro-EGCG, of itself, was completely inactive in inhibiting the chymotrypsin-like activity of the purified 20S of proteasome. On the other hand, Pro-EGCG is equally potent to, if not more potent than, (−)-EGCG in inhibiting the proteasomal activity in intact Jurket cells, suggesting again that Pro-EGCG can be converted to EGCG inside the cells [169].

The *in vivo* activity of Pro-EGCG has also been examined in animal models. Intragastric administration of Pro-EGCG to CF-1 mice resulted in higher bioavailability of EGCG in plasma compared with administration of equimolar doses of EGCG [172]. To investigate the potential efficacy of Pro-EGCG *in vivo*, breast cancer MDA-MB-231 tumors were induced in nude mice, followed by treatment with Pro-EGCG or (−)-EGCG for 31 days. Results

showed a significant inhibition of breast tumor growth by Pro-EGCG, compared with (−)-EGCG [173]. Superior efficacy of Pro-EGCG as compared to EGCG was also observed on their inhibitory effect on androgen-independent prostate cancer, CWR22R, xenograft model in nude mice [174]. In mouse models of xenograft of human endometrial cancer, Pro-EGCG inhibited tumor angiogenesis through down-regulation of vascular endothelial growth factor A (VEGFA) and hypoxia inducible factor 1 alpha (HIF1α) in tumor cells and chemokine (C-X-C motif) ligand 12 (CXCL12) in host stroma [175]. To evaluate the potential of cancer preventive effects, dietary administration of Pro-EGCG and EGCG in dextran sulfate sodium (DSS)-induced colitis in mice was studied. The results indicated that Pro-EGCG administration was more effective than EGCG in preventing the shortening of colon length, the formation of aberrant crypt foci (ACF) and lymphoid nodules (LN) in mouse colon stimulated by DSS [176]. In a mouse model of endometriosis, both EGCG and pro-EGCG significantly decreased the growth of endometrial implants and reduced the lesion size and weight, as well as inhibited functional and structural microvessels in the lesions. However, the inhibition by pro-EGCG in all the angiogenesis parameters was significantly better than that by EGCG [177].

8. Conclusion

Accumulating evidence has supported the potential roles of GTPs and its major constituent EGCG in chemopreventive and therapy of various cancers and other diseases. Unfortunately, intervention studies have not yet confirmed these health benefits of GTPs. Nanoparticle-based delivery techniques hold great promise for addressing issues related to poor stability, solubility and bioavailability encountered in medical applications of GTPs. However, there is still much more to study in terms of optimizing the performance of nanoparticle-based delivery systems for GTPs in the future. Using the pro-drug approach, Pro-EGCG, the octaacetate of EGCG, has been found to have superior stability *in vitro* and better activities and efficacy than EGCG in a number of animal models *in vivo*. It offers the potential to be further developed into a therapeutic agent.

9. Expert opinion

The key findings in the green tea field so far include that GTPs possess potent anticarcinogenic activities by interfering with the initiation, development, and progression of cancer; EGCG suppresses the angiogenesis signaling pathway and inhibits neovascularization, which can contribute to not only its anticancer activities, but also the growth inhibition of experimental endometriosis in mice [113, 116]. Another key finding in this field is that GTPs have numerous conformations due to the presence of the galloyl group, enabling them to interact with various types of molecules, such as nucleic acids and proteins. For instance, EGCG is capable of entering into nucleus and binding to both DNA and RNA in CpG-rich regions [178]. Although the exact mechanism by which the EGCG accumulates in nucleus remains unclear, these interactions might play an important role in regulating gene functions. EGCG is also found to bind with a variety of proteins, such as histidine-rich glycoproteins, kinases, anti- and pro-apoptotic proteins, insulin-like growth factor receptor, proteasomes, and so on. The interactions between EGCG and these targets may play regulatory roles in multiple signaling events. Moreover, EGCG has cell type-and

environment-specific responses. Therefore, it is important in the future to identify more molecular targets or biomarkers that respond to physiologically effective concentrations of EGCG. Such future studies will contribute an in-depth understanding of the chemopreventive and therapeutic mechanism of GTPs towards various cancers, as well as will provide a better guidance for the targeted therapy of cancers with GTPs as clinical therapeutic agents in the future.

There are some weaknesses or challenges related to GTPs that have to be overcome before their clinical use for human health benefits. The poor bioavailability is still a major challenge for the development of GTPs as a therapeutic agent for clinical application. In order to maximize chemopreventive and therapeutic efficiency of GTPs (mainly EGCG) as those observed in various *in vitro* cellular assays, continuous efforts aiming at improving their bioavailability should be made in the future. The use of pro-drug such as Pro-EGCG may help in resolving the poor bioavailability issue even though further studies are clearly required. In addition, by using various polymeric carrier systems, *i.e.* nanoparticles, liposomes, solid liposome nanoparticle and micelles, some advancement has been achieved in enhancing bioavailability and stability of GTPs. Nanoparticles-based molecular targeted cancer therapy is a promising strategy to overcome the lack of specificity of conventional chemotherapeutic agents. It should provide more specific targeting to tumor tissues via improved pharmacokinetics and pharmacodynamics and active intracellular uptake [179]. Ultimately, active targeting via the inclusion of a specific ligand on the nanoparticles is envisioned to provide the most effective therapy, and some ligand-targeted nanotherapeutics are either approved or under clinical evaluation [180]. As far as the biological targets are concerned, tumor-associated antigens appeared to be suitable biological targets for therapeutic intervention. The translation of these bioconjugates into clinical practice is expected soon, even though there is continuing interest in developing a localized therapeutic option for treatment of various cancers [156].

Although these drug carrier systems provide us with more options for targeted anticancer therapies, a big challenge associated with it is to understand the intrinsic characteristics of these carriers that determine their interactive actions with target organs/cells or various biological systems. The knowledge is needed in the field is determination of pharmacokinetic and therapeutic effectives of the loaded drugs as well as their disease-targeting selectivity. Therefore, in the coming years researchers should put more effort on developing novel GTPs (*e.g.*, EGCG)-loaded carriers that are able to target disease tissues selectively and specifically. In addition, one would expect to see the progress in determination of the actual magnitude and pharmacological mechanisms of GTPs encapsulated in nanoparticles, as compared to those free, in order to address newly emerging safely issues associated with the potential “local overdose” effect for nanotechnology-enabled encapsulation system.

Another big challenge in cancer chemotherapy field is time-dependent multidrug resistance to chemotherapy and radiotherapy, along with treatment discontinuation caused by various side effects. One future strategy to overcome this shortcoming is to develop novel combination treatments of chemotherapy and bioactive dietary compounds. Multidrug chemotherapy has become a research focus in the recent years because of better therapeutic

efficiency and lower adverse effects compared to the conventional monotherapy. More future attention should be paid to the combinations of GTPs or EGCG as adjuvant with chemotherapy or radiotherapy. It should be noted that antagonistic effects could be generated when GTPs are combined with other therapeutic drugs in some cases. However, in most of cases additive or synergistic interaction between EGCG and conventional anticancer agents could be generated that should enhance the therapeutic efficiency and reduce adverse side effects of conventional anticancer drugs. For example, combinations of green tea extract or EGCG with some conventional anticancer drugs, such as cisplatin, tamoxifen, *etc*., have exhibited some encouraging preclinical data, suggesting that the combinations are more effective than monotherapy [181]. However, there are only few clinical studies on such combinations. More efforts are needed in the future to search for promising combinations of EGCG with other anticancer agents preclinically and in clinical studies to validate such combinations for cancer therapy. In addition, the inconsistent results of the cellular assays, animal experiments and human intervention trials imply that it may be unwise to extrapolate the results of *in vitro* studies to *in vivo* situation. Taken into accounting the confounding and modulating factors that affect the *in vivo* anti-cancer performance of GTPs (e.g. quantities of tea polyphenol, duration time of tea intake, varied aetiology in different populations and population heterogeneity, and so on), well-designed cohort studies and human intervention trials to minimize the undesired influence of these factors are meaningful and needed. Studies on the selection of approximate dosages and biomarkers for clinical trials treated with GTPs are needed in the future.

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Article highlights

- Accumulating evidence from cellular, animal and epidemiological studies has supported the diverse health benefits of green tea polyphenols and its major constituent EGCG.
- The beneficial effects of green tea polyphenols are limited by their poor stability, absorption and bioavailability.
- The metabolic biotransformation of green tea catechins affects their absorption, distribution, metabolism, excretion and toxicity, as well as overall efficiency in disease prevention and therapy.
- Effects of EGCG are dependent on cell types and environments.
- Nanoparticle-based delivery techniques could improve stability, solubility and bioavailability of green tea polyphenols.
- The use of Pro-EGCG as a prodrug of EGCG offers improved stability, bioavailability and efficacy in animal models.

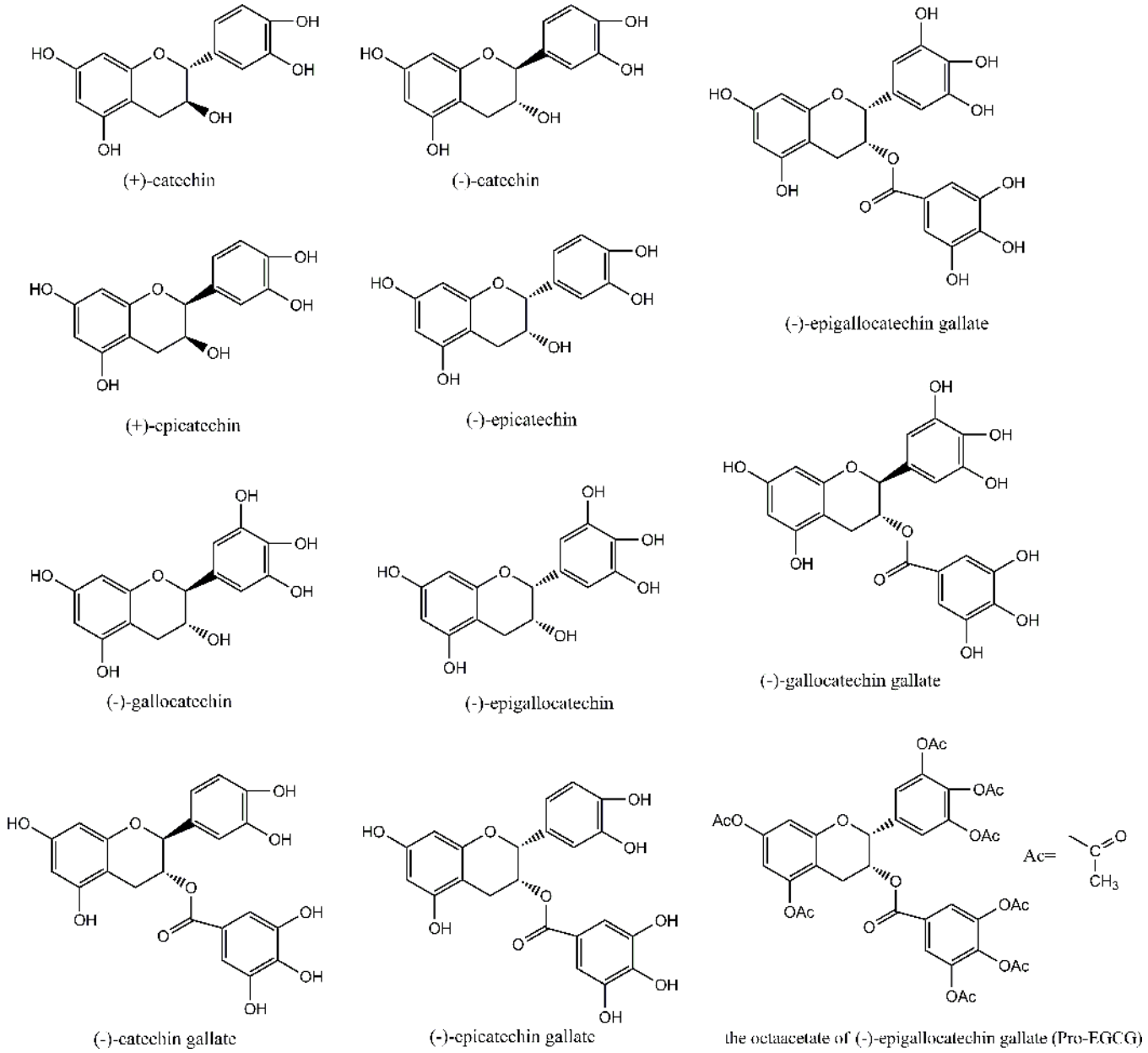

Figure 1.

Chemical structures of major tea catechins and the octaacetate of (−)-epigallocatechin gallate (Pro-EGCG).

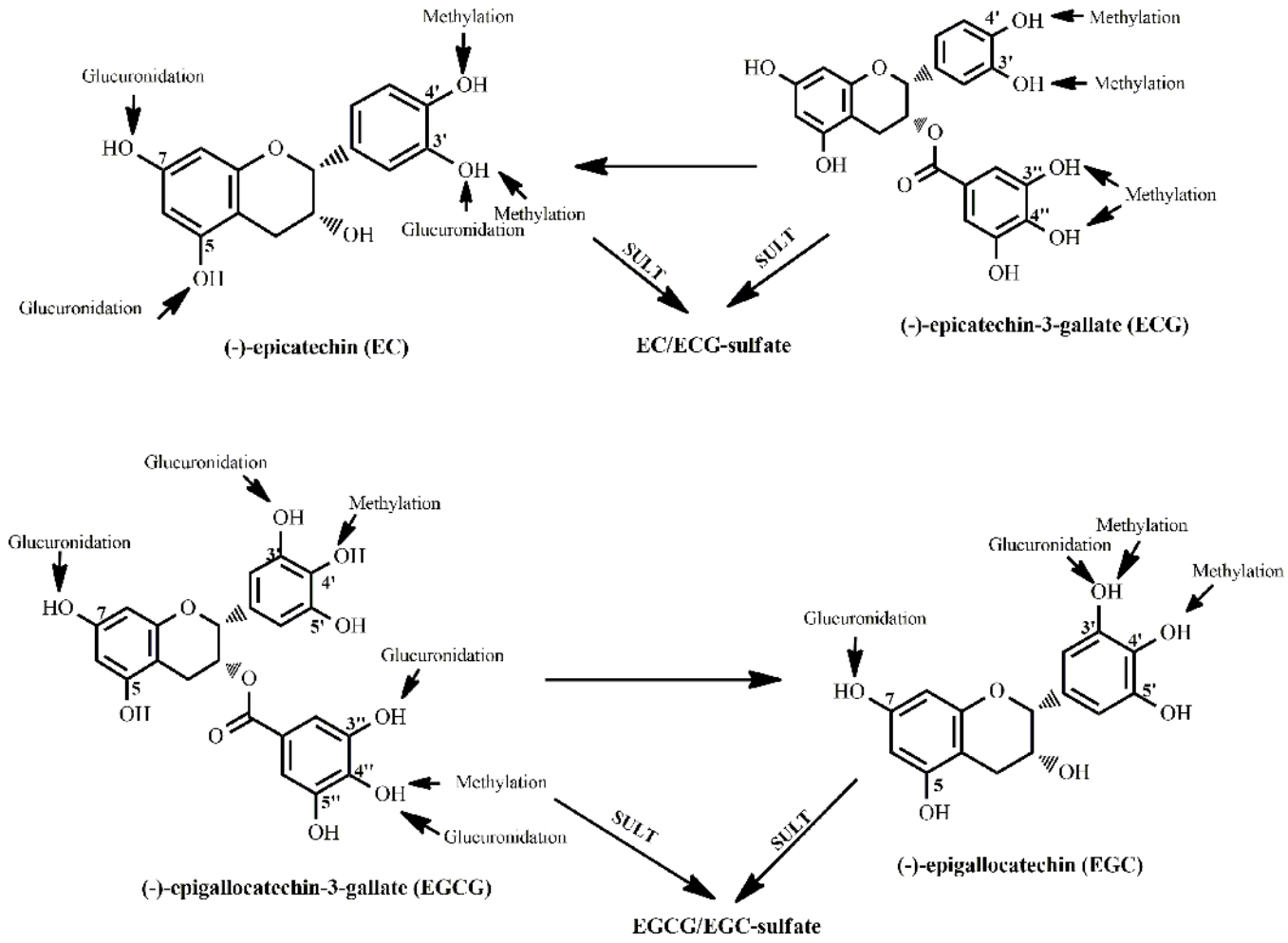

Figure 2.

Structure sites for methylation, glucuronidation and sulfation of major tea catechins by human and rodent enzymes.

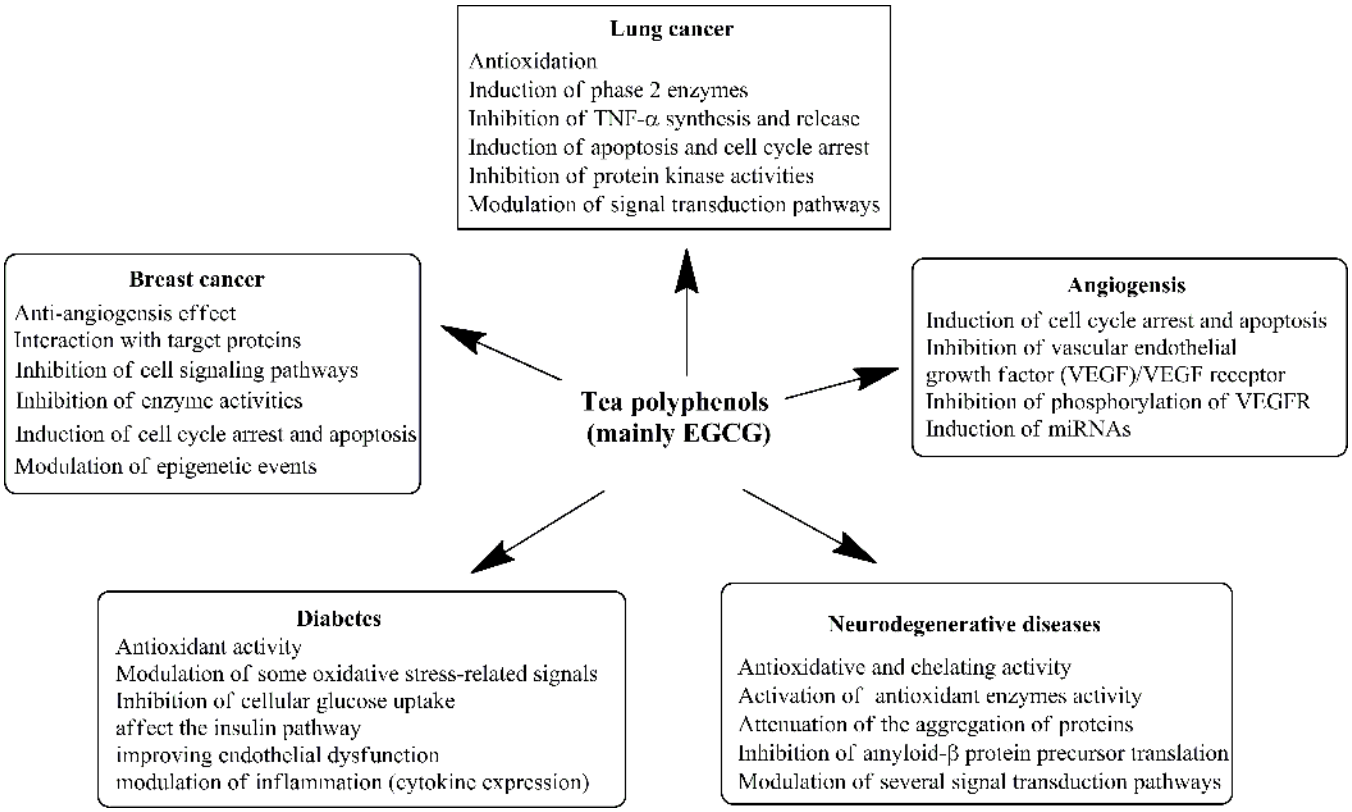

Figure 3.
Biological activities and physiological mechanisms of green tea catechins.

Table 1

Summary on the anticancer effects of green tea polyphenols against prostate cancer in experimental systems.

Models		Compounds used	Concentration	Methods	Results	Conclusion	References
Cell lines	LNCaP CV1 22Rv1 C4–2	EGCG	0–60 μM	*In silico* modeling, MTT assay, transient transfection and reporter assay, fluorescence resonance energy transfer assay, immunohistochemical and RT-PCR analysis	Inhibited cell growth, androgen (AR)-mediated transcriptional activation, AR nuclear translocation and protein expression. ↓ miRNA-21, ↑ miRNA-330.	Antiandrogenic action	[53]
	PC-3 LNCaP	EGCG + quercetin	EGCG 40 μM quercetin 20 μM	Cellular absorption analysis, MTT assay, flow cytometry analysis	Inhibited cell proliferation, induced cell cycle arrest and apoptosis in PC-3 cells, stronger antiproliferative activity in LNCaP cells.	Quercetin enhanced the anticancer effect of EGCG via increasing the cellular uptake and decreasing methylation of EGCG.	[54]
	LNCaP	Tea polyphenols	20–80 mg/mL	Proliferation assay, methylene blue staining and quantification, DNA fragmentation assay, cell death assay, western blot, ChIP assay, cell cycle analysis and apoptosis detection	Promoted apoptosis regardless of p53 status, ↑ FAS through activation of c-jun-N-terminal kinase, ↑ FADD phosphorylation, caspase-8 activation.	Induced prostate cancer cell death by two distinct mechanisms regardless of p53 status.	[55]
	LNCaP PCa DU145 PrEC	Tea polyphenols EGCG	1–10 μg/mL	DNA methyltransferase activity assay, HDAC activity assay, bisulfite treatment and methyl-specific PCR, genomic bisulfite sequencing, western blot, fluorescence immunocytochemistry, ChIP assay.	Caused re-expression of GSTP1, demethylation in the proximal GSTP1 promoter and regions distal to the transcription factor binding sites. ↓ the mRNA and protein levels of MBD1, MBD4 and MeCP2, ↑ acetylated histone H3 and H4, ↓ MBD2 association with Sp1 binding sites.	GTP has dual potential to alter DNA methylation and chromatin modeling	[56]
	LNCaP PrECs	EGCG	100 μM	Western blot	Recapitulated ATM expression and activity, ↓ the fusion transcript appearance.	Therapeutic effect of EGCG in mitigating the exacerbation of the disease with reference to the fusion transcripts.	[57]
	DU145 LNCaP	EGCG	10–80 mg/mL	DNA ladder assay, confocal microscopy analysis, flow cytometry analysis, cell cycle analysis, Western blot.	Caused cell cycle arrest, apoptosis, a dose-dependent increase of p53 in LNCaP cells of induction of cyclin kinase inhibitor WAF1/p21.	Antiproliferative effects against both androgen-sensitive and androgen-insensitive human PCA cells, and this effect is mediated by deregulation in cell cycle and induction of apoptosis.	[58]

Models		Compounds used	Concentration	Methods	Results	Conclusion	References
	KYSE HT-29 PC3	EGCG	5–50 μM	DNA methyltransferase assay, *In Silico* molecular modeling studies, Bisulfite modification and methylation-specific PCR, RT-PCR, Western blot	Caused a reversal of hypermethylation of $p16^{INK4a}$, retinoic acid receptor β, O^6-methylguanine methyltransferase, and human *mutL* homologue 1. Reactivated some methylation-silenced genes.	Inhibited DNMT activity and reactivated methylation-silenced genes in cancer cells.	[59]
	PC-3 LNCaP	Tea polyphenols	10–80 mg/mL	HDAC enzyme activity, RT-PCR, Western blot, ChIP assay, apoptosis analysis	Inhibited class I HDAC enzyme activity, ↑ acetylated histone H3 in total cellular chromatin, ↑ expression of p21/waf1 and Bax at the protein and message levels.	Anticancer effects of GTP may be due, in part, to HDAC inhibition.	[60]
	LNCaP DU145	EGCG	5–40 μg/mL	Cell cycle analysis, apoptosis analysis, morphological analysis, Western blotting, immunoprecipitation	↑ protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, ↓ protein expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6. ↑ the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, ↓the binding of cyclin E toward cdk2.	EGCG caused cell cycle arrest, which may be an irreversible process ultimately leading to apoptotic cell death.	[4]
	LNCaP	EGCG	20–80 μm	TUNEL assay, ELISA, DEVDase assay, reporter assays	Induced stabilization of p53, ↑ its transcriptional activity, its downstream targets p21/ WAF1 and Bax. This altered expression of Bcl-2 family members triggered the activation of initiator caspases 9 and 8.	EGCG induces apoptosis by shifting the balance between pro- and antiapoptotic proteins in favor of apoptosis.	[61]
	LNCaP	EGCG	20–40 μM	MTT assay, fluorescence microscopy analysis, Western blotting, gelatin zymography, invasion assay, Wound healing assay.	Enhanced apoptotic activity in LNCaP cells. ↑ poly(ADP-ribose) polymerase cleavage and modulation of pro- and antiapoptotic Bcl2 family of proteins, regulated death-inducing signaling cascade complex.	EGCG sensitizes TRAIL-resistant LNCaP cells to TRAIL-mediated apoptosis through modulation of intrinsic and extrinsic apoptotic pathways.	[62]
	DU145 LNCaP	EGCG	10–40 mg/mL	Western blotting	↓levels of PI3K and phospho-Akt, ↑ Erk1/2 in both DU145 and LNCaP cells.	Modulation of the constitutive activation of PI3K/Akt and Erk1/2 pathways by EGCG	[63]
	RWPE-1, PC-3	EGCG	0–50 μM	MTS assay, Immunoblot detection	inhibited PC-3 cell proliferation, activation of the extracellular signal-regulated kinase (ERK1/2) pathway	ERK1/2 activation via a MEK-independent, PI3K-dependent signaling pathway is partially responsible for the	[64]

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Models		Compounds used	Concentration	Methods	Results	Conclusion	References
						antiproliferative effects of EGCG.	
Animal experiments	PC-3 C57BL/SV129	EGCG	20 or 100 mM, 100 mg/kg by oral gavage	Reporter gene assays, cell viability assays, RT-PCR assays, promoter analysis, microarray data analysis	Attenuated AP-1 activation, ↓Nrf2-dependent genes, Conserved TFBS signatures were identified in the promoter regions of Nrf2, AP-1, ATF2, and ELK-1.	The anticancer effects was mediated via concerted modulation of Nrf2 and AP-1 pathways in the prostate.	[65]
	LNCaP PC-3 CWR22Rv1 mice	EGCG Tea polyphenols	EGCG (10–40 μM, 0.1% GTP	Cell growth and viability, fluorescence microscopy analysis, Western blotting, ELISA	Inhibited cell growth and induced apoptosis, ↓ peroxisome proliferator activated receptor γ, ↑ tumor growth inhibition, ↓prostate-specific antigen levels, insulin-like growth factor-I levels.	Green tea polyphenols and selective cyclooxygenase-2 inhibitors inhibited the growth of human prostate cancer cells both *in vitro* and *in vivo.*	[66]
	C57BL TRAMP mice	Tea polyphenols	0.1%	Immunoblot analysis, densitometric analysis	↓NF-κB, IKKα, IKKβ, RANK, NIK and STAT-3. ↓levels of transcription factor osteopontin.	Inhibition of osteopontin and NF-κB signaling may contribute to induction of apoptosis.	[47]
	C57BL/6 TRAMP mice	Tea polyphenols	0.1%	Magnetic resonance imaging, Insulin-like growth factor-I and insulin-like growth factor binding protein-3 assay, immunoblotting and apoptosis detection.	Inhibited CaP development. Delayed primary tumor incidence and tumor burden. Inhibitied serum insulin-like growth factor-I and restorated insulin-like growth factor binding protein-3 levels, ↓protein expression of proliferating cell nuclear antigen in the prostate.	GTP inhibited the growth and progression of CaP in TRAMP mice.	[67]
	TRAMP mice	EGCG	0.06%	Apoptosis analysis, immunohistochemistry, immunoblot analyses, ELISA	Inhibited early but not late stage PCa, reduced cell proliferation, induced apoptosis, ↓androgen receptor, insulin-like growth factor-1 (IGF-1), IGF-1 receptor, phospho-extracellular signal-regulated kinases 1 and 2, cyclooxygenase-2 and inducible NO synthase.	EGCG suppressed prostate cancer without toxicity.	[68]
	C57BL/6 TRAMP mice	Tea polyphenols	0.1%	Ultrasound imaging, ELISA kits, immunoblot analysis, histology and immunohistochemical analysis.	Inhibited IGF-I and its downstream targets including phosphatidylinositol 3-kinase, pAkt, and phosphorylated extracellular signal-regulated kinase	Chemopreventive potential of GTP decreases with advancing stage of the disease.	[69]

Table 2

Summary of the listed references on the development of nanoparticle-based delivery systems of green tea polyphenols.

Delivery systems	Drugs	Coating materials	Methods	Advantages	References
Nanomicell	Tea polyphenols	Gelatin, dextran, genipin		Sustained release of tea polyphenol *in vitro*. Comparable or stronger cytotoxicity against MCF-7 cells than free tea polyphenol.	[133]
Nanoliposome	EGCG	Chitosan, soy lecithin, cholesterol		Enhanced stability, sustained release, increased intracellular content in MCF7 cells, induced apoptosis, inhibited proliferation.	[134]
	EGCG	Phospholipid S75, cholesterol, Tween 80		Sustained release, improved stability in simulated intestinal fluid, slowed the degenerations of *in vitro* antioxidant activities of EGCG.	[135]
	EGCG	Carboxymethyl chitosan, hexadecyl dimethylammonium chloride, cholesterol		Good in vitro digestion stability, Sustained release, slowed the degradation of EGCG in simulated intestinal fluid.	[136]
	Tea polyphenol	Phospholipid, cholesterol, Tween 80	Ethanol injection method with dynamic high- pressure microfluidization	Retained antioxidant activities, sustained release property, improved the stability of tea polyphenol in alkaline solution.	[137]
Solid lipid nanoparticles	Green tea extract	Tween 20/80, synperonic PE/F68, phosphatidylcholine	high shear homogenization technique	High antioxidant activity, highly efficient against *E. coli* bacteria.	[138]
	EGCG	Phosphatidylcholine, kolliphor HS15, α-tocopherol acetate		Increased EGCG stability, sustained release, high binding affinity to and uptake by macrophages, increased macrophage EGCG content, and decreased macrophage MCP-1 mRNA levels and protein secretion.	[139]
Nanoemulsion	EGCG	Carrageenan, β-lactoglobulin	High-pressure homogenization	Enhanced *in vitro* anticancer activity.	[140]
	Tea extract	Cholesterol, phytosterol, cetech-3, cetyl phosphate, glycerine	Sprayed through a high-pressure emulsifier	Increased bioavailability and hypocholesterolemic effects *in vivo*.	[141]
	Catechins	Soy protein, oil		Improved the stability, bioaccessibility and permeability of green tea catechins.	[142]
Lipid nanoparticle	EGCG	Glycerol, cationic lipid, Softisan®100, soybean phosphatidylcholine	Multiple emulsion technique	Confirmed the possibility of lipid-based systems for EGCG ocular drug delivery.	[143]
	EGCG	Glycerol, cationic lipid, Softisan®100, soybean phosphatidylcholine	Double-emulsion technique	Prolonged EGCG release, ability to reach both anterior and posterior segment of the eye, safety and non-irritant nature.	[144]

Delivery systems	Drugs	Coating materials	Methods	Advantages	References
Multiple emulsion	Catechin, curcumin	Olive oil, polyglycerol polyricinoleate, gelatin	Two-step emulsification method	Co-loading of curcumin and catechin did not have adverse effects on either compound's stability or bioaccessibility.	[145]
Polyelectrolyte complex nanoparticles	EGCG	Polylactic acid, polyethylene glycol		Biological effectiveness with over 10-fold dose advantage for exerting its proapoptotic and angiogenesis inhibitory effects.	[130]
	EGCG	Gelatin, glutaraldehyde	two-step desolvation method	Blocked hepatocyte growth factor (HGF)-induced intracellular signaling in MBA-MD-231 cells.	[146]
	Tea catechins	Chitosan, poly(γ-glutamic acid)	Polyelectrolyte self-assembly method	pH-responsive, retained the activity of tea catechins, increased the paracellular transport.	[147]
	EGCG	Chitosan, polyaspartic acid	Polyelectrolyte self-assembly method	pH-responsive, improved effectiveness of EGCG against rabbit atherosclerosis.	[148]
Hydrogel nanoparticles	Tea catechins	Chitosan, sodium tripolyphosphate	Ionic gelation	Controlled release of tea catechins.	[149]
	Tea polyphenols	Carboxymethyl chitosan, chitosan hydrochloride	Ionic gelation	Sustained release, antitumor activities towards HepG2 cancer cells.	[150]
	EGCG	Chitosan, pentasodium tripolyphosphate hexahydrate	Ionic gelation	a slow release in acidic pH and faster release in simulated intestinal fluid, improved therapeutic benefit against PCa tumors.	[151]
	EGCG	Chitosan, pentasodium tripolyphosphate hexahydrate	Ionic gelation	Induction apoptosis, inhibited cell cycle.	[152]
	Green tea extract	Chitosan, sodium tripolyphosphate	Ionic gelation	Enhanced uptake in HepG2 cells, removed the extracellular collagen caused by CCl4 in the hepatic fibrosis rat liver.	[153]
Polymeric nanoparticles	EGCG, paclitaxel	Poly-L-lactide-co-glycolic acid, casein	Emulsion–precipitation method	Sustained and sequential release of both the drugs in plasma, prolonged circulation, enhanced availability, sensitized Ptx resistant MDA-MB-231 cells to Ptx, induced apoptosis, inhibited NF-κB activation, downregulated the key genes related to angiogenesis, tumor metastasis and survival.	[154,155]
	EGCG	Poly-L-lactide-co-glycolic acid, PEG polymer, pseudomimetic dipeptide		Selective *in vitro* efficacy against PSMA-expressing PCa cells.	[156]