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ORIGINAL PAPER

Green tea catechins: a fresh flavor to anticancer therapy

Yang Yu · Yuan Deng · Bang-min Lu · Yong-xi Liu · Jian Li · Jin-ku Bao

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Abstract Green tea catechins have been extensively studied for their cancer preventive effects. Accumulating evidence has shown that green tea catechins, like (−)-epigallocatechin-3-gallate, have strong anti-oxidant activity and affect several signal transduction pathways relevant to cancer development. Here, we review the biological properties of green tea catechins and the molecular mechanisms of their anticancer effects, including the suppression of cancer cell proliferation, induction of apoptosis, and inhibition of tumor metastasis and angiogenesis. We summarize the efficacy of a single catechin and the synergetic effects of multiple catechins. We also discuss the enhanced anticancer effects of green tea catechins when they are combined with anticancer drugs. The information present in this review might promote the development of strategy for the co-administration of green tea catechins with other anticancer drugs to increase the potency of currently available anticancer medicine. This new strategy should in turn lower the cytotoxicity and cost of anticancer treatment.

Keywords Green tea catechins · EGCG · Anticancer mechanisms · Synergetic effects · Therapeutic agents

Abbreviations

ACE	Angiotensin-converting enzyme
AP-1	Activator protein-1
Apaf-1	Apoptosis protease activating factor-1
ARE	Antioxidant response elements
Bad	Bcl2 antagonist of cell death
Bax	Bcl-2 associated x protein
Bcl-2	B cell lymphoma-2
Bid	BH3-interacting domain death agonist
CDKs	Cyclin-dependent kinases
CIs	Confidence intervals
CLL	Chronic lymphocytic leukemia
COX-2	Cyclooxygenase-2
CSCs	Cancer stem cells
Cyt-c	Cytochrome-c
EC	(−)-Epicatechin
ECG	(−)-Epicatechin-3-gallate
EGC	(−)-Epigallocatechin
EGCG	(−)-Epigallocatechin-3-gallate
ERK	Extracellular-regulated protein kinase
FADD	Fas-associated protein with death domain
FOXO	Forkhead box protein O
GCG	(−)-Gallocatechin gallate
GST	Glutathione *S*-transferases
HBP1	HMG box-containing protein 1
HGF/SF	Hepatocyte growth factor/scatter factor
Htert	Human telomerase reverse transcriptase
IAP	Inhibitor of apoptosis proteins
IGF	Insulin-like growth factor
IGFBP3	Insulin-like growth factor binding protein 3
IKK	Inhibitor of nuclear factor kappa-B kinase

Yang Yu and Yuan Deng have contributed equally to this work.

Y. Yu · Y. Deng · B. Lu · Y. Liu · J. Bao (✉)
School of Life Sciences and Key Laboratory of Bio-resources, Ministry of Education, Sichuan University, Chengdu 610064, China
e-mail: baojinku@scu.edu.cn

J. Li (✉)
Department of Biological Sciences, Ohio University, Athens, OH 45701, USA
e-mail: jl147107@ohio.edu

IL-6	Interleukin 6
IL-8	Interleukin 8
iNOS	Inducible nitric oxide synthase
JNK	c-jun N-terminal kinase
MAPK	Mitogen activated protein kinases
mdm2	Murine double mimute 2
MEK3	Mitogen-activated protein kinase 3
MEKK1	Mitogen-activated protein kinase kinase 1
MMPs	Matrix metallo proteinases
MT1-MMP	Membrane-type 1 matrix metalloproteinase
NF-κB	Nuclear factor-κB
nHDFs	Neonatal human dermal fibroblasts
Nrf2	Nuclear factor erythroid 2-related factor
OR	Odds ratio
PCNA	Proliferating cell nuclear antigen
PI3K	Phosphoinositide 3-kinases
Poly E	Polyphenon E
PRAP	Poly(ADP-ribose) polymerase
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinases
RR	Relative risk
SOD	Superoxide dismutase
Stat3	Signal transducer and activator of transcription 3
TCOP	Tea catechin oxy-polymers
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumor necrosis factor α
TRAIL	TNF-related apoptosis-inducing ligand
VEGF	Vascular endothelial growth factor
uPA	Urokinase-type plasminogen activator
XIAP	X-linked inhibitor of apoptosis protein

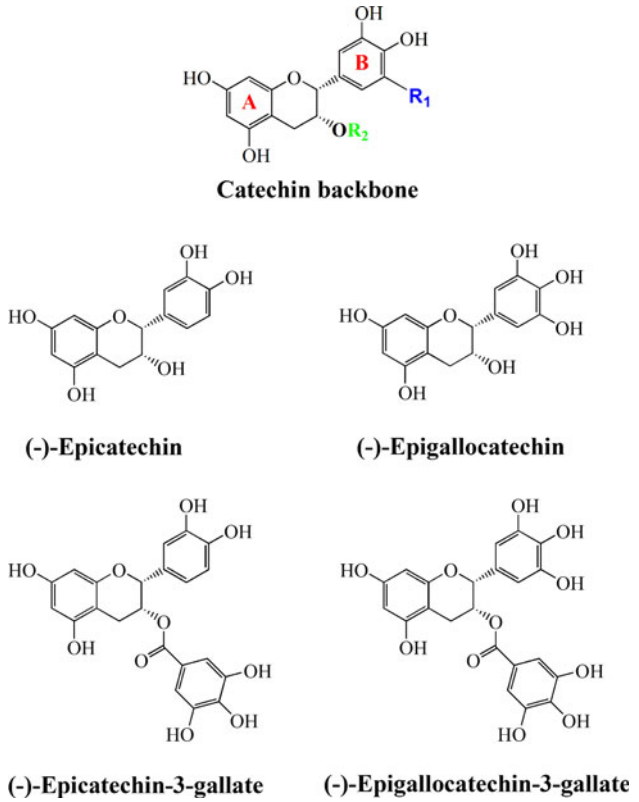

Fig. 1 Structure of the four main catechins. Polyphenolic structure is a common feature of green tea catechins. The four main catechins, including EGCG, EGC, ECG and EC, have the same catechin backbone, which is featured by a di-hydroxyl or tri-hydroxyl substitutions on the B ring and a *m*-5,7-dihydroxyl substitutions on the A ring

Introduction

Green tea is derived from the dried leaves of *Camellia sinensis* and is one of the most commonly consumed beverages all around the world. Green tea has been studied extensively for its cancer preventive effects. As the second most popular beverage in the world, these studies have great impact on the combat against cancer. Accumulated data from different disease models has verified the antitumor property of green tea extracts, especially green tea catechins. Green tea extracts contain primarily catechins (monomeric flavonoids) (~70 %), minor flavanols (mainly quercetin, kaemfferol, myricetin, and their glycosides) (~10 %), and polymeric flavonoids (~20 %) [1]. The major catechins in green tea are (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG) [2]. Of note, EGCG is the major catechin that accounts for 50 to 80 % of the total catechins in green tea [3]. EGCG is also the most potent compound in green tea extract, because it affects a range of signaling and metabolic pathways that may result in cancer cell growth inhibition, apoptosis, and inhibition of tumor angiogenesis and metastasis [2, 4, 5].

Polyphenolic structure is a common feature of green tea catechins. The four main catechins, including EGCG, EGC, ECG and EC, have the same catechin backbone, which is featured by a di-hydroxyl or tri-hydroxyl substitutions on the B ring and a *m*-5,7-dihydroxyl substitutions on the A ring [2] (Fig. 1). The polyphenolic structure makes catechins strong antioxidants and metal ion chelators (discussed below). The polyphenolic structure of green tea catechins makes them good donors for hydrogen bonding, which increases the water solubility of EGCG and contributes to EGCG's high affinity to proteins and nucleic acids [6].

In this review, we compile the recent the experimental findings and results from human epidemiological studies on the cancer prevention activity of green tea catechins. We compare the differences in the biological activity of various green tea catechins and discuss their synergetic effect on

cancer prevention and inhibition. We also discuss the molecular mechanism of green tea catechins anticancer activity, including the influences on the key events relevant to cancer initiation and progression. Based on their synergistic effects with other anticancer compounds, we discuss and propose the potentiality of green tea catechins as an effective adjuvants to cancer treatment.

Differences in biological activities and synergetic effects

Each catechin monomer has its unique bioactivity, bioavailability, metabolism pattern, and physiological pharmacokinetic [7]. It has been reported that tea polyphenols inhibit the growth of human lung cancer cell line PC-9, with the order of potency: EGCG > ECG > EGC ≫ EC [8]. In study done with human prostate cancer DU145 cell line, the efficiencies of different green tea catechins in growth suppression, apoptosis induction, reactive oxygen species (ROS) formation and mitochondrial depolarization are similar, i.e. ECG > EGCG > EGC > EC [9]. Differences in biological activity may be related to different chemical structures of green tea catechins. For instance, gallate moiety is present in ECG and EGCG but not in EGC and EC, while the 5-hydroxyl group is in EGC but not in EC.

Catechins from green tea extracts exert synergistic or additive effects on cancer prevention. EC has weak or no activity when used alone. Upon combination with EGCG, synergistic inhibition of cell growth and induction of apoptosis were observed in HT29 cells, colon cancer cells, lung cancer cells etc. [10, 11]. Masahito Shimizu et al. reported the effect of increasing concentrations of EC (1–100 μg/mL) in combination with 0–10 μg/mL of EGCG on induction of apoptosis. When EC was used alone, significant increase in apoptosis was only observed at the concentration of 100, and 10 μg/mL of EGCG caused statistically insignificant increase in apoptosis. However, the combination of as little as 1 μg/mL of EC and 10 μg/mL of EGCG caused a two to three folds of increase in apoptosis [12]. Co-treatment with ECG and EC, EGC and EC synergistically induced apoptosis in human lung cancer cell line PC-9, mediated by enhanced incorporation of tea polyphenols into the cells. Studies also showed a synergistic effects by co-treatment with EC and other tea polyphenols on apoptosis, growth inhibition, and tumor necrosis factor α (TNF-α) release in PC-9 Cells [8]. The potency order observed was: EGCG + EC > ECG + EC > EGC + EC.

Polyphenon E (Poly E), a standardized and well-characterized decaffeinated extract of green tea, contains about 60 % EGCG, 7 % EC, 12 % EGC, 1 % ECG, and 2 % GCG [13]. Both Poly E and EGCG have bioactivities including antioxidant modulation of enzyme systems form metabolizing chemical carcinogens, inhibition of nitrosylation reactions, scavenging of activated metabolites of chemical carcinogens, inhibition of tumor promotion, and induction of apoptosis [14]. In terms of cancer prevention effect, Shimizu et al. [12] reported that Poly E was better than EGCG alone, because the mixture of catechins exert synergistic cancer cell growth inhibitory effects. EGCG is the most powerful active compound in Poly E. However, pure EGCG may be ineffective when it is administered by diet, because of its instability and rapid degradation. The presence of other tea catechins might affect absorption, biological activity, and other properties of EGCG in Poly E and it has been shown that there are synergistic effects among EGCG, EGC, ECG and EC [15]. For example, even though EGCG only constitutes 60 % of Poly E, same concentration of Poly E and EGCG had comparable cancer cell growth inhibitory effect, which was due to the synergistic effects of other component in Poly E [12].

Catechins: from bench to clinic

Different catechins inhibit cancer cell growth by targeting different pathways. Understanding the specific mechanism has become important and valuable. Until now, cancer-preventive effect of tea polyphenols, especially EGCG, has been demonstrated by epidemiological, preclinical, and clinical studies. In cell lines, studies have shown that green tea catechins can affect a range of signaling and metabolic pathways [2]. These molecular events may result in cancer cell growth inhibition, apoptosis, and inhibition of invasion, angiogenesis and metastasis [2, 16]. Inhibition of tumorigenesis by tea extracts and tea polyphenols has also been demonstrated in different animal models, including those for cancer of the skin, lung, esophagus, stomach, colon, bladder, liver, pancrease, prostate, and mammary glands and so on [2].

Although the epidemiological studies of green tea have been reported in many literatures, the preventive effect of tea consumption on the risk of human cancer is inconclusive [17]. This is mainly because of the lack of accuracy in assessing tea consumption, the different lifestyles associated with tea consumption, the individual differences in genetic polymorphism, variation of etiological factors for cancer in different populations, and some other confounding factors. Only when these factors can be effectively managed, a clearer relationship between tea consumption and the risk of cancer can be accurately evaluated in epidemiological studies [2].

The quantity and ways of tea consumption are closely related to its effect on cancer development. The lack of a preventive effect of tea consumption against tumor formation observed in many studies is likely due to the low quantity of tea consumed. For example, a studies found a reduced risk of stomach cancer with intake of green tea

(relative risk (RR)/odds ratio (OR) = 0.86, 95 % confidence interval (CI) 0.74–1.00) through a meta-analysis [18]. It was also reported that there were significant correlation between high/medium/low green tea consumption and non-drinking risk of esophageal cancer among female (High: RR/OR = 0.32, 95 % CI 0.10–0.54. Medium: RR/OR = 0.43, 95 % CI 0.21–0.66. Low: RR/OR = 0.45, 95 % CI 0.10–0.79) [19]. In a case control study on esophageal cancer in the south China, a significantly increased risk of esophageal cancer was observed in participants who drank tea at high temperature, especially among drinkers and smokers [20].

Genetic polymorphism in the population may also affect the relationship between tea consumption and the risk of cancer. For example, a nested case control study of Singapore Chinese found that the intaking frequency of green tea was associated with the risk of breast cancer (P for trend = 0.039) among women with high-activity angiotensin-converting enzyme (ACE) genotype. However, there was no association between intake frequencies of green tea and risk of breast cancer among those women with low-activity ACE genotype [21]. Another study evaluated the effects of smoking, green tea consumption, as well as *IGF1*, *IGF2*, and IGF- binding protein 3 (*IGFBP3)* polymorphisms, on lung cancer risk. An elevated risk was observed in smokers who never drank green tea, as compared to smokers who drank green tea more than one cup per day (OR = 13.16, 95 % CI = 2.96–58.51). Interaction between smoking and green tea consumption on lung cancer risk was also observed. Besides, among green tea drinkers who drank more than one cup per day, *IGF1* $(CA)_{19}/(CA)_{19}$ and $(CA)_{19}/X$ genotypes carriers had a significantly reduced risk of lung cancer (OR = 0.06, 95 % CI = 0.01–0.44) compared with GF1 X/X carriers [22].

In recent years, clinical trials are ongoing, which use systematic and phased approach along with pre-defined protocols to investigate the efficacy of polyphenol or EGCG in cancer prevention. Phase I clinical trials with green tea catechins study the toxicity and bioavailability of green tea [23]. In one study, Sherry Chow and colleagues found that it was safe for healthy individuals to take green tea polyphenol products as much as EGCG contained in 8-16 cups of green tea once a day or in divided doses twice a day for 4 weeks. But the oral bioavailability of tea catechins was low in humans [24]. Their lab also reported that greater oral bioavailability of free catechins can be achieved by taking the Polyphenon E capsules after overnight fast. Polyphenon E containing 800 mg of epigallocatechin gallate was well-tolerated when taken under the fasting condition. The same dosage is also expected to give optimal biological effects of tea catechins [15].

The current Phase II clinical trial was designed to collect preclinical and epidemiologic data and evaluate patients' proclivities of restoring to green tea catechin as an alternative medicine [25]. A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma found that green tea carried limited antineoplastic activity, as defined by a decline in PSA levels [26]. Results from Phase IIa chemoprevention trial of green tea polyphenols carried out in high-risk individuals have demonstrated the relative safety of green tea catechins consumption in human subjects [25, 27]. However, the specific mechanism of catechins has not been solved completely and the low bioavailability of catechins in human body is still the major obstacle for clinical application. Moreover, there are some other factors also limit the application of green tea catechins [28, 29]. Completion of phase II clinical trials will provide critical information and will move the study forward to phase III clinical trials leading to the development of green tea polyphenols based chemopreventive agent for cancer [30] (Fig. 2) (some latest information about the clinical studies can be found in the website: www.Clinicaltrials.gov).

Molecular mechanisms of green tea catechins in cancer prevention

The anticancer effects of green tea catechins have been demonstrated both in vitro and in vivo [2, 5, 31–33]. The molecular mechanisms involved are complicated. These various mechanisms can be classified into three categories: the antioxidant/pro-oxidant activity of catechins, the influence on enzyme activities, and the modulation of specific molecular targets or signaling pathways relevant to tumor development. In most cases, green tea catechins may affect one or more targets (Table 1), and they have synergetic effects when combined with other anticancer drugs. Importantly, the selective effects observed in many studies indicate that green tea catechins may inhibit cancer development without affecting normal cells [34]. The bioavailability of catechins limits their biological activity in vivo. We use EGCG as an example to illustrate the major anticancer mechanisms of green tea catechins, including their impacts on multiple molecular targets and important cell signaling pathways.

Antioxidant/pro-oxidant activity in cancer prevention

Direct antioxidant effects

Numerous studies have demonstrated that tea catechins and polyphenols are effective scavengers of ROS, which can cause oxidant DNA damage and promote carcinogenesis

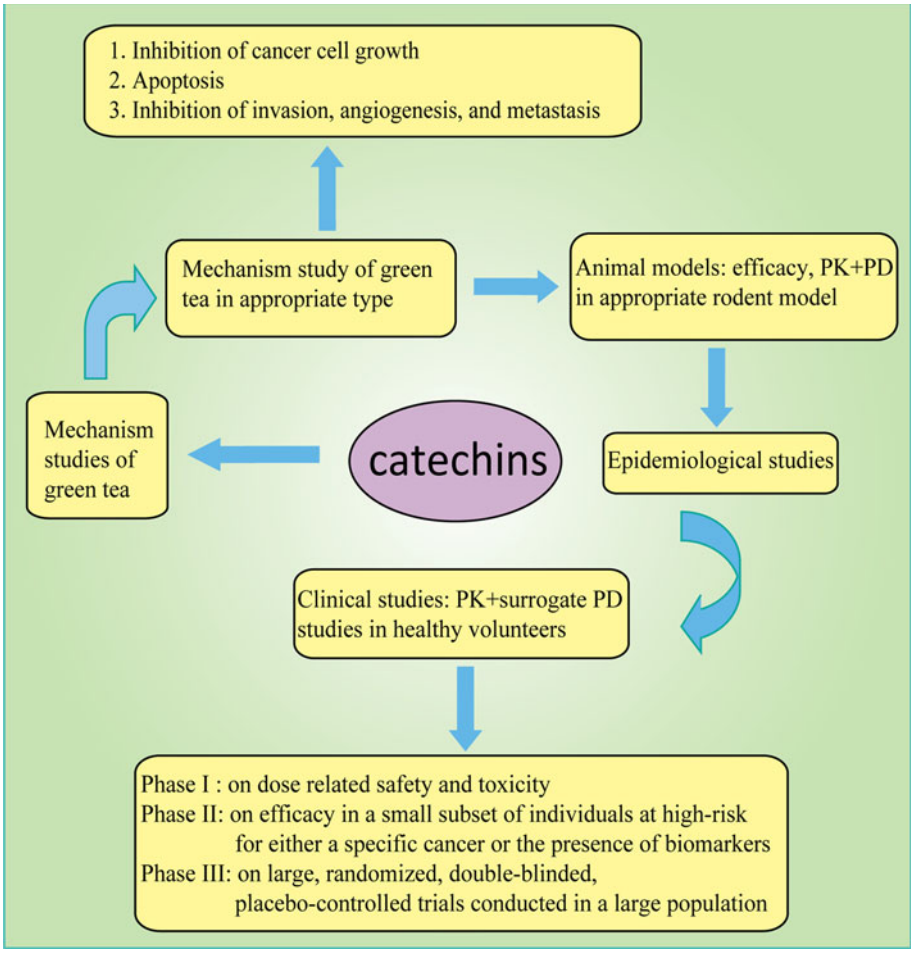

Fig. 2 The procedure of catechins: from bench to clinic. *PK* pharmacokinetics, *PD* pharmacodynamics. Epidemiological studies indicate that the worldwide incidence and mortality of cancer patients who consumed green tea differ greatly [136–139]. The phase I studies of EGCG almost had been completed. Phase I clinical trials with green tea catechins studying on the bioavailability and toxicity of green tea in humans [140, 141]. A large number of clinical phase II experiments are ongoing and some of them have been successful [25–27, 142, 143]. The phase III clinical studies with catechins or EGCG are studying onlarge, randomized, double-blinded, placebo-controlled trails that were conducted in a large population [144]

[7, 35, 36]. This antioxidant activity has been found both in vitro and in vivo. For example, treatment with EGCG can significantly reduce ROS generation and protect human bladder urothelial cells against H_2O_2-induced acute oxidative damage [36]. Another study found that administration of green tea catechins (500 mg/day) to healthy individuals for 4 weeks caused an 18 % decrease in plasma oxidized low density lipoprotein compared to controls [37]. Tea catechin oxy-polymers, product of tea catechins oxidation by H_2O_2, has stronger free radical scavenging ability [7]. Additionally, metal chelation is another antioxidant mechanism of catechins, such as the inhibition of copper-mediated LDL oxidation in vitro [36].

Inhibition of redox-sensitive transcription factors and "pro-oxidant" enzymes

Inhibiting the activation of the redox-sensitive transcription factors, like nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), is an essential mechanism of the antioxidant function of green tea catechins [38, 39]. NF-κB can be stimulated by ROS and then activates the transcription of multiple inflammatory genes [40]. Studies have found that green tea catechins can interrupt the translocation of NF-κB into the nucleus via inhibiting the phosphorylation of IκB, an NF-κB inhibitor [7]. The inhibition of "pro-oxidant" enzymes is another antioxidant mechanism of green tea catechins. Inducible nitric oxide synthase (iNOS), which could be stimulated by endotoxins or cytokines, can synthesize large amounts of nitric oxide and result in damage of DNA and proteins. Green tea catechins, especially EGCG, can inhibit the lipopolysaccharide-induced iNOS gene expression through the inhibition of NF-κB activation [36].

Phase II and antioxidant enzymes

Phase II detoxification enzyme is a group of enzymes that can promote the excretion of potentially toxic or carcinogenic chemicals. For example, glutathione *S*-transferases (GST), a family of phase II enzymes, can catalyze the conjugation of glutathione to electrophiles, reducing their ability to react with damage nucleic acids and proteins. Previous studies have found that feeding mice with green tea polyphenols in their drinking water for up to 30 days

Table 1 Multiple targets of EGCG

Anticancer effects	Molecular targets	References
1.Antioxidants	ROS; metal chelation; NF-κB; iNOS; H_2O_2; GST; SOD; Nrf2;	[5, 35, 43, 105, 122]
2.Cell cycle arrest	Cyclin D, cyclin E; CDK1; CDK2; CDK4; CDK6; Ras; PCNA; p16; p18; p21; p27; p53; mdm2; hTERT	[5, 52, 54, 55, 57, 123]
3.Inhibition of proliferation	PI3 K; AKT; ERK; EGFR; c-fos; AP-1; NF-κB; IKK; COX-2; JNK; Ras; MEKK1; MEK3; p38; TNF- α;	[31, 35, 45, 58, 74, 79, 82, 98, 124–126]
4.Induction of apoptosis	ROS; caspase-3; caspase-8; caspase-9; cyt-c; Bad; Bcl-2; Bcl-xL; Bid; c-myc; c-IAP1; c-IAP2; survivin; XIAP; Akt;	[10, 34, 79, 80, 82, 83, 112, 122, 126, 127]
5.Inhibition of metastasis	MMP-2; MMP-9; uPA; TIMP-2; MT1-MMP; Met; cGMP; urokinase; NO; ephrin-A1; ERK-1/2;	[82, 88, 125]
6.Inhibition of angiogenesis	VEGFR; VEGF; ErbB2; ErbB3; FOXO; ERK-1/2; Ephrin-A1; WARS; Wnt; PI3K; IL-6; IL-8; Stat3; MT1-MMP; MMP-2;	[82, 86, 87, 94, 97, 128]

could significantly increase GST activity in liver and small intestine [41]. The induction of phase II detoxification enzymes by EGCG are mainly regulated by nuclear factor erythroid 2-related factor (Nrf2), a redox-sensitive transcription factor binding to the cis–acting regulatory elements called antioxidant response elements (ARE) to start antioxidant enzymes gene expression [42]. Recent studies have demonstrated that green tea catechins or EGCG can upregulate antioxidant enzymes, such as superoxide dismutase (SOD) and catalase via the Nrf2–ARE signaling [43].

Pro-oxidant activities

Conversely, studies have demonstrated that EGCG are unstable in cell culture and will be oxidized to generate ROS [7]. When HT29 cells are exposed to McCoy's 5A medium, treatment with 50 μM of EGCG results in up to 23 μM of H_2O_2 and this may promote apoptosis [35]. However, this auto-oxidation may not happen in vivo due to the presence of some more active anti-oxidative agents such as SOD, glutathione peroxidase, glutathione, and ascorbic acid [35]. The pro-oxidant effects of EGCG in vivo needs to be further investigated.

Inhibition of cancer cell proliferation

Unrestricted proliferation, which is mainly due to loss of cell cycle control and disregulated growth signaling pathways (Fig. 3), allows cancer cells to outgrow and form tumors [44]. The anti-proliferation potential of EGCG against various cancer types has been validated in cell cultures, animal tumor bioassay systems, and human epidemiologic studies [45–47].

Induction of cell cycle arrest

Cell cycle progression consists four distinct phases, G1, S, G2, and M. It is timely regulated by cyclin-dependent kinases (CDKs) and their cyclin subunits at the G1/S and G2/M checkpoints [47–49]. The G1/S checkpoint is regulated by CDK4-cyclin D, CDK6-cyclin D, and CDK2-cyclin E [50]. CDK2- cyclin A controls the S-phase while the G2/M transition is regulated by CDK1 in combination with cyclins A and B. INK4 and CIP/KIP families, including p21cip1/WAF1, p27kip1, and p57kip2, inhibit a broad range of CDKs [51]. Studies done with cell culture have shown that EGCG can suppress tumor growth by blocking cell cycle progression and altering the expression level of cell cycle regulatory proteins, such as cyclin D1, cyclin E, CDKs, p21/WAF1/CIP1 and p27/KIP1 as well as causing Rb hypophosphorylation [45, 51–54].

EGCG can affect the cell cycle at low concentration in neonatal human dermal fibroblasts (nHDFs) [55]. At the concentration of 200 μM, EGCG slightly reduced the proportion of cells in S and G2/M phases, whereas the proportion of cells in G0/G1 phase was found to be increased. At the concentration greater than 400 μM, cells could not enter the S phase. EGCG was found to translocate from cytoplasm into the nucleus of nHDFs and data has shown that some cell cycle-related genes, such as cyclin A2, cyclin B, and CDK1, were significantly down-regulated in response to EGCG treatment [55], suggesting that EGCG might induce cell cycle arrest at the transition between G1 and S phase.

In colorectal cancer, oncogenic Ras mutation is a hallmark, which contributed to the neoplastic transformation of intestinal epithelial cells [53]. Ras proteins are membrane-bound GDP/GTP-regulated switch molecules that connect signal transduction from cell membrane to nucleus.

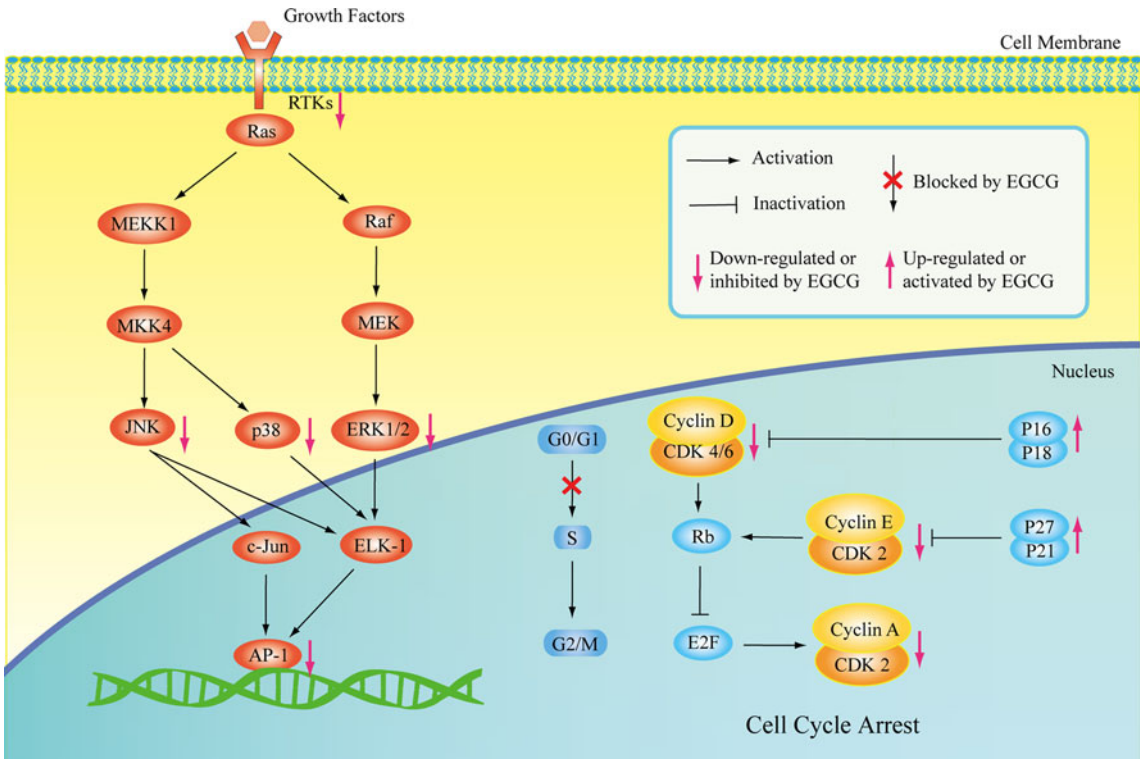

Fig. 3 Cell cycle arrest and growth inhibition. EGCG, major catechin from green tea, can affect the activities of multiple proteins involved in cell cycle progression. EGCG blocks the binding of EGF to its receptor. Factors involved in cell growth signaling pathway, including RTKs, MEK, ERK1/2, JNK, p38, and ELK-1 were down regulated by EGCG treatment. EGCG blocks the transition from G0/G1 to S phase. The activity of Cyclin D, CDK4/6, Cyclin E, CDK2, Cyclin A, and CDK2 are downregulated by EGCG. EGCG upregulates the activity of p16, p18, p21, and p27

Oncogenic Ras mutations result in constitutive activation of this small GTPase, which sequentially activates multiple downstream targets [56]. Green tea catechins or EGCG can inhibit Ras-induced transformation in intestinal epithelial cells. EGCG could inhibit cyclin D1 expression in colorectal cancer cell line, sequentially suppress cell proliferation induced by oncogenic Ras and block cell cycle transition at G1 phase [53]. The presence of oncogenic Ras accelerated the G1/S transition and treatment with EGCG led to a dose-dependent decrease of oncogenic Ras expression by cyclin D in the S and G2/M phases of the cell cycle [56].

Green tea catechins also have positive effect on prostate cancer prevention through cell cycle arrest at G0/G1 phase. Treatment with EGCG can cause the up-regulation of WAF1/p21, KIP1/p27, INK4a/p16 and INK4c/p18 and the down- regulation of cyclin D1, cyclin E, CDK2, CDK4, and CDK6 in prostate cancer cells (LNCaP and DU145) [45, 52, 54]. This cell cycle arrest is mainly due to the increased binding of cyclin D1 to WAF1/p21 and KIP1/p27, and the decreased binding of cyclin E to CDK2 [57]. Similar conclusions were found in breast cancer MDA-MB-231 cells. The amount of cells at G1 phase increased when treated with high concentrations (>50 μg/mL) of catechins, and analysis from western blot illustrated the decreased expression of cyclin D, cyclin E, CDK 4, CDK 1 and PCNA [45]. At the concentration of 30 μM, EGCG can inhibit the activity of CDK2 and CDK4 and trigger the cell cycle arrest in the G0/G1 phase in MCF-7 breast cancer lines [45]. In vascular smooth muscle cells (VSMC), studies show that EGCG also have negative effect in TNF-induced VSMC carcinogenesis by increasing the expression of p21/WAF1 [52]. It was detected that the activity of cell cycle regulator cyclin D1/CDK4 and cyclin E/CDK2 was inhibited by EGCG, which was thought to be a significant mechanism of the proliferation inhibition in VSMC [52].

Another study indicates that EGCG could inhibit T cell proliferation at physiologically relevant concentrations of 2.5–10 μM without induction of cytotoxicity or apoptosis [58]. At low concentration, EGCG makes T cells mainly remain in G0–G1 phase and its supplementation result in lower IL-2 receptor expression and higher IL-2 accumulation, suggesting an impeded IL-2/IL-2 receptor signaling [58]. In addition, study shows that green tea catechins can block the cell cycle in human colon carcinoma LoVo cell lines [54]. After catechins treatment for 24 h, EGCG, EGC, and ECG arrested the cell cycle progression at G1 phase,

which resulted in a decrease in the proportion of cells in S and M/G2 phases and a concomitant increase in G1 phase. EC resulted in S phase arrest [54].

Human telomerase reverse transcriptase (hTERT) is a critical element for cellular proliferation. One study also shows that at concentration of 10–30 μM, EGCG and EGC treatment are able to repress mRNA expression and promote activity of hTERT in carcinoma cells in a dose-dependent manner [59]. Another study indicate that EGCG covalently modifies the telomerase activity by the galloyl radical which formed by the auto-oxidation of EGCG [46].

Modulation of growth signaling pathways

EGCG has been shown to modulate the activities of various receptor tyrosine kinases and multiple signal transduction pathways that were related to cancer cell proliferation. Recently, it is reported that EGCG has multiple targets in various cancer cells. Importantly, EGCG is capable of selectively inhibiting cell proliferation without adversely affecting normal cells [34]. EGCG can affect the proliferation and differentiation of human eosinophilc leukemic cell line [60], making them to differentiate into mature, eosinophil-like cells and thus significantly inhibiting the proliferation of EoL-1 cells in a dose-dependent manner [60]. The mechanisms of EGCG in inhibiting proliferation are important for its potential applications in cancer prevention and treatment. Here we discuss the effects of EGCG on some signal transduction pathways related to the cancer cell proliferation.

Inhibition of mitogen activated protein (MAP) kinases and AP-1

MAPKs, including ERK, JNK, and p38, are one group of the critical targets of EGCG for cancer prevention and therapy [61]. Activation of the MAPK pathways may cause the induction of phase II detoxifying enzymes and activation of a variety of transcription factors, such as ELK and c-Jun, a component of AP-1 [61]. Thus, a series of cascade reaction may lead to changes in the expression of genes that play critical roles in cancer cell proliferation [62]. It has been reported that EGCG has the potential to inhibit oxidative stress-mediated phosphorylation of MAPK signaling pathways [32]. EGCG inhibits the phosphorylation of ERK1/2 and suppresses the activity of p38 MAPK in human fibrosarcoma HT1080 cells [63]. EGCG can also inhibit the phosphorylation of JNK [64].

AP-1 transcription factor is a heterodimer protein complex composed Jun and Fos, which are members of the basic region leucine zipper protein superfamily, activating transcription factor proteins [65]. High AP-1 activity contributes to the tumor promotion and progression of various types of cancers [32]. EGCG has been shown to effectively inhibit arsenite-induced AP-1 transcription activity and AP-1 DNA binding activity [39].

Inhibition of epidermal growth factor receptor (EGFR)-mediated pathways

EGFR (ErbB-1, HER1 in humans) is a cell surface glycoprotein receptor belonging to the receptor tyrosine kinases (RTK) superfamily, which includes EGFR (erbB1), HER2 (neu/erbB2), HER3 (erbB3), and HER4 (erbB4). Overexpression of EGFR produces a neoplastic phenotype in tumor cells [5]. Therefore, the blockage of EGFR signaling pathway may lead to apoptosis in cancer cells. Recently, EGCG has been shown to inhibit the activation of the EGFR, HER2, and multiple downstream signaling pathways in colon cancer cell lines [5]. EGCG inhibits the binding of EGF to EGFR and the subsequent dimerization and activation of the EGFR by altering membrane organization [62]. EGCG inhibits EGFR signaling pathway, most likely through the direct inhibition of ERK1/2 and Akt kinases, suggesting that blocking the EGFR signaling by EGCG would potentially inhibit both cancer cell proliferation and angiogenesis [66]. Besides, it has been reported that EGCG can blind to a specific metastasis associated 67 kDa laminin receptor that is expressed in a variety of tumor cells [67]. Using a subtraction cloning strategy involving cDNA libraries constructed from cells treated or untreated with all *trans*-retinoic acid, it was observed that the anticancer action of EGCG was mediated by laminin receptor that allowed EGCG to bind to the cell surface [32].

Inhibition of Insulin-like growth factor (IGF)-I mediated signal transduction pathway

The IGF/IGF-1R system, which includes the ligands IGF-1/2, their receptor IGF-1R, and the ligand controlling proteins IGFBPs, plays an important role in regulating cell proliferation, differentiation and apoptosis [68]. IGF could be a target for cancer chemoprevention because the increased levels of IGF-I is associated with an increased risk of cancer. It has been shown that the binding of free IGFs to IGF-I receptor results in auto-phosphorylation of intra-molecular receptor and phosphorylation of critical downstream targets [68]. This leads to the activation of several signaling pathways, including the PI3K/Akt pathway and the Ras/MAPK pathway, thus inducing activation of specific genes involved in DNA synthesis and cell proliferation [32]. EGCG can result in substantial reduction of IGF-I level and increase of IGFBP-3 level in TRAMP mice [62]. Therefore, targeting the IGF-I signaling pathway by EGCG might be an effective strategy for cancer treatment.

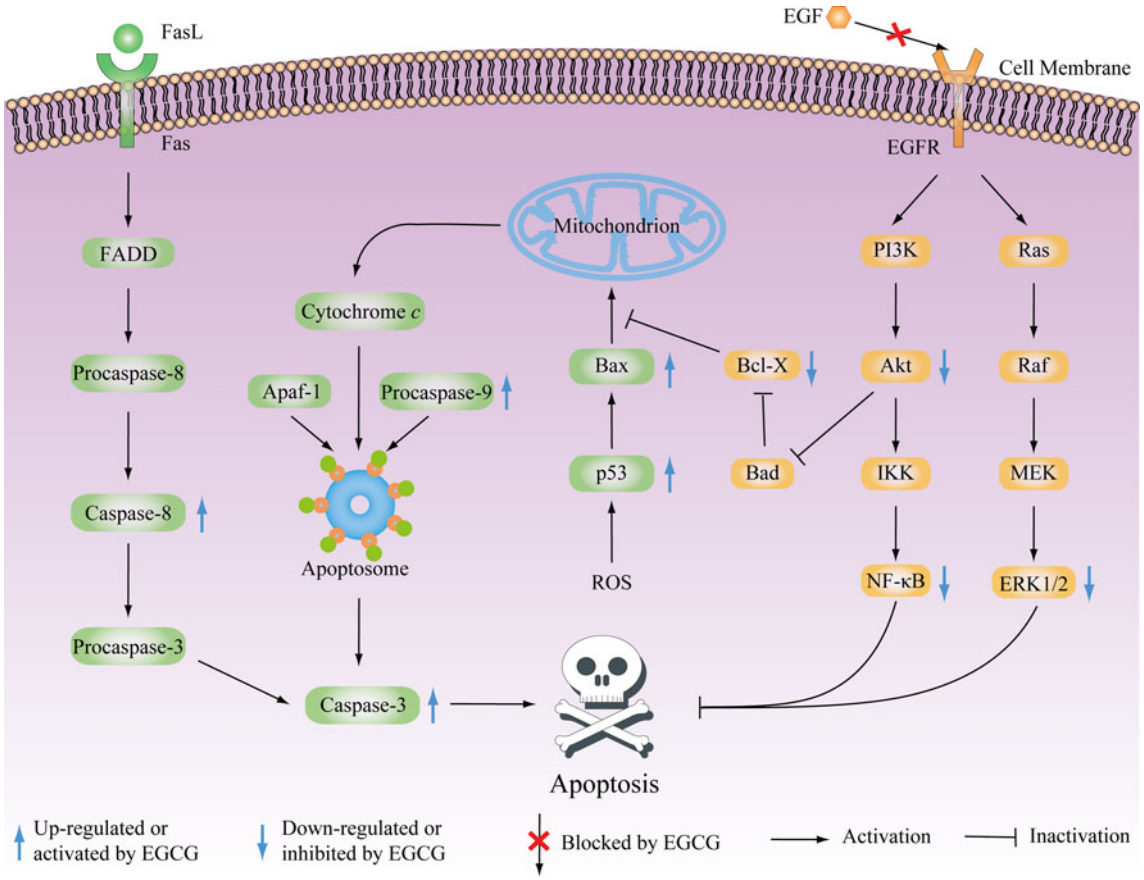

Fig. 4 Induction of cancer cell apoptosis. EGCG upregulates the activities of Fas (TNFR), Caspase-8, p53, Caspase-9, Bax, and Caspase-3. EGFR, Akt, Bcl-X, ERK1/2, IAPs, NF-κB, AP-1 activities are downregulated

Inhibition of enzyme activities

EGCG could inhibit the phosphorylation of JUK, JUN, MEK1, MEK2, ERK1, ERK2 and ELK1 in JB6 epidermal cell lines [69]. This inhibition was associated with the inhibition of AP-1 transcriptional activity or cell transformation [70]. In vitro, kinase assays suggested that EGCG inhibited MEK1 phosphorylation by decreasing its association with the kinase RAF1. EGCG seems to inhibit the phosphorylation of ElK1 by competing with the binding site for ERK1 and ERK2 [2].

The proteasome, a multi-catalytic enzyme found in all eukaryotic cells, is recognized as a novel and promising target for chemotherapy. A number of proteasome components, including cyclin proteins, cyclin-dependent kinase inhibitors, p53, Bcl-2, and IκB among others, have been identified [71]. These proteins have various functions like regulation of the cell cycle, protection from apoptosis, and transcriptional regulation. It has been reported that EGCG and ECG potently inhibited the chymotryptic activity of the 20S proteasome, thereby inhibiting cell proliferation [62].

Induction of apoptosis

Programmed cell death (apoptosis), which contains a series of intricate pathways and enzymes, plays a critical role in cell survival and proliferation. Accumulating evidence shows that uncontrolled proliferation of transformed cells due to lack of programmed cell death is closely associated with tumorigenesis [72]. There are two major pathways that initiate apoptosis, receptor mediated pathways (extrinsic pathways) and mitochondrial mediated pathways (intrinsic pathways). In addition, the cell survival pathways, such as PI3K/Akt, NF-κB, and MAPKs in cancer cells, may trigger apoptosis as well [73] (Fig. 4). The ability to induce cancer cell apoptosis of EGCG has been demonstrated in many studies. For example, treatment of EGCG in mice can significantly reduce the ultraviolet B-induced tumors [74]. Another study found that 20–40 μg/mL of EGCG causes more than 80 % cell death in chronic lymphocytic leukemia cells through apoptosis [75]. Importantly, EGCG triggers apoptosis without affecting normal cells, and it was reported that EGCG can

induce cell survival via anti-apoptosis in human epidermal keratinocytes.

Intrinsic and extrinsic apoptotic pathways

The increase of the membrane permeability of mitochondria is the key step of intrinsic pathway and pro-apoptotic factors, including procaspases, cytochrome c, apoptosis protease activating factor-1, will be released from mitochondria [76]. These factors may form a supramolecular complex termed apoptosome to activate caspases. Death receptor pathways including fas and TNF receptors can also activate caspases [77].

Activation of caspases, increase in the Bax/Bcl-2 ratio, and the cleavage of poly(ADP-ribose) polymerase (PRAP) play important roles during apoptosis [78]. In T24 human bladder cancer cells,the treatment of EGCG caused an increase in Bad and a decrease in Bcl-xL protein levels, and the 89 kDa cleaved PARP fragment was detected in EGCG-treated samples [79], suggesting that EGCG can initiate cancer cell death through these intrinsic apoptosis events. Similar conclusion has been found in human epidermoid carcinoma A431 cells with EGCG (10–40 μg/mL). EGCG treatment resulted in a significant time-dependent increase in the active form of caspase-3, caspase-8 and caspase-9, and the full-size PARP (116 kDa) protein was cleaved to an 85 kDa fragment [80]. EGCG also activates the intrinsic apoptosis pathway by stabilizing and activating p53 and increasing Bax in a p53-dependment manner.

TNF family ligands, associated with cell surface death receptors, trigger extrinsic apoptotic pathways. The activation of APO-1 receptor (Fas) leads to the recruitment of Fas-associated protein with death domain (FADD), which results in the activation of caspase family [77]. Studies have demonstrated that EGCG can be a potent Fas inhibitor [81]. Treatment of 40 μg/mL EGCG can inhibit Fas activity by 50 % and induce apoptosis in human LNCaP cells [81]. Another study has reported that TNF-related apoptosis-inducing ligand will enhance apoptotic progress in combination with EGCG [82]. EGCG can modulate both intrinsic and extrinsic pathways in LNCaP cells. EGCG not only increases the Bax/Bcl-2 ratio, but also decreases the activity of the inhibitor of apoptosis proteins (IAP), such as XIAP, and cIAP1 [83]. EGCG also upregulates the expression of DR4 and inhibits the expression of FADD in a dose-dependent manner [82].

Inhibition of cell survival pathways

Inhibition of NF-κB signaling pathway

NF-κB is a family of closely related protein dimers or oxidative stress-sensitive transcription factors that bind to a conserved sequence motif called the κB site [5]. NF-κB plays a critical role in the regulation of a variety of important genes in inducing apoptosis and inhibiting proliferation. NF-κB is sequestered in the cytoplasm in an inactive form through the interaction with IκB [38]. Phosphorylation of IκB by IκB kinase causes ubiquitination and degradation of IκB, thus releasing NF-κB that then translocates to the nucleus [32]. It has been known that EGCG treatment could lead to a significant dose- and time-dependent inhibition of activation and translocation of NF-κB to the nucleus by suppressing the degradation of IκB in the cytoplasm [34]. EGCG could also inhibit the ATP-induced activation of NF-kB and the activation of NF-κB induced by IL-1 [63]. EGCG stabilizes p53 and negatively regulates NF-κB activity, leading to the change of the Bax/Bcl-2 ratio in a manner that favors apoptosis. EGCG induces apoptosis in human prostate carcinoma LNCaP cells by the negative regulation of NF-κB activity, decreasing the expression of the pro-apoptotic protein Bcl-2 [34].

The PI3K/Akt and GSK-3 pathway

Mitotic or stress-activated pathways, such as the extracellular-regulated protein kinase (ERK) and Akt pathways, are critical to apoptosis [84]. Akt is involved in phosphorylation of Bad to its anti-apoptotic isoform, Bad-P. Studies have shown that EGCG treatment can inhibit PI3K/Akt pathways [79]. EGCG treatment (20–40 μg/mL) resulted in an appreciable down-regulation of phospho-PDK1, phospho-Thr308-Akt, and phospho-Ser473-Akt [79]. This inhibition of Akt phosphorylation may contribute to the inactivation of Akt cell survival pathways and results in apoptosis. In addition, EGCG can also trigger apoptosis via affecting GSK-3, a key enzyme involved in the inhibition of oxidative- stress-induced apoptosis [33].

Inhibition of cancer metastasis

Metastasis is one of the most serious challenges for cancer treatment. Many types of cancer metastasize to specific organs with an incidence much higher than that would be expected from the circulatory pattern between the primary tumor site and the secondary organs [85]. Therefore, inhibition of metastasis is one of the most important issues in cancer research. Evidences have suggested that consumption of green tea catechins can reduce cancer metastasis in vitro and in vivo [86–89].

Matrix metallo proteinases (MMPs), which can degrade the surrounding extracellular matrix, contribute to tumor cell migration [90]. Highly metastatic cancer cells secrete large amounts of MMPs (especially MMP-2 and MMP-9)

and studies have shown that EGCG can inhibit the activity of MMP-2 and MMP-9 [87]. Simultaneously, EGCG can also upregulate the expression of MMPs inhibitors, TIMP1 and TIMP2, which provide another way to suppress the activity of MMPs [87]. EGCG inhibits the activity of pro-MMP2 by membrane-type MMP [62]. At the concentration of 40 μM, EGCG can impede cell invasion by 48 % in LNCaP cells [82]. Decreased expression of MMP-2 and MMP-9 was also found in ultraviolet (UV)-induced skin cancer cells with topical application of EGCG [88].

Suppression of MMPs, however, is not the only mechanism to inhibit tumor metastasis by EGCG. Interfering with the hepatocyte growth factor/scatter factor (HGF/SF) signaling pathways may also inhibit cancer cells invasion. HGF/SF and its receptor, Met, is reported to be associated with human tumorigenesis and metastasis [91]. In one study, EGCG was found to inhibit HGF/SF-induced cell scattering and uPA activation with an IC50 of 15.8 μg/mL [91]. Another in vitro assay showed that B16F10 melanoma cells migration was abolished by EGCG in a dose-dependent manner [92]. These migration inhibition effects are due to the blockage of HGF/SF-induced tyrosine phosphorylation of Met, which further interferes with the paracrine or autocrine of HGF/SF-Met signaling.

EGCG might also inhibit cancer metastasis in many other ways. For example, sustained production of nitric oxide (NO) has a positive association with tumor metastasis, and treatment with EGCG can reduce the migration of 4T1 cells in a concentration-dependent manner with clear reduction of NO production [93]. EGCG resulted in a dose-dependent decrease in the levels of cGMP, and thus providing another way to inhibit 4T1 cells migration [93]. Urokinase, a hydrolase that facilitates degradation of the basement membrane and extracellular matrix, is frequently overexpressed in many types of cancers [86]. EGCG can directly bind to urokinase, block the histidine 57 and serine 195 residues of the urokinase catalytic triad, and interfere with its ability to recognize its substrates, thereby inhibiting its enzyme activity and the degradation of extracellular matrix [86].

As a tyrosine kinase inhibitor, EGCG has been demonstrated to be an effective inhibitor of ephrin-A1-mediated cell migration. EGCG can inhibit the ephrin-A1-mediated phosphorylation of EphA2 and ERK-1/2 [94]. At the concentration of 2 μM, EGCG suppressed ephrin-A1-induced migration by about 40 % on human umbilical vein endothelial cells (HUVECs). At the concentration of 5 μM, the suppression effect will be 80 %, and these are not due to the cytotoxicity of EGCG [94]. These anti-metastasis effects of EGCG mentioned above have been found in many types of cancer cells, which suggest the possible application of EGCG in the treatment of tumor metastasis. However, the general applicability of tea catechins for cancer metastasis prevention in vivo remains to be further investigated.

Inhibition of tumor angiogenesis

Angiogenesis, an essential machinery for tumor cells to satisfy their needs for nutrients and oxygen, is critical for cancer proliferation and metastasis. Angiogenesis is consisted of a series of strategies including basement membrane degradation, endothelial proliferation and migration, extracellular matrix remodeling, and vascular tube formation [95]. EGCG has been demonstrated that can prevent the growth of new blood vessels in animals which may benefit for cancer treatment [4].

Tumor cells can switch their gene-expression programs to produce angiogenic factors like the vascular endothelial growth factor (VEGF). VEGF is an angiogenic protein that has specific mitogenic and chemotactic effects on vascular endothelial cells, including the activation of NF-κB-inducing kinase, anti-apoptosis, angiogenesis and the modulation of some relevant pathways [75]. A microarray analysis has shown that 291 genes of endothelial cells were up-regulated and 51 were down-regulated by VEGF [43]. Tea catechins, especially EGCG, demonstrated anti-angiogenesis activity via the modulation of VEGF signaling, such as the inhibition of the phosphorylation of VEGF receptor, VEGFR [75]. The activity of VEGFR-2 has positive correlation with tumor angiogenesis, and VEGF-induced tyrosine phosphorylation of VEGFR-2 can be inhibited by three types of green tea catechins, EGCG, CG and ECG [96], which eventually results in the inhibition of tyrosine kinase activity of VEGFR-2 and the suppression of angiogenesis. EGCG itself can also block the binding of VEGF to its receptor, VEGFRs, which provides another way to suppress VEGF-induced angiogenesis. The gene expression of VEGF can also be modulated by green tea catechins [97]. Erk-1 and Erk-2, together with the transcription factor AP-1, have been reported to be involved in the overexpression of VEGF in tumor cells [11]. Previous studies have shown that EGCG inhibited the activation of Erk-1 and Erk-2, and suppressed the DNA binding activity of AP-1 to VEGF gene promoter [86].

Another member of RTKs family, ephrin (Eph), emerges as a critical mediator of angiogenesis. Ephrin-A1, as mentioned above, is critical for endothelial cell migration by inducing the tubular structure formation of HUVECs [94]. EGCG can significantly inhibit ephrin-A1-induced tubular structure formation at concentrations of 5 μM and completely abolish the effect of ephrin-A1 at the concentration of 20 μM [94]. The ephrin-A1-mediated activation of ERK-1/2 pathways is strongly inhibited by EGCG, which further suppresses the expression of VEGF.

As a key stage of tumor angiogenesis, endothelial cell proliferation and migration is an important topic in cancer chemoprevention studies. Two important pathways involved in endothelial cell proliferation are affected by

EGCG: Wnt signaling pathway and Id pathway [98]. The negative regulation on endothelial cell proliferation is not confined to modulation of signaling pathways. Interference of enzymes involved in angiogenesis is another main strategy contributes to the anti-angiogenesis activity of green tea catechins [86]. WARS is an angiogenesis enzyme, which inhibits VEGF-induced ERK activation and endothelial cell migration by forming a complex with VE-cadherin. EGCG can up-regulate WARS activity but has no effect on the expression of some well known genes that are involved in the classic angiogenesis signaling pathway, such as PI3 kinase and dual specificity phosphatase [98].

Interleukins, such as IL-6 and IL-8, is another class of factors that contributes to tumor angiogenesis, which are often overexpressed in cancer cells. IL-6 increases VEGF mRNA expression by 3.1 folds and upregulates VEGF secretion by 2.8 folds. In human gastric cancer (AGS) cells, treatment with EGCG inhibited IL-6 induced VEGF expression and angiogenesis via suppressing the activity of the signal transducer and activator of transcription 3 [99]. In another study, 0.5 μM of EGC significantly reduced IL-8 production, representing the most effective IL-8 inhibitor of green tea catechins. Suppression of MMPs also contributes to anti-angiogenesis [100]. For example, inhibition of Membrane-type 1 matrix metalloproteinase (MT1-MMP) and MMP-2 is closely related to the anti-angiogenesis activity of green tea catechins [87].

Targeting cancer stem cells

Human cancer has been recognized as heterogeneous population of cells and only a minority of them, termed cancer stem cells (CSCs), have the capacity of self-renewal, differentiation, and tumor formation initiation [101]. CSCs have been identified as tumor initiating cells in a variety of tumors including the tumors in breast, brain, colon, liver, ovarian and prostate. Different from the differentiated tumor cells, CSCs have shown resistance to many cytotoxic agents resulting in the tumor relapse and metastasis. This suggests that targeting CSCs may be an effective strategy to eliminate tumor recurrence and metastasis.

Recent studies have demonstrated that EGCG has the potential of inhibiting tumor initiation and invasion by targeting the CSCs. In the human prostate cancer cell lines, EGCG can reduce the cell viability of CSCs, and inhibit the growth of tumor spheroid and colony formation in a dose-dependent manner [102]. EGCG can induce apoptosis in CSCs through intrinsic apoptotic pathway associated with the activation of caspase-3/7, inhibition of XIAP, Bcl-2 and survivin in the human prostate CSCs [103].

Wnt signaling pathway is highly activated in the CSCs and controls the self-renewal of CSCs at various organ sites. EGCG was shown to block Wnt signaling pathway in the human breast cancer in a dose-dependent manner [104]. The reduction of CSCs is mainly due to the induction of HMG box-containing protein 1 transcriptional factor, a Wnt signaling suppressor [102].

Green tea catechins as effective adjuvants to therapeutic agents

The poor bioactivity and bioavailability of green tea catechins in vivo is the main difficulty for poly E or EGCG human administration. In order to solve this problem, we can enhance the systemic availability of poly E or EGCG by increasing single oral administration dosage [14, 15, 105]. When Poly E was administered to empty stomach after overnight fast, there was a remarkable increase in the blood levels of free EGCG, EGC, and ECG, compared to the subjects who took poly E with food. Clinical studies support the concept that combinations (such as Poly E) of chemopreventive compounds may be superior to such agents as single treatment [105]. Therefore, combinations of natural or synthetic agents for cancer prevention and treatment might be more effective and have fewer side effects [105]. The combination of catechins or EGCG with therapeutic drugs such as nonsteroidal anti-inflammatory drugs (sulindac and tamoxifen) or with drugs used in chemotherapy (5-fluorouracil oxaliplatin, or paclitaxel) is a promising strategy for anticancer therapeutics (Table 2).

Tamoxifen is an anti-estrogenic compound used to prevent breast cancer. It was reported that the combination of EGCG with tamoxifen induced apoptosis in PC-9 cells more strongly than EGCG alone or tamoxifen alone [8]. The combination of EGCG and tamoxifen also triggered the inhibition of TNF-α release from BALB/3T3 cells treated with okadaic acid, a tumor promoter, along with the inhibition of cell growth in human breast cancer cell line MCF-7 [8, 106]. There are also some laboratories reported that the combination of EGCG and tamoxifen together inhibited the proliferation of ER-positive and ER-negative human breast cancer cells, and inhibited the growth of xenografts of MCF-7 and MDA-MB-231 in nude mice more strongly than tamoxifen alone [106–108].

Sulindac is an agent used for suppression of colon adenoma formation in familial adenomatous polyposis [109]. Its usage is limited because of its side effects [110]. The combination of sulindac with EGCG may overcome this side-effect [8]. The combination synergistically inhibits the growth of mouse colon adenocarcinoma cell line, Colon26, more strongly than EGCG alone or sulindac alone [106]. The mechanism of EGCG and sulindac is

Table 2 Catechins combining with anticancer drugs

Catechins	Anticancer drugs	Types of cancer	Effects	References
EGCG	Tamoxifen	Breast cancer, spontaneous mammary tumors (MCF-7, PC-9)	Synergistic growth inhibition, inducing apoptosis,inhibiting invasion	[8, 106, 108, 129]
EGCG	Sulindac	Colon adenoma	Enhancing apoptosis	[130]
EGCG	Celecoxib	Lung and colon tumors	Inducing apoptosis	[112, 131]
EGCG or EC	Curcumin	Oral epithelial cells; lung cancer cells (PC-9); breast cancer cell	Enhance the inhibition of growth and induce apoptosis	[10, 116]
EGCG	EC	Colon cancer cells(HT29 cells), lung cancer cells	Inhibiting Growth, inducing apoptosis as well as inhibition of tumor angiogenesis and metastasis.	[8, 10, 11]
EGCG	5-Fluorouracil	Head and neck squamous cell carcinoma (YCU-N861,YCU-H891) hepatocellular carcinoma cell colon cancer cells	Synergistic growth inhibition	[132]
EGCG	Taxol	Head and neck squamous cell carcinoma (YCU-H891) breast carcinoma (BT-474)	Synergistic growth inhibition	[120, 121]
EGCG	Doxorubicin	Hepatocellular carcinoma (BEL-7404DOX)	Synergistic growth inhibition	[133]
EGCG	Gefitinib	Lung cancer (PC-9, A549)	Synergistic growth inhibition	[134]
EGCG	Erlotinib	Head and neck squamous cell carcinoma (Tu177, Tu212, 886LN, SQCCY1, SQCCY38)	Synergistic growth inhibition	[135]

related to GADD153 in cell. The combination of EGCG and sulindac can induce the upregulation of GADD153 and p21 genes dramatically [31]. However, the expression of those genes was not affected by either EGCG or sulindac alone. The combination can induce the down regulation of T plasminogen activator, TIMP3, IL-1β, and integrin β4 genes [111].

Celecoxib, a selective cyclooxygenase 2 inhibitor, is known to have anti-inflammatory activity and to induce apoptosis in various types of cancer cells [112]. It was reported that co-treatment with EGCG plus celecoxib strongly induced the expression of GADD153 both at mRNA level and protein level in PC-9 cells, while neither EGCG nor celecoxib alone did [113]. Comparing with the combination of EGCG and sulindac, the combination of EGCG and celecoxib did not induce the expression of other apoptosis related genes, such as p21 and GADD145. Some relevant research indicated that GADD153 expression was mediated through the ERK signaling pathway [114].

Curcumin, a phenolic compound isolated from the plant *Curcuma longa (Linn)*, is traditionally well known to have therapeutic effects on various types of diseases [10]. When curcumin is combined with EC or EGCG, it can overcome the low bioavailability of curcumin, enhance the inhibition of growth, and induce apoptosis [10, 115]. The combination of EGCG and curcumin showed synergistic interactions in growth inhibition and could increase sigmoid capacity of the dose–effect curves in human oral epithelial cells [116], EGCG and curcumin combination also provided higher efficacy in inhibiting ERa breast cancer cell growth in vitro and in vivo [116]. In another study, athymic nude female mice were implanted with MDA-MB-231cells and treated with curcumin, EGCG, EGCG plus curcumin. Tumor volume in the EGCG plus curcumin treated mice decreased 49 % compared to vehicle control mice, which correlated with a 78 ± 6 % decrease in levels of VEGFR-1 protein expression in the tumor [115]. The combination of curcumin with EC significantly increased the inhibition of cell growth compared with curcumin or EC alone in human lung cancer cell lines PC-9 and A549 [10]. Curcumin and EC combination increased both apoptosis and the expression of GADD153 and GADD45 genes, associated with enhanced protein expression [10].

The enhanced anticancer effects of the combination of EGCG with anticancer drugs, such as 5-FU, taxol, doxorubicin, gefitinib, and erlotinibect have been reported, indicating a great potential of EGCC in anticancer therapy [117–121].

Concluding remarks

Green tea catechins, especially EGCG, exhibit strong anticancer activity by targeting several cell signaling pathways that are relevant to cancer development, resulting in the suppression of tumor growth, induction of apoptosis, and inhibition of metastasis and angiogenesis. Identification of and characterization of novel molecular targets of

tea polyphenols is paramount to the development of cancer prevention and treatment strategy using green tea/synthetic (−)-EGCG analogs.

Currently, most anticancer drugs available are expensive and somewhat toxic to the non-cancer cells. Green tea catechins appear to target specifically to the cancer cells, supporting the development of green tea catechins as a potential anticancer agents. However, there are not enough evidences obtained from human studies for the anticancer effects of green tea catechins. This is mainly because of the low physiological concentration of catechins in human body after administration, the disturbance of many other dietary constituents or food items, the lack of accuracy in assessing tea consumption, the different lifestyles associated with tea consumption, the individual differences in genetic polymorphism, variation of etiological factors for cancer in different populations, and some other confounding factors in epidemiological studies. Even though there are many epidemiological and clinical studies of green tea, most of the human trials had been carried out were pilot studies. Therefore, future studies should focus on the bioavailability and biological activity of green tea catechins in vivo, improving the absorption and metabolism of tea catechins, setting a standard for assessing tea consumption, understanding the different etiological factors and effective targets of different cancer cells, exploring the combination between tea catechins and other effective anticancer drugs to increase the effect of cancer treatment and decrease the toxicity. Moreover, more definitive information on the cancer-preventive activity of tea catechins need to be obtain from well-designed cohort studies and human intervention trials.

We review the synergetic anticancer effect of green tea catechins in combination with other anticancer drugs. These observations are important because most of the anticancer drugs currently available are expensive and toxic to normal cells. The co-administration with catechins might lower the required dosage of anticancer drugs, which in turn should lower the cytotoxicity and cost of anticancer treatment. Because of this reason, it is of great interests for the future green tea catechins studies to focus on how to increase their synergetic effect and reduce the side-effects of anticancer drugs.

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References

1. Sang S, Lambert JD, Ho C-T, Yang CS (2011) The chemistry and biotransformation of tea constituents. Pharmacol Res 64:87–99
2. Yang CS, Wang X, Lu G, Picinich SC (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9:429–439
3. Feng WY (2006) Metabolism of green tea catechins: an overview. Curr Drug Metab 7:755–809
4. Cao Y, Cao R (1999) Angiogenesis inhibited by drinking tea. Nature 398:381
5. Singh BN, Shankar S, Srivastava RK (2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol 82:1807–1821
6. Yang CS, Lambert JD, Ju J, Lu G, Sang S (2007) Tea and cancer prevention: molecular mechanisms and human relevance. Toxicol Appl Pharm 224:265–273
7. Higdon JV, Frei B (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci 43(1):89–143
8. Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H (1999) Synergistic effects of (−)-epigallocatechin gallate with (−)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. Cancer Res 59:44–47
9. Hu B, Wang L, Zhou B, Zhang X, Sun Y, Ye H et al (2009) Efficient procedure for isolating methylated catechins from green tea and effective simultaneous analysis of ten catechins, three purine alkaloids, and gallic acid in tea by high-performance liquid chromatography with diode array detection. J Chromatogr A 1216:3223–3231
10. Saha A, Kuzuhara T, Echigo N, Suganuma M, Fujiki H (2010) New role of (−)-epicatechin in enhancing the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin. Cancer Prev Res 3:953–962
11. Jung YD, Kim MS, Shin BA, Chay KO, Ahn BW, Liu W et al (2001) EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. BRIT J Cancer 84:844
12. Shimizu M, Deguchi A, Lim JT, Moriwaki H, Kopelovich L, Weinstein IB (2005) (−)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. Clin Cancer Res 11:2735–2746
13. Hirose M, Mizoguchi Y, Yaono M, Tanaka H, Yamaguchi T, Shirai T (1997) Effects of green tea catechins on the progression or late promotion stage of mammary gland carcinogenesis in female Sprague-Dawley rats pretreated with 7, 12-dimethylbenz (a) anthracene. Cancer Lett 112:141–147
14. Yan Y, Cook J, McQuillan J, Zhang G, Hitzman CJ, Wang Y et al (2007) Chemopreventive effect of aerosolized polyphenon E on lung tumorigenesis in A/J mice. Neoplasia 9:401
15. Chow HH, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM et al (2005) Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of polyphenon E in healthy individuals. Clin Cancer Res 11:4627–4633
16. Chen D, Wan SB, Yang H, Yuan J, Chan TH, Dou QP (2011) EGCG, green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment. Adv Clin Chem 53:155
17. Johnson J, Bailey H, Mukhtar H (2010) Green tea polyphenols for prostate cancer chemoprevention: a translational perspective. Phytomedicine 17:3–13

18. Myung SK, Bae WK, Oh SM, Kim Y, Ju W, Sung J et al (2009) Green tea consumption and risk of stomach cancer: a meta-analysis of epidemiologic studies. Int J Cancer 124:670–677
19. Zheng P, H-m Zheng, X-m Deng (2012) Green tea consumption and risk of esophageal cancer: a meta-analysis of epidemiologic studies. BMC gastroenterol 12:165
20. Chen Z, Chen Q, Xia H, Lin J (2011) Green tea drinking habits and esophageal cancer in southern China: a case–control study. Asian Pac J Cancer Prev 12:229–233
21. Yuan J-M, Koh W-P, Sun C-L, Lee H-P, Mimi CY (2005) Green tea intake, ACE gene polymorphism and breast cancer risk among Chinese women in Singapore. Carcinogenesis 26:1389–1394
22. Lin IH, Ho ML, Chen HY, Lee HS, Huang CC, Chu YH et al (2012) Smoking, green tea consumption, genetic polymorphisms in the insulin-like growth factors and lung cancer risk. PLoS ONE 7:e30951
23. Kumar N, Shibata D, Helm J, Coppola D, Malafa M (2007) Green tea polyphenols in the prevention of colon cancer. Front Biosci 12:302–309
24. Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA et al (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 9:3312–3319
25. Tsao AS, Liu D, Martin J, Tang XM, Lee JJ, El-Naggar AK et al (2009) Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer Prev Res 2:931–941
26. Jatoi A, Ellison N, Burch PA, Sloan JA, Dakhil SR, Novotny P et al (2003) A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. Cancer 97:1442–1446
27. Luo H, Tang L, Tang M, Billam M, Huang T, Yu J et al (2006) Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. Carcinogenesis 27:262–268
28. Zhang T, Zhang J, Derreumaux P, Mu Y (2013) Molecular mechanism of the inhibition of EGCG on the Alzheimer's Aβ1-42 dimer. J Phys Chem B 117:3993–4002
29. Chen D, Pamu S, Cui Q, Chan TH, Dou QP (2012) Novel epigallocatechin gallate (EGCG) analogs activate AMP-activated protein kinase pathway and target cancer stem cells. Bioorgan Med Chem 20:3031–3037
30. Thomasset SC, Berry DP, Garcea G, Marczylo T, Steward WP, Gescher AJ (2007) Dietary polyphenolic phytochemicals—Promising cancer chemopreventive agents in humans? A review of their clinical properties. Int J Cancer 120:451–458
31. Yang CS, Wang X (2010) Green tea and cancer prevention. Nutr Cancer 62:931–937
32. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H (2006) Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. Cancer Res 66:2500–2505
33. Surh Y-J (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780
34. Hastak K, Gupta S, Ahmad N, Agarwal MK, Agarwal ML, Mukhtar H (2003) Role of p53 and NF-κB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. Oncogene 22:4851–4859
35. Lambert JD, Elias RJ (2010) The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 501:65–72
36. Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 133:3275S–3284S
37. Inami S, Takano M, Yamamoto M, Murakami D, Tajika K, Yodogawa K et al (2007) Tea catechin consumption reduces circulating oxidized low-density lipoprotein. Int Heart J 48:725–732
38. Yamamoto Y, Gaynor RB (2001) Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J Clin Invest 107:135–142
39. Dong Z, W-y Ma, Huang C, Yang CS (1997) Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (−)-epigallocatechin gallate, and theaflavins. Cancer Res 57:4414–4419
40. Karin M, Cao Y, Greten FR, Li ZW (2002) NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2:301–310
41. Khan SG, Katiyar SK, Agarwal R, Mukhtar H (1992) Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res 52:4050–4052
42. Reuland DJ, Khademi S, Castle CJ, Irwin DC, McCord JM, Miller BF et al (2013) Upregulation of phase II enzymes through phytochemical activation of Nrf2 protects cardiomyocytes against oxidant stress. Free Radic Bio Med 56:102–111
43. Na H-K, Surh Y-J (2008) Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol 46:1271–1278
44. Blagosklonny M (2005) Carcinogenesis, cancer therapy and chemoprevention. Cell Death Differ 12:592–602
45. Thangapazham RL, Singh AK, Sharma A, Warren J, Gaddipati JP, Maheshwari RK (2007) Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells in vitro and in vivo. Cancer Lett 245:232
46. Ju J, Hong J, Zhou JN, Pan Z, Bose M, Liao J et al (2005) Inhibition of intestinal tumorigenesis in Apcmin/+ mice by (−)-epigallocatechin-3-gallate, the major catechin in green tea. Cancer Res 65:10623–10631
47. Huh SW, Bae SM, Kim YW, Lee JM, Namkoong SE, Lee IP et al (2004) Anticancer effects of (-)-epigallocatechin-3-gallate on ovarian carcinoma cell lines. Gynecol Oncol 94:760
48. Stillman B (1996) Cell cycle control of DNA replication. Science 274:1659–1663
49. Graña X, Reddy EP (1995) Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene 11:211
50. Nigg EA (1995) Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. BioEssays 17:471–480
51. Massagué J (2004) G1 cell-cycle control and cancer. Nature 432:298–306
52. Kim CH, Moon SK (2005) Epigallocatechin-3-gallate causes the p21/WAF1-mediated G(1)-phase arrest of cell cycle and inhibits matrix metalloproteinase-9 expression in TNF-alpha-induced vascular smooth muscle cells. Arch Biochem Biophys 435:264–272
53. Peng G, Wargovich MJ, Dixon DA (2006) Anti-proliferative effects of green tea polyphenol EGCG on Ha-Ras-induced transformation of intestinal epithelial cells. Cancer Lett 238:260–270
54. Tan X, Hu D, Li S, Han Y, Zhang Y, Zhou D (2000) Differences of four catechins in cell cycle arrest and induction of apoptosis in LoVo cells. Cancer Lett 158:1–6
55. Han DW, Lee MH, Kim HH, Hyon SH, Park JC (2011) Epigallocatechin-3-gallate regulates cell growth, cell cycle and phosphorylated nuclear factor-κB in human dermal fibroblasts. Acta Pharmacol Sin 32:637–646
56. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3:11–22
57. Agarwal R (2000) Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 60:1051–1059

58. Wu D, Guo Z, Ren Z, Guo W, Meydani SN (2009) Green tea EGCG suppresses T cell proliferation through impairment of IL-2/IL-2 receptor signaling. Free Radic Bio Med 47:636–643
59. Lin SC, Li WC, Shih JW, Hong KF, Pan YR, Lin JJ (2006) The tea polyphenols EGCG and EGC repress mRNA expression of human telomerase reverse transcriptase (hTERT) in carcinoma cells. Cancer Lett 236:80–88
60. Baatout S, Derradji H, Jacquet P, Mergeay M (2005) Increased radiation sensitivity of an eosinophilic cell line following treatment with epigallocatechin-gallate, resveratrol and curcuma. Int J Mol Med 15:337–352
61. Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421
62. Khan N, Mukhtar H (2008) Multitargeted therapy of cancer by green tea polyphenols. Cancer Lett 269:269–280
63. Sarkar FH, Li Y, Wang Z, Kong D (2009) Cellular signaling perturbation by natural products. Cell Signal 21:1541–1547
64. Lambert JD, Yang CS (2003) Mechanisms of cancer prevention by tea constituents. J Nutr 133:3262S–3267S
65. Miner JN, Yamamoto KR (1992) The basic region of AP-1 specifies glucocorticoid receptor activity at a composite response element. Gene Dev 6:2491–2501
66. Yang CS, Lambert JD, Hou Z, Ju J, Lu G, Hao X (2006) Molecular targets for the cancer preventive activity of tea polyphenols. Mol Carcinog 45:431–435
67. Tachibana H, Koga K, Fujimura Y, Yamada K (2004) A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 11:380
68. Samani AA, Yakar S, LeRoith D, Brodt P (2007) The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev 28:20–47
69. Yang CS, Sang S, Lambert JD, Hou Z, Ju J, Lu G (2006) Possible mechanisms of the cancer-preventive activities of green tea. Mol Nutr Food Res 50:170–175
70. Chung JY, Huang C, Meng X, Dong Z, Yang CS (1999) Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transformed cells structure–activity relationship and mechanisms involved. Cancer Res 59:4610–4617
71. Smith DM, Wang Z, Kazi A, Li L-H, Chan T-H, Dou QP (2002) Synthetic analogs of green tea polyphenols as proteasome inhibitors. Mol Med 8:382–392
72. Gusman J, Malonne H, Atassi G (2001) A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. Carcinogenesis 22:1111–1117
73. Gosslau A, Chen KY (2004) Nutraceuticals, apoptosis, and disease prevention. Nutrition 20:95–102
74. Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS et al (2002) Topical applications of caffeine or (−)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. P Natl Acad Sci USA 99:12455–12460
75. Lee YK, Bone ND, Strege AK, Shanafelt TD, Jelinek DF, Kay NE (2004) VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia. Blood 104:788–794
76. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–776
77. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Bi 15:269–290
78. Salvesen GS, Dixit VM (1999) Caspase activation: the induced-proximity model. P Natl Acad Sci USA 96:10964–10967
79. Qin J, Xie LP, Zheng XY, Wang YB, Bai Y, Shen HF et al (2007) A component of green tea, (−)-epigallocatechin-3-gallate, promotes apoptosis in T24 human bladder cancer cells via modulation of the PI3K/Akt pathway and Bcl-2 family proteins. Biochem Bioph Res Co 354:852–857
80. Gupta S, Hastak K, Afaq F, Ahmad N, Mukhtar H (2004) Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappa B and induction of apoptosis. Oncogene 23:2507–2522
81. Brusselmans K, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV (2003) Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. Int J Cancer 106:856–862
82. Siddiqui IA, Malik A, Adhami VM, Asim M, Hafeez BB, Sarfaraz S et al (2008) Green tea polyphenol EGCG sensitizes human prostate carcinoma LNCaP cells to TRAIL-mediated apoptosis and synergistically inhibits biomarkers associated with angiogenesis and metastasis. Oncogene 27:2055–2063
83. Roy AM, Baliga MS, Katiyar SK (2005) Epigallocatechin-3-gallate induces apoptosis in estrogen receptor–negative human breast carcinoma cells via modulation in protein expression of p53 and Bax and caspase-3 activation. Mol Cancer Ther 4:81–90
84. Shankar S, Chen Q, Srivastava RK (2008) Inhibition of PI3K/AKT and MEK/ERK pathways act synergistically to enhance antiangiogenic effects of EGCG through activation of FOXO transcription factor. J Mol Signal 3:7
85. Dorsam RT, Gutkind JS (2007) G-protein-coupled receptors and cancer. Nat Rev Cancer 7:79–94
86. Jung YD, Ellis LM (2001) Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. Int J Exp Pathol 82:309–316
87. Yamakawa S, Asai T, Uchida T, Matsukawa M, Akizawa T, Oku N (2004) (−)-Epigallocatechin gallate inhibits membrane-type 1 matrix metalloproteinase, MT1-MMP, and tumor angiogenesis. Cancer Lett 210:47–55
88. Khan N, Mukhtar H (2010) Cancer and metastasis: prevention and treatment by green tea. Cancer Metast Rev 29:435–445
89. Garbisa S, Biggin S, Cavallarin N, Sartor L, Benelli R, Albini A (1999) Tumor invasion: molecular shears blunted by green tea. Nat Med 5:1216
90. Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2:161–174
91. Bigelow R, Cardelli J (2006) The green tea catechins,(−)-epigallocatechin-3-gallate (EGCG) and (−)-epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. Oncogene 25:1922–1930
92. Suzuki Y, Isemura M (2001) Inhibitory effect of epigallocatechin gallate on adhesion of murine melanoma cells to laminin. Cancer letters 173:15–20
93. Punathil T, Tollefsbol TO, Katiyar SK (2008) EGCG inhibits mammary cancer cell migration through inhibition of nitric oxide synthase and guanylate cyclase. Biochem Biophys Res Co 375:162–167
94. Tang FY, Chiang EP, Shih CJ (2007) Green tea catechin inhibits ephrin-A1-mediated cell migration and angiogenesis of human umbilical vein endothelial cells. J Nutr Biochem 18:391–399
95. Fassina G, Venè R, Morini M, Minghelli S, Benelli R, Noonan DM et al (2004) Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. Clin Cancer Res 10:4865–4873
96. Lamy S, Gingras D, Béliveau R (2002) Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. Cancer Res 62:381–385

97. Kondo T, Ohta T, Igura K, Hara Y, Kaji K (2002) Tea catechins inhibit angiogenesis in vitro, measured by human endothelial cell growth, migration and tube formation, through inhibition of VEGF receptor binding. Cancer Lett 180:139–144
98. Liu L, Lai CQ, Nie L, Ordovas J, Band M, Moser L et al (2008) The modulation of endothelial cell gene expression by green tea polyphenol-EGCG. Mol Nutr Food Res 52:1182–1192
99. Zhu BH, Chen HY, Zhan WH, Wang CY, Cai SR, Wang Z et al (2011) (−)-Epigallocatechin-3-gallate inhibits VEGF expression induced by IL-6 via Stat3 in gastric cancer. WJG 17:2315–2325
100. Tang FY, Meydani M (2001) Green tea catechins and vitamin E inhibit angiogenesis of human microvascular endothelial cells through suppression of IL-8 production. Nutr Cancer 41:119–125
101. Kim YS, Farrar W, Colburn NH, Milner JA (2012) Cancer stem cells: potential target for bioactive food components. J Nutr Biochem 23:691–698
102. Li Y, Wicha MS, Schwartz SJ, Sun D (2011) Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds. J Nutr Biochem 22:799–806
103. Tang S-N, Singh C, Nall D, Meeker D, Shankar S, Srivastava RK (2010) The dietary bioflavonoid quercetin synergizes with epigallocathechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition. J Mol Signal 5:14
104. Curtin JC, Lorenzi MV (2010) Drug discovery approaches to target Wnt signaling in cancer stem cells. Oncotarget 1:552
105. Bode AM, Dong Z (2009) Epigallocatechin 3-gallate and green tea catechins: united they work, divided they fail. Cancer Prev Res 2:514–517
106. Suganuma M, Saha A, Fujiki H (2011) New cancer treatment strategy using combination of green tea catechins and anticancer drugs. Cancer Sci 102:317–323
107. Scandlyn M, Stuart E, Somers-Edgar T, Menzies A, Rosengren R (2008) A new role for tamoxifen in oestrogen receptor-negative breast cancer when it is combined with epigallocatechin gallate. BRIT J Cancer 99:1056–1063
108. Sartippour MR, Pietras R, Marquez-Garban DC, Chen HW, Heber D, Henning SM et al (2006) The combination of green tea and tamoxifen is effective against breast cancer. Carcinogenesis 27:2424–2433
109. Boolbol SK, Dannenberg AJ, Chadburn A, Martucci C, Guo XJ, Ramonetti JT et al (1996) Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 56:2556–2560
110. Cruz-Correa M, Hylind LM, Romans KE, Booker SV, Giardiello FM (2002) Long-term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. Gastroenterology 122:641–645
111. Fujiki H, Suganuma M, Kurusu M, Okabe S, Imayoshi Y, Taniguchi S et al (2003) New TNF-α releasing inhibitors as cancer preventive agents from traditional herbal medicine and combination cancer prevention study with EGCG and sulindac or tamoxifen. Mutat Res-Fund Mol M 523:119–125
112. Kim SH, Hwang CI, Juhnn YS, Lee JH, Park WY, Song YS (2007) GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells. Carcinogenesis 28:223–231
113. Sharafkhaneh A, Velamuri S, Moghaddam SJ, Badmaev V, Dickey B, Kurie J. (2008) Natural Agents for Chemoprevention of Lung Cancer. Lung Cancer:441
114. Suganuma M, Kurusu M, Suzuki K, Tasaki E, Fujiki H (2006) Green tea polyphenol stimulates cancer preventive effects of celecoxib in human lung cancer cells by upregulation of GADD153 gene. Int J Cancer 119:33–40
115. Somers-Edgar TJ, Scandlyn MJ, Stuart EC, Le Nedelec MJ, Valentine SP, Rosengren RJ (2008) The combination of epigallocatechin gallate and curcumin suppresses ERα-breast cancer cell growth in vitro and in vivo. Int J Cancer 122:1966–1971
116. Khafif A, Schantz SP, Chou TC, Edelstein D, Sacks PG (1998) Quantitation of chemopreventive synergism between (−)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. Carcinogenesis 19:419–424
117. Amin AR, Khuri FR, Shin DM (2009) Synergistic growth inhibition of squamous cell carcinoma of the head and neck by erlotinib and epigallocatechin-3-gallate: the role of p53-dependent inhibition of nuclear factor-κB. Cancer Prev Res 2:538–545
118. Hou Z, Sang S, You H, Lee MJ, Hong J, Chin KV et al (2005) Mechanism of action of (−)-epigallocatechin-3-gallate: auto-oxidation–dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Res 65:8049–8056
119. Hwang JT, Ha J, Park IJ, Lee SK, Baik HW, Kim YM et al (2007) Apoptotic effect of EGCG in HT-29 colon cancer cells via AMPK signal pathway. Cancer Lett 247:115–121
120. Chen H, Landen CN, Li Y, Alvarez RD, Tollefsbol TO (2013) Epigallocatechin gallate and sulforaphane combination treatment induce apoptosis in paclitaxel-resistant ovarian cancer cells through hTERT and Bcl-2 down-regulation. Exp Cell Res 319:697–706
121. Luo T, Wang J, Yin Y, Hua H, Jing J, Sun X et al (2010) (−)-Epigallocatechin gallate sensitizes breast cancer cells to paclitaxel in a murine model of breast carcinoma. Breast Cancer Res. doi:10.1186/bcr2473
122. Kweon MH, Adhami VM, Lee JS, Mukhtar H (2006) Constitutive overexpression of Nrf2-dependent heme oxygenase-1 in A549 cells contributes to resistance to apoptosis induced by epigallocatechin 3-gallate. J Biol Chem 281:33761–33772
123. Levites Y, Amit T, Youdim MBH, Mandel S (2002) Involvement of protein kinase C activation and cell survival/cell cycle genes in green tea polyphenol (−)-epigallocatechin 3-gallate neuroprotective action. J Biol Chem 277:30574–30580
124. Urusova DV, Shim JH, Kim DJ, Jung SK, Zykova TA, Carper A et al (2011) Epigallocatechin-gallate suppresses tumorigenesis by directly targeting Pin1. Cancer Prev Res 4:1366–1377
125. Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M (2010) Micronutrient synergy—a new tool in effective control of metastasis and other key mechanisms of cancer. Cancer Metast Rev 29:529–542
126. Balasubramanian S, Eckert RL (2007) Keratinocyte proliferation, differentiation, and apoptosis–differential mechanisms of regulation by curcumin, EGCG and apigenin. Toxicol Appl Pharm 224:214–219
127. Manna S, Banerjee S, Mukherjee S, Das S, Panda CK (2006) Epigallocatechin gallate induced apoptosis in Sarcoma180 cells in vivo: mediated by p53 pathway and inhibition in U1B, U4-U6 UsnRNAs expression. Apoptosis 11:2267–2276
128. Katiyar S, Elmets CA, Katiyar SK (2007) Green tea and skin cancer: photoimmunology, angiogenesis and DNA repair. J Nutr Biochem 18:287–296
129. Suganuma M, Sueoka E, Sueoka N, Okabe S, Fujiki H (2000) Mechanisms of cancer prevention by tea polyphenols based on inhibition of TNF-α expression. Biofactors 13:67–72
130. Ohishi T, Kishimoto Y, Miura N, Shiota G, Kohri T, Hara Y et al (2002) Synergistic effects of (−)-epigallocatechin gallate with sulindac against colon carcinogenesis of rats treated with azoxymethane. Cancer Lett 177:49–56
131. Boreddy SR, Srivastava SK (2012) Pancreatic cancer chemoprevention by phytochemicals. Cancer Lett 334:86–94
132. Ha J, Zhao L, Zhao Q, Yao J, Zhu BB, Lu N et al (2012) Oroxylin A improves the sensitivity of HT-29 human colon

cancer cells to 5-FU through modulation of the COX-2 signaling pathway. Biochem Cell Biol 90:521–531
133. Sadzuka Y, Sugiyama T, Hirota S (1998) Modulation of cancer chemotherapy by green tea. Clin Cancer Res 4:153–156
134. Chang CM, Chang PY, Tu MG, Lu CC, Kuo SC, Amagaya S et al (2012) Epigallocatechin gallate sensitizes CAL-27 human oral squamous cell carcinoma cells to the anti-metastatic effects of gefitinib (Iressa) via synergistic suppression of epidermal growth factor receptor and matrix metalloproteinase-2. Oncol Rep 28:1799–1807
135. Liang Y-C, Lin-Shiau S-Y, Chen C-F, Lin J-K (1997) Suppression of extracellular signals and cell proliferation through EGF receptor binding by (−)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. J Cell Biochem 67:55–65
136. Sun C-L, Yuan J-M, Koh W-P, Mimi CY (2006) Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis 27:1310–1315
137. Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K (2000) Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 13:49–54
138. Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E (1997) Why drinking green tea could prevent cancer. Nature 387:561
139. Imai K, Suga K, Nakachi K (1997) Cancer-preventive effects of drinking green tea among a Japanese population. Prev Med 26:769
140. Crew KD, Brown P, Greenlee H, Bevers TB, Arun B, Hudis C et al (2012) Phase IB randomized, double-blinded, placebo-controlled, dose escalation study of polyphenon e in women with hormone receptor–negative breast cancer. Cancer Prev Res 5:1144–1154
141. Pisters KM, Newman RA, Coldman B, Shin DM, Khuri FR, Hong WK et al (2001) Phase I trial of oral green tea extract in adult patients with solid tumors. J Clin Oncol 19:1830–1838
142. Shanafelt TD, Call TG, Zent CS, Leis JF, LaPlant B, Bowen DA et al (2012) Phase 2 trial of daily, oral polyphenon E in patients with asymptomatic, Rai stage 0 to II chronic lymphocytic leukemia. Cancer 119(2):363–370
143. Moyers SB, Kumar NB (2004) Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. Nutr Rev 62:204–211
144. Webb T (2000) Green tea experiments in lab, clinic yield mixed results. J Natl Cancer I 92:1038–1039