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Cancer prevention by tea: animal studies, molecular mechanisms and human relevance

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Abstract

Extracts of tea, especially green tea, and tea polyphenols have been shown to inhibit the formation and development of tumours at different organ sites in animal models. There is considerable evidence that tea polyphenols, in particular (–)-epigallocatechin-3-gallate, inhibit enzyme activities and signal transduction pathways, resulting in the suppression of cell proliferation and enhancement of apoptosis, as well as the inhibition of cell invasion, angiogenesis and metastasis. Here, we review these biological activities and existing data relating tea consumption to human cancer risk in an attempt to understand the potential use of tea for cancer prevention.

Dietary factors can substantially influence human cancer risk. Many food items, beverages and dietary constituents have reported cancer-preventive or anticancer activities. Tea, a commonly consumed beverage derived from the dried leaves of the *Camellia sinensis* plant, has been studied extensively for its health benefits, including cancer prevention. These studies are important because tea is consumed by a large proportion of the world's population and is the most popular beverage after water. Therefore, tea is the major source of dietary flavonoids (or polyphenols) in many populations. Tea is a unique food item in that the major constituents, especially those in green tea, are well characterized, allowing their biological activities to be thoroughly studied. The relationship between tea consumption and human cancer can be investigated using retrospective and prospective studies in which tea consumption is assessed by dietary recalls or by specific urinary markers.

Although different aspects of tea and cancer prevention have been covered by previous reviews^{1–10}, this Review critically assesses existing data and discusses our current understanding of how tea constituents might prevent cancer. We use selected recent findings to illustrate the types of laboratory results that are available. We also briefly review the results of human epidemiological and intervention studies, interpret these results on the basis of our understanding of the biochemical properties of tea constituents (BOX 1) and discuss possible mechanisms by which tea polyphenols prevent cancer development. Finally, we discuss the opportunities and challenges presented by the study of tea for cancer prevention. We hope that this Review will have wide-ranging implications, as many of the issues discussed here might also be applicable to studies of other dietary materials.

Tea constituents and their biochemical properties

The major catechins (a group of polyphenols) in green tea are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epicatechin (BOX 1). Tea catechins are characterized by the dihydroxyl or trihydroxyl substitutions on the B ring and the *m*-5,7-dihydroxyl substitutions on the A ring¹¹. The B ring seems to be the principal site of antioxidant reactions^{12,13} and the antioxidant activity is further increased by the trihydroxyl structure in the D ring (gallate) in EGCG and ECG. The polyphenolic structure allows electron delocalization, conferring the ability to quench free radicals. Tea preparations have been shown to react with reactive oxygen species (ROS) such as superoxide radical, singlet oxygen, hydroxyl radical, peroxy radical, nitric oxide, nitrogen dioxide and peroxynitrite^{11–13}. Among tea catechins, EGCG is the most effective in reacting with the majority of ROS. Tea polyphenols are also strong chelators of metal ions; the chelation of free metal ions prevents the formation of ROS from the auto-oxidation of many compounds.

The vicinal dihydroxy or trihydroxy structures not only contribute to the antioxidative activity of tea polyphenols, but also increase the susceptibility of these compounds to air oxidation under alkaline or neutral pH. In the case of EGCG, auto-oxidation generates superoxide anion and hydrogen peroxide and leads to the formation of dimers, such as theasinensins¹⁴. These reactions occur under cell culture conditions, and we propose that this is due to superoxide anion-catalysed chain reactions because EGCG can be stabilized by the addition of superoxide dismutase (SOD).

At a glance

- Tea, made from the dried leaves of the plant *Camellia sinensis*, is the most popular beverage worldwide after water.
- Tea and tea polyphenols have been shown to inhibit tumour formation and growth in different animal models for human cancer. The inhibition is associated with decreased cell proliferation, increased apoptosis and suppression of angiogenesis.
- Tea polyphenols are antioxidants, but they can also generate reactive oxygen species. The major polyphenol from green tea, (–)-epigallocatechin-3-gallate, has been shown to bind directly to several receptors and signalling molecules, and to inhibit the functions of key receptors, kinases, proteinases and other enzymes.
- Epidemiological studies, however, have not yielded conclusive results on the cancer-preventive effect of tea consumption in humans, possibly owing to different confounding factors. Some human cancer prevention trials with green tea polyphenol preparations have shown promising results.
- Well-designed epidemiological studies and intervention trials are needed to evaluate the cancer-preventive activities of tea and tea polyphenols in humans.
- Many issues raised and the experience gained from studies on tea and cancer prevention may be applicable to studies on other dietary constituents.

The polyphenolic structure of tea polyphenols also makes them good donors for hydrogen bonding. For example, hydrogen bonding of water molecules to EGCG forms a large hydration shell, which reduces the absorption of EGCG¹⁵ (BOX 2). This hydrogen bonding capacity also enables tea polyphenols to bind strongly to proteins and nucleic acids (discussed below). How these effects contribute to the cancer-preventive effect of green tea is partly determined by the bioavailability of these compounds *in vivo*^{16–21} (BOX 2).

Inhibition of tumorigenesis in animal models

Cancer prevention by tea and tea components has been studied in many different animal models of carcinogenesis (reviewed in ^{REFS 3,10}) (TABLE 1). In a review of the 147 papers published up to December 2008, 133 described cancer-preventive or inhibitory effects; we briefly discuss some of these effects below.

Inhibition of lung tumorigenesis

Administration of green tea, black tea, green tea polyphenols, EGCG or theaflavins during the initiation or promotion stage of tumorigenesis inhibited 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in rats, mice and hamsters^{22–30} (BOX 3). In a recent study, A/J mice were treated with a single dose of NNK and kept for 20 weeks to allow lung adenomas to develop. Mice were then given 0.5% polyphenon E (PPE), or 0.044% caffeine, in their drinking fluid for 32 weeks; this treatment reduced the progression of lung adenomas to adenocarcinomas³⁰. PPE or caffeine treatment was shown to inhibit cell proliferation, increase apoptosis and decrease levels of the phosphorylated transcription factors TUN, ERK1 and ERK2 in adenocarcinomas. However, in normal lung tissue, neither agent had any significant effect on the levels of cell proliferation or apoptosis. Treatment of A/J mice with green or black tea for 60 weeks also inhibited the spontaneous formation of lung tumours³¹. In a metastasis model, oral administration of green tea infusion reduced the number of murine Lewis lung carcinoma cell colonies³². These results suggest that tea preparations exert an inhibitory effect at all stages of lung carcinogenesis. However, high doses of tea polyphenols are required to produce a cancer-preventive effect, possibly owing to the relatively low bioavailability of EGCG.

A new approach for cancer prevention is the use of polyphenols in combination with other agents. For example, we recently demonstrated the synergistic inhibitory action of a combination of PPE and the lipid-lowering agent atorvastatin against NNK-induced lung carcinogenesis in A/J mice³³. The synergistic action of this combination was also demonstrated in human lung cancer H1299 and H460 cells. In both the cell lines and the mouse model, downregulation of the anti-apoptotic proteins MCL1 and BCL-X_L and induction of apoptosis were associated with the synergistic inhibitory action³³. The possible synergistic actions between atorvastatin and tea in humans warrant future investigation.

Lu *et al.*³⁴ recently analysed the gene expression changes caused by the administration of green tea or PPE in the inhibition of NNK- or benzo[a]pyrene (B[a]P)-induced lung tumorigenesis in A/J mice. The authors found that the differential expression of 88 genes in tumours compared with normal tissues was reversed by green tea or PPE treatment. These authors also identified a classifier of 17 genes that was altered by tea and PPE treatments in both normal lungs and lung adenomas; they suggested that these genes could be used as markers for tea exposure. Additional studies are needed to verify the results and relate these gene expression changes to protein levels.

Inhibition of oral-digestive tract tumorigenesis

The cancer-preventive activities of tea and tea constituents have also been studied in different models of oral–digestive tract carcinogenesis (reviewed in ^{REF. 10}). Tea preparations have been shown to inhibit chemically induced oral carcinogenesis in a hamster model and oesophageal carcinogenesis in a rat model^{35,36}, as well as tumorigenesis in rat stomach and forestomach induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)³⁷. A recent study showed that 0.05% black tea polyphenols (polyphenon B) given through the diet effectively inhibited tumour formation in a similar model³⁸; this inhibition was associated with reduced cell proliferation, infiltration and angiogenesis, as well as increased apoptosis.

In the *Apc*^{Min/+} mouse model of intestinal tumorigenesis, EGCG solution (0.02–0.32%, weight:volume), given as the sole source of drinking fluid, inhibited the spontaneous development of small intestinal tumours in a dose-dependent manner³⁹. Inhibition of tumour multiplicity was associated with increased expression of the cell signalling protein epithelial cadherin (E cadherin) and decreased levels of nuclear β -catenin, MYC, phospho-Akt, phospho-ERK1 and phospho-ERK2. Caffeine was not effective in inhibiting tumorigenesis in this animal model. We also compared the efficacy of EGCG as a pure compound with a defined catechin mixture, PPE, in the *Apc*^{Min/+} mouse model⁴⁰. Total tumour multiplicity was decreased by both dietary PPE (0.12%) and the corresponding amount of dietary EGCG (0.08%). Although PPE seemed to be more effective than EGCG, the difference was not statistically significant. Additional studies are required to further elucidate whether PPE or other green tea catechin preparations are more effective at inhibiting tumorigenesis than EGCG.

Box 1

Constituents in green and black tea

Originating in China and South East Asia, tea plants have been cultivated for thousands of years. The leaves were initially used for medicinal purposes and only later as a popular beverage. Cultivated in more than 30 countries, ~3.8 million tonnes of tea are produced annually. The composition of tea varies with climate, horticultural practices, variety and the age of the leaves. The different production methods alter the chemical composition of the dried tea leaves. Green tea, which accounts for 20% of world tea consumption, is prepared by pan-frying or steaming and then drying the tea leaves to inactivate polyphenol oxidase and other enzymes. These processes preserve the characteristic tea polyphenols known as catechins, which account for 10–15% of the weight of the dried leaves. The major catechins are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epicatechin (see the figure). EGCG is the most abundant catechin, and may account for 50–75% of the catechins. Catechin, catechin gallate, gallic catechin, gallic catechin gallate, epigallocatechin digallates, epicatechin digallate, methylepicatechin and methyl EGC are present in smaller quantities. Flavonols, including quercetin, kaempferol, myricetin, and their glycosides, are also present in tea. A typical cup of green tea, brewed with 2.5 g of tea leaves in 250 ml of hot water, contains 620–880 mg of water-extractable materials of which about one-third are catechins and 3–6% is caffeine.

Black tea, which accounts for 78% of world tea consumption, is prepared by crushing the tea leaves and causing enzyme-catalysed oxidation and polymerization of tea catechins in a process commonly known as fermentation. This process results in the formation of oligomers, such as theaflavins (see the figure) and polymers known as thearubigins, which account for 2–6% and 15–20%, respectively, of the dry weight of the black tea infusion. Theaflavins are characterized by the benzotropolone ring structure and a bright red or orange colour, and contribute to the unique taste of black tea. Thearubigins, which have higher molecular weights, are poorly characterized chemically and biochemically. Oolong tea, which accounts for 2% of world tea consumption, is made by a delicate procedure that crushes only the rim of the tea leaf in a short fermentation process. The product retains higher levels of catechins and contains newly formed oligomers of catechins, such as theasinensins.

A review of the literature indicates that the colon cancer prevention activity of tea and tea polyphenol preparations has been consistently demonstrated in mouse models, but results from studies in rat models have been inconsistent (reviewed in ^{REF. 10}). We recently examined the effect of PPE on the development of aberrant crypt foci (ACF) and adenocarcinoma in the colons of rats treated with azoxymethane. The treatment of rats with PPE (0.24% in the diet) for 8 weeks decreased the total number of ACF per rat by 36.9%. In ACF with high-grade dysplasia the inhibitory activity of PPE was associated with decreased levels of nuclear β -catenin and cyclin D1, and increased retinoid X receptor- α staining⁴¹. Treatment with 0.24% PPE for 34 weeks decreased the incidence of adenocarcinoma by 60% and the multiplicity of adenocarcinoma and adenoma by 80% and 45%, respectively (C.S.Y. *et al.*, unpublished observations). In a model using a food carcinogen, PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine), to induce colon carcinogenesis in rats, treatment with EGCG during the post-initiation stage for 15 weeks reduced ACF formation by 71% compared with water-treated controls⁴². Conversely, in rats given PhIP and a high-fat diet, subsequent administration of tea (2%) or caffeine (0.065%) in drinking water, in the post-initiation stage, significantly increased colon tumour incidence⁴³. This suggests that caffeine can promote colon tumorigenesis under these experimental conditions.

Inhibition of prostate tumorigenesis

Liao *et al.* have demonstrated the growth-inhibitory activity of EGCG when administered by intraperitoneal injection to mice with human prostate cancer xenografts⁴⁴. Mukhtar *et al.* have also shown that administration of a green tea polyphenol infusion (0.1% in drinking fluid) to transgenic adenocarcinoma of the mouse prostate (TRAMP) mice for 24 weeks markedly inhibits prostate cancer development and distant site metastases^{45,46}. In the mice receiving tea polyphenols cell proliferation was decreased and apoptosis was increased, insulin-like growth factor 1 (IGF1) level was decreased, and IGF binding protein 3 (IGFBP3) was restored to

normal levels in both serum and the dorso-lateral prostate^{45,46}. This modulation of IGF1 and IGFBP3 levels was associated with reduced levels of phosphatidylinositol 3-kinase (PI3K), and of the phosphorylated forms of Akt, ERK1 and ERK2. The green tea polyphenol treatment also significantly decreased levels of angiogenic and metastatic markers, such as vascular endothelial growth factor A (VEGFA), matrix metalloproteinase 2 (MMP2) and MMP9. These results suggest that the inhibition of VEGFA, MMPs and the IGF1 signalling pathway contributes to the cancer prevention activity of green tea polyphenols.

Possible cancer prevention by tea in humans

The effects of tea consumption on the risk of human cancer have been investigated in many epidemiological studies, but the results have been inconclusive (reviewed in ^{REF 10}). A literature search in the PubMed database for papers published up to December 2008 showed 127 case-control studies and 90 cohort studies on the relationship between tea consumption and the risk for colon, lung, stomach, breast, prostate, ovarian, pancreatic, kidney, bladder and other cancers (TABLE 2). Of these studies, only 51 case-control studies and 19 cohort studies showed an inverse association between tea consumption and cancer risk, suggesting a cancer-preventive effect of tea, whereas other studies showed no such association. One overall impression is that the cancer-preventive effect is seen more frequently in case-control studies than in prospective cohort studies. Cancer risk reduction is also observed more frequently in studies on green tea than in those on black tea, which is probably because many polyphenols in black tea are poorly, or not, bioavailable (BOX 2).

Box 2

Absorption and metabolism of tea polyphenols

After drinking tea only a certain percentage of the polyphenols is absorbed, with those appearing in the blood and tissues considered bioavailable. The bioavailabilities of tea polyphenols with large molecular masses, such as theaflavins (564–868 Da), are low. (–)-Epigallocatechin-3-gallate (EGCG; 458 Da) also has limited bioavailability and the smaller molecules, (–)-epigallocatechin (EGC) and (–)-epicatechin (306 Da and 290 Da, respectively), have higher bioavailabilities. For example, after drinking an equivalent of two cups of green tea, it takes 1.5–2.0 hours for the catechins to reach peak values in the blood, and the peak plasma levels for EGCG, EGC and (–)-epicatechin are 0.26, 0.48 and 0.19 μM , respectively, although the EGCG content in tea is much higher than those of EGC and (–)-epicatechin. EGCG clears from the blood with an elimination half-life of 2.0–3.5 hours. The detailed pharmacokinetics of tea catechins in humans and rodents have been studied^{16–20}. The blood levels of EGCG in humans due to tea consumption or in animals receiving tea preparations in cancer prevention studies are generally lower than 0.5 μM . When large pharmacological doses of polyphenols are orally administered, peak plasma concentrations of 5–7 μM are observed in humans and mice. EGCG is excreted mainly through the bile to the faeces, with little excreted through the urine, whereas the smaller molecule EGC is excreted to the urine. Therefore, the urinary EGC and related metabolites can be used as an exposure marker for tea consumption.

Inside the body, tea polyphenols are readily methylated by *S*-adenosyl-methionine, which is catalysed by the enzyme catechol-*O*-methyltransferase (COMT). They are also catalysed by UDP-glucuronyltransferase (UGT) and sulphotransferase (SULT) to form corresponding glucuronide and sulphate conjugates of catechins²¹. In addition, ring-fission metabolites, such as 5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone (M4), and 5-(3',4'-dihydroxyphenyl)- γ -valerolactone, are formed. These metabolites are produced by intestinal microflora, as humans and mice do not have the enzymes to catalyse the fission of the C ring and the hydrolysis of the ester bond in EGCG. These pathways are illustrated

in the figure, using EGCG as an example. All these metabolites have biological activities, but the activities were lower than that of EGCG in cancer cell growth inhibition assays *in vitro*.

Confounding and modulating factors

The inconsistent results of the epidemiological studies were probably due to different confounding factors, difficulties in quantifying tea consumption, varied cancer aetiology in different populations and population heterogeneity. When these factors were effectively managed, a clearer relationship between tea consumption and cancer risk was observed in several studies; some examples are discussed below.

Cigarette smoking and alcohol consumption are important interfering factors. For example, in a case-control study on oesophageal cancer in Shanghai by Gao *et al.*⁴⁷, a protective effect of green tea consumption was observed in women, mostly non-smokers, with an odds ratio (OR) of 0.50 and a 90% confidence interval (CI) of 0.30–0.83. However, no protective effect was found in men, who were mostly smokers. In a subset of subjects who neither smoked tobacco nor drank alcohol, oesophageal cancer risk among tea drinkers was significantly decreased in both men (OR = 0.43; 95% CI = 0.22–0.86) and women (OR = 0.40; 95% CI = 0.20–0.77). In Asia, tea drinking is commonly associated with cigarette smoking in men. Although many studies tried to correct for smoking, the interaction between these two factors is difficult to assess.

The quantity and quality of the tea consumed affects the outcome of epidemiological studies. The lack of a preventive effect of tea consumption against cancer formation observed in many studies may be due to the low quantity of tea consumed. In some studies in Japan, daily

consumption of ten cups of tea was required for the cancer-preventive effect⁴⁸. In the study by Gao *et al.*⁴⁷, the quantity of green tea consumption was reported as grams of dry tea leaves consumed, and they found a protective effect against oesophageal cancer in women who consumed ≥ 150 g of tea per month (an equivalent of 2–3 cups of tea per day). Similarly, in a case–control study in nearby Zhejiang province, consumption of ≥ 250 g of dried green tea leaves per annum was associated with a reduced risk for breast cancer⁴⁹. As the habit of tea drinking in these areas is known, reporting grams of tea consumed may be a good way of assessing tea consumption. Objective measurements of biomarkers that reflect exposure to tea, such as urinary catechins and their metabolites^{50,51}, may also be useful. For example, in a nested case–control study in the Shanghai cohort, we used the presence of urinary EGC as an indicator of tea consumption and found that it was inversely associated with gastric cancer risk⁵⁰. The protective effect was primarily observed in subjects with lower levels of serum carotenoids, suggestive of an antioxidative mechanism for the action of green tea⁵⁰. In a similar study in this Shanghai cohort, urinary EGC (or EGC plus its metabolite, 4'-methyl-EGC) was inversely associated with colon cancer risk⁵¹. In a recent nested case-control study in a large population-based prospective study in Japan, plasma levels of EGC were associated with a decreased risk of gastric cancer in women, but not in men⁵². Once again, it is plausible that these results reflect a higher proportion of smokers in the male population compared with the female population⁵².

Genetic polymorphism in the population may also affect the relationship between tea consumption and cancer risk. For example, a population-based case–control study of women of Asian descent living in Los Angeles, United States, found a significantly reduced risk of breast cancer among green tea drinkers who carried at least one low-activity allele of catechol *O*-methyltransferase^{53,54}. Individuals with low-activity alleles of this enzyme may have prolonged exposure to catechins and their cancer-preventive functions. Effects of genetic polymorphism on lung cancer prevention by tea have also been reported⁵⁵.

Intervention studies

Well-designed intervention studies may provide a clear demonstration of the cancer-preventive effects of tea preparations. Early studies have shown the chemopreventive effects of tea in human oral precancerous mucosa lesions⁵⁶ and human cervical lesions⁵⁷. In a recent double-blinded study in Italy, 30 men with high-grade prostate intraepithelial neoplasia (PIN) were given 600 mg of green tea catechins daily for 12 months. Only 1 patient developed prostate cancer, whereas 9 of the 30 patients with high-grade PIN in the placebo group developed prostate cancer⁵⁸. These results are promising and would have a large impact if they could be reproduced in similar trials with larger numbers of subjects. In a recent study in Japan, supplementation of green tea extract (1.5 g per day for 12 months) in patients who had colorectal adenomas removed by polypectomy was shown to reduce the development of metachronous colorectal adenomas compared with a group of patients who did not take green tea extract⁵⁹. These patients are regular tea drinkers (average of 6 cups per day); the dose-response result of this study is difficult to explain and further studies are needed. The human trials cited above are all pilot studies; additional studies with larger numbers of patients for a longer duration are required to obtain more explicit information. More than 20 human trials with tea polyphenol preparations are ongoing or are planned (see clinical trials website in Further information); hopefully these studies will yield clear, consistent conclusions.

Mechanisms for cancer prevention by tea

Polyphenols, especially EGCG, have received most of the attention in studies of active tea constituents that can inhibit carcinogenesis, although the inhibitory activity of caffeine has also been demonstrated in lung and skin carcinogenesis models^{23,30,60}. The mechanisms of action of caffeine in skin carcinogenesis models have been previously discussed by Conney *et al.*⁶⁰

and so are not addressed here. This section discusses cancer-preventive mechanisms of the major tea polyphenol, EGCG.

Many mechanisms have been proposed based on studies with EGCG in cell lines. However, the concentrations of EGCG used in some of the cell culture experiments (20–100 μM) are higher than the plasma and tissue concentrations observed in humans after drinking two or three cups of green tea or in mice in cancer prevention experiments (usually $<0.5 \mu\text{M}$)²¹. It remains unclear whether the information obtained from cell lines with high EGCG concentrations can be extrapolated to cancer prevention in animals. In addition, many proposed mechanisms that are based on cell line studies are likely to be more relevant to therapeutic activity, rather than the cancer-preventive effect of tea.

As a chemical, EGCG can exert its actions by serving as an antioxidant or a pro-oxidant. It can also bind to target molecules and trigger cascades of signalling or metabolic pathways that lead to the inhibition of carcinogenesis. An assessment of our current understanding of the cancer prevention mechanisms is given below.

Antioxidation, pro-oxidation and carcinogen elimination

Although tea polyphenols are strong antioxidants *in vitro*, their antioxidative effects *in vivo* can only be demonstrated in certain studies. The bioavailability of tea polyphenols apparently limits the biological activity *in vivo*. Administering EGCG to old rats reduced oxidative stress, and the EGCG-treated animals had decreased levels of lipid peroxidation and protein carbonylation, as well as increased levels of antioxidants and antioxidant enzymes in the liver, skeletal muscle and brain^{61,62}. However, no effects were observed in young rats, suggesting that the antioxidative effects of EGCG are only apparent in the presence of excessive oxidative stress. Similar conclusions have also been drawn from other studies in both animals and humans (reviewed in REF. 63). Supplementation of the diets of healthy human volunteers with tea catechins (500 mg per day) for 4 weeks resulted in an 18% decrease in plasma oxidized low-density lipoprotein compared with the control⁶⁴. Similarly, supplementation of the diets of patients on haemodialysis with green tea catechins (455 mg per day) for 3 months decreased plasma hydrogen peroxide, C-reactive protein and several pro-inflammatory cytokines compared with placebo-treated controls⁶⁵. The antioxidative activity of tea polyphenols could decrease oxidative DNA damage, and this has been shown in human and animal models. For example, in a randomized intervention study, supplementation of the diets of heavy smokers with 4 cups of decaffeinated green tea (73.5 mg of catechins per cup) for 4 months was found to reduce urinary 8-hydroxydeoxy-2'-deoxyguanosine (8-OHdG) levels by 31% compared with the control group⁶⁶. Similarly, administration of green tea to smokers for 4 weeks reduced the number of 8-OHdG-positive cells to 50% of the pretreatment level⁶⁷. As endogenously formed ROS are also important in promoting carcinogenesis, tea polyphenols may have important roles in quenching these species at different stages of carcinogenesis.

Box 3

A lung tumorigenesis model in A/J mice

Tobacco carcinogens 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[*a*]pyrene (B[a]P) are commonly used to induce lung cancer in mice^{22–28}. A/J mice have been used extensively in these studies because they are prone to the development of lung tumours. The following figure illustrates that after treatment (intraperitoneal injection) of the mice with one dose of NNK, lung tumours develop in almost all the mice in 16–20 weeks. Inhibition of tumorigenesis has been demonstrated when tea preparations are administered to the mice in drinking water for 3 weeks, starting 2 weeks before the NNK treatment²². NNK is metabolically activated to electrophiles that alkylate DNA, and

mutational activation of the Ras oncogenes is a key step in the initiation of tumorigenesis. In the second version of this model, tea preparations are administered 1 week after the NNK treatment until the termination of the experiment. Inhibition of tumorigenesis has also been observed²². The action of tea preparations in this case is considered to be exerted during the post-initiation stage. In the third version, the mice are kept for 16–20 weeks to allow tumours to develop, and they are then treated with tea preparations for another 30–40 weeks to allow the tumours to progress to adenocarcinoma. This is considered a progression model and the inhibitory effect of tea preparations has also been demonstrated³⁰. B[a]P or a combination of B[a]P and NNK has also been used to induce lung tumours in the A/J mice³⁴. Multiple lower doses of these carcinogens administered in longer treatment periods have been used by some researchers²³.

Conversely, tea catechins can also be oxidized to generate ROS, which are readily observed in cell culture medium, and lead to cell death^{14,68}. After entering the cells, EGCG may also produce ROS by an unknown mechanism and the role of these ROS in cancer prevention is under investigation in our laboratory. ROS may also activate the nuclear factor erythroid 2-related factor 2 (NRF2, also known as NFE2L2) antioxidant-responsive element pathway to activate antioxidant and detoxifying enzymes⁶⁹. This activation appears to occur *in vivo*: oral gavage of EGCG (200 mg per kg) to C57B1/6J mice upregulated gene expression of γ -glutamyltransferase, glutamate cysteine ligase and haemoxygenase 1 in the liver and colon⁷⁰. Similarly, treatment of human volunteers for 4 weeks with 800 mg PPE per day increased glutathione *S*-transferase P activity in blood lymphocytes⁷¹. This effect was greatest in individuals with the lowest tertile baseline glutathione *S*-transferase P activity (80% increase compared with the baseline). Correspondingly, in a recent intervention study in a high aflatoxin exposure area in China, a 3-month supplementation with 500 or 1,000 mg green tea polyphenols per day increased the median urinary aflatoxin B1-mercapturic acid levels by more than tenfold compared with the baseline⁷². Conversely, there have been case reports showing that excessive amounts of tea extracts (taken as a dietary supplement for the purpose of weight reduction) induce liver toxicity⁷³, which is probably caused by a pro-oxidant mechanism⁷⁴. It remains to be determined whether the activation of NRF2 by ROS overlaps with the toxic effect of tea polyphenols.

Binding to molecular targets

The eight phenolic groups of EGCG can serve as hydrogen bond donors to many biomolecules. Previously, EGCG has been shown to bind to salivary proline-rich proteins, fibronectin,

fibrinogen and histidine-rich glycoproteins, and more recently to proteins such as the 67 kDa laminin receptor^{75,76} and Bcl-2 proteins⁷⁷ (FIG. 1).

Binding of EGCG to the 67 kDa laminin receptor with a dissociation constant (K_d) value of 0.04 μM was observed using a surface plasmon resonance assay⁷⁵. Expression of the metastases-associated 67 kDa laminin receptor increased the responsiveness of MCF-7 cells to low micromolar concentrations of EGCG⁷⁵. A recent study that used NMR spectroscopy showed the direct binding of tea polyphenols to the BH3 pocket of anti-apoptotic Bcl-2 proteins — inhibition constant (K_i) = 0.33–0.49 μM ⁷⁷. However, higher EGCG concentrations were needed to induce apoptosis.

Using an EGCG–Sepharose 4B column, two-dimensional electrophoresis and matrix-assisted laser desorption/ionization-time-of-flight mass spectroscopy, Dong *et al.* identified vimentin⁷⁸, insulin-like growth factor 1 receptor (IGF1R)⁷⁹, FYN⁸⁰, glucose-regulated protein 78 kDa (GRP78)⁸¹ and ZAP70⁸² as high-affinity EGCG binding proteins. All of these proteins were demonstrated to be important for the inhibitory activity of EGCG in cell lines, but higher EGCG concentrations than the K_d values were needed. For example, vimentin binds to EGCG with a K_d of 3.3 nM, and functional studies showed that EGCG inhibited the phosphorylation of vimentin at serine 50 and serine 55 by cell division cycle 2 (CDC2) with IC_{50} = 17 μM . The difference in effective concentrations is probably due to the nonspecific binding of EGCG to other proteins, which compete with the target protein. Vimentin knockdown by small interfering RNA in JB6 C141 cells resulted in a lower proliferation rate, and the cells became less responsive to inhibition by EGCG. IGF1R activation can induce cell proliferation and survival, as well as inhibit apoptosis, transformation, metastasis and angiogenesis in different types of cancer⁸³. EGCG also inhibits IGF1R phosphorylation and increases expression of transforming growth factor- β 2 (TGF β 2) in human colon cancer SW837 cells⁸⁴, which is consistent with our observations in HRAS-transformed human bronchial epithelial 21BES cells⁸⁵. The physical binding of EGCG to nucleic acids suggests that DNA and RNA can also be binding targets of green tea catechin⁸⁶. However, the relevance of this proposed binding depends on whether the catechins can bind selectively to specific nucleic acid species in cancer or premalignant cells without affecting normal cells.

In general, if a biological effect of a compound can be observed *in vitro* at concentrations lower or similar to those observed *in vivo*, then the event can occur *in vivo*. Therefore, the discovery of the high-affinity EGCG-binding proteins discussed above is promising. The general applicability of these mechanisms for cancer prevention remains to be investigated.

Inhibition of enzyme activities

In collaboration with Z. Dong, we previously observed that EGCG, at concentrations of 5–20 μM , inhibited the phosphorylation of JNK (JUN N-terminal kinase), JUN, MEK1, MEK2, ERK1, ERK2 and ELK1 (Ets-like protein 1) in JB6 epidermal cell lines^{87–89}. This inhibition was associated with the inhibition of AP1 transcriptional activity or cell transformation. *In vitro* kinase assays suggested that EGCG inhibited MEK1 phosphorylation by decreasing its association with the kinase RAF1. Moreover, EGCG seems to inhibit the phosphorylation of ELK1 by competing with the binding site for ERK1 and ERK2 (REF 89). Inhibition of phosphorylation of JUN, ERK1 and ERK2 by EGCG or green tea polyphenols was also observed in lung carcinogenesis models³⁰. Cyclin-dependent kinase 2 (CDK2) and CDK4 were also inhibited by 30 μM EGCG in MCF-7 breast cancer lines, and this was associated with cell cycle arrest in G0 and G1 (REF. 90).

EGCG has also been reported to inhibit the chymo-tryptic activity of 20S proteasomes⁹¹. Treatment of LNCaP prostate cancer cells with EGCG resulted in G0 and G1 cell cycle arrest⁹² and accumulation of p27 and inhibitor of nuclear factor- κB (I κB)^{91,93,94}, both of which

are targets for proteasomes. The difference of effective concentrations in cell-free systems ($IC_{50} = 0.09\text{--}0.2\ \mu\text{M}$) and in cell lines ($IC_{50} = 1\text{--}10\ \mu\text{M}$)⁹¹ suggests that EGCG may bind nonspecifically to proteins or other macromolecules in the cells and therefore lower the effective concentration of EGCG at the active site of the proteasome. In HT1080 human fibrosarcoma cells, EGCG and other catechins affect MMPs directly; the activity of secreted MMP2 and MMP9 was inhibited by EGCG with IC_{50} values of 8–13 μM in zymographic analysis^{95,96}. EGCG also increased the expression of the tissue inhibitor of MMPs (TIMP1 and TIMP2) at lower concentrations ($\sim 1\ \mu\text{M}$), which provides an additional mechanism to suppress the activity of MMPs⁹⁶. Additionally, EGCG inhibited the activation of pro-MMP2 by membrane-type MMP (also known as MMP14)⁹⁷. These activities may contribute to the reported inhibition of metastasis and invasion following treatment of tumour-bearing mice with green tea or EGCG⁹⁸. Further *in vivo* studies are needed to generate more direct evidence for this mechanism.

We reported that EGCG inhibited DNA methyltransferase ($K_i = 7\ \mu\text{M}$) in KYSE 510 human oesophageal cancer cells and this resulted in the demethylation of the hypermethylated promoters of the tumour suppressor gene *INK4A*, retinoic acid receptor- β , and the DNA repair genes *MLH1* and methylguanine methyltransferase; increases in gene expression were seen⁹⁹. Reactivation of some of these genes was also observed in HT29 colon and PC3 prostate cancer cells. EGCG has also been reported to inhibit dihydrofolate reductase¹⁰⁰, glucose-6-phosphate dehydrogenase¹⁰¹ and glyceraldehyde-3-phosphate dehydrogenase¹⁰². These enzyme inhibition results are biochemically interesting but it remains to be determined whether these activities can be demonstrated *in vivo* at a non-toxic dose of EGCG and how this enzyme inhibition might prevent cancer formation.

Inhibition of receptor-dependent signalling pathways and angiogenesis

Members of the epidermal growth factor receptor (Egfr) family are frequently overexpressed in human cancers and are associated with poor prognosis¹⁰³. Many studies have demonstrated the inhibitory effects of EGCG on the Egfr signalling pathways^{14,104–107}. For example, it has been shown or suggested that EGCG can inhibit EGFR tyrosine kinase activity ($IC_{50} = 1\text{--}2\ \mu\text{M}$)¹⁰⁴; inhibit the phosphorylation of EGFR, possibly by interfering with the binding of EGF to EGFR¹⁰⁴; alter lipid organization in the plasma membrane (lipid rafts) and inhibit EGF binding to EGFR¹⁰⁶; and induce internalization of EGFR into endosomes¹⁰⁷. Inhibition of EGFR signalling has also been shown to decrease the production of VEGFA in cancer cells¹⁰⁸. However, it is not known whether these actions of EGCG occur *in vivo*. The concept that EGCG may affect lipid rafts is interesting, although it remains to be demonstrated *in vivo* whether EGCG also alters the lipid rafts of normal cells and what concentration of EGCG is required to exert a desirable effect.

Deregulation of the hepatocyte growth factor (HGF)–HGFR pathway occurs in several types of human cancers and can lead to increased tumorigenesis and metastasis¹⁰⁹. HGFR (a receptor tyrosine kinase also known as MET) and HGF have key roles in epithelial–mesenchymal transition, which is associated with tumour invasion¹¹⁰. It has been shown in MDA-MB-231 cells that the HGF-induced phosphorylation of HGFR and AKT1 was completely blocked by 0.6 μM EGCG, and that cell invasion was significantly decreased by 5 μM EGCG¹¹¹. In a study with FaDu hypopharyngeal carcinoma cells, 1 μM EGCG prevented HGF-induced motility in an *in vitro* wound healing assay¹¹².

Growth factors — such as VEGFA, fibroblast growth factor 2 (FGF2), IGF and EGF — serve as chemical stimuli to initiate angiogenesis, which provides nutrients for tumour growth. EGCG has been reported to decrease the RNA and peptide levels of VEGFA^{108,113}. In addition, EGCG (0.5–10 μM) disrupted VEGFA-induced VEGFR2 dimerization and decreased the downstream PI3K activity in human umbilical vein endothelial cells¹¹⁴. We have previously

observed the downregulation of VEGFA expression and suppression of angiogenesis by treatment with green tea (0.6% green tea solid in drinking fluid) in the NNK-induced lung tumorigenesis model²⁸. Also, in a murine gastric tumour model, EGCG (1.5 mg per day per mouse, intraperitoneally for 28 days) suppressed VEGFA protein expression and tumour microvessel density¹¹⁵. In a recent report, EGCG (0.01% in drinking water) was shown to decrease FGF2 levels in lysates from intestinal tumours of *Apc*^{Min/+} mice¹¹⁶. Inhibition of FGF2 is also expected to contribute to an anti-angiogenic effect¹¹⁷.

Overview of cancer prevention mechanisms

As tea constituents, or even pure EGCG, have broad cancer-preventive effects in different animal models, it is likely that multiple molecular mechanisms, rather than a single receptor or molecular target, are involved. Even in a single experimental system, multiple molecular targets may still be involved. Among the mechanisms reviewed above and shown in FIG. 1, those with low effective concentrations *in vitro* are likely to occur *in vivo*. It is reasonable to suggest that EGCG initially binds to one or more of the target proteins, which may be transmembrane receptors, kinases or other enzymes. These actions may inhibit key signalling and metabolic pathways that are essential for the development of tumours, or may induce apoptosis of the premalignant or malignant cells. It remains a challenge to demonstrate the specific molecular events that are responsible for cancer prevention in animal models and humans. An interesting phenomenon that has been observed in different laboratories is the greater susceptibility of cancer cells to the inhibitory effect of EGCG and other chemopreventive agents than normal and non-transformed cells. A possible explanation for this phenomenon is the concept of oncogene addiction, as proposed by Weinstein and Joe¹¹⁸. According to this concept, the rapid growth of cancer cells depends on the aberrant activity or overexpression of a few oncogenes; if EGCG blocks the activity of one or two of these oncogenes, the cell growth would be severely inhibited and apoptosis would occur. Conversely, growth and survival of normal cells depends on the expression of many sets of pathways: when one or two of them are inhibited by EGCG, it would not significantly affect cell survival. The proposed cancer prevention mechanisms involving antioxidative and pro-oxidative actions and elimination of environmental carcinogens also need to be further investigated.

Concluding remarks

As discussed in this Review, the inhibitory activities of tea and tea polyphenols against carcinogenesis have been established in different animal models. Studies in cell lines have also demonstrated that tea polyphenols can affect a range of signalling and metabolic pathways. These molecular events may result in cancer cell growth inhibition, apoptosis and inhibition of invasion, angiogenesis and metastasis. On the basis of these results, we think that tea, which is readily available and widely consumed, has a high potential for application in the prevention of human cancer. Nevertheless, the cancer-preventive activity of tea has not been consistently observed in studies in humans. This may be due to the relatively low levels of tea consumption by some human populations (compared with animal studies) and the various confounding factors in epidemiological studies in different populations. The differences between *in vitro* and *in vivo* studies and the difficulties in extrapolating results from animal studies to humans also exist in cancer prevention studies with many other dietary constituents or food items. The lessons learned from studies on tea may be applicable to research on other cancer chemopreventive agents. Bioavailability is an issue with polyphenolic compounds. For agents that are not well absorbed systemically, direct contact with the digestive tract may be important for the cancer-preventive activity and this should also be applicable to the activities of EGCG and polyphenols in black tea. This concept is consistent with the results from studies on prevention of oral, oesophageal and colon cancer by black raspberry powder (which contains anthocyanins and ellagitannins) conducted by G. Stoner and colleagues^{119,120}. More definitive

information on the cancer-preventive activity of tea polyphenols will emerge from well-designed cohort studies and human intervention trials. Knowledge gained from the studies on the biological properties and activities of tea polyphenols reviewed in this article will be useful in the design of prospective studies and in the selection of the agent, dosage and biomarkers for intervention trials.

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Glossary

Dietary recalls	A human dietary assessment procedure in which subjects are asked to recall their food consumptions over a certain time frame
Polyphenon E	A standard green tea polyphenol preparation containing 65% (–)-epigallocatechin-3-gallate
Atorvastatin	An inhibitor of 3-hydroxy-3-methyl-glutaryl CoA reductase and a commonly used cholesterol-lowering drug with the trade name Lipitor
Aflatoxin	A carcinogenic toxin produced by fungi that induces hepatic damage and leads to liver cancer
IC ₅₀	The concentration of a drug giving a 50% inhibition of the activity of a target enzyme
Lipid raft	A dynamic assembly of plasma membrane enriched in certain cholesterol, sphingolipids, saturated phospholipids and cell signalling proteins

Figure 1. Possible targets for the cancer preventive activity of (-)-epigallocatechin-3-gallate (EGCG)

Some of these are direct targets through binding; others are affected indirectly. The reported effective concentrations, in IC_{50} , K_i (inhibition constant) or K_d (dissociation constant) are shown in μM . All these are from studies *in vitro*. When two values are given, the first value is from cell-free systems and the second value is from studies in cell lines. 67LR, 67 kDa laminin receptor; CDK, cyclin-dependent kinase; DNMT, DNA methyltransferase; EGFR, epidermal growth factor receptor; GRP78, glucose-regulated protein 78kDa; HGFR, hepatocyte growth factor receptor; IGF1R, insulin-like growth factor 1 receptor; MMP, matrix metalloproteinase; ROS, reactive oxygen species; VEGFA, vascular endothelial growth factor A.

Table 1
Inhibitory effects of tea and tea constituents in animal models*

Site	Number of studies showing inhibitory effects	Numer of studies showing no inhibitory effects
Lung	20 (1)	2
Oral cavity	6	0
Oesophagus	4	0
Stomach	9	0
Small intestine	8	1
Colon	11 (3)	6
Skin	27 (1)	0
Prostate	4 (5)	0
Breast	10 (8)	0
Liver	7	1
Bladder	3 (1)	0
Pancreas	2 (2)	0
Thyroid	1	0

* The data were obtained by a literature search of PubMed from 1965 to 2008 of animal carcinogenesis models. The number of xenograft studies is shown in parentheses.

Table 2

Studies on tea consumption and the risk of human cancer*

Site	Type of tea	Cohort studies		Case-control studies	
		Risk reduction	No risk reduction	Risk reduction	No risk reduction
Colon	Green	2	5	4	3
	Black	2	8	4	12
Lung	Green	0	3	2	3
	Black	0	8	6	4
Stomach	Green	2	6	8	8
	Black	0	6	4	5
Oesophagus	Green	0	2	3	4
	Black	0	1	2	1
Breast	Green	3	4	3	0
	Black	1	8	1	9
Prostate	Green	2	0	2	0
	Black	1	2	1	3
Ovaries	Green	1	0	1	0
	Black	2	3	0	6
Pancreas	Green	0	2	1	1
	Black	0	4	1	3
Kidney and bladder	Green	0	1	0	4
	Black	1	4	2	5
Other	Green	0	2	5	1
	Black	2	3	1	3

* These data were obtained by a literature search of Pubmed from 1965 to 2008. The number of studies showing risk reduction or no risk reduction are shown.