Chapter 4
Cancer Prevention with Green Tea Polyphenols

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Abstract Consumption of green tea (*Camellia sinensis*) has been suggested to have beneficial health effect, including cancer prevention. Extensive studies have established that the active cancer preventive constituents in green tea are a group of polyphenols. Green tea polyphenols display anticancer activity in many organ sites in different experimental models in rodents and in cultured cell lines *in vitro*. Treatment with green tea polyphenols leads to the inhibition of cancer cell proliferation, cancer-associated angiogenesis, and metastasis, as well as the induction of cancer cell apoptosis. Experimental studies demonstrate that these activities are likely resulted from the antioxidant activity and the direct binding of green tea polyphenols to proteins, resulting in the modulation of multiple cellular signaling pathways. The findings of polyphenol binding proteins reveal the mechanisms of the effectiveness and specificity of the anticancer actions. However, the inverse association between of green tea consumption and cancer risk is supported by epidemiological studies, but not all. This inconsistence may due to the lower blood and tissue levels of polyphenols from green tea drink, and may also depend on various etiology factors. Using much higher doses, results from some interventional studies support the safety and effectiveness of green tea polyphenols in cancer prevention. Well-designed clinical studies are required to fully evaluate the usefulness of green tea polyphenols in cancer prevention.

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W.C.S. Cho (ed.), *Cancer Chemoprevention and Treatment by Diet Therapy*, Evidence-based Anticancer Complementary and Alternative Medicine 5,
DOI 10.1007/978-94-007-6443-9_4,

4.1 Introduction

The consumption of green tea, a beverage derived from the dried leaves of the *Camellia sinensis* plant, has a long history in Asia and is believed to have beneficial health effects such as preventing cancer, diabetes, neurodegeneration, eliminating toxic chemicals, anti-aging, improving cardiac function (Weisburger 1999, 2003; Yang et al. 2002; Higdon and Frei 2003; Lambert et al. 2005a; Yang et al. 2009a, b). Recent years, its cancer preventive activity has drawn the most attentions. Since 2000, there are over 2,000 research publications associated to tea and cancer. Extensive studies have established that there are two types of chemicals in green tea constituents, polyphenols and caffeine, responsible for cancer preventive activities (Conney et al. 2007; Yang et al. 2009a, b). The cancer preventive activity of polyphenols is the focus of this chapter.

Green tea catechin are a group of polyphenols, including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), (−)-epicatechin (EC) and other minor catechins (Lambert et al. 2007; Yang et al. 2009a, b, 2011; Sang et al. 2011). Among them, EGCG is the most abundant and active constituent in green tea. The anticancer activity of green tea polyphenols has been demonstrated in many types of cancers in experimental modeling systems range from different cancer cells cultured *in vitro* to various animal models (Yang et al. 2002, 2011). Treatment with green tea polyphenols leads to the direct effects on cancer cells such as the inhibition of tumor cell growth and the induction of tumor cell apoptosis (Yang et al. 2002, 2009b, 2011; Lambert et al. 2005a). Such effects have been reported to involve not only the regulation of specific genes, but also the modulations of multiple cellular signaling pathways (Yang et al. 2002, 2009b, 2011; Lambert et al. 2005a). The cancer preventive activity may be a result of combinatory effects on multiple targets, although the relative importance of the different pathways may depend upon the cellular context. Despite a large body of experimental evidences supporting the anticancer activity of green tea polyphenols, epidemiology studies conducted to determine the potential inverse association between the consumption of green tea and human cancer risk has shown inconsistent results. Some epidemiological studies conclude that green tea consumption is associated with reduced cancer risk, whereas some studies suggest that green tea is not associated with the reduced cancer risk (Boehm et al. 2009; Yang et al. 2009b). Albeit there are only a few clinical studies on the application of green tea polyphenols in cancer prevention, it is suggested that higher doses of green tea polyphenols can be tolerated and effective in cancer prevention. Here, we review the experimental evidences of the biological activity of green tea polyphenols and discuss the existing data from epidemiology and clinical studies regarding whether green tea polyphenol is effective in reducing human cancer risk in order to understand the potential application of green tea polyphenols for cancer prevention.

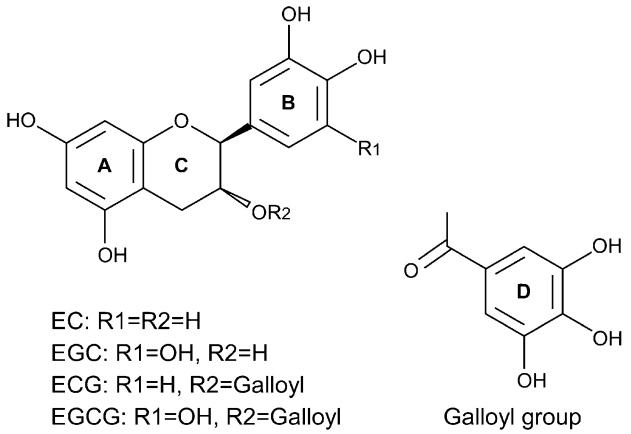

Fig. 4.1 The structures of the major green tea polyphenols

4.1.1 Tea Constituents and Their Biochemical Properties

A typical cup of green tea, brewed with 2.5 g of dry tea leaves in 250 mL hot water, contains 620–880 mg of water extractable chemicals among which tea polyphenols account for 30–42 % (Balentine et al. 1997). The major polyphenols are four catechins, EGCG, EGC, ECG, and EC, and their structures are shown in Fig. 4.1. The water extractable fraction is the green tea extract (GTE) and has often been used for numerous experimental studies in earlier years. Because EGCG is accounted for 50–80 % of the total catechins in tea, it is considered as the major tea catechin and purified EGCG has been used in substantial amount of studies. Thus the bioactivities of tea polyphenols are often represented by the activity of EGCG in many studies.

A well-defined activity of green tea polyphenols is the antioxidant activity, which is associated with the multiple phenolic groups on each ring: dihydroxyl or trihydroxyl substitutions on the B ring and the *m*-5,7-dihydroxyl substitutions on the A ring (Fig. 4.1) (Balentine et al. 1997). The B ring is the principle site of antioxidant reactions (Valcic et al. 2000; Meng et al. 2002), while the trihydroxyl D ring (gallate) of EGCG or ECG provides the extra potentials for antioxidant reactions. The polyphenolic structures allow electron delocalization and give green tea polyphenols ability to quench free radicals. Indeed, it is demonstrated that tea polyphenols are able to trap reactive oxygen and nitrogen species (RONS) including superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite (Balentine et al. 1997; Valcic et al. 2000; Meng et al. 2002). EGCG has the most hydroxyl groups (a total of eight hydroxyl groups) and is the most potent in reacting with RONS. Besides the direct mechanism to quench free radicals, green tea polyphenols are strong chelators of metal ions. Chelation of free metal ions by green tea polyphenols

prevents the formation of reactive oxygen species (ROS) from the auto-oxidation of many compounds that requires metal ions.

However, the vicinal dihydroxy or trihydroxy structure of tea polyphenols not only contributes to the anti-oxidative activity, but also increases the susceptibility of these compounds to air oxidation under alkaline or neutral pH. This is a particularly important feature of EGCG as the auto-oxidation of EGCG in solution generates superoxide anion and hydrogen peroxide and leads to the formation of several unstable intermediates including EGCG quinone, quinone-dimer, and theasinensins (Yang et al. 2009b; Lambert and Elias 2010). It is worth noting that, in the cell culture experiment, the auto-oxidation of EGCG is enhanced in cell culture medium containing metal ions. For example, a half-life of EGCG less than 30 min was observed in McCoy's 5A medium, commonly used for colon cancer cell line culture (Sang et al. 2007). By a real-time mass analysis, the kinetic of EGCG auto-oxidation at the concentrations of 50 and 200 μM in a Tris–HCl buffer (pH 7.2) has been elucidated (Sang et al. 2007). It has been proposed that oxygen reacts with EGCG to produce EGCG radical ($EGCG^{\cdot}$) and superoxide radical ($O_2^{-}\cdot$), both of which are unstable and active. This reaction is probably catalyzed by metal ions such as Cu^{2+} or Fe^{2+}. Then $O_2^{-}\cdot$ reacts with another EGCG molecule to produce EGCG· and hydrogen peroxide (H_2O_2). EGCG· reacts with oxygen to produce EGCG quinone and generate $O_2\cdot^{-}$. $O_2\cdot^{-}$ can then react with another molecule of EGCG for the propagation of a chain reaction of EGCG auto-oxidation (Fig. 4.2), which produces significant amount of ROS.

ROS generated by the auto-oxidation of EGCG is given more attentions in our experimental system using cultured cells. We have found that EGCG is very effective in killing cultured cells as analyzed by cytotoxic or cell proliferation assays with effective concentration as low as 5 μM. This is due to the cytotoxicity of quinone and ROS. However, when we add superoxide dismutase (SOD) into the medium prior to the addition of EGCG to block $O_2^{\cdot-}$, the cell killing effect is significantly reduced. The half maximal inhibitory concentration (IC_{50}) of EGCG required for inhibiting cell proliferation at 48 h is determined to be around 30–50 μM. This issue is particularly important for evaluating the results of the studies on cell signaling mechanism using cultured cells since ROS is able to cause changes on variety of cell signaling pathways. For example, ROS contributes to the inactivation of epidermal growth factor receptor (EGFR) (Naasani et al. 2003; Hou et al. 2005). Thus, we strongly suggest the addition of SOD to remove $O_2\cdot^{-}$ and catalase to remove H_2O_2 for the prevention of EGCG auto-oxidation when EGCG is studied in cultured cells *in vitro*. This addition reduces the cytotoxic effect but does not change the biological action of EGCG. In our recent study identifying *miR-210* up regulated by EGCG, we found no difference in the upregulation of *miR-210* by EGCG in lung cancer cells in the presence or absence of SOD and catalase (Wang et al. 2011). On the other hand, the effect associated with the ROS produced by the EGCG auto-oxidation, such as its inhibition on TGF (Vittal et al. 2004) and EGF (Hou et al. 2005), is prevented by the addition of SOD. Thus, the addition of SOD and catalase to remove ROS in the medium can distinct whether the action is mediated by ROS produced by the auto-oxidation of EGCG.

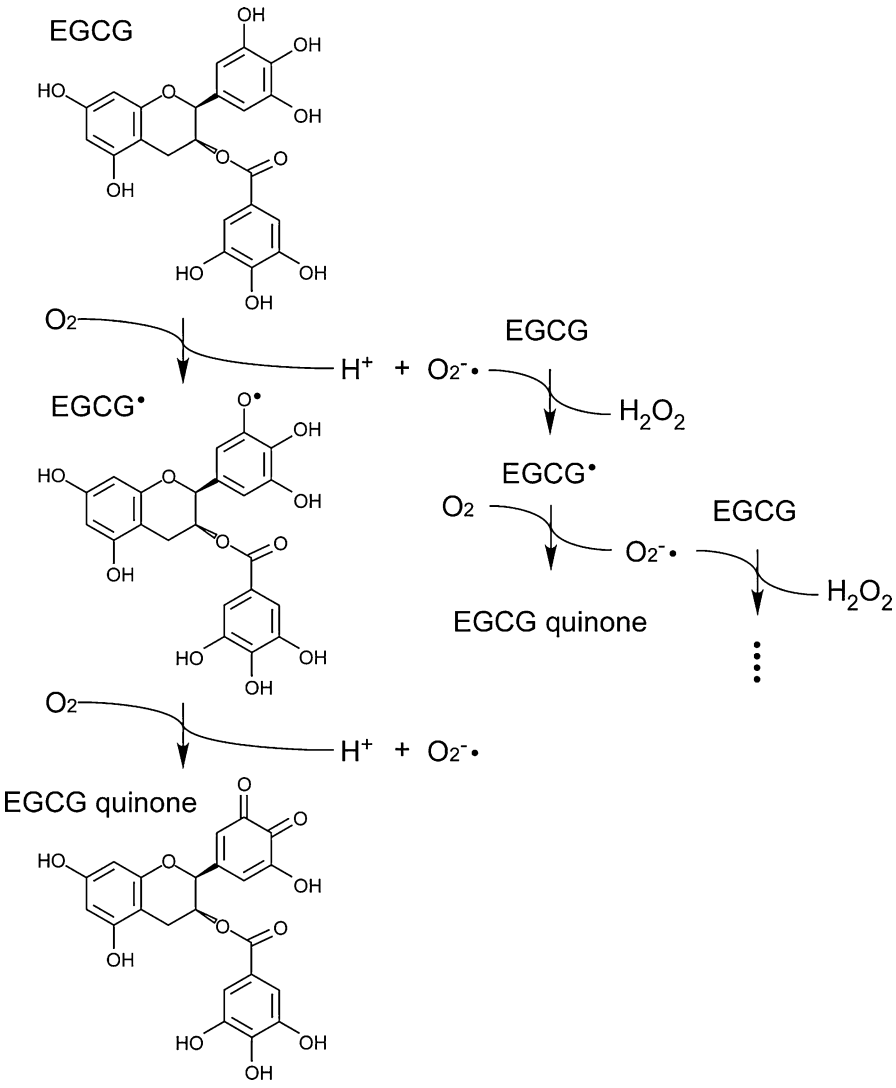

Fig 4.2 The auto-oxidation reaction of EGCG

4.1.2 Green Tea Polyphenol Biotransformation and the Activities of Metabolites

After drinking green tea, it takes about 1–1.5 h for green tea polyphenols to reach peak levels in the blood. When drinking an equivalent of two cups of green tea, the peak values of EGCG, EGC, and EC were 0.26, 0.48 and 0.19 μM, respectively (Lee et al. 2002). Considering the application of EGCG as the cancer prevention

agent, pharmacological doses have been studied and the highest blood peak value reported were as high as 7 μM (Lee et al. 2002). In most animal and clinical studies, the peak blood levels were usually below 1 μM and the half life of EGCG was approximately 2–3.5 h (Lee et al. 2002; Lambert et al. 2008). These observed peak blood values of EGCG were lower than the concentrations of EGCG that were used in most mechanistic studies, which often are about 10–100 μM, in cell culture. Although local concentration of EGCG in cancer tissue may be different, these data about the peak blood levels should be used as references for designing experiments and evaluating the results from *in vitro* studies.

After ingestion, green tea polyphenols have been found to undergo extensive biotransformation, including methylation, glucuronidation, and sulfation, as well as the microbial metabolism (i.e. ring fission) in the digestion tracts (Yang et al. 2002; Feng 2006; Sang et al. 2011). In the plasma, most EGCG is unconjugated (Chow et al. 2001), whereas most of ECG, EGC and EC are in the glucuronidated or sulfated (Lee et al. 1995; Zhu et al. 2000; Yang et al. 2002). However, it is less clear how these processes affect the anticancer activity of green tea polyphenols. Some *in vitro* experimental results suggest that the inhibitory activities of EGCG metabolites on cancer cell growth are less effective than EGCG (Lambert et al. 2005b, 2006; Nakagawa et al. 2007). Thus, it is reasonable to assume that the anticancer activities of green tea polyphenols are not due to their metabolites. EGCG is mainly excreted through bile, whereas EGC is excreted in urine. Since the bioavailability of polyphenols is a key parameter for understanding this biological effect, to measure tea polyphenols and their metabolites may provide useful information.

4.2 Application of Green Tea Polyphenols in Cancer Prevention

Cancer prevention by green tea polyphenols has been extensively studied for many years from *in vitro* and *in vivo* models as well as epidemiology and clinical studies. The overall conclusions are that green tea polyphenols are effective in inhibiting or preventing cancer progression in the majority of animal models. However, not all epidemiology studies support the inverse association between green tea consumption and cancer risk.

4.2.1 Inhibition of Tumorigenesis in Animal Studies

Green tea polyphenols display inhibitory activity against carcinogenesis in animal models at many organ sites, including lung, oral cavity, esophagus, stomach, small intestine, colon, skin, prostate, breast, liver, bladder, pancreas and thyroid (Yang et al. 2009b, 2011; Yang and Wang 2011). Among the models, lung, colon, prostate, breast, and skin cancers have been investigated extensively and will be discussed in details as examples of our current understanding of this subject.

4.2.1.1 Prevention of Lung Carcinogenesis by Green Tea Polyphenols

Administration of green tea polyphenols has been demonstrated to be effective in inhibiting lung carcinogenesis in 19 out of 21 studies using mice, rats, and hamsters (Ju et al. 2007; Yang et al. 2009b). Among these animal models, the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or benzo[*a*]pyrene (B[*a*]P)-induced lung carcinogenesis draws the most attentions. NNK and B[*a*]P are the major carcinogens found in cigarette smoke and used to mimic cigarette smoke to induce lung cancer. In A/J mice, NNK treatment induces lung carcinogenesis with the development of adenoma within 20 weeks and the progression of adenoma to adenocarcinoma between 20 and 50 weeks (Hoffmann et al. 1996). When 0.5 % green tea polyphenol extract was given to A/J mice bearing NNK-induced lung tumors as drink fluid for 32 weeks, adenoma progression to adenocarcinoma was inhibited (Lu et al. 2006a). Further, EGCG has been demonstrated to inhibit the xenograft tumors of human lung cancer cell lines H1299 and H460 in nude mice (Li et al. 2010). Apoptosis specific in tumors and not in normal lung tissues was induced by EGCG treatment while pro-proliferation signaling (i.e. c-Jun and phospho-ERK1/2) in tumors were reduced (Lu et al. 2006a; Li et al. 2010). Differential gene expression had also been profiled in tumors from the mice treated with green tea polyphenol (Lu et al. 2006b). Together with other studies on lung cancer, green tea polyphenols display multiple activities in inhibiting different aspects of lung carcinogenesis in this experimental model.

4.2.1.2 Prevention of Colon Carcinogenesis by Green Tea Polyphenols

The cancer preventive activity of green tea polyphenols is also demonstrated in different colon cancer animal models (Ju et al. 2007; Yang et al. 2009b). First, EGCG significantly inhibits colon tumorigenesis in $Apc^{min/+}$ transgenic mouse model. Administration of $Apc^{min/+}$ transgenic mouse with 0.02–0.32 % EGCG as the drink fluid shows a dose-dependent inhibition on the tumorigenesis in the small intestine (Ju et al. 2005; Hao et al. 2007). EGCG treatment leads to reduced Wnt signaling activity as indicated by the increased level of E-cadherin, decreased level of nuclear β-catenin, and reduced level of Wnt target such as c-myc, and pro-proliferation signaling such as phospho-Akt and phospho-ERK1/2 (Ju et al. 2005). Second, EGCG inhibits the chemical carcinogen induced colon cancer in rodent models. The incidence of aberrant crypt foci (ACF), representing colonic premalignant lesion, in azoxymethane (AOM)-treated F334 rats is reduced significantly by 0.01 % EGCG in drinking water (Ohishi et al. 2002). Besides, 0.1 % EGCG in drinking water further inhibits the high-fat diet enhanced incidence of ACF in the AOM-treated CF-1 mice (Ju et al. 2003). However, the involved mechanism remains unclear.

4.2.1.3 Prevention of Prostate Carcinogenesis by Green Tea Polyphenols

Green tea polyphenols display inhibitory activity in mouse prostate cancer models in several studies (Ju et al. 2007; Yang et al. 2009b). In study using the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, 0.1 % green tea polyphenols in drinking water is reported to be effective in inhibiting tumor incidence, burden, and metastasis (Gupta et al. 2001; Caporali et al. 2004). Similar to other cancer models, the pro-proliferation signalings (i.e. phospho-Akt and phospho-ERK1/2) are reduced in the prostate cancer of TRAMP model (Adhami et al. 2004). In this model, IGF-1 is reduced and IGFBP3 is increased (Adhami et al. 2004), which suggest that green tea polyphenols block IGF-1 signaling. However, these studies may not be sufficient to support the inhibitory activity of green tea polyphenols in prostate carcinogenesis. Prostate cancer developed in TRAMP model is androgen-independent and most of them are endocrine origin, whereas human prostate cancer is epithelial cell origin and hormone dependence in the earlier stage (Shen and Abate-Shen 2010). Thus, a proper model is necessary for addressing these issues for determining the effectiveness of the inhibition on prostate carcinogenesis.

4.2.1.4 Prevention of Breast Cancer by Green Tea Polyphenols

The studies of the anticancer activity of green tea polyphenols in mammary cancer in animal models are found to be somewhat inconsistent (Ju et al. 2007; Yang et al. 2011). Some studies show the anticancer activity of green tea polyphenols while others suggest no effect. The overall results can be seen in three different categories: potent inhibitory activity, partial inhibitory effect on certain aspect of mammary tumor, and no effect. It has been suggested that the poor bioavailability of green tea polyphenols in mammary gland tissues might be the reason behind these differences (Yang et al. 2011). Therefore, green tea polyphenols may not be able to target mammary cancer directly. The observed anticancer activity could be an indirect effect resulted from the inhibition of green tea polyphenols on other aspects such as inflammation or oxidative stress.

4.2.1.5 Prevention of Skin Cancer by Green Tea Polyphenols

Skin cancer can be treated by topical application, which overcomes the possible poor bioavailability as proposed in mammary gland tissue described above. Green tea polyphenols can be used more frequently and at higher concentrations. As matter of fact, this approach is very effective in treating mouse skin cancer by EGCG. For instance, topical application of EGCG to the skin in SKH-1 mice treated by UVB results in the reduction of tumor incidence, multiplicity, and size (Lu et al. 2002, 2005). Interestingly, the treatment of green tea polyphenols is found

to also decrease adipose tissue in skin and the inhibition of UVB-induced skin cancer appears to be associated to the reduction of adipose tissue (Lu et al. 2001). It remains to be determined whether this phenomenon is a coincidence or an indirect inhibition of skin cancer.

4.2.2 Epidemiology Studies on the Association Between Consumption of Green Tea and Cancer Risk

Many epidemiology studies, including both cohort and case–control studies, have investigated the cancer preventive activity of green tea against different types of cancers. Most of the studies on possible inverse association between green tea consumption and cancer risk have been conducted in Asian countries such as Japan and China, where green tea is widely consumed. Based on the quality of these studies as assessed by several systematic analyses (Liu et al. 2008; Zhou et al. 2008; Boehm et al. 2009; Myung et al. 2009; Sasazuki et al. 2012), we selected 18 cohort and 28 case control studies and compiled a summary in Table 4.1. About half of these studies were focused on cancers in digestive tract, especially gastric cancer.

Among the studies on gastric cancer, the conclusion from the cohort studies except one found no association between gastric cancer risk and green tea consumption. The meta-analyses on the available data conclude that there is no sufficient evidence to support the inverse association (Liu et al. 2008; Zhou et al. 2008; Sasazuki et al. 2012). However, a recent meta-analyses on selected six cohort studies including more than 218,000 Japanese aged 40 or older and over 3,500 incident stomach cancer cases found the statistically significant, inverse association between green tea consumption and stomach cancer risk in nonsmoker women but not in men. A significantly decreased risk was observed for nonsmoker women with consumption of $\geq$5 cups/day (Inoue et al. 2009).

There are about half of the case–control studies listed in Table 4.1 supporting the inverse association between green tea consumption and gastric cancer risk. Apparently, the studies supporting the inverse association are conducted in China, while the similar studies conducted in Japan do not support the inverse association. Possible error resulted from random events can be ruled out because the sample numbers in all the related studies were sufficient. Inconsistence resulted from the tea composition can also be ruled out because it is generally accepted that the green tea consumed in Japan and China contains the same tea constituents. Perhaps, more details involving the life styles are necessary in order to understand the inconsistence between Chinese and Japanese studies.

The discrepancy can be also found from the studies on the inverse association between green tea consumption and the risk of breast, colorectal, lung, pancreatic, and prostate cancers in Table 4.1. We cannot speculate whether other factors are involved. However, the results from the studies on oral/esophageal and ovarian cancers appear to be consistent. In oral and esophageal cancers, the inverse

Table 4.1 Epidemiology studies on the association between green tea consumption and reduced risk of cancer

Cancer type	Study type	Country	Participants	Association			References
				Women	Men	All	
Breast	Cohort	Japan	488,989	No			Key et al. (1999)
		Japan	35,004	No			Suzuki et al. (2004)
		Japan	63,257	No			Inoue et al. (2008)
	Case control	USA	1,095	Yes			Wu et al. (2003)
		China	2,018	Yes			Zhang et al. (2007)
Colorectal	Cohort	Japan	65,915			No	Suzuki et al. (2005)
		China	69,710	Yes			Yang et al. (2007)
		Singapore	61,320	No	Inverse	Inverse	Sun et al. (2007)
	Case control	Japan	1,324			Yes/no[c]	Kato et al. (1990)
Gastric	Cohort	USA[a]	11,907	No	Inverse		Galanis et al. (1998)
		Japan	26,311			No	Tsubono et al. (2001)
		Japan	44,930	No	No		Fujino et al. (2002)
		Japan	72,851	No	No		Hoshiyama et al. (2002)
		Japan	65,915			No	Koizumi et al. (2003)
		Japan	72,273	Yes	No		Sasazuki et al. (2004, 2008)
	Case control	Japan	376			No	Tajima and Tominaga (1985)
		Japan	4,855			No	Kato et al. (1992)
		Japan	1,336			No	Inoue et al. (1994)
		China	1,422			Yes	Yu et al. (1995)
		China	2,575	Yes	Yes		Ji et al. (1996)
		Japan	2,991			Yes	Kono et al. (1988)
		China	816			Yes	Ye et al. (1998)
		Japan	22,834			No	Inoue et al. (1998)
		Japan	29,506			No	Huang et al. (1999)
		Japan	732			Yes	Setiawan et al. (2001)
		China	1,043			Yes	Mu et al. (2003)

Leukemia/lymphoma	Case control	China	217			Yes	Zhang et al. (2008a, b)
		China[b]	889			Yes	Kuo et al. (2009)
Lung	Case control	China	1,320	Yes			Zhong et al. (2001)
		China	244			No	Bonner et al. (2005)
Oral and esophageal	Cohort	Japan	78,950			Inverse	Ishikawa et al. (2006)
		Japan	50,221	Yes	No	Yes	Ide et al. (2007)
	Case control	China	2,454	Yes	No		Gao et al. (1994)
		China	418			Yes	Wang et al. (1999)
		China	703	Yes	No		Wang et al. (2007)
Ovarian	Case control	China	706	Yes			Zhang et al. (2002)
		USA	2,017	Yes			Song et al. (2008)
Pancreatic	Cohort	Japan	102,137			No	Luo et al. (2007)
		Japan	77,850	No	No	No	Lin et al. (2008)
	Case control	Japan	213			Yes	Goto et al. (1990)
		Japan	248			Inverse	Mizuno et al. (1992)
		China	3,818	Yes	Yes	Yes	Ji et al. (1997)
Prostate	Cohort	Japan	19,561		No		Kikuchi et al. (2006)
		Japan	49,920		Yes		Kurahashi et al. (2008)
	Case control	Japan	280		No		Sonoda et al. (2004)
		China	404		Yes		Jian et al. (2004)

[a]Only including Japanese residents in Hawaii, USA
[b]Only in Taiwan, China
[c]Yes in colon cancer but no in rectal cancer

association has been found in women, but not men in both Japan and China. Results from the two case control studies on leukemia in China are also consistent. Therefore, based on the current data, it is safe to propose that the consumption of green tea can be cancer preventive, but whether it is effective may be related to the etiology of certain cancer.

It should be pointed out again that green tea polyphenols in the subjects of these studies are from the daily consumption and can only reach ~0.1 μM in the blood. The dose of green tea polyphenols found to be effective in animal studies are at a much higher level (>1 μM in the blood). Considering that the bioavailability is a key issue, the intake level of green tea polyphenols should be documented in the future case–control and cohort studies. This can be done by indirectly monitoring the metabolites in urine based on our knowledge about polyphenol metabolism. Such data would be helpful to classify the subjects according to bioavailability levels and to rule out the possible difference in the composition of green tea when similar studies from different area or time are applied for comparison. Given that etiology factors are often related to life-style which could be very different depending on geography or culture, to collect different data for further study such as meta-analysis should involve these records or the related etiology study result. In one example described above, although several meta-analyses found no association between gastric cancer risk and green tea consumption when all studied were combined, the statistically significant inverse association is clear in nonsmoker women (Inoue et al. 2009).

4.2.3 Clinic and Intervention Studies

Limited clinical and intervention studies have been conducted to further explore the application of green tea polyphenols against cancer. In these studies, patients or healthy personals are given the higher doses of green tea polyphenols resulting in the blood levels higher than levels obtained from usual tea consumption. While most results are positive, a clear conclusion in support of anticancer effect cannot be reached. This might due to the fact that the numbers of people in these studies are often very small and the duration of treatment is short compared to that in animal studies. Here, we discuss a few studies in order to understand the opportunity for the application of green tea polyphenols in cancer prevention.

Healthy person can be benefited by the antioxidant activity of green tea polyphenols. Supplementation of green tea polyphenols (500 mg/day) in the diet of healthy persons for 4 weeks reduced oxidized low-density lipoproteins in blood by 18 %, compared to the placebo (Inami et al. 2007). When the similar dose (455 mg/day) was given to patients on haemodialysis for 3 months, plasma hydrogen peroxide, hypochlorous acid, C-reactive protein, and pro-inflammatory cytokines were significantly reduced (Hsu et al. 2007). These results support the concept that green tea polyphenols can improve the antioxidant activity in our body and prevent the damage due oxidative stress.

Some intervention studies provide suggestive evidences for the application of green tea polyphenols against cancer in high risk population. In a double-blind, placebo-controlled study, 60 volunteers with high-grade prostatic intraepithelial neoplasia (HG-PIN) were randomized to receive three capsules (200 mg of green tea polyphenols each; a total of 600 mg/day) or placebo for 1 year. One subject was diagnosed with prostate cancer among 30 men receiving green tea polyphenols (incidence = ~3 %), whereas nine cancers were found among 30 men receiving placebo (incidence = 30 %) (Bettuzzi et al. 2006). The 30 % incident rate in the placebo group was consistent with the clinical data that about 30 % HG-PIN patients develop advanced cancer. This result strongly supports that green tea polyphenols are effective in treating premalignant lesions and preventing its development to advanced tumor. A 2-year follow-up study on a subset of these 60 patients showed a promising protective effect against prostate cancer development (Brausi et al. 2008). In another study on 26 patients receiving green tea polyphenols (1.3 g/day containing 800 mg EGCG) for an average of 35 days during the interval between positive biopsies and radical prostatectomy, the application of green tea polyphenols reduced the levels of cancer-associated biomarkers such as PSA, HGF, VEGF, IGF-1 and IGF-1:IGF binding protein 3 ratio (McLarty et al. 2009). Similarly, in a phase 2 randomized trial consist of 41 patients with high-risk oral premalignant lesions (11 receiving placebo, 11 receiving 500 mg GTE/m^2, 9 receiving 750 mg GTE/m^2, and 10 receiving 1 g GTE/m^2 for 12 weeks), biomarkers such as VEGF and cyclin D1 were significantly reduced in the lesion biopsies (Tsao et al. 2009). This result is consistent with the finding in another randomized, placebo-controlled phase 2 trial that 3 g/day of green tea extract reduced the size of oral mucosa leukoplakia, a precancerous lesion, in 37.9 % patients (Li et al. 1999). In a Japan trial comprised of 136 patients with colorectal adenomas first removed by endoscopic polypectomy and confirmed the clean colon 1 year later (71 receiving with 1.5 g GTE/day for 12 months and 65 as control), the incidence of adenomas at the end-point colonoscopy was 31 % in the control group and 15 % in the GTE group (Shimizu et al. 2008a).

However, there are also studies showing negative results of green tea polyphenols against cancer. An earlier phase 2 trial on 42 patients with hormone-independent prostate cancer showed that receiving green tea polyphenols (a total 6 g/day) for a month increased PSA levels by 43 % (Jatoi et al. 2003). In a recent intervention study randomizing 50 prostate cancer patients scheduled to undergo radical prostatectomy, patients receiving 800 mg EGCG for 3–6 weeks before surgery show favorable when compared to placebo but not statistically significant changes in PSA, IGF, and oxidative DNA damage (Nguyen et al. 2012).

Taken together, the available clinical studies support the cancer preventive activity of green tea polyphenols, but these studies are rather preliminary since the size of the trials is small and the long-term effect is unknown. Albeit, green tea supplement already became the most commonly used product for self-treating breast cancer survivors in Canada (Boon et al. 2007). Now, various green tea

products are available over counter and self-treatment may run ahead of our knowledge. The opportunity is on the horizon, but the challenge is how to better design and conduct further clinical study to clearly address whether green tea polyphenols are applicable against cancer. A larger-scale study such as phase 3 trial is necessary to determine the efficacy. Given the fact that we know more about the metabolism of green tea polyphenols, the intake level should be monitored by examining the urine samples frequently. More, future clinical study should integrate the experimental study to apply biomarkers to determine the short-term responsiveness in addition to long-term effect. Furthermore, we suggest to preserve blood, urine, and tissue samples for "-omic" studies. The genomic and proteomic studies using these materials will provide data for understanding the molecular mechanism in-depth. These materials will also be useful to better categorize cancers by the genetic or epigenetic features, the so-called molecular pathological characteristics. Although personalized medicine has not been established in this field, the responsiveness could be possibly associated to specific subtype of cancer if the "-omic" data is available. Based on our knowledge from experimental studies, it is anticipated that a well-designed phase 3 clinical study could determine the efficacy of green tea polyphenols in cancer prevention and clarify whether its application depends on the types or stages of cancer.

4.3 Mechanisms of Tea Polyphenols in Cancer Prevention

To better understand the cancer preventive activity of green tea polyphenols found in animal studies and to promote them for human cancer prevention, substantial studies have been conducted to uncover the mechanism at the cellular and molecular levels. Experimental results collectively show that the treatment of animal cancer models or cancer cells *in vitro* with green tea polyphenols leads to wide range of responses. It has been reported that green tea polyphenols enhance detoxification to prevent the carcinogen-induced genetic and epigenetic damage (Na and Surh 2006, 2008; Chow et al. 2007), alter epigenetic modification on chromosome such as reducing DNA hypermethylation-associated tumor suppressor silence (Fang et al. 2003; Navarro-Peran et al. 2005; Gao et al. 2009; Choudhury et al. 2011; Nandakumar et al. 2011; Wong et al. 2011), inhibit tumor cell growth by inducing cell cycle arrest and apoptosis (Yang et al. 2009b; Singh et al. 2011), decrease inflammation (Hong et al. 2001; Na and Surh 2006; Pan et al. 2011), and inhibit tumor-associated angiogenesis (Noonan et al. 2007; Yang et al. 2009b; Singh et al. 2011). These activities are consequences of the direct scavenge of ROS and the physical interactions on proteins with various functions. The cancer preventive activity could be resulted from a combinatory effects on multiple targets. The actions of green tea polyphenols mediated through different mechanisms directly lead to the

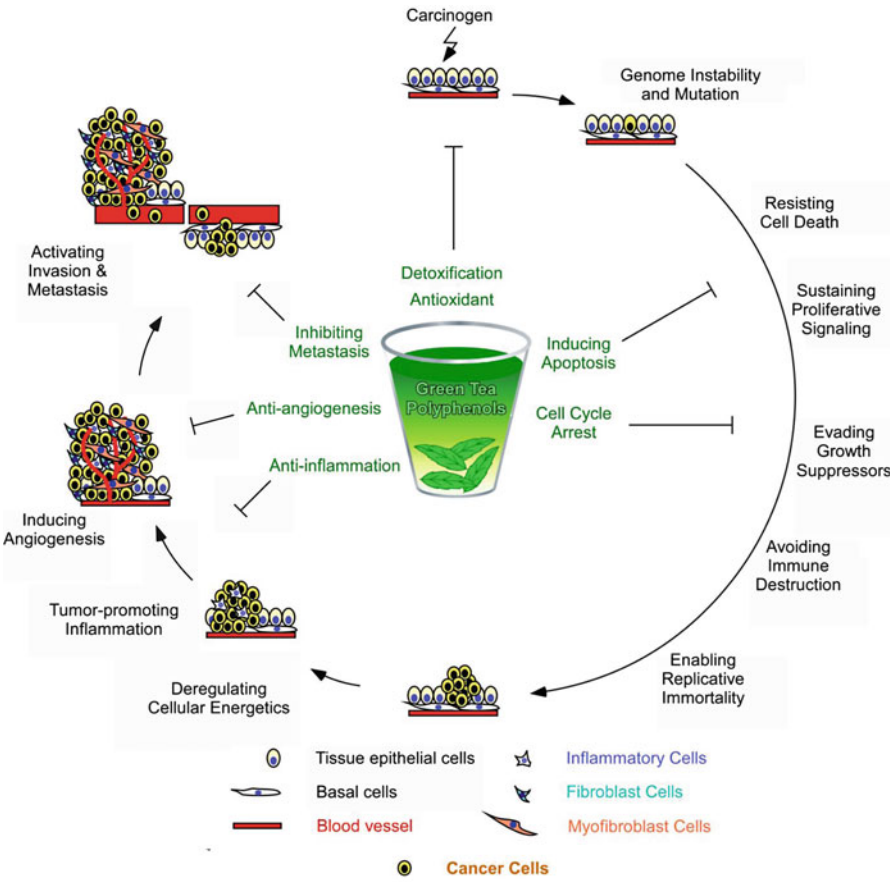

Fig. 4.3 Green tea polyphenols display inhibitory activity on multiple cancer hallmarks

inhibitions on several aspects in carcinogenesis (Fig. 4.3). These aspects are part of the key elements promoting human cancer and also referred to as cancer hallmarks (Hanahan and Weinberg 2011). Compared with the inhibitors designed for targeting specific hallmarks, green tea polyphenols are not potent inhibitors. But, perhaps, it is the inhibitions on multiple hallmarks that lead to the overall anticancer activity of green tea polyphenols albeit most of these inhibitory actions are weak (Fig. 4.3). It is also possible that a specific event/hallmark targeted by EGCG plays dominant role in a specific cancer. To associate any cellular and molecular mechanism to the anticancer activity of EGCG and its application should be carefully evaluated by its effective concentration at the levels compatible to the achievable blood levels in human. In the following, we will discuss the mechanism generally accepted in this field.

4.3.1 Antioxidant Activity

Green tea polyphenols are very sensitive to oxidation reaction. For decades, the antioxidant activity is believed to be the major biological activity of tea polyphenols (Yang et al. 2009a, b; Singh et al. 2011). For example, supplementation of green tea polyphenols (500 mg/day) in diet for healthy individuals for 4 weeks reduces oxidized low-density lipoproteins in blood by 18 % (Inami et al. 2007). In the experimental model, administration of EGCG to aging rats decreases the aging associated oxidative stress and lipid and protein damages (Senthil Kumaran et al. 2008; Srividhya et al. 2008). The similar effect could also protect cells from oxidative DNA damage. It has been reported that the supplement of four cups of green tea with 73.5 mg polyphenols to heavy smokers for 4 months reduced the urinary level of 8-hydroxydeoxy-2′-deoxyguanosine (8-OHdG) by 31 % (Schwartz et al. 2005).

In addition, the indirect antioxidant mechanism, which includes the induction of antioxidant enzymes (i.e. catalase, SOD, etc.) and phase 2 conjugating enzymes (i.e. glutathione-*S*-transferases, glucuronidase, and sulphotransferases), has been proposed for green tea polyphenols (Na and Surh 2008). In a trial with 42 volunteers receiving green tea extract containing 800 mg/day for 4 weeks, glutathione-*S*-transferases activity and glutathione-*S*-transferases-pi (GSTP1) level in blood lymphocytes were increased significantly in individuals with low baseline enzyme activity/level (Chow et al. 2007). Since some of these enzymes are regulated by transcriptional regulator Nrf2, which is responsive to cellular reactive oxygen level, it has been suggested that EGCG enhances Nrf2 activity (Na and Surh 2008). However, this issue remains to be further clarified. EGCG generates ROS *via* auto-oxidation reaction, as discussed above (Fig. 4.2). It is possible that Nrf2 as the ROS sensor is activated by ROS generated by EGCG. In fact, we have found Nrf2 activity is upregulated in lung cancer cell H1299 carrying Nrf2 binding site-luciferase reporter treated by EGCG, but such an upregulation is abolished when catalase and SOD are added to culture medium. Therefore, it is unlikely that the antioxidant activity of green tea polyphenols involves Nrf2. Whether the indirect antioxidant mechanism is activated through other mechanism remains to be determined.

4.3.2 Direct Binding to Proteins

One major mechanism of the cancer preventive activity of green tea polyphenols is attributed to binding to proteins. Phenolic groups, for example in EGCG, offer hydrogen bond donor to mediate interactions with other molecules. To date, EGCG has been demonstrated to bind physically to a panel of proteins with different affinities. These proteins are featured with varieties of functions involved in cellular signaling, proliferation, apoptosis, structure, and etc. (Singh et al. 2011; Yang and

Wang 2011). They underline the involvement of multiple mechanisms, but make it hard to recognize the important one. Sometime, high concentration of EGCG (i.e. ~100 μM) is needed to validate the influence of polyphenols on the functions of these proteins in cells. Such high concentrations raise questions about the physiological relevance of these targets. Indeed, to what degree the binding proteins contributing to the *in vivo* anticancer activity remains to be determined. Here, we briefly review the proteins with the high-affinity binding EGCG.

Using EGCG-sepharose 4B column, Dong and colleagues have identified a group of proteins that include intermediate filament vimentin, non-receptor tyrosine kinases Fyn and ZAP70, cell signaling regulators GRP78, and Ras-GTPase-activating protein SH3 domain-binding protein 1 (Ermakova et al. 2005, 2006; He et al. 2008; Shim et al. 2008, 2010). Since the binding affinities for these proteins range from 3.3 to 0.7 μM, they are generally considered as the high-affinity binding proteins. Among them, EGCG binds vimentin with $K_d = 3.3$ nM. However, the role of EGCG binding remains unclear since there is no phenotypic change found in mice with vimentin null mutation (Colucci-Guyon et al. 1994). EGCG binding to Fyn and ZAP70 results in the loss of kinase activity of Fyn and ZAP70. But the inhibition of Fyn and ZAP70 are unlikely to directly mediate the cancer preventive activity of EGCG because these two protein kinases are hematopoietic-cell specific. On the other hand, SH2 domains of Fyn and ZAP70 are the physical binding sites for EGCG, suggesting that EGCG may target other SH2 domain proteins expressed in cancer cells, a possibility that should be explored further. EGCG was also reported to bind and inhibit IGF-1R but with $K_d = 14$ μM (Li et al. 2007). Although the K_d is higher, it is consistent with the findings of IGF signaling inhibition by EGCG administrated in a colon carcinogenesis model (Shimizu et al. 2008b) and a liver carcinogenesis (Shimizu et al. 2011) in db/db mice and a prostate carcinogenesis in TRAMP mice (Gupta et al. 2001). Thus, both *in vitro* and *in vivo* experimental studies support that IGF signaling is an EGCG target.

A recent important finding is the interaction of EGCG with peptidyl prolyl *cis/trans* isomerase (Pin1). Dong and colleagues demonstrated the physical interaction by X-ray crystal structure of EGCG-Pin1 complex at 1.9 Å resolution (Urusova et al. 2011). This interaction inhibits isomerase activity of Pin1 by preventing the access of its catalytic domain to the substrates (Urusova et al. 2011). Since NF-κB and the AP-1 member c-Jun are Pin1 substrates, this result suggests how EGCG indirectly regulates AP-1 and NF-κB activities, which are critical regulators in both cancer and inflammatory cells. Furthermore, this mechanism is demonstrated to be the mechanism, at least partially, for EGCG to inhibit the growth of colon cancer cells in a xenograft model (Urusova et al. 2011).

Another new finding revealed recently is that EGCG binds and stabilizes HIF-1α and upregulates *miR-210* expression (Wang et al. 2011). There are several studies on the regulation of EGCG on HIF-1α but whether EGCG downregulates or upregulates HIF-1α activity remains controversial. By microRNA expression profile analysis of lung cancer cells treated by EGCG, *miR-210* is found to be the only microRNA upregulated by EGCG treatment (Wang et al. 2011). Further study reveals that EGCG is likely to bind the key Proline residues in the

oxygen-dependent regulatory domain of HIF-1α and prevent the modification of Proline and subsequent proteasome-mediated degradation. Since *miR-210* displays suppressor activity in tumor initiation, presumably by regulating the expressions of more than 50 genes (Huang et al. 2009), the upregulation of *miR-210* provides an additional mechanism for EGCG to target multiple genes indirectly. However, whether this is the case *in vivo* needs to be further investigated in the animal model.

Besides the above mentioned targets, EGCG has been reported to bind other proteins and affect their functions. Some of them, such as 67-LR, glucose-6-phosphate dehydrogenase and HGF receptor/c-met, also provide the mechanism for EGCG to interfere with the cellular signaling, metabolism, and inflammatory regulation (Yang and Wang 2011). In addition, EGCG is found to alter plasma membrane through affecting the protein distribution and function in lipid raft, resulting in indirect influence on the activity of EGFR (Adachi et al. 2007), c-Met (Duhon et al. 2010), and 67-LR (Fujimura et al. 2005) at relatively lower concentrations in different cancer cell lines. Therefore, EGCG can display activities on different pathways through these proteins. A combinatory effect of these activities may lead to inhibition of cancer initiation, progression or metastasis (Pan et al. 2011; Singh et al. 2011; Yang and Wang 2011).

4.3.3 Induction of Cell Cycle Arrest and Apoptosis

The anticancer activity resulted from the treatment of green tea polyphenols has been found to be associated to the reduced cell proliferation and increased apoptosis (Yang et al. 2009b; Singh et al. 2011). For example, in the mouse lung cancer models, treatment of green tea polyphenols leads to the significantly reduction of cell proliferation marker Ki-67, pro-proliferation signaling such as the phosphorylation of Akt and ERK1/2, and the increase of cleaved caspase-3, an apoptosis index (Lu et al. 2006a; Li et al. 2010). In various cancer cells cultured *in vitro*, EGCG has been reported to trigger cell cycle arrest by modulating the levels of cyclin D1, cdk4, cdk6, p21/WAF1/CIP1, and p27/KIP1 as well as p53, and induce apoptosis by increasing levels of pro-apoptotic regulators, Bax, Bak, Bcl-XS, and PUMA, and decreasing levels of anti-apoptotic regulators, Bcl-2 and Bcl-XL (Yang et al. 2009b; Singh et al. 2011). Cell cycle arrest and induction of apoptosis are likely to be resulted from the actions of green tea polyphenols on the targets such as the inhibition on the activities of NF-κB, Ap-1 transcriptional factors, Akt and MAP kinases (Yang et al. 2009b). More, they could be combinatory effects resulted from multiple upstream events targeted by green tea polyphenols.

4.3.4 Anti-angiogenesis

The anticancer activity of green tea polyphenols also involves the inhibition on the growth of tumor-associated blood vessels. Such an anti-angiogenesis activity has been reported to be mediated by the downregulation of VEGF in cancer cells or by direct inhibition on endothelial cells. When cancer cell lines (HeLa, HepG2, and SW837) were treated with EGCG, the hypoxia-induced stabilization of HIF-1α and upregulated expression of VEGF were reduced (Zhang et al. 2006; Shimizu et al. 2010). However, these data are controversial to the results supporting that EGCG can direct bind and stabilize HIF-1α (Thomas and Kim 2005; Weinreb et al. 2007; Wang et al. 2011). Our expression profiles on the EGCG-treated lung cancer H460 cells also show that treatment with EGCG upregulates VEGF and other HIF-1α targets (unpublished data). Thus the anti-angiogenesis *via* the downregulation of VEGF by EGCG may depend on the cells or cell culture conditions. When endothelial cells such as HUVECs were treated with EGCG, the activity of FOXO was upregulated, resulting in the reduced HUVEC migration and capillary tube formation (Shankar et al. 2008), suggesting the direct inhibitory activity of EGCG on endothelial cells. This result is consistent with the finding that EGCG enhances the phosphorylation and phosphorylation-dependent transcriptional activity of FOXO at lower concentration (i.e. 1 μM) (Anton et al. 2007; Bartholome et al. 2010). Thus, the anticancer activity of green tea polyphenols in animal models involves the anti-angiogenesis via directly modulating the activity of FOXO in endothelial cells.

4.3.5 Other Potential Mechanisms

In addition to above discussed mechanisms, experimental results suggest the involvement of other mechanisms such as anti-inflammation and anti-metastasis. The inhibition on NF-κB and AP-1 transcriptional factors supports that green tea polyphenols play regulatory roles in inflammatory response (Singh et al. 2011). For example, EGCG reduces the virus-induced inflammation mediated through quenching the RONS which activates NF-κB (Lee et al. 2004). Inhibitions on AP-1 transcriptional factors are the downstream events of the inhibition on PI3K/Akt and MAP kinases (Singh et al. 2011). More, as discussed above, the negative regulation on NF-κB and AP-1 factors could be mediated through the inhibition on Pin1 by EGCG (Urusova et al. 2011). Green tea polyphenols are also reported to be effective on inhibiting tumor metastasis in the mouse model using Lewis lung carcinoma cells, which mimic lung metastasis after injected through tail veins. The total number of tumor colonies in lung, an index of metastatic Lewis lung carcinoma cells, has been found reduced significantly by oral administration of green tea polyphenols (Sazuka et al. 1995). In an *in vitro* assay to measure the metastasis potentials of cancer cells, treatment with EGCG reduces the invasive

characteristics of B16 melanoma cells (Watanabe et al. 2012), which might be associated to the reduced matrix metalloproteinases in cancer cells after EGCG treatment (Deng and Lin 2011). In addition to these mechanisms discussed, it can be expected that further in-depth studies on each of these specific directions will uncover more details of the action of green tea polyphenols in cancer prevention. Nevertheless, experimental results support that the anticancer activity of green tea polyphenols is mediated through multiple mechanisms.

4.4 Prospective

In summary, preclinical studies using animal models, molecular and cellular approaches demonstrate the anticancer activities of green tea polyphenols. In addition, limited clinical studies support the cancer preventive effect, despite the mixed results yielded from epidemiology studies. Our understanding of the roles of green tea polyphenols remains incomplete. Further clinical study should integrate new advancements and technologies to systematically monitor the intake, blood and urine levels of green tea polyphenols and metabolites in subjects as well as to determine the short-term responsiveness with biomarkers and correlate it to disease causes, progression, and other aspects. Since the "-omic" technologies are ready for better characterization of individual case based on its genetic and epigenetic background, the tissue sample should be ensured and properly preserved for future extraction of DNA/RNA/protein for -omic analyses.

Furthermore, the combination of green tea polyphenols with other agents or medicines also provides an opportunity to explore the potentials for more effective prevention or treatment. In NNK-induced A/J mouse lung carcinogenesis model, green tea polyphenols display significant inhibition on adenoma progression to adenocarcinoma but not on the induction of adenoma (Lu et al. 2006a). When 0.25 % green tea polyphenols was used with 200 ppm atorvastatin (trade name Lipitor) to treat NNK-induced A/J mouse, the tumor multiplicity was reduced by 56 % and the tumor burden was reduced by 55 % at 20 weeks after NNK-treatment (Lu et al. 2008). When 0.25 % GTE or 200 ppm atorvastatin was used alone, there was no effect significant effect on tumor multiplicity at this stage. Higher dose, 0.5 % GTE or 400 ppm atorvastatin, reduced the tumor burden by 22 % compared with the NNK control group, but no effect on tumor multiplicity (Lu et al. 2008). This work strongly suggests the synergistic effect of polyphenols and atorvastatin on cancer prevention. Other preclinical studies support the synergy of EGCG with other agents, such as taxane (Stearns and Wang 2011), curcumin (Yunos et al. 2011), COX-2 inhibitors (Suganuma et al. 2011), doxorubicin (Stearns et al. 2010), luteolin (Amin et al. 2010), erlotinib (Amin et al. 2009), and sulforaphane (Nair et al. 2008). An exciting advance reported recently is that the combination of EGCG with phosphodiesterase 5 inhibitor (i.e. Vardenafil) significantly potentiates the EGCG induced apoptosis of cancer cells expressing high level of 67-LR (Kumazoe et al. 2013). Low dose of EGCG (i.e. 1 μM) which can be reached in plasma is

effective to induce cancer cell death in animal model when administrated with Vardenafil. These combinations effectively inhibit tumor growth in animal model or cancer cell *in vitro* presumably by targeting different aspects of tumor simultaneously, multiple components in one pathways, or same protein through different mechanisms. Such applications show advantages to overcome several limitations of one agent, which is consistent with the concept to treat cancer with multiple agents to improve the effectiveness, to reduce the side effect or toxicity, and to reduce the possibility of drug resistance (Glickman and Sawyers 2012). For example, the synergistic effect of EGCG and taxane can reduce the toxicity of taxane by using lower dose of taxane (Stearns and Wang 2011). More, the combination of EGCG and erlotinib could be more potent in inhibiting lung cancer with EGFR mutations but also make drug resistance resulted from addition EGFR mutation much less possible by simultaneously targeting EGFR with different mechanisms (Amin et al. 2009). These findings will need to be further investigated to uncover the mechanisms and be validated in both animal and clinical studies. Nevertheless, it opens a new page for the potential application of green tea polyphenols against cancer.

Acknowledgment This work was supported by NIH grants CA120915, CA122474, and CA133021.

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